Bioregulation in Gastrointestinal Tract – Mechanism of Maintaining Mucosal Integrity
T. Arakawa
Professor and Chairman, Department of Gastroenterology, Osaka City University Postgraduate School of Medicine

Peptic ulcer disease (PUD) is a most common disease of the stomach and duodenum, which arises under the disturbance of bioregulation. Most of such ulcers recur spontaneously after healing. This may be related to the concept of quality of ulcer healing (QOUH) as shown by pathological illness of regenerated mucosa of healed experimental ulcer (Tarnawski A et al. J Clin Gastroenterol 1991). In clinical studies, we found that two components impair QOUH. One is the decrease in mucosal prostaglandins (PGs) and the other is Helicobacter pylori infection (Arakawa T et al. Eur J Gastroenterol Hepatol 1993). Reason why the eradication of H. pylori much contributing to decrease in recurrence of PUD may be due to improvement of QOUH. Some gastroprotective drugs such as reboxipide, prostaglandin inducer, also improve QOUH and decrease in future recurrence of ulcer (Higuchi K, Arakawa T et al. Dig Dis Sci 1998). In an experimental model we have proven that low dose of indomethacin during ulcer healing increase in incidence of future recurrence of gastric ulcer, which is completely reversed by co-administration of PG analogue (Arakawa T et al. Dig Dis Sci 1996). The common phenomenon of these two component impairing QOUH is increment of inflammatory cells in interstitial space beneath ulcer scar area. Therefore, the inflammation at the ulcer scar site may play a key role in future ulcer recurrence. We have found that interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) induce the recurrence of ulcer within 48 h after the injection in an chronic ulcer model (Watanabe T, Arakawa T et al. Am J Pathol 1997. Watanabe T, Arakawa T et al. Am J Physiol 2004). As the initial event after the injection of IL-1β or TNF-α, massive infiltration with macrophages occurs at the ulcer scar area, since it is induced by the mRNA expression of monocytic chemotactic protein (MCP)-1, a C-C chemokine. This is followed by infiltration with neutrophils attracted by cytokine-induced neutrophil chemoattractant (CINC)-2α and MIP-2, C-X-C chemokines. Anti-neutrophil antiseraum prevents recurrence of ulcer. The only abnormality present at the ulcer scar site before injection of IL-1β or TNF-α was the increase in number of resident macrophages. IL-1β and TNF-α may stimulate resident macrophages at the scar site, and these activated macrophages produce MCP-1, which attracts other macrophages. These macrophages acting in concert generate large amounts of cytokines and chemokines, which further promote inflammation. All these inflammatory mediators result in a massive accumulation of neutrophils, which may cause the recurrence of ulcer by injuring the regenerated tissue and destabilizing scar. This cytokine network is also activated in NSAIDs-induced gastric injury (Watanabe T, Arakawa T et al. Am J Physiol 2004), suggesting that inflammatory cytokines are a key mediator of gastric ulceration. Gastric acid plays a permissive role in inducing ulcer recurrence via maintaining or enhancing inflammatory responses, because PPs inhibit ulcer recurrence induced by cytokines along with decrease in expression of inflammatory mediators (Watanabe T, Arakawa T et al. Am J Physiol 2004). Acid also plays a crucial role in the pathogenesis of gastro-esophageal reflux disease. It can increase expression of genes encoding cytokines or TNF-α and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in the ulcerated tissue (Watanabe T, Arakawa T et al. Am J Physiol 2004). Since NOS inhibitor prevented such COX-2 thus exerts dual actions to gastro-intestinal tract in inflammatory conditions. Recent advance in gastrointestinal endoscopy including capsule endoscopy and double-balloon endoscopy enables us to examine the entire small intestine. Studies using these endoscopies have identified that small intestinal lesions are not rare, and that NSAIDs are, to some extent, causally involved in intestinal ulcers. Pretreatment with antibiotics prevents NSAIDs-induced small intestinal injury in rats and mice, suggesting involvement of enteric bacteria in the pathogenesis of this injury. Innate immunity is the first line of defense against pathogens and is initiated rapidly after recognition of microbial products by receptors such as the Toll-like receptors (TLRs). Among them, TLR4 is a signaling receptor for lipopolysaccharide (LPS). Upon activation by LPS recognition, TLR4 signals activate the nuclear factor-κB (NF-κB) pathway, a critical regulator of many proinflammatory genes. Expression of TLR4, cytokines, and inducible nitric oxide synthase (iNOS), which were mainly detected on macrophages in the small intestinal epithelium, was increased during development of indomethacin-induced injury in rats. Pretreatment with LPS inhibited the damage, accompanied by a decrease in the expression of TLR4. Furthermore, the intestinal damage was almost completely inhibited in TLR4−/− mice, accompanied by decrease in expression of cytokines and iNOS. Probiotic Lactobacillus casei strain Shirota inhibited the damage without affecting TLR4 expression, probably through inhibition of NFκB pathway. Molecules involved in inflammation such as MCP-1, NFκB, or TLR4 may be new possible targets for treatment of gastrointestinal ulcer disease.

Historical Notes on the Acetic Acid Ulcer Models
S. Okabe, K. Amagase
Department of Pharmacology, Donkai Women’s College, Kyotanabe, Department of Applied Pharmacology, Kyoto Pharmaceutical University, Yamashina, Kyoto, Japan

Four types of acetic acid ulcer, a chronic ulcer model, were devised to examine the ulcer healing process, screening anti- ulcer drugs and adverse effects of various anti-inflammatory drugs (steroidal and non-steroidal drugs). Due to its easiness and consistence for inducing round and deep ulcers in the stomach, duodenum and esophagus, acetic acid ulcer are produced in mice, Mongolian gerbils, rats, guinea pigs, cats, dogs and miniature pigs. The models have frequently been used by basic and clinical scientists nearly 40 years in the world. These ulcer models highly resemble human ulcers in terms of both the pathology and the healing process. Characteristic feature of acetic acid ulcer is that endoscopical observation for more than 1 year revealed that once healed ulcers spontaneously relapse without any treatment. Indomethacin significantly delays the healing of acetic acid ulcers, probably by the reduction of endogenous prostaglandins and inhibition of angiogenesis in the ulcerated tissue. In Mongolian gerbils, Helicobacter pylori significantly delayed the healing of acetic acid ulcers and caused relapse of the healed ulcers at high incidence. Antisecretory drugs (gastric proton pump inhibitors, histamine H_{2}-blockers, prostaglandins, and mucosal defense drugs (sucralfate), epidermal growth factor, β-fibroblast growth factor, vascular endothelial growth factor significantly enhanced the healing of acetic acid ulcers. Gene therapy with epidermal growth factor applied to acetic acid ulcers in rats was found to be effective to enhance ulcer healing. Since NOS inhibitor prevented the ulcer healing, NO might be involved in the mechanism underlying ulcer healing. We conclude that acetic acid ulcer models are quite useful ulcer models for the various studies related to gastro-duodenal ulcers.
Gastric ulcer healing is a complex process involving inflammation, re-epithelialization, formation of granulation tissue, angiogenesis, interactions between various cells and matrix, remodeling and scar formation [1]. All these processes are regulated by the cytokines and growth factors acting through respective signaling pathways. In the ulcer margin the predominant players are EGF, HGF, COX2, which activate EGF receptor, MAPK, PI3K/Akt, Rho/Rac, tension and focal adhesion kinase (FAK) signaling pathways. These events trigger and promote cell proliferation, migration, and re-epithelialization and gastric gland reconstruction within the scar. Our recent study [2] indicates that serum response factor (SRF) that regulates transcription of immediate-early gene and muscle genes, plays a major role in gastric ulcer healing. Gastric ulceration triggers activation of SRF gene, which promotes migration and proliferation of epithelial and smooth muscle cells essential for re-epithelialization and restoration of muscular structures [2]. Other important factor playing a permissive role in ulcer healing is surviving [3, 4]. It promotes cell proliferation and inhibits apoptosis [3, 4]. Our most recent data indicate that IGF-1 plays an important role in ulcer healing [5]. Gastric ulceration activates IGF-1 gene expression in epithelial cells of the ulcer margin and in turn IGF-1 activates Cox2 expression, actin polymerization, epithelial cell migration and proliferation mainly through the PI3K and partly through MAPK signaling pathways [5]. G-protein to G-protein activation FAK, SCR, tension and Rho/Rac signaling are crucial for re-epithelialization [6, 7]. Gastric or esophageal ulceration also induce VEGF and its receptor (VEGFR2) in granulation tissue and in epithelial cells of ulcer margin via hypoxia inducible factor 1α (HIF-1α) [8]. VEGF and angiopoietin-2 activate angiogenesis, which is essential for the restoration of microcirculscule and delivery of oxygen and nutrients to the healing site. Our recent data showed that SRF is a critical requirement for VEGF signaling in endothelial cells and VEGF-induced angiogenesis, and that VEGF activates VEGFR expression in endothelial cells via MAPK and Rho signaling [5]. Angiogenesis in granulation tissue is initiated by endothelial cells of preexisting vessels, but recent studies indicate that incorporation of bone marrow-derived endothelial precursor cells may also contribute to angiogenesis and complement the sprouting angiogenesis [10]. Bone marrow derived stem and progenitor cells also contribute to epithelial and connective cells restoration during ulcer healing [11, 12]. TGFβ plays important role in remodeling phase of ulcer healing [13]. Activation of particular growth factor and cytokine genes occurs in a well orchestrated, sequential and orderly manner with some overlap. Some of the growth factors affect not only mesenchymal but also epithelial cells, thus providing coordination for local paracrine interactions between epithelial and mesenchymal components of ulcer healing, and also serve as a back-up system. Different events occurring at different time trigger the release of chemical signal that modulate the cell migration, proliferation, and differentiation of cells and the synthesis and degradation of ECM proteins in orderly and complementary manner. These proteins, in turn, directly affect cellular events and modulate cell responsiveness to growth factors. The quality of ulcer scar is determined by proper interactions between above growth factors, cytokines and mesenchymal and epithelial cells. Esophageal ulcer healing follows the same general pattern of healing as gastric ulcers, but in addition IGF, CREB and EP2 receptors appear to play more important roles [14, 15].

References

JS-2
Bone Marrow Cell Transfer as a New Modality for Ulcer Healing
T. Nichida1, S. Tsuji1, H. Iijima1, H. Murata1, N. Hayashi2, S. Kawanaka1
1Departments of Gastroenterology and Hepatology, 2Clinical Laboratory Sciences, Osaka University Graduate School of Medicine

Background and Aim: Bone marrow (BM) has been found to be a source of multi-potent stem cells that could distribute into various organs including gut. We have reported that BM-derived adult cells have a potential to differentiate to myofibroblasts. However, therapeutic potential of BM-derived cells in intractable ulcers has not yet demonstrated. The aim of this study was whether BM cells could promote ulcer healing in mice. Methods: BM cells and gastric myofibroblasts were prepared from GFP transgenic mice. BM-cells were incubated in dishes and the cells attaching the dishes were collected and designated as BM cells. Gastric ulcers were induced by serosal application of acetic acid on anterior wall of the stomach in C57/B6 mice. Then, the ulcers were implanted with cultured BM cells (4 × 106 cells), and PBS as the control in the serosal surface where gastric ulcers were implanted. Results: Healing of the acetic acid ulcer was significantly promoted by injection of cultured BM cells than the control on day 7, but not on day 3. The products of the long and short diameter of ulcer area were representative as ulcer index. The origin and phenotypes of implanted cells were examined by fluorescence immunochemistry for GFP, α-smooth muscle actin (α-SMA), vimentin, and other surface marker. Results: Healing of the acetic acid ulcer was significantly promoted by injection of cultured BM cells than the control on day 7, but not on day 3 (ulcer index 4 ± 5 vs. 11 ± 2 ± 8 mm2, p = 0.0140). In healing ulcers, GFP + cultured BM cells were observed in submucosal layers. These cells were positive for α-SMA, GFP + have spindle shape, and considered to be differentiated mesenchymal cells. GFP + stromal cells were not seen in ulcerated area in mice transplanted with myofibroblasts. Conclusions: Cultured BM cells could be a new source of mesenchymal cells that could differentiate mesenchymal cells to myofibroblasts.
Ghrelin produced in the gastric mucosa and orexin-A (OX-A) presented in enteric nerves of GI tract and endocrine cells, stimulate food intake but their role in gastric mucosal defense and ulcer healing remains unknown. We compared the effect of ghrelin and OX-A applied intraperitoneally (i.p.) or intracerebroventricularly (i.c.v) on gastric mucosal lesions induced by 75% ethanol or 30 min of ischemia followed by 3 h of reperfusion and in the healing of acetic acid gastric ulcers without or with 1) inhibition of NO-synthase by L-NNA (20 mg/kg i.p.); 2) cutting of vagal nerves (bilateral vagotomy) and 3) blockade of sensory nerves by capsaicin acid gastric ulcers without or with 1) inhibition of NO-synthase by L-NNA (20 mg/kg i.p.); 2) intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) on gastric mucosal lesions induced by ethanol or I/R produced typical gastric erosions accompanied by the rise in plasma ghrelin levels and gastric mucosal expression of ghrelin mRNA. Ghrelin and OX-A (1-60 mg/kg i.p.) or (100-2,500 mg/kg i.v.) dose-dependently attenuated gastric ulcers induced by ethanol or I/R while raising plasma ghrelin and OX-A levels, GFB and luminal NOx concentration. Daily administration of both hormones (20 μg/kg/day i.p.) accelerated ulcer healing, increased GFB at ulcer margin and mucosal expression of CGRP. The protective and hyperemic effects of ghrelin and OX-A were abolished by vagotomy and significantly attenuated by L-NNA treatment and capsaicin denervation. The addition to exogenous CGRP (100 μg/kg s.c.) or L-arginine (200 mg/kg i.g.) to ghrelin and OX-A in capsaicin-dener- vated or L-NNA-treated animals restored the protective and hyperemic effects of OX-A against ethanol and I/R-induced gastric injury. We conclude that orexigenic peptides exert a potent gas- troprotective and ulcer healing actions via mechanism involving an activation of brain-gut axis and hyperemia mediated by NO and sensory neuropeptides.

**JS-3**

**Orexigenic Hormons in the Mechanism of Gastroprotection and Ulcer Healing. Role of Brain-Gut Axis**


Department of Physiology Jagiellonian University Medical College, Cracow, Poland; 1Department of Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany

**JS-4**

**Experimental Study of Rebamipide on Mechanism of Gastric Ulcer Healing**

Q. Zhu, J. Li, H. Cao

Department of Gastroenterology, Rui Jin Hospital, Shanghai Jiao Tong University Medical College

**Aim:** To investigate the mechanism of gastric ulcer healing, and to evaluate the effect of Rebamipide and its combination on functional re-establishment of gastric mucosa. **Methods:** Forty Sprague-Dawley rats were divided randomly into 4 groups with 10 in each group. gastric ulcer model was induced by exposing the acetic acid on serosa of rats stomach. Rebamipide 60mg/kg/day (group 1), Omeprazol 40mg/kg/day (group 2), Rebamipide 60mg/kg/day and Omeprazol 10mg/kg/day combination (group 3) as well as Saline 2ml/day (group 4) were adminis- trated for 7 days, then all rats were sacrificed for further investigation. Size and focial gastric layer structure of the ulcer were understood in vitro under miniature probe ultrasonography (Fr 20MHz), corresponding mucosal sections were prepared for histo-pathological examination, meanwhile levels of IL-8, PGE2 and MDA were evaluated by ELISA and TBA method. **Results:** Comparing to control group, the maximal diameter of ulcer in Rebamipide group, Omeprazol group and combination group were significantly reduced (P < 0.05). In Rebamipide and its combination group, ultrasonography revealed that layer echo structure of gastric wall was partially rebuilt; histo-pathologically, degree of ulcer range, inflammatory infiltration were less important comparing with control group. Meanwhile, levels of MDA and IL-8 in corresponding mucosal sections were significantly decreased, while PGE2 level was significantly increased in each of the treatment group. In addition, IL-8 level was most significantly decreased and PGE2 level was most significantly increased in combination group; however, there was no significant difference between Rebamipide group and Omeprazol group. **Conclusion:** The present study indicated a close relationship between mucosal function and healing quality of gastric ulcer, the build-up of gastric mucosal function influenced QOUH directly, and in some degree was reflected indicated a close relationship between mucosal function and healing quality of gastric ulcer, the cutting of vagal nerves (bilateral vagotomy) and blockade of sensory nerves by capsaicin acid gastric ulcers without or with 1) inhibition of NO-synthase by L-NNA (20 mg/kg i.p.); 2) cutting of vagal nerves (bilateral vagotomy) and 3) blockade of sensory nerves by capsaicin (125 mg/kg s.c.). The area of gastric lesions was measured by planimetry, the gastric blood flow (GFB) was determined by H2-gas clearance technique and gastric content was collected for measure- ment of luminal NOx levels. Exposure to ethanol or I/R produced typical gastric erosions accompanied by the rise in plasma ghrelin levels and gastric mucosal expression of ghrelin mRNA. Ghrelin and OX-A (1-60 mg/kg i.p.) or (100-2,500 mg/kg i.v.) dose-dependently attenuated gastric ulcers induced by ethanol or I/R while raising plasma ghrelin and OX-A levels, GFB and luminal NOx concentration. Daily administration of both hormones (20 μg/kg/day i.p.) accelerated ulcer healing, increased GFB at ulcer margin and mucosal expression of CGRP. The protective and hyperemic effects of ghrelin and OX-A were abolished by vagotomy and significantly attenuated by L-NNA treatment and capsaicin denervation. The addition to exogenous CGRP (100 μg/kg s.c.) or L-arginine (200 mg/kg i.g.) to ghrelin and OX-A in capsaicin-dener- vated or L-NNA-treated animals restored the protective and hyperemic effects of OX-A against ethanol and I/R-induced gastric injury. We conclude that orexigenic peptides exert a potent gas- troprotective and ulcer healing actions via mechanism involving an activation of brain-gut axis and hyperemia mediated by NO and sensory neuropeptides.

**JS-5**

**Aggravation of Non-Steroidal Anti-Inflammatory Drug (NSAID)-Induced Small Intestinal Ulceration in Adjuvant-Induced Arthritic Rats: Involvement of Increased Expression of Toll-Like Receptor (TLR)-4**

S. Rato, K. Amagase, H. Nishio, K. Fukushima, K. Takeuchi

Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Kyoto, Japan

**Background and Aim:** We previously reported that NSAID-induced gastric lesions were markedly aggravated in adjuvant-induced arthritic (AA) rats. In the present study, we exam- ined the intestinal ulcerogenic property of NSAID in AA rats, in comparison with normal animals. **Methods:** Arthritis was induced in male DA rats by injection of Freund's complete adjuvant into the right hind paw. Two weeks later, the animals were given indomethacin, and the intesti- nal mucosa was examined for lesions 6 and 24 h later. The expression of iNOS and TLR-4 was determined by Western blotting. **Results:** Oral administration of indomethacin produced mul- tiple hemorrhagic lesions in the small intestine. The severity of lesions being apparently increased in AA rats in comparison with normal rats. Nω-ω-tartro-L-arginine methyl ester and aminoguanidine when given once 6 h after indomethacin significantly prevented these lesions in normal rats, but failed to suppress the aggravated lesions in AA rats. Aminoguanidine when given twice, 30 min before and 6 h after indomethacin, significantly prevented the severity of lesions in AA rats. Moreover, the occurrence of lesions was apparently accelerated in AA rats in comparison with normal rats, the severe lesions being observed even 6 h after indomethacin in AA rats. These lesions in either normal or AA rats were totally prevented by dexamethasone and ampicilin. The expression of iNOS and TLR-4 was only slightly detected in the small intestine of normal rats, but was enhanced and clearly observed in that of AA rats under basal conditions (without indomethacin). **Conclusion:** These findings suggest that the ulcerogenic response to indomethacin in the small intestine, similar to the stomach, is markedly aggravated in AA rats. Especially, the lesions are more rapidly occurred in AA rats than those in normal rats. This phe- nomenon may be due to increased expression of TLR-4 in the small intestine during arthritis.

**JS-6**

**A Topical Treatment of Inflammatory Bowel Disease**

A. Sugitachi, K. Otsuka, Y. Akiyama, T. Itabashi, N. Uesugi, G. Wakabayashi

Department of Surgery 1, Department of Clinical Pathology, Iwate Medical University, Morioka, Japan

The authors devised muco-adhesive solutions in an attempt to topically treat inflammatory bowel disease and then, evaluated the efficacy of the agents using rat colitis models. Two differ- ent types of the solutions were prepared: namely, solution A was made of deacetylated chitosan (DAC) alone, and solution B was made of DAC and 5-amino-salicylic acid (5-ASA). The ade- siveness of each solution was measured ex vivo in our own method. Rat colitis models were induced by intracolonic application of 10% acetic acid solution. Each devised solution was given as a daily enema for 7 days from the next day of the colitis induction. The efficacy of the treat- ment was evaluated by survival time of the injured rats and their histopathological grading scores. Some were periodically sacrificed to observe the extent of the colonial inflammation. The adhe- siveness of solution A was stronger than that of solution B. The all animals had severe ulcerative colitis by the acetic acid solution. Three of 8 animals treated with solution A survived for longer than four weeks. Seven of 11 animals treated with solution B also survived for longer than 4 weeks. All 10 non-treated animals died within 5 days. Histopathology of the colons from the long-survived rats showed favorable repair of the mucosa and significant proliferation of fibrob- last in the submucosal layers, while that from the animals died in an early stage demonstrated severe ulceration and transmural necrosis of the colonic walls. Our devised solutions, especially solution B, suggested to enhance the healing activity at the seriously damaged site in ulcerative colitis, and intracolonie application of the agent would be clinically useful in topical treatment of inflammatory bowel disease.
JS-7
The Plant Sterol Guggulsterone Inhibits NF-κB Signaling and Gene Expression by Blocking IkB Kinase Activity in Intestinal Epithelial Cells, and Ameliorates DSS Colitis in Mice
J.S. Kim, J.H. Cheon, H.C. Jung, I.S. Song
Department of Internal Medicine, Seoul National University College of Medicine

Background and Aims: The plant sterol guggulsterone has been shown to have anti-inflammatory properties. It remains unknown, however, whether guggulsterone is effective for the treatment of inflammatory bowel disease (IBD). Therefore, we investigated anti-inflammatory effects of guggulsterone on intestinal epithelial cells (IEC) and on experimental murine colitis models, and elucidated its molecular mechanisms. Methods: Human Caco-2 cells and rat non-transformed IEC-18 cells were stimulated with IL-1β or LPS with/without guggulsterone. The effects of guggulsterone on NF-κB signaling in IEC were examined by ICAM-1 real-time RT-PCR, NF-κB transcriptional activity assay, Western blotting for IkB phosphorylation/degradation, electrophoretic mobility shift assay, and in vitro IkB kinase (IKK) assay. For in vivo study, dextran sulfate sodium (DSS)-treated mice were fed with/without guggulsterone. Colitis was quantified by disease activity index and evaluation of macroscopic and microscopic findings. Phosphorylation of IkB and IKK in colon mucosa was assessed by Western blotting and immunohistochemistry. Results: Guggulsterone significantly inhibited LPS- or IL-1β-induced ICAM-1 gene expression, NF-κB transcriptional activity, IkB phosphorylation/degradation, and NF-κB DNA binding activity in IEC. Moreover, guggulsterone strongly blocked IKK activity. Administration of guggulsterone significantly reduced the severity of DSS-induced murine colitis as assessed by clinical disease activity score, colon length, and histology. Furthermore, tissue upregulation of IkB and IKK phosphorylation induced by DSS was attenuated in guggulsterone-treated mice. Conclusion: Guggulsterone blocks NF-κB signaling pathway by targeting IKK complex in IEC and attenuates DSS-induced acute murine colitis, which suggests that guggulsterone could be an attractive therapeutic option in the treatment of IBD.

JS-8
Cell Membrane Fluidity Assays of Fluorescence Anisotropy and Excimer Formation in Indomethacin-Treated Gastric Epithelial Cell-Line RGM-1
T. Kaneko, H. Matsui, O. Shimokawa, A. Yanaka, I. Hyodo, A. Tsuji, K. Hirano
Graduate School for Comprehensive Human Sciences, University of Tsukuba and Tsukuba Research Laboratory, Hamamatsu Photonics Ltd.

Background and Aims: Gastric complications of indomethacin, a non steroidal anti-inflammatory drugs (NSAIDs), involves reactive oxygen species (ROS) formation, which induces cell membrane lipid peroxidation and gastric mucosal injuries. When peroxidized by ROS, the amounts of unsaturated fatty acids in cell membrane lipid bilayers are reportedly decreased, which affects cell membrane fluidity. We thus investigate the cell membrane fluidity change after indomethacin treatment both with fluorescence anisotropy and with excimer formation using fluorescence indicators decanoylaminofluorescein (DAF) and 1,3-di(1-pyrenyl) propane (PC3P), respectively, in gastric epithelial RGM-1 cell line. Methods: Indomethacin-pretreated RGM-1 cells were treated with DAF and PC3P and both their fluorescence intensity ratios were measured and calculated with an image intensified CCD (IICCD)-mounted fluorescence microscope and an image-processor. Indomethacin-pretreated RGM-1 cells were also treated with another fluorescence indicator, diphenyl-1-pyrenylphosphine (DPPP), which reacts with membranous organic hydroperoxides and hydrogen peroxide to emit fluorescence, and their fluorescence intensities were measured with the previous system. Results: After indomethacin treatment the fluorescence intensities of DPPP in RGM-1 were increased in a time- and dose-dependent manner, indicating that the fluidity of cell membrane was decreased by the indomethacin treatment. Conclusion: We demonstrated indomethacin treatment decreased cell membrane fluidity in a gastric epithelial cell line. Together with the result of DPPP fluorescence observation, the fluidity change reflects compositional changes of membranous lipid bilayers induced by lipid peroxidation. We thus propose the fluorescence observation of the cell membrane fluidity can apply to the detection of cellular pathophysiological changes in NSAID-induced gastric lesions.
Background and Aims: Previous studies demonstrated preventive effects of Escherichia coli strain Nissle 1917 (EcN) on acute and chronic intestinal inflammation in different murine models of colitis. However, little is known whether EcN shows protective effects against acute gastric stress injury and this effect is mediated by its anti-inflammatory and vasodilatory effects. Gastric mucosa adapts to damaging action of aspirin (ASA) and the formation of ‘aspirin-triggered lipoxin LXA4’ (ATL) due to acetylation of cyclooxygenase (COX)-2 by ASA was recently proposed to explain this phenomenon. The effects of NO-aspirin and EcN on ASA on gastric mucosal adaptation have been little studied. Gastric adaptation was induced in rats by repeated exposure to 3.5h of water and immersion stress (WRS) and then sacrificed immediately after stress exposure. In order to study the involvement of prostaglandins, non-selective COX-inhibitor (5mg/kg i.p.) was given with immersion stress (WRS) and then sacrificed immediately after stress exposure. The expression of proinflammatory cytokines was associated by increase in gastric mucosal mRNA expression for IL-1

Methods: Rats were divided in following groups: 1) vehicle; 2) EcN 10^1 CFU/ml; 3) EcN 10^4 CFU/ml and 4) EcN 10^8 CFU/ml. One hour later, the rats were exposed to 3.5h of water and immersion stress (WRS) and then sacrificed immediately after stress exposure. The expression of proinflammatory cytokines (IL-1)

Results: Exposure to WRS resulted in the development of acute gastric stress erosions (mean lesion number ~22). Pre-treatment with EcN caused a significant and dose dependent reduction of stress lesions and increase in gastric mucosal blood flow. This effect was completely abolished by indomethacin. The exposure to WRS was associated by increase in gastric mucosal mRNA expression for IL-1. EcN pre-treatment caused a significant down-regulation of mRNA expression for IL-1. Conclusion: E. coli Nissle shows protective effects against acute stress-induced gastric mucosal injury and this effect is due to its anti-inflammatory and vasodilatory effects. The gastroprotective effects of EcN is completely abolished by COX-inhibitors what indicates the role of prostaglandins in this effect. EcN may be potentially effective agent in the prevention of acute gastric damage in humans.

I-O-1-1

Effect of E. coli Nissle Gastric Mucosal Lesions Induced by Stress Exposure

P.C. Konturek, T. Brzozowski, G. Burnat, R. Pajdo, E.G. Hahn, S.J. Konturek
First Department of Medicine, University Erlangen-Nuremberg, Germany

I-O-1-2

Is the Gastric Adaptation to Aspirin Influenced by Coxiës and Nitric Oxide (NO)-Releasing Aspirin? Importance of Cyclooxygenase-2 (COX-2)/Lipoxigenase-5 (5-LOX) Products

Department of Physiology Jagiellonian University Medical College, Cracow, Poland; 1Department of Medicine I, University of Erlangen-Nuremberg, Germany

Gastric mucosa adapts to damaging action of aspirin (ASA) and the formation of ‘aspirin-triggered lipoxin LXA4’ (ATL) due to acetylation of cyclooxygenase (COX)-2 by ASA was recently proposed to explain this phenomenon. The effects of NO-aspirin and EcN on ASA on gastric mucosal adaptation have been little studied. Gastric adaptation was induced in rats by repeated exposure to acidified ASA (100mg/kg/day, i.g. 5 days) with or without NO-ASA (40mg/kg,i.g.), rofecoxib and celecoxib (10mg/kg i.g.) or indomethacin (5mg/kg i.p.). Area of gastric lesions was determined by planimetry, the gastric blood flow (GBF) was assessed by H2-gas clearance method. The measurements of ATL and LTBl levels (ELISA) and malondialdehyde (MDA) concentration as an index of lipid peroxidation, COX-1 and COX-2 mRNA (RT-PCR) were assessed in the gastric mucosa. ASA produced gastric lesions and decreased GBF while raising mucosal ATL, LTBl and MDA contents but 5 times ASA reduced the lesion area and mucosal LTBl levels while raising the GBF and mucosal ATL levels. At day 5, NO-ASA significantly attenuated ASA damage with the extent similar to that in vehicle and accompanied by the rise in GBF, ALT, luminal NO and an inhibition of mucosal LTBl and MDA levels. Coxibs and Indomethacin significantly augmented ASA damage and produced a further fall in the GBF, mucosal ATL levels and dramatic rise in LTBl4 and MDA levels. Expression of mRNA for COX-2 was absent in the intact gastric mucosa but strongly upregulated in ASA-treated mucosa with and without NO-ASA treatment. We conclude that: 1) ATL-induced by ASA, play an important role in the mechanism of gastric adaptation to continued ASA-treatment; 2) NO-ASA does not interfere with the adaptation to ASA but coxibs impair this adaptation to ASA due to suppression of ATL and the enhancement in mucosal 5-LOX products, LTBl4 and lipid peroxidation.

I-O-1-3

cDNA Microarray Analysis Reveals Anti-Inflammatory Effects of Lansoprazole, a Proton Pump Inhibitor, Against Indomethacin-Induced Intestinal Mucosal Injury in Rats

H. Ishikawa,1 N. Yoshida,2 M. Okumura,3 S. Akagiri,1 T. Takagi,1 O. Handa,1 K. Matsuhashi1, S. Adachi1, H. Shibuki1, S. Kokura1, N. Nakabe1, K. Kamada1, Y. Naito1, T. Yoshikawa1
1Inflammation and Immunology, 2Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyto Prefectural University of Medicine, Kyoto, Japan

It is well known that indomethacin produces gastrointestinal inflammation and ulceration through a diverse number of mechanisms. We have already reported that lansoprazole, a proton pump inhibitor, have anti-inflammatory effects as well as inhibition of acid secretion. The purpose of the present study is to evaluate the biological effects of lansoprazole by using cDNA microarray analysis in experimental rat model on gastrointestinal damage in vivo. Male ICR rats were administered indomethacin (10mg/kg) s.c., killed 3h and 24h later under deep ether anesthesia, and both the jejunum and ileum were removed. Lansoprazole was given subcutaneously (5mg/kg) just after following indomethacin administration. To evaluate the severity of the intestinal mucosal damage by indomethacin, we measured the concentrations of thiobarbituric acid-reactive substances (TBARS), myeloperoxidase (MPO) activity and the content of cytokine-induced neutrophil chemotactic-1 (CINC-1) in the intestinal mucosa. To investigate the molecular mechanisms underlying the effects of lansoprazole on this model, we examined gene expression profiles in mucosal cells which were collected by laser capture microdissection method. Preparation of cRNA and target hybridization was performed according to the Affymetrix GeneChip Eukaryotic Small Sample Target Labeling Assay Protocol (Version II). The gene expression profile was determined by the the GeneChip (Rat Toxicology U34 array, Affymetrix) microarray system. Single administration of indomethacin at 10mg/kg provoked severe hemorrhagic lesions in the small intestine, mostly the jejunum and ileum. The concentrations of TBARs, the levels of MPO activities and the content of mucosal CINC-1 in the intestinal mucosa significantly increased 24h after indomethacin administration and these increased levels were significantly inhibited by the treatment with lansoprazole. Microarray analysis revealed that lansoprazole restored the increased expression of genes for enzymes involved in oxidative stress, such as metallothionein, catelase and superoxide dismutase, in mucosal cells after 3h of injection of indomethacin. Lansoprazole also suppressed the upregulated expression of genes for cytochrome c oxidase, N-acetyltransferase-2 and transferrin. Furthermore, some genes for enzymes involved in anti-apoptosis, protein folding and DNA-binding protein were downregulated by indomethacin treatment, and lansoprazole was able to restore these changes. These observations clearly illustrate that lansoprazole protects against acid-unrelated intestinal injury induced by indomethacin. It was suggested that oxidative stress and apoptosis were involved in the pathogenesis of indomethacin-induced enteritis.

I-O-1-4

Role of Toll-Like Receptor 4 in Indomethacin-Induced Small Intestinal Damage

T. Watanabe, K. Higuchi, T. Tanigawa, E. Sasaki, M. Shiba, K. Tominaga, Y. Fujimura, N. Oshitani, T. Arakawa
Department of Gastroenterology, Osaka City University, Graduate School of Medicine, Osaka, Japan

Background: In a previous study, we demonstrated that enteric bacteria play an important role in indomethacin-induced small intestinal damage. Toll-like receptor (TLR) 4 is a signaling receptor for lipopolysaccharide (LPS). Upon activation by LPS recognition, TLR4 signals to activate the nuclear factor-kappaB pathway, a critical regulator of many proinflammatory genes. Aim: We investigated role of TLR4 in indomethacin-induced small intestinal damage. Method: Rats without fasting were administered 10mg/kg indomethacin by gavage, and intestinal damage was evaluated 3, 6, and 24h later. LPS from Escherichia coli (0.3mg/kg), a selective inducible nitric oxide synthase (iNOS) inhibitor, aminoguanidine (20mg/kg), anti-neutrophil serum, and anti-collagen (an antibodies, 800mg/kg) were also administered prior to or after indomethacin challenge. Additionally, TLR4-deficient mice were given indomethacin. Intestinal mucosa were subjected to measurement of myeloperoxidase activity (a marker of neutrophil infiltration), immunohistochemical staining, Western blot analysis, and assay of mRNA levels of cytokines, iNOS, and TLR4 by real-time RT-PCR. Results: Intestinal damage were observed from 3h, and reached peak by 24h. The levels of mRNAs for TNF-α and iNOS, and the myeloperoxidase activity were increased...
in a time-dependent manner. TLR4 was expressed on inflammatory cells and a few epithelial cells. By 24 h, aminoguanidine and anti-neutrophil serum inhibited intestinal damage by 60% and 47%, respectively. Administration with LPS 1 h after indomethacin significantly increased the damage, whereas pretreatment with LPS inhibited it, accompanied by decrease in the expression of TLR4 as assessed by RT-PCR and Western blot. Amphicillin also inhibited the damage by 94%, and decreased the number of Bacteroidaceae and Enterobacteriaceae which can produce LPS in small intestinal content. In TLR4-deficient mice, the intestinal damage was inhibited by 90%, comparing to wild-type mice. Conclusion: TLR4 signaling may play a crucial role in the pathogenesis of indomethacin-induced small intestinal damage.

I-O-1-5 Role of Dietary Fiber, Bile Acids and Intestinal Motility in the Formation of Intestinal Lesions Induced by Indomethacin in Cats
H. Satoh, N. Otsuka, S. Shiozata
Veterinary Pharmacology Faculty of Agriculture, Tottori University

Background and Aim: Recently we reported that dietary fiber (DF) played some role in the formation of intestinal lesions induced by indomethacin (IM) in rats, cats and dogs. In this study, the role of DF (soluble or insoluble), bile acids and intestinal motility in the lesion formation was investigated in cats. Methods: Several types of diets containing various percentage of DF were given to cats twice a day during the experiment. IM was administrated orally once a day after a morning meal for 3 days. In some experiments, either cholestyramine (bile acid-binding resin) or atropine was administered twice a day just before each meal during the treatment with IM. Mucosal lesions in the small intestine were examined 24 h after the final dose of IM. In another experiment, the intestinal motilities were recorded in conscious cats implanted with force transducers in the small intestine. Results: 1) Many lesions were produced by IM (3 mg/kg) in the middle and lower intestine in cats given dry containing 4% DF, the mean lesion areas (MLA) were 8.4 ± 2.7 cm². The lesions were markedly decreased in cats given canned diets containing 0.4% DF (MLA: 0.4 ± 0.2 cm²). Whereas the lesions were increased significantly in animals given canned diet supplemented with 3% cellulose (insoluble fiber), but not with 3% pectin (soluble fiber). 2) The lesion formation by IM in cats given dry foods was significantly prevented by treatment with cholestyramine (300 mg/kg, p.o.) or atropine (0.3 mg/kg, s.c.). 3) IM (1–3 mg/kg, p.o.) dose-dependently increased the intestinal motilities in cats, and the effects were blocked by pretreatment with atropine (0.3 mg/kg, s.c.). Conclusion: It could be concluded that all insoluble dietary fiber, bile acids and intestinal motility play an important role in the formation of intestinal lesions by IM.

I-O-2 Mucosal Defense 1 Chairpersons: C.H. Cho, S. Watanabe

I-O-2-6 Involvement of K/1.1 and Na/1.5 in Proliferation of Gastric Epithelial Cells
Department of Pharmacology and Research Centre of Infection and Immunology, Faculty of Medicine, The University of Hong Kong, Hong Kong, China

In the present study, patch clamp experiments demonstrated the expression of multiple ionic currents, including a Ba²⁺-sensitive inward rectifier K⁺ current (Iₖ₄.₃), a 4-aminopyridine-(4-AP) sensitive delayed rectifier K⁺ current (Iₖᵥ), and a nifedipine-sensitive, tetrodotoxin-resistant inward Na⁺ current (Iₖᵥ) in the non-transformed rat gastric epithelial cell line RGM-1. RT-PCR revealed molecular identities of mRNAs for the functional ionic currents, including K₁.₂ for Iₖᵥ, K₁.₁, K₁.₆, and K₂.₁ for Iₖᵥ, and Na₁.₅ for I₆.₇/₉. Pharmacologic blockade of K₁.₂ or Na₁.₅, but not K₁.₁, suppressed RGM-1 cell proliferation. To further elucidate which subtypes of the ion channels were involved in cell proliferation, RNA interference was employed to knockdown specific gene expression. Downregulation of Kv₁.₁ or Nav₁.₅ by RNA interference was employed to assess the role of the ion channels were involved in cell proliferation, RNA interference was employed to

I-O-2-7 Effects of Repetitive Deformation on Intestinal Epithelial Cells
M.D. Basson
Department of Surgery, Wayne State University and John D. Dingell VA Medical Center

The intestinal mucosa is subject to repetitive deformation, shear stress, and pressure in vivo during peristalsis, villous motility, and the passage of luminal nutrients. Such forces may be markedly reduced in abnormal states such as fasting, postoperative ileus, or sepsis, and may be accompanied by intestinal mucosal atrophy, loss of barrier integrity, and poor mucosal healing, despite adequate parenteral nutrition. However, the effect of such forces upon intestinal epithelial cells remains poorly understood. Repetitive deformation can be applied to intestinal epithelial monolayers by culture on a flexible membrane repetitively deformed by the application of a vacuum below the membrane (Flexcell, McKeepo, PA). We studied the effects of an average 10% strain applied at 10 cycles/min on the proliferation, differentiation, and motility of human Caco-2 intestinal epithelial cells, primary human intestinal epithelial cells, and non-transformed rat IEC-6 cells. In some variants, we varied the matrix protein substrate on which the cells were cultured or added fibronectin to the culture medium to mimic the deposition of fibronectin common to inflammatory states. Proliferation was assessed by cell counting and β-hymidine update over 24–72 h differentiation by measurement of intestinal brush border enzyme specific activity, and migration by rates of circular wound closure in the cell monolayer over 6–72 h. On a collagen I, collagen IV, or laminin substrate, repetitive strain promotes the proliferation of sparsely cultured intestinal epithelial cells by a pathway involving FAK, Src, ERK, and PKC. Intestinal epithelial differentiation is also promoted by repetitive deformation on a cationic transduction substrate. However, repetitive strain induces a very different response in confluent wounded monolayers. Wound closure was significantly stimulated in intestinal epithelial monolayers on fibronectin, independently of proliferation, while wound closure is actually inhibited on a collagen substrate. Although the signals responsible for this pathway are incompletely understood, we have established that both ERK activation and MLCK activation are required for the effect, as each is activated, and blocking either prevents the effect. The seeming paradox that ERK activation yields disparate results in these two situations may be explained by differences in ERK subcellular relocation in response to strain. Confocal imaging after immunocytochemistry suggests that repetitive deformation causes ERK to relocalize to the intestinal epithelial cell’s migrating lamellodiploid edge on fibronectin substrate, but not on collagen. These results suggest that changes in tissue fibronectin levels in inflammatory states may trigger a switch between a mitogenic and motogenic response to repetitive deformation in intestinal epithelial cells. Under normal circumstances, repetitive deformation seems tropic for the gut mucosa, maintaining proliferation and differentiation in the intestinal epithelium. However, acute or chronic changes in fibronectin in pathophysiologic states may shift the cellular response to that stimulus epidendritic shear migration and wound closure instead of proliferation and differentiation. Conversely, if strain itself is diminished in pathophysiologic states, these pathways may be similarly ablated, leading to loss of mucosal integrity.

I-O-2-8 Over-Expression of a 72-kDa Heat Shock Protein Protects Rat Gastric Mucosal Cells from H₂O₂-Induced Necrosis and Apoptosis
Department of Gastroenterology, Akita University

Background: We have demonstrated the importance of a 72-kDa heat shock protein (HSP72) for gastric and colon mucosal protection in vitro and in vivo experiments. However, the problem of our previous studies was that it was impossible to exclude a possible contribution of other cytoprotective factors other than HSPs, since systemic stress including heat shock, chemicals or stress-related hormones had to be applied to induced HSPs in those experimental systems. Therefore, in order to solve this problem, we have established HSP72-overexpressing gastric epithelial cells by transfecting full length of human HSP72 DNA, and we successfully cloned HSP72-highly expressed gastric epithelial cells that expressed approximately 500% of HSP72 compared with vector-control cells. We examined the cytoprotective function of these cells against H₂O₂-induced necrosis and apoptotic cell death. Methods: The cationic liposome-mediated method was employed for transfection and the resistance to genetin was used for selection of successfully transfected cells. To examine anti-necrotic ability, these cells were challenged with high concentration of H₂O₂ (0–50mM) and the cell viability was measured by WST-1 assay. To examine anti-apoptotic ability, these cells were treated with low concentration of H₂O₂ (0–1mM) for 24. Fluorescence staining for DNA was performed using Hoechst 33342 to quantify histone-complexed DNA fragments. Further, we assessed the levels of Pol-ADP-Ribose-Polymerase (PARP) using western blot. Results: H₂O₂ induced necrotic and apoptotic cell injury to pBK-CMV12 in a concentration-dependent manner. This phenomenon was significantly diminished in 7018-RGM1 compared to pBK-CMV12. The numbers of apoptotic cells were reduced in 7018-RGM1 morphologically and suppressed significantly genomic DNA fragmentation. The detection
I-O-2-9 Carbonic Anhydrase and Alkaline Phosphatase Coordinate Adaptation to Acidosis in Rat Duodenum

Y. Akiba, M. Mizumori, C. Supuran, P.H. Guth, E. Engel, J.D. Kaunitz
Department of Medicine, West Los Angeles VA Medical Center, CURE/UCLA, Brentwood Biomed Res Inst, USA; Università di Firenze, Firenze, Italy

Duodenal brush border membrane (BBBM) has multiple highly-expressed eoto-enzymes, membrane-bound carbonic anhydrase (CA) and intestinal alkaline phosphatase (IAP). Since CA activity affects bicarbonate secretion and AP hydrolyses organic phosphates at alkaline pH, we hypothesized that these enzymes co-regulate duodenal bicarbonate secretion (DBS). We examined the effect of CA and AP inhibitors on AP activity in BBM and DBS in rat. BBM AP activity was measured in situ in duodenal frozen sections with the fluorogenic substrate ELF®-97 phosphate. Sections were reacted with ELF phosphate in pH 8.6 buffer. ELF fluorescence intensity was measured ±IAP inhibitors L-cysteine, L-phenylalanine, or sodium tungstate (0.1–10mM), or tissue non-specific AP inhibitor levamisole (0.1–10mM). We further examined the effect of high pH 6.4, PCO₂ = 206 mm Hg) or low pH 6.4 buffer, PCO₂ = 0 mm CO₂ on AP activity ± cell-permeant CA inhibitor methazolamide (1 mM) or novel cell-impermeant CA inhibitors (1 μM). DBS through duodenal loop was measured with pH-stat or with flow-through pH and CO₂ electrodes. The loop was perfused with high CO₂ solution or pH 3.2 saline ± cell-impermeant CA inhibitors (0.1 μM) or AP inhibitors (10μM). ELF fluorescence appeared intensely on BBM of villous cells, and was less intense in the mucus gel layer. IAP inhibitors dose-dependently inhibited ELF®-based AP activity, levamisole had lesser effect. High CO₂ solution enhanced BBM AP activity compared with low CO₂ solution. CA inhibitors reduced the CO₂-related augmentation of AP activity. Augmented DBS by high CO₂ or acid perfusion was reduced with methazolamide, whereas cell-impermeant CA inhibitors and AP inhibitors enhanced the DBS response. ELF fluorescence enabled us to develop a simple in situ duodenal AP assay. Interaction between CA and IAP suggests that these enzymes coordinate and negatively regulate DBS. Regulation of DBS is a novel and incompletely studied function of IAP.

I-O-3-11 Regulation of Intestinal Motility by Gastrointestinal Hormonal Factors such as Ghrelin, Motilin and Leptin

J. Beck
Department of Gastroenterology, University Hospitals Leuven, Belgium

The gastrointestinal tract plays a key role in the control of food intake. Hence, it is no surprise that orexigenic and anorexigenic peptides have a major impact on gastrointestinal motility. Receptors for these peptides are present both in the brain and on the enteric nervous system. Leptin is an anorexigenic peptide involved in the long-term regulation of food intake. Several studies have reported that leptin, especially when administered centrally, inhibits gastric emptying in animals. Ghrelin is an orexigenic peptide that is released from the gastric mucosa. Recent studies in animals have shown that ghrelin stimulates upper gastrointestinal motility through the vagus and the enteric nervous system. Although closely related, the motilin and the ghrelin receptor are differentially expressed and the effects of these peptides on gastrointestinal motility are specific and mediated through separate receptors. Orexin A is another peptide that plays a role in the regulation of food intake, arousal, and energy balance. Obestatin is a peptide that is also derived from proghrelin, which suppresses appetite and inhibits gastrointestinal motility in animal models. Previous studies had already established that motilin induced an activity front of the migrating motor complex in man, increased tone of the proximal stomach, and enhanced gastric emptying in health and in gastroparesis. In healthy volunteers, we studied the effects of administration of ghrelin on interdigestive motility. Ghrelin had a strong stimulatory effect on gastric contractility, and induced an activity front of the migrating motor complex, with gastric antral peristalses. This was accompanied by a prolonged stimulatory effect on tone of the proximal stomach. Ghrelin-induced activity fronts are not accompanied by a motilin peak, but ghrelin did increase pancreatic polypeptide levels. We confirmed a therapeutic potential of ghrelin receptor agonism by demonstrating that administration of ghrelin enhanced gastric emptying and improved meal-related symptoms in patients with idiopathic gastroparesis. Orexin A was shown to decrease the gastric emptying rate in man. Studies of the effects of obestatin in man are in progress. The discovery of ghrelin and obestatin is likely change our understanding of the role and control of gastrointestinal motility and appetite control, and may lead to potential new therapeutic approaches.

I-O-3-12 Effect of Central Thyrotropin-Releasing Hormone (TRH), an Ulcerogenic Factor in the Brain, on Hepatic Regulations

M. Yoneeda, T. Shinhada, A. Terano, H. Hirashita
Department of Gastroenterology, Dokkyo University, School of Medicine

TRH is well recognized as neurotransmitter of the central nervous system and acts in the certain brain nuclei to induce several physiological alternations. In the gastrointestinal tracts, central TRH stimulates gastric acid secretion, gastric motility, and induces gastric ulceration through vagal nerves. From some animal studies, TRH in the brain is demonstrated to play a role in stress-induced gastric mucosal damage. The liver is also richly innervated and the autonomic nervous system controls hepatic physiological functions. In this study, the effect of central TRH on hepatic physiological and pathophysiological regulation was investigated. Methods: Male Wistar rats were used throughout the studies. Hepatic microcirculations: Rats were intracerebrally injected with TRH analog (RXT73768, 5-100ng) under urethane anesthesia and hepatic blood flow was monitored by laser Doppler flowmeter. Portal blood flow and portal pressure were also measured simultaneously. Hepatic proliferation: Rats were intracerebrally injected with the TRH analog (1-100ng) and hepatic proliferation was assed by thymidine incorporations at 6-72h after TRH administration. Experimental liver injury: Rats were intracerebrally injected with the TRH analog (1-10ng), and then CCl4 (2.0ml/kg) was administered. The liver injury was assessed 24h after CCl4 by serum ALT levels and liver histology. Results: Central injection of TRH dose-dependently stimulates hepatic blood flow concomitantly with a decrease in portal pressure and an increase in portal blood flow. Hepatic proliferation was dose-dependently stimulated by intracerebral injection of TRH. Moreover, central injection of TRH dose-dependently inhibited CCl4-induced liver injury assessed by serum ALT and hepatic histological changes. These hepatic changes induced by central injection of TRH was abolished by vagotomy. In contrast, treatment of rats with propranolol, an alpha-adrenergic factor, acts in the brain and regulates hepatic physiological and pathophysiological regulations.

I-O-3-10 Corticotropin Releasing Factor (CRF) 1 and 2 Receptor Subtype Actions on the Gastrointestinal Tract

Y. Taché
CURE: Digestive Diseases Research Center, Center for Neurovisceral Sciences and Women’s Health, Division of Digestive Diseases, David Geffen School of Medicine at UCLA, VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA

Stress alters gastrointestinal motor function through mechanisms that involve CRF receptor-mediated actions. Peripheral injection of CRF ligands such as CRF and urocortin 1 reproduce stress response by inhibiting gastric function while stimulating colonic propulsive motor activity. CRF₁ mRNA is detected within the whole gut and expressed with the highest levels in the esophagus and the colon, which is similar to that found in CRF₂. The CRF₂ gene pattern distribution differs from that of CRF₁ which was expressed at the highest levels in the ileum and colon and very low to undetectable levels in the esophagus and gastric corpus. Peripheral injection of CRF results in a CRF₂-mediated decrease in intraluminal gastric pressure, closing of lower esophageal sphincter and increase in gastric mucosal blood flow. By contrast, in the colon, CRF stimulates the peristaltic reflex by activating cholinergic and nitricergic myenteric neurons, mucus secretion and motility. CRF₂ receptors are localized on colonic myenteric neurons, and selective CRF₂ antagonists prevent peripheral CRF- and water avoidance stress-induced colonic myenteric and motor responses. This contrasts with colonic CRF₁ receptor that when activated increases colonic blood flow, and suppresses exogenous CRF or stress-induced CRF₂-mediated stimulation of colonic mucus secretion, myenteric and motor activity and visceral hyperalgesia. These data provide morphological and functional evidence for a distinct role of CRF receptors in the upper and lower gut to regulate the secretory, neuronal, vascular and motor activity and indicate that CRF₁ receptor drives while CRF₂ damps the colonic responses to stress.
I-O-3-13
Hypothalamic-Pituitary-Adrenocortical Axis: the Hidden Gold in Gastric Mucosal Homeostasis
L. Filaretov\(^1\), T. Podgorna\(^1\), P. Bobryshev\(^1\), T. Bagaeva\(^1\), P. Poprydzhin\(^1\), A. Tanaka\(^2\), H. Shirahama\(^2\), K. Takemus\(^2\)
\(^1\)Laboratory of Experimental Endocrinology, Pavlov Institute of Physiology, St. Petersburg, Russia; \(^2\)Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Missasagi, Yamashina, Kyoto, Japan

It is well known that glucocorticoids hormones released during activation of hypothalamic-pituitary-adrenocortical (HPA) axis provide life-saving protective processes mounted up the body to overcome negative effects of stress stimuli. In spite of knowledge about important contribution of glucocorticoids hormones to general body homeostasis, for a long period the contribution of ghrelin released during activation of HPA axis to gastric mucosal homeostasis was hidden. Moreover, for many years the activation of HPA axis has been considered as an ulcerogenic hormonald component of brain-gut axis. Our results, however, suggest that glucocorticoids produced during acute activation of HPA axis are important gastroprotective factors. Various ulcerogenic stimuli induce an increase in glucocorticoid production that in turn helps the gastric mucosa to resist against a harmful action of ulcerogenic stimuli. Beneficial influence of glucocorticoids on the healing of injured gastric mucosa as well as their contribution to the realization of phenomenon of adaptive gastric cytoprotection have been also demonstrated. There are multiple targets for glucocorticoids to exert their beneficial influence on gastric mucosa. The gastroprotective action of glucocorticoids is accounted for maintaining the local defensive factors and inhibiting the pathogenic elements. Anti-inflammatory properties of glucocorticoids contribute to their gastroprotective action. The contribution of glucocorticoids to gastric mucosal homeostasis is tightly related with their contribution to general body homeostasis. Various links of general homeostasis can be the primary targets of the glucocorticoid action. The beneficial action of glucocorticoids on gastric mucosal blood flow can be provided through its beneficial influence on such general physiological parameter as systemic blood pressure. Maintenance of glucose as well as temperature homeostasis by glucocorticoids hormones could be considered as baseline of their action on various local gastric targets. Thus, glucocorticoids released during activation of HPA axis may contribute to protection of gastric mucosa through their contribution to general body homeostasis.

I-O-3-14
Mosapride Citrate Inhibits Gastric Distension-Induced Visceral Pain Behavior and Visceromotor Response in Conscious Rats
H. Kaneko
Department of Pathophysiology and Therapeutics, Aichi Medical University College of Nursing

Mosapride enhances gastric emptying and alleviates symptoms in patients with functional dyspepsia (FD). Recently, gastric hyperalgesia as well as delayed gastric emptying has been proposed as the possible pathogenesis for FD. Aims: To establish the novel rat model for measuring gastric visceral pain, and to examine the action of mosapride. Methods: A flexible latex balloon was surgically placed into the stomach, thereafter a force transducer was implanted on the abdominal striated muscle to measure the visceromotor response. The distension-induced abdominal muscle contractions were measured 1h before and 1 or 2 h after intragastric (ig) administration of mosapride, itopride (a prokinetic agent; dopamin D2 blocker and anticholinesterase inhibitor) or morphine. Results: When infusion of air volume amounted to 8-12 ml, abdominal striated muscle contractions were induced prior to occurrence of a typical pain behavior (writhe phenomenon). Mosapride (3-10mg/kg, ig) significantly inhibited distension-induced abdominal muscle contractions. Conclusions: Mosapride, but not itopride, inhibited the visceromotor response by gastric distension, mediated via a 5-HT4 receptor in part. This study was performed by the collaboration with Yasuhiro Seto, Katsuyoshi Kawashima, Naoyuki Yoshida, PhD, at Dainippon-sumitomo Pharmaceutical Company, Japan.

I-O-3-15
Ghrelin, Energy Balance and Gastrointestinal Motility
A. Azakami\(^1\), M. Fujimura\(^2\), A. Inou\(^3\)
\(^1\)Department of Behaviorai Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan; \(^2\)Department of Anatomy, Shiga University of Medical Science, Shiga, Japan

Ghrelin, an endogenous ligand for growth hormone secretagogue receptor (GHS-R), was identified in the stomach. Gastric peptide ghrelin has two major molecular forms: acylated ghrelin and desacyl ghrelin. We examined the effects of the gastric peptide ghrelin on energy balance and the gastrointestinal motility. Acylated ghrelin exhibited gastroprokinetic activity with structural resemblance to motilin and potent orexigenic activity through action on the hypothalamic NPY and Y1 receptor, which was lost after vagotomy. Acylated ghrelin decreased gastric vagal afferent discharge. Ghrelin gene expression in the stomach was increased by fasting. Peripherally administered acylated ghrelin blocked IL1β-induced anorexia and produced positive energy balance by promoting food intake and decreasing energy expenditure. On the other hand, intraperitoneal administration of desacyl ghrelin decreased food intake in food-deprived rats and mice. Desacyl ghrelin-overexpressing mice exhibited a decrease in body weights, food intake and fat pad mass weight. Both intracerebroventricular (ICV) and intravenous (IV) administration of desacyl ghrelin disrupted the fasting motor activity in the antrum but not in the duodenum in conscious rats. Changes in the gastric motility induced by IV administration of desacyl ghrelin was completely antagonized by ICV administration of selective CRF2 receptor antagonist and non-selective CRF receptor antagonist, however CRF receptor antagonist did not have effects. Intraperitoneal administration of desacyl ghrelin did not enhance c-fos expression in the nucleus of the solitary tract, the inverse effects of these two peptides, suggest that the stomach might be involved as an endocrine organ in the regulation of the energy balance and gastrointestinal motility.

I-O-3-16
Acid Exerts Anti-Proliferative Effects via p53 in Non-Neoplastic Barrett’s Cells
Department of Medicine, University of Texas Southwestern Medical Center at Dallas and the Dallas VA Medical Center, Dallas, Texas, USA

Introduction: The observation that acid has pro-proliferative effects on Barrett’s cancer cells and on Barrett’s mucosal explants is a basis for the common clinical practice of prescribing high-dose antisecretory therapy routinely for patients with Barrett’s esophagus. Those model systems may not be appropriate for determining the effects of acid on benign Barrett’s epithelial cells, however. We studied the effects of acid on proliferation and apoptosis in a non-neoplastic, telomerase-immortalized Barrett’s epithelial cell line. Methods: Barrett’s cells were treated with two 3-minute exposures to acidic media, 60 min apart. Cell growth was determined using cell counts, proliferation was studied by flow cytometry, cell viability was determined by trypan blue staining, and apoptosis was assessed by TUNEL and Annexin V. The expression levels of p53 and p21 were determined by Western blotting. Infection with p53 siRNA was used to study the effect of p53 inhibition on total cell numbers after acid exposure. Results: Acid exposures significantly decreased total cell numbers at 24h without affecting either cell viability or apoptosis. In control cells, the peak fraction of cells in G2/M was seen at 6h. In contrast, acid exposures delayed the G2/M peak to 12h. Acid exposures increased expression of p53, but not p21. Uninfected and empty vector infected cells both exhibited a significant decrease in cell number following the acid exposures. In contrast, cells containing p53 siRNA exhibited no significant change in cell number after the acid exposures. Conclusions: In non-neoplastic Barrett’s epithelial cells, multiple acid exposures have an anti-proliferative effect that can be blocked by inhibiting p53 expression. This study elucidates a molecular pathway whereby acid exposure has potentially beneficial, anti-proliferative effects on benign Barrett’s epithelial cells and suggests that the common clinical practice of prescribing antisecretory therapy in dosages beyond those required to heal esophagitis could be detrimental.

I-O-4-17
Nrf2 Induced H+–K+–ATPase in MNNG-Induced Transformed RGM-1 Cells and in Gastric Epithelial RGM-1 Cells
Graduate School for Comprehensive Human Sciences, Department of Gastroenterology, University of Tsukuba

Background and Aims: We have recently treated a gastric epithelial cell line RGM-1 with a carcinogenic agent N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and established a transformed cell line, RGM-1. RGM-1 acquired tumor-genesis and anchorage independence, none of which RGM-1 had. Moreover, we founded that H+–K+–ATPase (proton pump) was expressed in RGM-1. The expression of proton pump was regulated by a couple of transcriptional factor, GATA4 and GATA6. In blood cells differentiation, the cooperation between members of both GATA family and CNC family such as Nr2 plays important roles. Thus Nr2 and GATA4/6
may play an important role to induce proton pump. To clarify the hypotheses, we first investigated the expression of Nr2f2 in RGM-1 and RGK-1. We then investigated the effects of an electro- 


troph, DEM which activates Nr2f2 on the proton pump mRNA expression. Moreover we investigated the effect of siRNA for Nr2f2 whether it restrained the expression of both HO-1 and proliferation. We used transfected RGM-1, RGK-1 (GH cells from human gastric cancer tissue: AGS and MKN45. Expression of Nr2f2, proton pump, and HO-1 were investigated with Western blotting analysis and RT-PCR. Cells pretreated with siRNA were also investigated.

**Results:** In RGK-1 cells, proton pump, Nr2f2 and HO-1 were expressed without DEM. In RGM-1, these proteins were not expressed without DEM. However, the DEM treatment induced expression of them. SRNA treatment decreased Nr2f2 expression as well as the expression of both HO-1 and proton pump.

**Conclusion:** These result suggested that Nr2f2 plays an important role to induce proton pump. Moreover, RGM-1 was suggested to be a gastric stem cell which can be differentiated with the Nr2f2 activation.

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**I-O-4-18**

**Heightened Malignant Potentials by a Putative Differentiation Regulator Musashi-1 in AGS Human Gastric Cancer Cells**

H. Muneta, T. Tajiri, M. Nishiyama, K. Tsutsui, H. Eguchi, S. Kawano, N. Hayashi

1Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2Department of Clinical Laboratory Science, School of Allied Health Sciences, Osaka University Graduate School

Musashi-1 (MSI-1) is an RNA-binding protein that putatively regulates cell differentiation. Previous study revealed that MSI-1 expression correlated with grades and stages of human gastric cancers. We found that MSI-1 expression was observed in the stomach, in particular, in Helicobacter pylori-infection infected gastric mucosa, but MSI-1 expression has been poorly investigated in gastric cancer. Here we aim to explore the involvement of MSI-1 in the development of gastric cancer in vitro in terms of proliferation, apoptosis, and colony formation. AGS human gastric cancer cells were transfected with MSI-1 siRNA and a stable transfectant (AGS-Msi-1 cells) was established. Western blotting analysis and RT-PCR. Cells pretreated with siRNA were also investigated. These results suggested that MSI-1 plays an important role in the development of gastric cancer.

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**I-O-4-19**

**Relationship between MUC5AC and ATBF1 in Gastric Cancer**


1Department of Internal Medicine and Bioregulation, 2Department of Molecular Neurobiology, Nagoya City University Graduate School of Medical Sciences

**Background and Aims:** MUC5AC expression in gastric carcinoma is known to correlate with poor prognosis. AT motif binding factor 1 (ATBF1) is a homeodomain transcription factor which was originally found to bind to AT motif in AFP promoter region and negatively regulates its transcription. In the present study, we investigated the role of ATBF1 in colon cancer. ATBF1 gene transfer may be a new candidate for treatment of colorectal cancer.

**Results:** ATBF1 binds to the AT motif like element in MUC5AC promoter region and negatively regulates its transcription in gastric cancer cells.

**Conclusion:** ATBF1 binds to the AT motif like element in MUC5AC promoter region and negatively regulates its transcription in gastric cancer cells.

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**I-O-4-20**

**A New Strategy for Colon Cancer Cell (HT-29) by Dominant Negative Gene Transfer of Apoptosis Signal-Regulating Kinase (ASK)-1**


1Department of Gastroenterology, 2Department of Pharmacology, Osaka City University Graduate School of Medicine, 3Laboratory of Cell Signaling, Graduate School of Pharmaceutical Sciences, University of Tokyo, CREST, Japan

**Background:** Apoptosis signal-regulating kinase (ASK)-1 is identified as a molecule which mediates pro-apoptotic signaling thereby inducing apoptosis for many types of cells. On the other hand, some reports indicated that ASK-1 induced the cellular differentiation and survival. These results suggest that ASK-1 had a broad range of biological activities depending on cell types and cellular context. In the present study, we investigated the role of ASK-1 in colorectal cancer cells.

**Materials and Methods:** We used the well-characterized colorectal cancer cell line: HT-29 in this study. Adenovirus vector carrying dominant-negative form of ASK-1 (AD-DN-ASK-1) was produced by PCR as previously reported method. Phosphorylation of ASK-1, c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) was analyzed by Western blot analysis. On day 5 after the transfection of AD-DN-ASK-1 or AD-LacZ, a control vector, the viable cell number was counted by the hemocytometer using a light microscope. On day 2 after the transfection of AD-DN-ASK-1 or AD- LacZ, human colon cancer cells were centrifuged and the cell pellet was performed to evaluate the apoptotic cell number. **Results:** Selective phosphorylation of ASK-1 at Thr-845, a kinase domain cite, but not Ser 83 nor 967 cites was induced in HT-29 cells by the serum stimulation in a time-dependent fashion. Its phosphorylated levels reached to the maximum levels (about 3-fold) compared to the control levels by 30 min. Using AD-LacZ, the accuracy of the transfection techniques into HT-29 cells was confirmed by the immunostaining. Gene transfer of AD-DN-ASK-1 inhibited the serum-induced phosphorylation of JNK and p38MAPK, which are downstream molecules of ASK-1. Infection with AD-DN-ASK-1 at an MOI of 30, 50, or 100 to HT-29 cells diminished the serum-induced proliferation in a dose-dependent fashion. The number of positive cells for Hoechst staining after the AD-DN-ASK-1 transfection was significantly higher than that of AD-LacZ-transfected cells. **Conclusion:** We obtained that AD-DN-ASK-1 gene transfer exerted a significant antitumor effect on colon cancer. AD-DN-ASK-1 gene transfer may be a new candidate for treatment of colorectal cancer.

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**I-O-5 IBD-Clinical**

**Chairpersons:** R.W.L. Leong, B.E. Sands

**1Department of Gastroenterology, 2Department of Pharmacology, Osaka City University Graduate School of Medicine, 3Laboratory of Cell Signaling, Graduate School of Pharmaceutical Sciences, University of Tokyo, CREST, Japan

**Background:** Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the digestive tract characterized by recurrent episodes of inflammation and ulceration in the gastrointestinal tract. There is a paucity of robust population-based epidemiological data on inflammatory bowel disease (IBD) in Australia. The absence of an Australian registry has prevented the characterization of both Crohn's disease (CD) and ulcerative colitis (UC). Published series derived from tertiary hospitals are biased towards more severe disease missing milder disease and understimating the IBD incidence and prevalence. Consistent with other reported Western series, there has been increasing hospitalisation of CD relative to UC from the 1960's onwards. In the past decade, hospital separations have doubled for CD with a smaller increase for UC. More recently, there has been a rise in paediatric CD with an incidence increasing from 0.1 to 2.0 per 100,000 over a 30 year period. An overrepresentation of females in CD is present with a female-to-male ratio of 2.1. A review of 997 IBD cases in central Sydney comprised of 42% CD, 53% UC, and 5% IBD-undifferentiated patients. With a median follow-up time of 6.5 years, the prevalence of CD strictures was 40%, fistulas 24% and perforations 20%. Colorectal cancer occurred in 2% of IBD patients (3% for UC and 0.5% for CD). The commonest extra-intestinal manifestation was arthritis in 13% of patients. The requirement for surgery for UC was 39% at 16 years. The IBD-related mortality rate was 1%. Migration studies have not been performed in Australia but parallel studies comparing Australians with Chinese IBD population revealed distinct differences in genotype, phenotype and severity. The allelic frequencies for the three CARD15/NOD2 single nucleotide polymorphisms are 0.11, 0.02, and 0.07. Asian CD is characterized by male predominance, less familial clustering and less strong association with smoking compared to Australian Caucasians. A population-based study may confirm an underestimation of the IBD burden in Australia. Of interest, migration studies may reveal important environmental risk factors that contribute to the development of IBD.
Long-Term Clinical course of Crohn
K Hirai, T Matsu
Department of Gastroenterology, Fukuoka University Chikushi Hospital

The aim of this study is to clarify the long-term clinical course of patients with Crohn’s disease of aphthous type (type A CD). Twenty-two patients (13 male, 9 female, observation period: 10.1 ± 6.2 years) diagnosed as type A CD at our hospital were studied. Incidence of progress to typical CD, initial clinical features, findings of X-ray examination, cumulative probability of surgery were investigated. We compared the initial clinical features and radiographic findings of the patients whose aphthous lesions progressed to typical lesions (longitudinal ulcers and cobble stone appearance) with patients whose lesions did not progress. Radiographic findings were analyzed concerning type and number of aphthous lesions and existence of edema. Results: Thirteen patients (59.1%) of 22 patients progressed to typical CD. Period of progress after initial diagnosis was 44.8 ± 37.8 months. Twelve patients (92.3%) of 13 patients progressed within 60 months (5 years) after diagnosis. There were no significant difference in inflammatory marker on laboratory examinations, activity index (CDAI) and the initial treatments were analyzed concerning type and number of aphthous lesions and existence of edema.

Discussion: In the present study, the %TR was proven to decrease in active UC. This observation supports recent studies that suppression of TR may play an important role in induction of aggressive immune responses in UC, and GCAP might have a potential to improve this autoimmune-disturbance by amplifying the TR expression. On the contrary, %CD28-CD4+ T-cells was elevated regardless of UC activity comparing with HV. Hence, CD28-CD4+ T-cells may include auto-reactive memory cells in UC and play a role in the recurrence of the disease.

Appendix is a Priming Site in the Development of Ulcerative Colitis
M. Matsuishita, K. Uchida, K. Okazaki
Department of Gastroenterology and Hepatology, Kansai Medical University Hakata Hospital

Aim: The role of the appendix has been highlighted in the pathogenesis of ulcerative colitis (UC). The aims of this study were to elucidate the immunomodulinal in the appendix of UC patients, and to clarify the role of the appendix in the development of UC. Methods: Colonoscopic biopsy specimens of the appendix, transverse colon, and rectum were obtained from 86 patients with UC. Active left-sided colitis (A-L; n = 25), A-L with appendiceal involvement (A-L/Ap; n = 10), inactive left-sided colitis (I-L; n = 14), and inactive left-sided colitis (I-L; n = 22), and from controls. In the isolated mucosal T cells, the CD4/CD8 ratio and proportion of activated CD4+ T cells were investigated, and compared with controls. Results: In the appendix, the CD4/CD8 ratio significantly increased in active UC compared with controls. The ratio significantly increased only in the appendix of UC patients. In the appendix, the CD4/CD8 ratio and proportion of activated CD4+ T cells were investigated, and compared with controls. Results: In the appendix, the CD4/CD8 ratio significantly increased in active UC compared with controls. The proportion of CD4+HLA-DR+ (mature activation antigen) T-cells significantly increased only in the appendix of UC patients. However, no significant difference was found in the proportion of CD4+HLA-DR+ (mature activation antigen) T-cells in the appendix of UC patients.

Discussion: It is suggested that the appendix is a priming site in the development of UC.
include celiac disease, allergic and eosinophilic enteropathies, lesions of the small bowel due to drugs and radiation, graft versus host disease and small bowel transplantation, as well as for the investigation of undiagnosed malabsorption, such as intestinal lymphangiectasia. Other studies have validated its role in children and established the safety of its introduction into the duodenum using a gastroscope in patients unable to swallow the capsule or for those with gastroparesis. Complications are rare and include capsule retention in diverticular or at stricture sites. A new patency capsule appears to diminish the risk of the latter problem in at risk patients. A double lens esophageal capsule has been shown to be promising for the evaluation of esophageal disorders, such as the presence of Barrett's esophagus and for varices.


1Ewha Womans University College of Medicine, 2Korea University College of Medicine, 3Yonsei University College of Medicine, 4Chung Ang University College of Medicine, 5Seoul National University College of Medicine, 6Inje University College of Medicine, 7the Catholic University of Korea, 8Hallym University College of Medicine, 9Apu University College of Medicine, 10Soon Chun Hyang University College of Medicine, Seoul, Korea

Background: Capsule endoscopy (CE) is approved for the evaluation of obscure GI bleeding and its use has increased in the assessment of patients with various small bowel disorders. The yield of CE for indications of disorders other than GI bleeding is not yet well described. The aim of the present study was to determine in which subgroup of patients with unexplained abdominal pain, CE would be a helpful evaluation tool. Methods: The results of CE in 110 patients (70 men, 40 women; mean age 50.8 ± 14.1 years) with unexplained abdominal pain from 12 tertiary referral centers between September 2002 and September 2004 were retrospectively analyzed. Results: The visualization of the small bowel to the cecum was achieved in 89.1% of the patients. Among the 110 cases revealed positive findings that explained the symptoms of the patient (diagnostic yield = 77.3%). Diagnosis included small bowel stricture (3), Crohn’s disease (3), small bowel tumor (2), radiation-induced enteritis (1), NSAID-induced enteropathy (1), ischemic ileitis (1), diffuse lymphangiectasia (1), and significant erosion or ulceration (5). By univariate logistic regression analysis, the positive findings of CE were significantly associated with weight loss (odds ratio (OR), 11.9; 95% CI [2.0, 70.6]), elevated ESR (>20 mm/h) (OR, 11.5; 95% CI [1.9, 65.3]), elevated CRP (≥4 mg/dl) (OR, 5.0; 95% CI [1.6, 15.9]), and hypoalbuminemia (albumin <3 g/dl) (OR, 23.1; 95% CI [2.4, 221.1]). By multivariate analysis, weight loss was found to be a significant risk factor for positive findings of CE (OR, 18.6; 95% CI [1.6, 222.4], p = 0.02). This study suggests that CE can be helpful in patients suffering from abdominal pain that cannot be explained by established examinations if the pain is accompanied by weight loss. Key words: Capsule endoscopy, Abdominal pain.

Oral Presentations

I-O-6-29 Double-Balloon Endoscopy for the Diagnosis and Treatment of Small Intestinal Disorders H. Kita, H. Yamamoto, T. Yano, T. Miyaya, M. Iwamoto, K. Sunada, M. Arashiro, Y. Hayashi, K. Ido, K. Sugano Department of Internal Medicine, Division of Gastroenterology, Jichi Medical University

Backgrounds: Double-balloon endoscopy (DBE) was developed by us based on a new insertion theory that enables the insertion of an endoscope into the distal portion of the small intestine. DBE consists of a dedicated endoscope that can mount a balloon at its distal end, an overtube with a balloon, and an air pump to inflate or deflate the balloon. DBE enables endoscopic scrutiny of the entire small bowel with intervention capabilities; targeted biopsy as well as endoscopic treatments including electrocoagulation, clip placement, balloon dilatation, and polypectomy are possible. Methods and Result: We performed 459 endoscopic examinations in 259 patients using the Fujinon double-balloon endoscopy system between September 2000 and December 2005. The spectrum of the small intestinal diseases in our experience was analyzed. Results: Of 259 patients, ulcerative and/or erosive lesions were found in 82 cases, including 18 cases of Crohn’s diseases, 8 cases of NSAIDs induced injuries, 4 cases of ulcers associated with Meckel’s diverticulum. Of 259 patients, tumors/polyps were found in 48 cases, including 9 cases of adenocarcinomas, 9 cases of GIST, and 3 cases of enteric invasion of the malignant tumor from other organs. We also found 27 cases of angiodysplasia in the small intestine. Endoscopic treatments, including hemostasis using clipping devices or electrocoagulation, polypectomy, endoscopic mucosal resection, balloon dilatation, and stent placement was successfully carried out in the small intestine. Balloon dilatation for strictures of Crohn’s disease was performed 21 times for 12 patients. Major complications such as perforations were not experienced from the endoscopic treatments. Conclusions: DBE is both feasible and useful technique for the diagnosis as well as treatment of small intestinal disorders.

I-O-6-30 Double-Balloon Endoscopy Preceded by Capsule Endoscopy for Optimal Diagnosis and Treatment of Obscure Gastrointestinal Bleeding S. Fujimori, T. Sea, K. Gudai, A. Ehara, T. Kobayashi, K. Mitsui, S. Tanaka, A. Tatsuguchi, Y. Sekita, K. Nagata, Y. Shibata, T. Kishida, K. Shikada Department of Medicine, Division of Gastroenterology, Nippon Medical School

Background: Double-balloon endoscopy has been proven an invaluable tool for total enteroscopy and the treatment of small intestinal diseases. However, it is difficult to determine, at the onset, the optimal approach or scope size. This study was to determine whether capsule endoscopy prior to double-balloon endoscopy could improve diagnosis and treatment in the setting of occult bleeding. Methods: We selected 24 patients (12 female, 12 male) with obscure gastrointestinal bleeding for our study. Female patients were 61.2 ± 17.5 years of age with a mean hemoglobin concentration of 8.5 ± 2.8 g/dl at the start of study; male patients were 54.2 ± 14.3 years of age with a mean hemoglobin concentration of 9.9 ± 3.4 g/dl. Prior to double-balloon enteroscopy subjects were first examined by capsule endoscopy. Results: Total small intestinal examination was successfully performed by capsule endoscopy in 17/24 (71%) patients, with suspected abnormalities found in 17/24 patients. Double-balloon endoscopy verified 11/17 (65%) of these suspected abnormalities as small intestinal lesions. The main diagnoses of these 11 patients consisted of 6 cases each of angiodysplasia, multiple red lesions, and gastrointestinal stromal tumors; and 1 case each of lymphangioma, inflammatory fibroid polyp, small intestinal ulcer, small intestinal erosion, and a small intestinal ulcer with NSAID-induced diaphragm disease. The remaining 2 patients were diagnosed with colonic diverticulum. There were no lesions found by double-balloon endoscopy in the remaining 11 patients. The angiodysplasia and polyp patients were successfully treated by double-balloon endoscopy. For seven patients we could choose the optimal approach, anal or oral; in four, the adequate scope size for treatment. Thus, capsule endoscopy data allowed for more efficient use of the double-balloon endoscopy. Conclusions: Capsule endoscopy yields valuable data that can guide our use of the double-balloon endoscopy for optimal diagnosis and treatment of patients with obscure gastrointestinal bleeding.

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I-O-6-31 Diagnosis Supporting System for Capsule Endoscopy Y. Yagi, V. Haisi, T. Echigo, R. Sagawa, K. Yagi, M. Shiba, K. Hijiguchi, T. Akiyama Kao Pharmaceutical University, Osaka City University Graduate School of Medicine

This paper presents a method for reducing diagnostic time by controlling frame rate adaptively in a capsule endoscopic image sequence captured during 8h. The video sequence would be
Risk Factors Related to Delayed Bleeding after Endoscopic Submucosal Dissection for Gastric Neoplasms
T. Sumiyoshi, H. Kondo
The Center for Digestive Diseases, Tonan Hospital

Background and Aim: Recently, endoscopic submucosal dissection (ESD) has been increasingly performed for the treatment of gastric neoplasms because it has advantages of higher en-block resection rate than conventional methods. However, delayed bleeding after ESD has often occurred as a major complication. The aim of this study is to evaluate risk factors related to delayed bleeding after ESD. Methods: We studied retrospectively a total of 206 patients who underwent ESD for solitary gastric neoplasms from October 2000 to December 2005. Delayed bleeding was defined that a hemostatic treatment such as endoscopic clipping and/or coagulation by hemostatic forceps was required after ESD. Among 15 patients with ulceration or ulcer scar with the lesion, scarring was achieved in 9 patients (60%), which was significantly low. (Study 2) Regenerative mucosa was hardly observed until 5 weeks after ESD. A mucosal defect was still observed in 2 patients who had severe fibrosis with the lesion due to previous peptic ulcer or submucosal invasion of the lesion. Conclusion: Preoperative fibrosis under the lesion may delay the healing process, but otherwise, gastric artificial ulcers after ESD would heal within 8 weeks.

Future Expectations in the Field of Endoscopic Resection for Early GI Cancers
T. Okano1, M. Yamamoto2, T. Okano3, K. Takasaki
1Department of Surgery, Institute of Gastroenterology, 2Institute of Advanced Biomedical Engineering and Science, Tokyo Women’s Medical University

In the field of endoscopic resection, remarkable progress with conventional endoscopic mucosal resection (EMR), named endoscopic submucosal dissection (ESD) has recently been developed. ESD allows for precise diagnosis and extensive en-block resections, thus decreasing the risk of recurrence. However, complications such as ulcerative stenosis due to extensive esophageal healing can still severely affect the quality of life (QOL) for patients. For improved treatment of early esophageal cancers we examined the transplantation of tissue engineered autologous oral mucosal epithelial cell sheets in an effort to reduce post-operative stenosis and inflammation. Autologous epithelial cells were isolated from specimens of oral mucosa harvested from beagle dogs, and cultured on temperature-responsive culture dishes for 2 weeks at 37°C. Oral mucosal epithelial cell sheets (24 x 24mm) were harvested by simply reducing the temperature to 20°C without the need for enzymatic treatments. Autologous epithelial cell sheets were then transplanted with endoscopic forceps onto an artificial esophageal ulcer after ESD. As deposited adhesive proteins were maintained on the basal side of the cell sheets, thus allowing for simple manipulation and transplantation without suture. Wound healing was accelerated in comparison to the control ESD group with complete re-epithelialization four weeks after transplantation. The present study with a clinically relevant canine model demonstrates the effectiveness of a novel endoscopic approach combined with tissue engineering for the potential treatment of esophageal cancers that can effectively enhance wound healing and possibly prevent post-operative esophageal stenosis. Additionally this novel method can be applied for the treatment of Barrett’s esophagus.

Comparison of Proton Pump Inhibitor with H2 Receptor Antagonist for Inhibition of Delayed Bleeding after Endoscopic Submucosal Dissection for Early Gastric Cancers: A Non-Blind Randomized Controlled Trial
Department of Gastroenterology, Osaka Medical Center for Cancer and Cardiovascular Diseases

Background and Aim: Preventive effect of proton pump inhibitor (PPI) and H2 receptor antagonist (H2RA) on bleeding after endoscopic mucosal resection (EMR) for early gastric cancer (EGC) has been examined by conventional methods. Introduction of endoscopic submucosal dissection (ESD) technique caused higher rate of delayed bleeding than the conventional methods. An aim of this study is to compare preventive effects of PPI with H2RA on bleeding from ulcer after ESD. Methods: This is a non-blind randomized controlled trial that was performed at an endoscopy unit in a cancer center between January and November 2005. A total of 120 patients (98 men and 22 women, mean age 68 years old) with EGC treated by ESD technique were enrolled. Median (range) tumor size was 15.2 (5-105) mm. Patients were randomized to two groups and received following treatments: rabeprazole 20 mg o.d. from 1 day before procedure (PPI group), and cimetidine 800 mg o.d. for 2 days followed by cimetidine 800 mg p.o. bid (H2RA group). Treatments were continued for 8 weeks. The rates of bleeding, that was defined as hematoctis and melena required endoscopic hemostasis, and decreased Hb of less than 2mg/dl, were measured as a primary endpoint. Results: No significant differences were observed in age, sex, history of peptic ulcer, status of Helicobacter pylori, mean tumor size, and location of the tumor between the groups. Bleeding rate of the PPI group (6.6%: 4/61) was less than that in the H2RA group (16.9%; 10/59: p = 0.067) although it did not reach to the significant level. Non-blinded design and a relatively small number of subjects seemed to be a limitation of the study. Conclusion: PPI could inhibit bleeding from ulcer after ESD for EGC compared with H2RA.
method are as effective as diluted epinephrine injection therapy.

**Aim:** The endoscopic injection therapy with diluted epinephrine (EIT) is the most widely and easily used procedure in the management for peptic ulcer bleeding. Argon plasma coagulation (APC) and hemoclipping (HEC) are the recent introduced endoscopic procedure. Thus we performed a prospective, randomized trial to compare the haemostatic efficacy between these three types of endoscopic therapy. **Methods:** Sixty patients with stigma of ulcer bleeding were randomly assigned to receive either EIT (n = 20), APC (n = 20) or HEC (n = 20) treatment during the period of Apr 2004 to Oct 2004. The three groups were matched for sex, age, site of bleeding, endoscopic finding, and initial hemoglobin at randomization. **Results:** Bleeding was initially controlled in 19 (95%) of the EIT group, 18 (90%) of the APC group, and 5 (4%) of the HEC group. Robleeding occurred in 2 (10%) of the EIT group, 0 (0%) of the APC group, and 1 (5%) of the HEC group. There was no emergent operation undergone in all the three groups. The stay in hospital was 6.7 days in EIT group, 5.6 days in APC group, and 5.4 days in HEC group. There was no mortality in all these groups. All the results of this study were no significant differences. **Conclusion:** The Argon plasma coagulation and hemoclipping method are as effective as diluted epinephrine injection therapy.

**I-O-7-37**

**Magnification Endoscopy in the Gastric Ulcer**

L. Xueling
Qi Lu Hospital of Shandong University, Jinan, Shandong, China

Recently, magnifying endoscope has been used more frequently in clinical for its developments in amplifying power, definition and operational capability. Magnifying endoscopy is helpful for more correctly distinguishing hyperplastic lesions from adenomatous and cancerous lesions, and for improving detection of early flat and depressed cancer. We observed the ulcer used magnifying endoscope. The margin is smooth or not. There is evidence of surrounding mucosal discoloration or nodularity. The generative epithelium at the border of the ulcer and the red scar. The digestion ulcer diagnosis standard includes: (1) Has the ulcer the typical symptom, or does not have the symptom, formerly or in family ulcer medical history. (2) In under the endoscope according to the ulcer shape (smooth, hollow, stration, frost spot, scar, mixed type ulcer and so on) may make the diagnosis. (3) The mucous membrane biopsies assumes the anxious chronic ulcer, has the model the ulcer histology characteristic, the decidable ulcer nature, the ulcer depth graduation, whether there is the Hp infect and the ulcer heals the quality and so on. Enhanced magnification endoscopy is an effective, readily available method that can be used to assist in target biopsies and endoscopic diagnosis leading to an endoscopic classification system. The technique is not difficult and adds only an additional 5 -10 min to a standard endoscopic procedure. The value of this technique is still being explored. Increased use of this technique will aid in the diagnosis of ulcer. Using enhanced magnification endoscopy at multiple centers in studies will help to standardize the endoscopic criteria for ulcer.

**I-O-8 Helicobacter pylori-Clinical**

**Chairpersons:** S.K. Lam, S. Takahashi

**I-O-8-38**

**Recent Advance in Pathogenesis and Treatment of Gastrudodenal Diseases Associated with Helicobacter pylori Infection**

S.K. Lam
Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong

Helicobacter pylori infection is a major cause of gastritis and peptic ulcer disease, and contributes to at least 50% of cases of gastric cancer. Although the underlying mechanisms have not been fully elucidated, accumulating evidence has revealed that H. pylori stimulates the production of a large amount of cytokines and chemokines by the inflammatory and epithelial cells within the gastric mucosa, leading to acute inflammation and chronic inflammation. Moreover, H. pylori infection induces apoptosis and subsequently increases cell proliferation of gastric epithelial cells and glands. The balance between apoptosis and cell proliferation is usually maintained in H. pylori-induced gastric inflammation. However, following the genetic and epigenetic alterations of related genes, the balance may be eventually lost. When apoptosis is dominant, ulceration or atrophy may develop. When cell proliferation is dominant, dysplasia or even gastric cancer may develop. Moreover, bacterial cytotoxicity and host genetic build-up both contribute significantly to the development of various gastrointestinal diseases associated with H. pylori infection. Eradication of H. pylori is the only feasible strategy to cure and prevent H. pylori-associated gastroduodenal diseases at present. Treatment with a single antimicrobial agent is essentially ineffective, and early reports of the relatively high efficiency of some dual therapies (e.g., omeprazole plus amoxicillin or clarithromycin) have not been reproducible. Over the past 15 years, several triple regimens including bismuth-based, proton pump inhibitor (PPI)-based and ranitidine bismuth citrate – based triple therapies, have been evaluated worldwide with eradication rates ranging from 70 to 95%, and thus used as the ‘standard’ ‘first-line’ regimens. Moreover, several regimens, including quadruple therapy (e.g., bismuth-based triple therapy plus a PPI, or H2 blocker), furazolidone-based, thio Rumfodolone-based, and rifabutin-based triple therapies have also evaluated and recommended as ‘alternative first-line’, or ‘rescue’ regimens. Recently, ‘multi-step’ and ‘sequential’ strategies have been reported with eradication rates of over 90%.

**I-O-8-39**

**Clinicopathologic Features of Gastrointestinal Cancer in Korea**

Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine

In Korea, gastric cancer is the most prevalent malignant neoplasm and the second leading cause of death by malignancy, although its incidence is slowly decreasing. We reviewed several recent reports to examine the clinicopathologic features of gastric cancer in Korea. Korean gastric cancer had a wide age distribution range from the thirties to the eighties with highest incidence in the seventies (33%). The male to female ratio was 2.2:1. Of a wide variety of etiologic risk factors, Helicobacter pylori infection may be one of the important risk factors in Korea, an area of high prevalence of H. pylori infection that approaches 80%. More than 50% were located in the lower third of stomach or the lesser curvature site. On histopathologic examination, adenocarcinomas accounted for more than 95% including 17% with signet-ring cell carcinoma and 2% with mucinous carcinoma. According to the UICC staging, the proportion was 29.3% for stage Ia, 13.9% for stage Ib, 14.8% for stage II, 13.2% for stage IIIa, 6.3% for stage IIIb, and 18.1% for stage IV. In early gastric cancer (EGC), type IIc was the most common (42.8%). Since EGC is known to have a highly favorable prognosis (5-year survival rate >90%), early detection is very important. Although the proportion of EGC reached almost 50% after the institution of mass screening in Japan, it was only 24-35% in Korea despite its increase during the last two decades. The symptoms of Korean gastric cancer patients were non-specific and vague; more than 80% of the symptomatic patients experienced epigastric pain and discomfort. Moreover, the proportion of the asymptomatic patients has increased up 17%. These features appear to support the usefulness of gastroscopy as a screening test for gastric cancer in Korea, an area of high incidence of gastric cancer.

**I-O-8-40**

**The Efficacy of a Clostridium Butyricum Preparation Concomitantly with Helicobacter pylori Eradication Therapy in Relation to Changes in the Intestinal Bacterial Flora**

K. Imase1, M. Takahashi2, A. Tanaka1, K. Iokunaga1, N. Aoki1, M. Tanaka1, H. Sugano1, H. Ishida1, S. Karna1, S. Takashashi1
1Third Department of Internal Medicine, 2Department of Infectious Disease, Kyorin University School of Medicine, 1Kyiyarian Pharmaceutical Co., Ltd.

H. pylori eradication therapy is currently used widely in clinical practice. But side effects, such as diarrhea and soft stools, occur in H. pylori eradication therapy. In this study, we administered a Clostridium butyricum MYIAR588 strain preparation (CBM588, Miyas BM® Tablet), concomitantly with H. pylori eradication therapy and investigated the changes in the intestinal bacterial flora in relation to improvement of the clinical symptoms. **Material and Methods:** Nineteen patients with H. pylori-positive peptic ulcer were divided into three groups: Group A (H. pylori eradication therapy without CBM588) group B (with regular dose of CBM588) and group C (with double dose of CBM588). The subjects were instructed to fill in a questionnaire everyday throughout the treatment. And the composition of the intestinal bacterial flora was analyzed by examining stool specimens. **Results:** The incidences of diarrhea and soft stool were reported 71% in Group A and 29% in Group B, while none of the patients in Group C reported these symptoms. Besides the changes in composition of the intestinal bacterial flora, obligate anaerobes decreased 3 days after the start of the H. pylori eradication therapy in Group A but remained stable in Group C. In addition the detection of Group C difficile enterotoxin (Toxin A) showed a tendency towards increase in Group A but was not detectable in Group C. **Conclusion:** Side effects of H. pylori eradication therapy, including diarrhea and soft stools, are considered attributable to disturbances in the composition of the intestinal bacterial flora caused by the antibiotics. These results suggest that these symptoms can be prevented by probiotics.
**I-O-9 Helicobacter pylori-Related Chronic Mucosal Inflammation in Gastric Remnant after Gastric Cancer Surgery**

*Department of Surgical Oncology, Osaka City University Graduate School of Medicine*

**Purpose:** The remnant stomach after surgery for gastric cancer is at high risk for the development of secondary primary cancers. We examined *H. pylori* infection in gastric remnant after distal gastrectomy for primary gastric cancer and investigated the relationship between *H. pylori* infection and chronic mucosal inflammation. **Methods:** One hundred-nine patients who had been performed gastrectomy were studied. Endoscopic findings and results from urease test, bacteriologic assessment, serological test, histopathological examination were analyzed. Twenty-seven *H. pylori*-positive patients were treated with amoxicillin 500mg, clarithromycin 800mg and rabeprazole 20mg daily for 7 days. **Results:** Seventy-one patients (65.1%) were judged positive for *H. pylori* infection. The prevalence of *H. pylori* infection was found to decrease significantly in older cases, cases that passed long after operation, cases with symptoms, or cases with severe reflux gastritis. On the other hand, histologically chronic and acute gastritis correlated significantly with *H. pylori* infection. *H. pylori* prevalence was the most frequent in the middle atrophic mucosa and was decreased along with the completion of atrophic change. Eradication was successful in 25 cases (92.6%), and eradication therapy resulted in a significant decrease in inflammatory cell infiltration of the mucosal layer. **Conclusions:** Persistence of *H. pylori*-related active gastritis after gastric cancer surgery was suggested in the gastric remnant of young patients with mild atrophic gastritis and without reflux gastritis. Eradication therapy was as effective and safe in the remnant stomach as in non-operated stomach to minimize the mucosal inflammation. **Chairpersons:** K.B. Hahn, N. Yoshida

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**I-O-9/44 Formation of MALT Lymphoma by Helicobacter Heilmannii Infection: Relation to Parietal Cell Apoptosis and Microcirculatory Alteration**

*M. Nakamura, S. Takahashi, H. Matsuzaki, T. Iizumoto*  
*Institute of Fermentation, Japan, Tokyo, Japan*

Helicobacter heilmannii has been reported to colonize the stomach, while its pathological significance is still controversial. Thus, the present study was undertaken to observe the localization of Helicobacter heilmannii-like organism (HHLO) in the fundic area. In addition, the microcirculatory alteration was studied. **Materials and Methods:** 1C5BL/6 mice, infected with HHLO for more than 6 months were used in the following experiments. The localization of the HHLO was observed by the indirect immunofluorescence method using monoclonal antibodies against Helicobacter pylori. Microvascular alteration was studied by intravenous injection of FITC-dextran (MW 50,000) and VEGF immunoreactivity. **Results:** HHLO in the fundic area of mice infected with HHLO for more than 6 months were observed in the body of the glandular lumen as well as in the mucous layer. Tunel method and caspase 3, 8 and 9 immunoreactivities revealed that about one third of the infected parietal cells cells fell into apoptosis. In addition, MALT lymphoma was formed in almost 10% of the mice infected for more than 6 months. Apoptotic parietal cells were recognized around the lymphoma by immunohistochemistry of caspase 3, 8 and 9 and electron microscopy. Many of the accumulated lymphocytes were immunoreactive to B220 and Bcl-2. Surrounding these proliferative lesion, the increase of microvascular distribution and permeability was observed. **Conclusion:** HHLO was shown to be related to the formation of MALT lymphoma and microcirculatory alteration was suggested to be related to the lesion formation.

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**I-O-45 Positive Crosstalk between Signaling Molecules in Helicobacter pylori-Induced Cyclooxygenase-2 Expression in Gastric Epithelial Cells**

*J.H. Seo, S.O. Cho, K.H. Kim*  
*Department of Pharmacology and Brain Korea 21 Project for Medical Science, College of Medicine, Department of Food and Nutrition, College of Human Ecology, Yonsei University, Seoul, Korea*

Previously we have shown that cyclooxygenase-2 (COX-2) was expressed in *H. pylori*-infected gastric epithelial cells. 2 NF-κB and 2 AP-1 consensus sites are present in promoter region of human COX-2 gene. COX-2 expression is reported to be mediated by mitogen-activated protein kinases

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**I-O-9-43 Chemoprevention of Gastric Cancer by Celemobin in H. pylori-Infected Mongolian Gerbils**

*Division of Gastroenterology, Nippon Medical School*

H. pylori infection is recognized surrounding the lymphoma by immunohistochemistry of caspase 3, 8 and 9 and electron microscopy. Many of the accumulated lymphocytes were immunoreactive to B220 and Bcl-2. Surrounding these proliferative lesion, the increase of microvascular distribution and permeability was observed. **Conclusion:** HHLO was shown to be related to the formation of MALT lymphoma and microcirculatory alteration was suggested to be related to the lesion formation.
I-O-9-46  Gastrin Enhances the Gastric Mucosal Injury Associated with Helicobacter pylori Infection in Mongolian Gerbils
T. Brzozowski, P.C. Konturek\(^1\), W. Bielskosi, D. Drozdowicz, R. Pajdow, K. Mach, \(\text{M. Jaros, S.K. Konturek, W.W. Pawlik}\)

Department of Physiology, Jagiellonian University Medical College, \(1\) Department of Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany

Hp-infection in Mongolian gerbils is an established experimental model of gastric carcinogenesis resulting from the long-term Hp-infection but the role of gastric and prolonged hipergastrinemia induced by omeprazole treatment in this model has been little studied. We examined the effects of intragastric inoculation of Mongolian gerbils with Hp strain (cagA\(^+\), vacA\(^+\), \(5 \times 10^6\) CFU/ml) treated daily for the 3 weeks with: 1) vehicle (saline); 2) omeprazole (17-10nmol/kg i.p.); 3) gastrin (40nmol/kg s.c.) and 4) omeprazole (10mg/kg). Some groups of animals received Lacidiolfi (Rossell, Ortalo, Canada) containing probiotics L. rhamnosus and L. acidophilus. At 4, 12, 30 and 60 weeks later, the gastric blood flow (GBF) was measured by H2-clearance technique and plasma gastrin levels (RIA) and expression of COX-2 mRNA (Western Blot) and apoptosis (Bax and Bcl2) were assessed. The viable Hp was analyzed by rapid urease test (CLO) and the density of Hp-colonization and histology of glandular mucosa were determined. The gastric Hp-infection was detected in all animals by histology, Hp-culture and CLO. By the treatment with Lacidiolfi, the GBF in Hp-infected gerbils treated with gastrins or omeprazole were significantly enhanced. Plasma gastrin, apoptosis and the expression of COX-2 protein in the gastric mucosa were markedly enhanced in Hp-infected gerbils treated with gastrins or omeprazole and these effects were significantly attenuated by the treatment with Lacidiolfi. The GBF in Hp-infected gerbils treated with gastrins or omeprazole was significantly lower than that in vehicle-treated animals. We conclude that: 1) gastrin and prolonged hypergastrinemia resulting from long term omeprazole treatment with the sub- sequent COX-2 induction, play a major role in the development of typical functional changes such as impairment of gastric mucosal microcirculation and apoptosis predisposing to precancerosis, and 2) probiotics are beneficial in counteracting of the pathologic aspects of Hp-infection.

II-O-1-48  The Novel Acid Pump Antagonist, Revapran, Protect NSAID-Induced Gastropathy through the Induction of Heat Shock Protein 27
M.S. Kawk, Y.-M. Na, S.J. Suk, D.J. Lee, D.W. Kim, Y. Cho, K.-B. Hahn

Genome Research Center for Gastroenterology, Ajou University Medical Center

Oral Presentations

I-O-1-47  Molecular Pharmacological Approach to Drug Actions on the Afferent and Efferent Fibres of the Vagal Nerve in the Gastric Mucosal Protection (Animal Experimental and Human Observations)
G. Mozsi, D. Andras
First Department of Medicine, Medical and Health Centre, University of Pe\'cs, Hungary

Background: The gastric mucosal protection is associated with the effects of drugs (compounds) acting on the afferent and/or efferent fibres of the vagal nerve. Aims: 1) To identify the dose-response curves of drugs (compounds) acting on the afferent (capacins, resinferatoxin) and efferent (atropine, Pirenepine, cimetidin, ranitidine, famotidine, omeprazole, esomeprazole) on gastric (basal and stimulated) acid secretion, chemicals-induced gastric mucosal damage in rats and in healthy human subjects; 2) To identify and to compare the ED50 values of these compounds. Materials and Methods: 1) The observations were carried out in rats (30 different models), in healthy human subjects. Different NSAIDs were used to produce gastric mucosal damage. Results: 1) ED50 values of the studied compounds were found: 3.27nmol/kg capacin for inhibition of gastric acid secretion; 0.03nmol/kg pyloric atropine and gastrins-induced rats and 0.054nmol/kg TX for inhibition the gastric mucosal damage in 20mg/kg indomethacin plus 4h pylorus-ligated rats, 20mg/kg indomethacin in 0.15N HCl for 4h pylorus-ligated rats. 2) The ED50 value of capacin is 0.327-2.616nmol/kg for inhibition of gastric acid output and indomethacen-induced gastric mucosal damage in healthy human subjects. 3) The ED50 values for atropine 3.45μmol/kg, 0.178nmol/kg for Pirenepine, 1.58-3.96nmol for cimetidine, 0.478-0.954nmol/kg for ranitidine, 0.119-0.237nmol/kg for famotidine and 57.9-115.8μmol/kg for omeprazole and esomeprazole. Conclusion: 1) The molecular concentrations (ED50) values of drugs (compounds) acting on the afferent nerves are 2-3 times less than those on the efferent nerves. (2) The stimulation of capacin-sensitive afferent nerves by capacin (RTX) represents the first line in the regulation of gastric secretion and development of gastric mucosal prevention in animals and humans. Grant: RET-II/08/2005.
II-O-1-50
α-Lactalbumin Suppresses Interleukin-6 Release after Intestinal Ischemia/Reperfusion via Nitric Oxide in Rats
M. Yamaguchi, M. Uchida
Meiji Daines Company

Background and Aims: Milk protein is one of the most trusted food ingredients with high nutritional value. Recent studies have offered considerable information about its biological properties and nutritional functions. In this study, we investigated the effect of milk proteins, casein, whey protein isolate (WPI) and its components, on the interleukin (IL)-6 release induced by the intestinal ischemia/reperfusion in rat. Methods: Male Sprague-Dawley rats were undernourished mesenteric artery occlusion for 45 min followed by reperfusion. Each milk proteins (300mg/kg) were injected into the duodenum 1 h before the induction of ischemia. IL-6 release in the blood was measured in course of time. The duration of the protective effect of αLA was also examined. Results: WPI had the suppressive effect against ischemia/reperfusion-induced IL-6 release (7.8 ± 2.2ng/ml vs. 18.1 ± 3.2ng/ml; p < 0.05), while casein showed no effect. Among the three major components contained in WPI, α-lactalbumin (αLA), β-lactoglobulin (βLG) and bovine serum albumin (BSA), αLA showed the most potent and dose-dependent suppressive effect on IL-6 release. αLA also had the suppressive activity on IL-6 release, while BSA showed no effect. As the interval between αLA pre-administration and ischemia/reperfusion was increased, the degree of protection decreased. However, the prophylactic protective effect of αLA persisted for at least 2 h until ischemia/reperfusion. Among these protocols, 1 h interval was the most effective. NG-nitro-L-arginine methyl ester, nitric oxide (NO) synthase inhibitor, significantly diminished the IL-6 release by IL-6 release by αLA. Conclusions: NO inhibits NF-κB transcriptional activation and subsequent expression of the pro-inflammatory cytokines, such as tumor necrosis factor-α, interferon-β, IL-8, IL-1β, IL-2 and IL-6. From these findings, the present results suggest that αLA has marked anti-inflammatory activity, at least in part, through NO/NF-κB-mediated mechanism.

II-O-2 Mucosal Defense 2
Chairpersons: J.D. Kaunitz, B.M. Peskar

II-O-2-53
Gastrointestinal Defense: Role of Endogenous Mediators
J.D. Kaunitz, T. Takeuchi, M. Mizumori, Y. Akiba
Greater Los Angeles VAMC, CURE, and UCLA Department of Medicine, Los Angeles, CA, USA

The duodenal absorbs ~450mmol of H+24h in order to prevent distal gut acidification. HCl in the duodenal lumen is neutralized by HCO3-- secreted by the pancreas and duodenal epithelium, generating extremely high luminal CO2 pressures (pCO2 > 30kPa) which dissipate by the proximal jejunum. Thus, the duodenum is the major site for intestinal H+ and CO2 absorption. We hypothesized that most of the excess duodenal luminal H+ was absorbed through neutralization of secreted HCO3--; yielding luminal CO2, which would then be transported across the apical membrane, entering the cytoplasm, and hydrated to H2CO3, which then dissociates in the cytoplasm to H+ and CO32--. H2CO3 – HCO3-- is then secreted into the lumen whereas H+ is transported into the submucosal space by membrane transport proteins. Luminally H+ is then transported through the apical membrane as CO2, but HCO3-- is simultaneously secreted in its anionic form. Luminally acid stress provokes several epithelial responses, such as acidification of the cytoplasm, and increased mucosal blood flow, HCO3-- secretion, and mucus secretion. Since these responses are likely dependent on acidification of the subepithelial space, and since elevated luminal CO2 or H+ provokes similar responses, luminal CO2 must be converted to subepithelial H+ and CO2, which then signals these protective mechanisms. Measurement of the movement of CO2 and H+ between lumen, mucosa, and the portal vein in the presence of absence CA inhibitors supported our hypothesis that luminal H+ neutralized by secreted HCO3--; is converted to CO2 prior to entry into the intracellular space. Since cellular CO2 is reconverted to H+ and HCO3-- which is then transported into the portal vein. Through the understanding of epithelial H+ and CO2 and its interconversion with CO2, we are working towards a clearer understanding of how normally fragile epithelial cells can withstand extremes of pH and pCO2 without damage.

II-O-2-54
TRPV-1 Knockout Paradoxically Reduces Gastrointestinal Injury by Unraveling Compensatory Protective Mechanisms in Mice
J. Meyrowitz, Y. Akiba, M. Mizumori, P.H. Guth, E. Engel, J.D. Kaunitz
Department of Medicine, West Los Angeles VA Medical Center, CURE/UCLA and Brentwood Biomed Res Inst, Los Angeles, USA

Capsaicin-sensitive afferent nerves coordinate upper gastrointestinal tract mucosal protective mechanisms through the release of calcitonin gene-related peptide (CGRP) and increasing prostaglandin (PG) synthesis. Capsaicin receptor TRPV-1 may serve as a primary acid sensor that activates capsaicin-sensitive nerves in response to luminal acidity. To evaluate the role of TRPV-1 in gastric mucosal protection, we examined its role in gastric injury using TRPV-1 knockout (KO) mice, hypothesizing that KO would increase injury susceptibility. Wild type C57BL/6 (WT) and KO mice were gavaged with 150mg HCl/60% ethanol. Pretreatment with CGRP receptor antagonists hCGRP (0.3mg/kg, i.v), selective cyclooxygenase (COX) inhibitor indomethacin (Indo, 5mg/kg, sc), nitric oxide synthase inhibitor L-NAME (20mg/kg, sc), TRPV-1 antagonist capsazepine (CPZ, 10mg/kg, sc), or capsaicin (10mg/kg, i.g) were given. Expression of acid-sensing ion channels (ASICs), COX, EPI or IP receptor, and calcu (pro-CGRP) gene in stomach, dorsal root ganglion (DRG) and nodose ganglion (NG) were analyzed by real-time RT-PCR. Unexpectedly, acid/ethanol treatment injured the gastric mucosa of KO mice significantly less than it
GST and HO-1 were more up-regulated than those at 4-week. 3) Treatment with NS-398 excreted by up-regulating nrf2-dependent antioxidant enzymes. We aimed to determine if endogenously generated 15-DPGJ2 contributes to gastric mucosal protection against oxidative stress, plays an important role in induction of antioxidant enzymes (GST, and HO-1) were analyzed by real-time RT-PCR. Mucosal level of 15-DPGJ2 was shown to exert anti-inflammatory action by activating PPAR γ. Although PGJ2 family has been shown to exert anti-inflammatory action by activating PPAR γ, its exact mechanisms by which 15-DPGJ2 protects cells against oxidative stress remains unclear. We have previously reported that nrf2 (NF-E2 p45-related factor-2), a transcription factor, which regulates cellular response against oxidative stress, plays an important role in induction of nrf2-dependent nuclear accumulation of Nrf2. This up-regulation appears to protect the gastric mucosa against oxidative injury. 15-deoxy PGJ2 (15-DPGJ2), which is induced apoptosis in vitro and in vivo and in vivo. During 8–16-week treatment, mucosal inflammation improved over time, accompanied by up-regulation of HO-1 and nuclear accumulation of Nrf2. Conclusions: Up-regulation of HO-1 by NSAIDs seems to be mediated by the p38 MAPK-dependent nuclear accumulation of Nrf2. This up-regulation appears to protect the gastric mucosa from NSAID-induced gastric lesions by inhibiting NSAID-induced apoptosis.

**Background and Aims:** Gastric mucosal cell death by non-steroidal anti-inflammatory drugs (NSAIDs) is suggested to be involved in NSAID-induced gastric lesions. Therefore, cellular factors that suppress this cell death are important for protection of the gastric mucosa from NSAID-induced lesions. 1) In nrf2−/− mice, 4-week treatment with NS-398 exacerbated the gastric mucosal injury in TRPV-1 KO can be explained by compensatory upregulation of CGRP release and COX-PG function. In KO, up-regulation of ASIC1 in NG and ASIC3 in DRG may serve as compensatory, alternative acid sensors in affenters that may coordinate CGRP release and PG synthesis.

**Results:** In cultured gastric mucosal cells, all NSAIDs tested up-regulated HO-1 at concentrations that did not affect cell viability. In rats, orally administered indomethacin up-regulated HO-1, induced apoptosis and produced lesions at gastric mucosa. An inhibitor of HO stimulated NSAID-induced apoptosis in vitro and in vivo and also stimulated NSAID-produced gastric lesions. Indomethacin activated the HO-1 promoter and caused nuclear accumulation of NF-E2-related factor 2 (Nrf2), a transcription factor for the HO-1 gene. Indomethacin stimulated phosphorylation of p38 mitogen activates protein kinase (MAPK), while p38 MAPK inhibitor suppressed the endogenous HO-1 up-regulation and nuclear accumulation of Nrf2. Conclusions: Up-regulation of HO-1 by NSAIDs seems to be mediated by the p38 MAPK-dependent nuclear accumulation of Nrf2. This up-regulation appears to protect the gastric mucosa from NSAID-induced gastric lesions by inhibiting NSAID-induced apoptosis.

**Background:** Persistent H. pylori infection causes gastritis, ulcers, and gastric cancer. However, cells can withstand stresses by inducing a variety of antioxidant enzymes, thereby prevents gastric mucosa from oxidative injury. 15-deoxy PGJ2 (15-DPGJ2), which is endogenously generated by COX-1 in inflamed tissues, affords strong cytoprotective activity against oxidative stress (Ann NY Acad Sci 2003;993:208–216). Although PGJ2 family has been shown to exert anti-inflammatory action by activating PPAR γ and by inhibiting NF-κB, the exact mechanisms by which 15-DPGJ2 protects cells against oxidative stress remains unclear. We have previously reported that nrf2 (NF-E2 p45-related factor-2), a transcription factor, which regulates cellular response against oxidative stress, plays an important role in induction of antioxidant enzymes in gastric mucosa during H. pylori infection. During P. aeruginosa infection (AGA 2003). In the present study, we aimed to determine if endogenously generated 15-DPGJ2 contributes to gastric mucosal protection via up-regulating nrf2-dependent antioxidant enzymes. Methods: Standardized mice model of Hp infection were set up by inoculating C57BL/6 female mice (12-weeks) with H. pylori-Sydney Strain. SS1. Mice were maintained with high salt diet (7.5% NaCl). Some mice were treated with either NS-389 and/or 15-DPGJ2. Mice were sacrificed at 4, 8, and 16-weeks later. Degree of gastritis was evaluated by updated Sydney system. DNA damage was evaluated by measuring mucosal level of 8-OHdG. Expressions of TNF-α, IL-1β, COX-2, and antioxidant enzymes (GST, and HO-1) were analyzed by real-time RT-PCR. Mucosal level of 15-DPGJ2 was evaluated using ELISA. Results: 1) In nrf2−/− mice, 4-week treatment with Hp + high salt diet caused severe inflammation in corpus mucosa, accompanied by marked elevation of TNF-α, IL-1β, COX-2 and 8-OHdG, while expressions of 15-DPGJ2, GST and HO-1 were not prominent. 2) During 8–16-week treatment, mucosal inflammation improved over time, accompanied by a gradual decrease in levels of TNF-α, IL-1β, COX-2, and 8-OHdG. In contrast, 15-DPGJ2, GST and HO-1 were more up-regulated than those at 4-week. 3) Treatment with NS-389 exacerbated the corpus gastritis at 4-week, and inhibited subsequent improvement of gastritis, whereas administration of exogenous 15-DPGJ2 in combination with NS-389 rescued the worsening effect of NS-389 on gastric mucosa. 4) The effects of NS-389 and 15-DPGJ2 were completely abolished in nrf2−/− mice treated with Hp and high salt. Conclusion: These results suggest that endogenous 15-DPGJ2 protects gastric mucosa against oxidative stress during Hp infection, at least in part by up-regulating nrf2-dependent antioxidant enzymes.

**Background and Aim:** We previously reported that both prostaglandin E2 and NOR-3, the NO donor, increased gastric HCO3− secretion, mediated intracellularly by Ca2+ and cyclic 3’,5’-guanosine monophosphate (cGMP), respectively though forskolin the stimulator of adenylate cyclase did not. However, there is no information about the isozymes of phosphodiesterase (PDE) involved in this process. In the present study, we examined the effects of various isozyme-selective PDE inhibitors on the HCO3− secretion in rat stomachs in vitro and mouse stomachs in vivo, and investigated which types of PDE isozymes are involved in the HCO3− response to NO. Methods: Male SD rats and Male DDDY mice were used. The rat stomach mounted on an ex-vivo chamber was perfused with saline under urethane anesthesia, while the mouse gastric mucosa was mounted on an Using chamber, and the tissue was bathed in saline on the mucosal side and HCO3−. Ringer’s solution on the serosal side. The HCO3− secretion was measured at pH 7.0 using a pH-stat method. Results: NOR-3 increased HCO3− secretion in a dose-dependent manner in both rat and mouse stomachs, and the response to NOR-3 was inhibited by methylene blue, but not verapamil. Sildenafil, the PDE5 inhibitor, caused a significant increase of the secretion of HCO3− and potentiated the response to NOR-3 in rat stomachs. Likewise, in the mouse stomach both vascupetine (PDE1 inhibitor) and zaprinast (PDE5 inhibitor), but not other PDE inhibitors, increased the HCO3− secretion and potentiated the response to NOR-3 at the dose that had no effect by itself on basal HCO3− secretion. RT-PCR analyses revealed the expression of PDE1 and PDE5 mRNAs in the mouse gastric mucosa. Conclusion: These results confirmed that NO stimulates gastric HCO3− secretion mediated intracellularly by cGMP, and further suggested that this action is regulated by both PDE1 and PDE5.

**Background:** Major pathways of arachidonic acid metabolism known to contribute to the maintenance of integrity and healing of ulcers in the gastric mucosa involve cyclooxygenase (COX-1 and COX-2) and 5-, 12- and 15-lipoxygenase (LO). In normal rat gastric mucosa, damage only occurs when both COX-1 and COX-2 are inhibited. In contrast, in rats subjected to ischemia-reperfusion, suppression of nitric oxide biosynthesis or denervation ofafferent neurons, isolated inhibition of either COX-1 or COX-2 induces marked damage. In rats with gastric ulcers, expression of COX-2 is increased in the ulcerated area whereas expression of COX-1 is decreased adjacent to the ulcer crater. Inhibition of COX-2 significantly delays gastric ulcer healing. In rats ischemia-reperfusion causes only minor damage in the absence of additional treatment. Co-administration of inhibitors of 5-LO (Aspirin), 12-LO (Ibuprofen) and 15-LO (Piroxicam) increases the ulcerogenicity of non-steroidal anti-inflammatory drugs. Thus, aspirin, indomethacin and paracetamol do not aggravate gastric mucosal damage during ischemia-reperfusion when given at low doses but substantial injury occurs when the same low doses of the drugs are administered together with the LO inhibitors or Boc-1. The findings show that arachidonic acid is metabolized by various enzymatic pathways to lipid mediators that are involved in gastric mucosal defense and acceleration of ulcer healing. These mediators act either separately or in concert depending on the pathophysiological background.
Ghrelin Attenuates the Experimental Induced Esophagitis

Methods: Twenty patients with reflux esophagitis were included in the study. The expression of ghrelin and its receptor was assessed by quantitative RT-PCR. In Wistar rats, the esophageal experimental ulcers were induced by serosal application of acetic acid on subdiaphragmatic portion of esophagus. Following experimental groups were included: (1) control (esophageal ulcers + placebo) and (2) esophageal ulcers + ghrelin 10 μg/kg/day s.c., three days. The ulcer area was assessed by planimetry and esophageal mucosal blood flow by H2 gas clearance method. In addition, in vitro experiments were performed in which OE-19 cells were incubated with ghrelin alone (150 and 450nM) or ghrelin with addition of TNF-α (10ng/ml) to simulate inflammatory state. The expression of COX-2, iNOS and IL-1β was assessed by quantitative RT-PCR.

Results: In humans with reflux esophagitis expression of ghrelin and a significant upregulation of ghrelin receptor were recorded in the esophageal mucosa as compared to controls. In rats with esophageal mucosa, the treatment with ghrelin reduced significantly the esophageal injury and this effect was accompanied by a significant rise in esophageal mucosal blood flow. The 'in vitro' experiments demonstrated expression of COX-2 and iNOS, and a significant increase in inducible NO synthase (iNOS) in esophageal cell line (OE-19).

Conclusion: Ghrelin represents a novel ulcer healing and anti-inflammatory factor for the esophageal mucosa. Its beneficial effects seems to be due to iNOS and increased generation of NO, contributing to anti-inflammatory effects of ghrelin.
II-O-3-63

Regulation of Gastric Ghrelin Secretion by Nitric Oxide (NO) Derived from Neuronal Nitric Oxide Synthase (nNOS)


1Department of Emergency Medicine, 2Department of Internal Medicine, 3Center for Integrated Medical Research, 4Department of Biochemistry and Integrative Medical Biology, School of Medicine, Keio University, Tokyo, 5Department of Biochemistry, National Cardiovascular Center Research Institute, Osaka, Japan

Background: Ghrelin is a gastrointestinal peptide with physiological roles, including stimulation of growth hormone release, food intake and gastric motility. Although in normal obese individuals, decreased plasma ghrelin levels have been reported, increased plasma and gastric ghrelin levels have been reported in patients with Prader-Willi syndrome (PWS) characterized by hyperphagia and obesity. This could be attributable to the lower level of insulin resistance in PWS. Nitric oxide (NO) induces adaptive relaxation of the esophageal sphincter or gastric fundus. NO is considered to be one of the primary mediators for meal ingestion. Thus, a relationship between NO and ghrelin is suggested. Using neuronal nitric-oxide-synthase-deficient (nNOS−/−) mice, the present study was designed to investigate the role of NO derived from nNOS in gastric ghrelin production and secretion. Method: Twelve week old male nNOS−/− mice (n = 10) and wild-type (WT) mice (n = 10) were examined after 18 hours fasting. The plasma and gastric ghrelin levels were measured by radioimmunoassay, and the level of proghrelin mRNA was examined by quantitative RT-PCR. Serum insulin levels were measured by ELISA. Gastric ghrelin-immunoreactive active cells were evaluated by immunohistochemistry. Results: In the nNOS−/− mice, the body weight, food intake, plasma and gastric levels of ghrelin, gastric levels of proghrelin mRNA and numbers of gastric ghrelin-immunoreactive cells were significantly increased compared with those in the WT. On the other hand, there were no significant differences between the two groups in mice in levels of serum insulin. Conclusion: The production and secretion of ghrelin from the stomach were significantly increased in nNOS−/− mice, which manifest significant body weight increase and lower levels of insulin resistance, as reported for PWS. The results suggest that NO derived from nNOS may regulate the relation between ghrelin and insulin.

II-O-3-64

TGF-β during Sporadic Human Colorectal Carcinogenesis: The Shift from an Epithelial to a Mesenchymal Signaling

K. Matsumoto, K. Gashaw

Department of Gastroenterology and Hepatology, Kansai Medical University, Moriyuchi, Osaka, Japan

Transforming growth factor-β (TGF-β) activates not only TGF-β type I receptor (TβRI) but also c-Jun N-terminal kinase (JNK), changing unphosphorylated Smad3 to its phosphoform. C-terminally phosphorylated Smad3 (pSmad3C) and linker phosphorylated Smad3 (pSmad3L). While TβRII/pSmad3C pathway inhibits growth of normal epithelial cells, JNK/pSmad3L-mediated signaling is involved in an invasion of the activated mesenchymal cells. During sporadic human colorectal carcinogenesis, TGF-β signaling confers a selective advantage upon tumor cells by shifting from the TβRII/pSmad3C pathway characteristic of mature epithelial cells to the JNK/pSmad3L pathway, which is more characteristic of the state of flux shown by the activated mesenchymal cells. JNK acts as a regulator of TGF-β signaling by increasing the basal level of pSmad3L available for action in the nuclei of invasive adenocarcinoma, in the meantime shutting down TGF-β-dependent nuclear activity of pSmad3C. Loss of epithelial homeostasis and acquisition of a migratory, mesenchymal phenotype are essential for tumor invasion. From the viewpoint of TGF-β signaling, a key therapeutic aim in cancer would be restoration of the lost tumor suppressor function observed in normal colorectal epithelial cells at the expense of effects promoting aggressive behavior in the adenocarcinoma. Specific inhibitors of the JNK/pSmad3L pathway might prove useful in this respect. In the case of molecularly targeted therapy for human cancer, pSmad3L and pSmad3C could be assessed as biomarkers to evaluate the likely benefit from specific inhibition of the JNK/pSmad3L pathway.

II-O-4-65

Hydrogen Sulfide: An Endogenous Mediator of Mucosal Defence and a Therapeutic Target

J.L. Wallace, T. Joh

1Department of Internal Medicine and Bioregulation, Nagoya City University Graduate School of Medical Sciences, Nagoya, 2Department of Molecular Medicine, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan

Hydrogen sulfide (H2S), like nitric oxide, is an endogenously produced gas with a wide range of biological actions. While high concentrations of H2S can be toxic, it is produced in various tissues in the body at concentrations exceeding 100 μM. In the context of gastrointestinal mucosal defence, it is important that H2S is a vasodilator, and can therefore increase mucosal blood flow. Moreover, H2S is a potent inhibitor of leukocyte adhesion to the vascular endothelium, and has been shown to reduce visceral pain. Most of these effects are mediated via actions on ATP-activated K+ channels, as indicated by the observation that they can be reversed by treatment with glibenclamide. We have demonstrated that one of the mechanisms through which NSAIDs cause gastric mucosal damage is by reduction of endogenous H2S synthesis, which can occur via two enzymes. In recent years, we have developed H2S-releasing derivatives of a number of drugs and have observed marked increases in their potency and or efficacy. For example, an H2S-releasing derivative of melamine, called Mel-H2S, was found to be significantly more effective than melamine in animal models of colitis. ATB-429, but not melamine, markedly suppressed the expression of a number of pro-inflammatory cytokines as well as reducing macroscopic and histological signs of inflammation and injury. Colorectal distension induces visceral pain in normal rats and rats with colitis. Treatment with ATB-429 was found to be significantly more effective than melamine in reducing visceral pain in that model, as well as reducing spinal c-Fos expression. These effects were inhibited by glibenclamide, again suggesting an effect mediated via ATP-activated K+ channels. These studies highlight the importance of hydrogen sulfide as an endogenous mediator of mucosal defence, and suggest that H2S-releasing drugs may have considerable utility for treatment of disorders of the gastrointestinal tract characterized by inflammation and pain.

II-O-4-66

Current Status of the Development and Use of NSAIDs

K.D. Rainford

Sheffield Hallam University, Sheffield, UK

The past year has seen major concerns about the safety of NSAIDs following the dramatic withdrawal worldwide of the COX-2 selective drug, rofecoxib on 29th September 2004, because of serious cardiovascular (CV) adverse events (AEs). Subsequently, celecoxib was withdrawn in 2005 for the same reason and concerns were then expressed that other NSAIDs may also exhibit, albeit variably, some CV risk. Additionally, increasing scepticism about whether the new generation of COX-2 selective agents, or coxibs, really have the clinically significant benefit of reducing serious and symptomatic gastrointestinal (GI) AEs compared with conventional NSAIDs especially those that are well known to have low GI ulcerogenicity (e.g. etodolac, ibuprofen, nabumetone, nimesulide). The publicity concerning the CV risks and the swift reaction of the drug regulators while justified has had a profoundly negative impact on the development and commercial development of other classes of NSAIDs. None-the-less there are interesting and important drugs in development that should be considered along with the development of therapeutic strategies to reduce ulcer incidence (e.g. use of PPIs, gastroprotective agents etc.) Unfortunately, the much heralded nitric oxide (NO) – releasing NSAIDs have had a setback since termination of the development of NO-naproxen (though this is still in some stage of development). However, other NO-releasing NSAIDs with differing chemistry are in progress and we await their introduction with interest. Better understanding of the kinetics of NO-release from NO-NSAIDs and the chemistry of linkers may give better drugs. The COX-LOX inhibitor, licofoxil, was also seen as a drug with novel potential pharmacologically but the clinical outcomes have not been so marked as anticipated. With some of these newly developed drugs there have often been issues relating to whether or not they were ideally formulated.

II-O-4-67

Wip1 Protects Hydrogen Peroxide-Induced Intestinal Epithelial Cell Injury

T. Oshima, M. Sasaki, H. Kataoka, T. Takeuchi, T. Joh

1Department of Internal Medicine and Bioregulation, Nagoya City University Graduate School of Medical Sciences Nagoya, 2Department of Molecular Medicine, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan

Background and Aim: Tight junctions create a paracellular permeability barrier, and though reactive oxygen species (ROS) have been implicated as mediators of inflammation in
inflammatory bowel diseases their effect on the function of colonic epithelial tight junctions remains unknown. In this study, we examined the effect of hydrogen peroxide (H2O2) on colonic mucosal barrier properties and tight junction organization and investigated the role of Wip1, a serine/threonine phosphatase. 

**Methods:** Human colonic epithelial cells, CaCo2, were used. Epithelial barrier integrity was determined by assessing trans-epithelial electrical resistance (TEER). To examine the effects of H2O2 on junctional proteins, immunoblotting and immunofluorescence staining were performed with claudins specific antibodies. In some experiments, cells were dual stained and samples were analyzed with a confocal laser scanning microscope. The mRNA level was analyzed by real-time PCR. The claudin-4-expressing cells were used as a positive control. 

Wip1 inhibitor and a MAP kinase inhibitor were used as a Wip1-specific inhibitor and a MAP kinase inhibitor, respectively. Wip1-expressing CaCo2 were produced by transfection with adenovirus vector containing the Wip1 gene. Results: H2O2 treatment of colonic epithelial monolayers caused a significant decrease in TEER. Oxidant-mediated permeability and phosphorylation of p38 MAP kinase were significantly attenuated by SB203580, but not by PD98059. Although the amount of tight junction protein was not altered with H2O2 stimulation, H2O2 changed the localization of claudin-4 protein from an NP-40 insoluble fraction to a soluble fraction. Interestingly, levels of claudin-4 mRNA were increased by H2O2 stimulation. Wip1 transfection significantly attenuated phosphorylation of p38 MAP kinase, oxidant-mediated permeability, and levels of claudin-4 mRNA. H2O2-induced changes in claudin-4 localization were abolished by SB203580 pretreatment as well as Wip1 transfection. 

**Conclusion:** This is the first study able to demonstrate that the change in claudin-4 localization is important for H2O2-induced colonic permeability and that Wip1 functions to protect colonic mucosal integrity by targeting claudin-4.

**II-O-4-68**

**Target Molecules of Molecular Chaperone (HSP70) in Injured Gastric Mucosal Cells – the Key Molecules of Gastric Mucosal Damage**


Department of Internal Medicine and Gastroenterology, Akita University School of Medicine

**Background:** Many recent studies, including ours, have indicated the importance of heat shock proteins (HSPs) in cytoprotection against cytotoxic agents and environmental stresses mediated by their function as a ‘molecular chaperone’. On the other hand, it has not been found the target molecule of HSPs in the damaged cells. As HSPs quickly recognize and bind to degenerated protein in the cells to maintain protein structure, the target molecules should be key molecules for the initiation and the pathogenesis of cellular damage. In the present study, gastric mucosal proteins that specifically bind to HSP70 were analyzed using HSP70-affinity chromatography. 

**Materials and Methods:** The gastric mucosa was removed from Sprague-Dawley rats (250–300 g) after exposure to WI-stress for 0, 1, 3 or 5 h. Soluble fraction of each gastric mucosa was applied to HSP70-affinity column separately. Non-specific binding was washed out by conditioning buffer containing 0.5 M NaCl. Specific HSP70-binding proteins were eluted by elution buffer containing 1 mM ATP. Eluted fractions were analyzed by SDS-PAGE and the molecular weights were calculated. Also, amino acid sequence of HSP70-binding proteins was analyzed. 

**Results:** Specific HSP70-binding proteins with molecular weight of 220-kDa and 45-kDa were eluted by ATP-containing buffer from HSP70-affinity column when mucosal homogenate of 1h-stress exposure was applied. Specific binding protein was not observed in control, 3 or 5 h-stress exposed mucosal homogenates. 

**Conclusions:** We found that specific HSP70-binding proteins were 220-kDa and 45-kDa proteins observed only in extremely early stage of stress-exposed gastric mucosa before occurrence of pathological change. In the pathogenesis of stress-induced mucosal damage, structurally degenerated these two proteins may be the key or initiation molecules which structural changes were firstly recognized by molecular chaperone. Amino acid sequence of these two proteins will be demonstrated.

**II-O-4-69**

**Role of Cell Membrane Composition Modification by ω-3 Polyunsaturated Fatty Acids in Stress-Related Ulcerogenesis**

**O.S. Zayachkivska**  

Department of Physiology, Lviv National Medical University, Lviv, Ukraine

**Background and Aim:** Many recent studies, including ours, have indicated the importance of heat shock proteins (HSPs) in cytoprotection against cytotoxic agents and environmental stresses mediated by their function as a ‘molecular chaperone’. On the other hand, it has not been found the target molecule of HSPs in the damaged cells. As HSPs quickly recognize and bind to degenerated protein in the cells to maintain protein structure, the target molecules should be key molecules for the initiation and the pathogenesis of cellular damage. In the present study, gastric mucosal proteins that specifically bind to HSP70 were analyzed using HSP70-affinity chromatography. Materials and Methods: The gastric mucosa was removed from Sprague-Dawley rats (250–300 g) after exposure to WI-stress for 0, 1, 3 or 5 h. Soluble fraction of each gastric mucosa was applied to HSP70-affinity column separately. Non-specific binding was washed out by conditioning buffer containing 0.5 M NaCl. Specific HSP70-binding proteins were eluted by elution buffer containing 1 mM ATP. Eluted fractions were analyzed by SDS-PAGE and the molecular weights were calculated. Also, amino acid sequence of HSP70-binding proteins was analyzed. Results: Specific HSP70-binding proteins with molecular weight of 220-kDa and 45-kDa were eluted by ATP-containing buffer from HSP70-affinity column when mucosal homogenate of 1h-stress exposure was applied. Specific binding protein was not observed in control, 3 or 5 h-stress exposed mucosal homogenates. Conclusions: We found that specific HSP70-binding proteins were 220-kDa and 45-kDa proteins observed only in extremely early stage of stress-exposed gastric mucosa before occurrence of pathological change. In the pathogenesis of stress-induced mucosal damage, structurally degenerated these two proteins may be the key or initiation molecules which structural changes were firstly recognized by molecular chaperone. Amino acid sequence of these two proteins will be demonstrated.

**II-O-4-70**

**Molecular Mechanisms of Mucosal Damage in the GI Tract**


VA Medical Center and University of California, Irvine, School of Medicine, Long Beach, CA, USA

The molecular mechanisms of cell and tissue injury cannot be separated from etiologic factors and cellular mechanisms/targets. As in any tissue injury, the major etiologic factors are ischemic (due to low blood flow), chemical and infectious in nature: physical and genetic factors are not frequently implicated in the etiology of mucosal injury. The molecular mediators of ischemic injury include vasoconstrictors like the most potent endothelin (ET)-1, 2 or 3 and the lack of vasodilability of vasodilators like NO. In chemical injury, main molecular players include the rapidly acting free radicals and the directly acting stable chemicals like HCl, ethanol and the mostly indirectly damaging NSAIDs. The major etiologic infectious agent in the upper GI tract is H. pylori with a growing list of potential molecular mediators, e.g., VacA, CagA and the expression of other molecules linked to the ‘pathogenetic island’ H. pylori genome. In the lower GI tract numerous Gram – + bacteria as well as immunologic factors seem to play a role in the pathogenesis of inflammatory bowel diseases. The GI tract is unique in the molecular pathogenetic sequence of events since the lack of protective chemicals, e.g., bicarbonate, NO, phospholipids, prostaglandins, sulfhydryls, angiogenic growth factors, is often sufficient to initiate extensive cell and tissue injury that could virtually never happen in the heart, kidney, lung, or brain. In the molecular pathogenesis enhanced expression and release of ET-1 is the most likely event (based on recent gene expression and related studies using ET-1 antagonists and antisense molecules), resulting in local ischemia that triggers the expression of hypoxia-inducible factors (HIF-α) and immediate early genes such as c-fos. Two other pathways seem to follow this event, or may run parallel with the vascular events: (1) oxidative injury from inside to epithelial and endothelial cells, and (2) external injury to epithelial cells by HCl and proteolytic enzymes, aggravating and extending the limited injury to a few cells to mucosal necrosis, i.e., superficial erosions and deep ulcers that trigger limited and localized inflammation in the stomach and duodenum but extensive inflammation in the lower GI tract.
The precise etiology of ulcerative colitis (UC), one major form of inflammatory bowel disease is still unclear. Resistance of colitis development in germ-free animals and inflammation induced by reintroduction of intestinal flora in these animals have indicated a critical role of microflora in the pathogenesis of UC. Recent studies have illustrated that cathelicidin, an antimicrobial peptide produced by leukocytes and most epithelial cells, can mediate innate defense against bacterial pathogens. To elucidate the relationship between cathelicidin and UC, BALB/C mice were induced with acute colitis by feeding dextran sulfate sodium (DSS) in drinking water for one week and synthetic cathelicidin (5 mg/kg) was administered intrarectally daily during colitis induction. All animals receiving DSS solution alone showed weight loss, diarrhea, blood clot around anus and significantly higher myeloperoxidase activity. Interestingly, intrarectal administration of cathelicidin ameliorated DSS-induced colitis. Upon examination of fecal microflora before and after colitis induction, about 30% increase in the aerobic population was detected per dry weight of feces and this significant increase disappeared when the mice were treated with cathelicidin. Cathelicidin was found to reduce apoptosis and promote angiogenesis in colon tissues. RT-PCR data showed that cathelicidin administration markedly up-regulated the expression of muc1 and muc2 in the colitis-induced groups. Results here suggest that angiogenesis in colonic tissues. RT-PCR data showed that cathelicidin administration markedly up-regulated the expression of muc1 and muc2 in the colitis-induced groups. Results here suggest that angiogenesis in colonic tissues. RT-PCR data showed that cathelicidin administration markedly up-regulated the expression of muc1 and muc2 in the colitis-induced groups. 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would facilitate PMN recruitment to the colonic mucosa. The aim of the present study was to evaluate the role of PECAM-1 (an adhesion molecule critical to PMN emigration) and chemokines in PMN recruitment using an in vitro model of the vascular/interstitial interface. Colonic lavage fluid (CLF) was collected from mice 2 days after induction of colitis by TNBS and used to study PMN migration across mouse mesenteric endothelial cell (MMEC) monolayers in cell culture inserts. CLF increased PMN transendothelial migration in a concentration-dependent manner. Although CLF contained LPS, the measured concentration of LPS could not promote PMN migration, indicating that LPS was not responsible for the enhanced PMN migration. The CLF-induced PMN transendothelial migration was inhibited by antibodies against LIX or KC, but not by antibodies against MIP-2. Antibodies against PECAM-1 could also inhibit PMN migration induced by CLF. The extent of mucosal damage in TNBS-induced colitis was dramatically reduced when PECAM-1−/− mice were used. CLF obtained from PECAM-1−/− mice exposed to TNBS was less potent in promoting PMN migration than that from WT mice. The concentrations of LIX and KC in CLF of colonic tissue of PECAM-1−/− mice was significantly less than those of WT mice. When PMN from PECAM-1−/− mice were used in the assays the extent of PMN migration was reduced. These findings indicate that CLF may provide a ‘window’ on the colonic interstitial milieu and that both the chemokines, LIX and KC, and the PECAM-1 adhesion molecule contribute to the PMN infiltration of the colonic interstitial in the TNBS model of IBD.

### IL-6-577
**Exclusive Expansion of CX3CR1+CD4+ T Cells in Inflammatory Bowel Disease**

**Background:** Fractalkine is a unique CX3C chemokine that mediates not only leukocyte migration but also adhesion. It has been reported that the interactions of fractalkine and its receptor CX3CR1 might contribute to the pathogenesis of some inflammatory diseases mainly by recruiting monocytes to the inflamed sites. In this study, we investigated whether this system is present in the intestine, and how this receptor system is recognized as an immunoregulatory pathway by suppressing excessive inflammation.

**Methods:** C57BL/6 mice (8 weeks aged) were used for this study. For induction of colitis, 1.5% dextran sulfate sodium (DSS) containing water was given for 10 days. The extent of mucosal damage in TNBS-induced colitis was dramatically reduced when PECAM-1−/− mice were used. CLF obtained from PECAM-1−/− mice exposed to TNBS was less potent in promoting PMN migration than that from WT mice. The concentrations of LIX and KC in CLF of colonic tissue of PECAM-1−/− mice was significantly less than those of WT mice. When PMN from PECAM-1−/− mice were used in the assays the extent of PMN migration was reduced. These findings indicate that CLF may provide a ‘window’ on the colonic interstitial milieu and that both the chemokines, LIX and KC, and the PECAM-1 adhesion molecule contribute to the PMN infiltration of the colonic interstitial in the TNBS model of IBD.

**Results:**

- Upregulated fractalkine may contribute to the pathogenesis of IBD through the emergence of specific Th1 and Th17 effector memory and cytotoxic T cells, which express CX3CR1 by chemotaxis assay.
- Conclusion: Uregulated fractalkine may contribute to the pathogenesis of IBD through the emergence of unique CX3CR1+CD4+ T cells that can act both as Th1 and cytotoxic T cells in the gut.

### IL-6-680
**SNPs and Gene Polymorphisms in Gastric Atrophy**

**Background:** Dietary fat intake is known to be closely related to the exacerbation of inflammation of bowel in inflammatory bowel diseases. Recently, expression of adiponectin and receptor system is recognized as an immunomodulatory pathway by suppressing excessive inflammation. In this study we investigated whether this system is present in the intestine, and how this system is affected by the induction of colonic inflammation or by dietary fat intake.

**Materials and Methods:** C57BL/6 mice (8 weeks aged) were used for this study. For induction of colitis, 1.5% dextran sulfate sodium (DSS) containing water was given for 10 days. Intestinal and mesenteric lymph nodes were removed and messenger RNA expression of adiponectin, adiponectin receptor1, 2, TNF-α and IFN-γ was analyzed by quantitative reverse transcription-PCR and immunohistochemistry. Chemotaxis assay was examined using Transwell system to confirm whether fractalkine actually attracts CX3CR1+CD4+ cells.

**Results:**

- Adiponectin expression was dramatically decreased in TNBS-induced colitis as compared to control mice.
- Significant upregulation of its receptors was observed in the mucosa of ileum and colon in the DSS treatment group.
- These receptors did not increase in the control mice.

**Conclusion:**

- Decreased expression of adiponectin in the colonic mucosa in inflamed mucosa or in high fat diet accompanying with TNF-α increase may possibly decrease the resistance of colonic mucosa against immune dysregulation in these conditions.
compared to the common (55%) of the with atrophy compared to those without (1 subject in the gastric atrophy group vs. 8 in the group

to clari-thromycin were major factors associated with the therapeutic success or failure of the eradication of H. pylori by the triple therapy with a PPI, clari-thromycin, and amoxicillin. Aim: We investigated whether individualized therapy based on these factors as determined by genetic testing would increase the cure rate of the initial eradication therapy in comparison with the standard therapy. Methods: First, a lansoprazole dosing schedule that would achieve sufficient inhibition of acid secretion to allow H. pylori eradication therapy in each CYP2C19 genotype group was determined in a 24-hour intragastric pH monitoring study. Next, 300 H. pylori-positive patients were randomly assigned to two treatment groups. Patients assigned to the standard regimen group were treated with lansoprazole 30 mg bid, clarithromycin 400 mg bid, and amoxicillin 750 mg bd for 1 week. Patients assigned to the tailored regimen group were treated with personalized regimens based on their CYP2C19 genotype and the H. pylori 23S rRNA genotype. The per patient drug cost required for successful eradication was calculated for each group.

Results: The eradication rate (ITT) in the tail-ored regimen group was 96.0% (144/150), significantly higher than in the standard regimen group (70.0%: 105/150) (p < 0.001). Drug costs per successful eradication in the tailored and standard regimen groups were $73.08 and $89.49, respectively. Conclusion: The pharma-cogenomics-based tailored treatment for H. pylori infection allowed a higher eradication rate at a reduced drug cost in comparison with the standard treatment.

Backgrounds: Proton pump inhibitors (PPI), such as omeprazole, lansoprazole, and rabeprazole, are metabolized by CYP2C19 in the liver. There are genetic differences in the activ-ity of this enzyme. We previously found that the CYP2C19 genotype status of patients and the sus-pceptibility of Helicobacter pylori (H. pylori) to clarithromycin were major factors associated with the therapeutic success or failure of the eradication of H. pylori by the triple therapy with a PPI, clarithromycin, and amoxicillin. The Pro12Ala polymorphism is associated with risk of gastric cancer in Japanese Population T. Tahara, T. Ariaawa, T. Shibata, M. Sakata, N. Maruyama, T. Nakano, Y. Kaminz, H. Fujita, S. Hasegawa, M. Nakamura, M. Nakamura, T. Mizuno, M. Naasaka, M. Iwata, K. Takahama, M. Watanabe, I. Hirata, H. Nakano Department of Gastroenterology, Fujita Health University, School of Medicine

Background: Experimental study shows that Peroxisome proliferators-activated receptor gamma (PPARγ) inhibits the growth and induces apoptosis of gastric cancer cells. A common polymorphism at codon 12 of this gene (Pro12Ala) has been shown to confer protection against diabetes and colorectal cancer. Aim: To study the association between PPARγ gene Pro12Ala polymorphism and gastric cancer in Japanese population. Methods: 415 patients enrolled in this study. The PPARγ Pro12Ala polymorphism was compared in patients divided into two groups: Gastric cancer (GC) cases (n = 214, mean age 65.0 [29-91], F:M = 0.29), and 201 patients without evidence of GC (mean age 64.0 [29-92], F:M = 0.32). PPARγ Pro12Ala polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism. Results: The frequency of Ala12 allele was significantly higher among GC patients (9.3%) than in control (4.0%; OR 2.49; p < 0.05). Ala12 allele was more strongly associated with risk of intestinal type histopathology (OR 3.14, p < 0.05). Interestingly, Ala12 homozygosity, a very rare genotype in Japanese, was found in one case in GC patients. Conclusion: Our study suggests the potential association between PPARγ Pro12Ala polymorphism and risk of gastric cancer in Japanese.
II-O-7 New Mechanisms of GI Drug 2
Chairpersons: P. Sikiric, T. Mitsuzhima

II-O-7-85
Fully Distended and Filled Stomach in Rat and Alcohol. Stomach, Esophageal and Duodenal Lesion and Omeprazole, Ranitidine, Atropine, Stable Gastric Pentadecapeptide BPC 157 (PLID116, PL14736, Pliva)
Department of Pharmacology, School of Medicine, University of Zagreb

Hyperemic response of left gastric artery along with stomach distention is studied in rat alone or with 96% alcohol ingestion (2 ml/kg/stomach) into fully distended and filled stomach (12 ml water, 12 ml of air) that increases damaging potential of gastro-esophageal and -duodenal reflux, presenting lesion in proximal esophagus and duodenal bulb besides stomach (as gray areas at 2, 5 and 15 min intervals, assessed (percentage of total area) at 2, 5 and 15 min intervals, digital compact camera, morphometry). Therapy (mg/kg, 2 ml/stomach) was immediately before. Results: Omeprazole (50) and stable gastric pentadecapeptide BPC 157 (0.01 i.m. in IIBS (PLID116, PL14736, Pliva) increased, ranitidine (50) and atropine (10) decreased presentation of left gastric artery major branches of exposed stomach (percentage of initial value, at 5sec intervals for 2 min). Alcohol antagonizes hyperemic response, an effect reversed with BPC 157. Lesion inhibition was in stomach (BPC 157), duodenum (BPC 157, omeprazole), esophagus (BPC 157, omeprazole, ranitidine, atropine). Conclusion: With increased hyperemic response, only BPC 157 protects stomach, esophagus and duodenum against damaging gastro-esophageal and -duodenal reflux.

II-O-7-86
 Genome Research Center for Gastroenterology, Ajou University Medical Center

Background and Aims: To assess the comparative efficacy and safety of revaprazan, a novel acid pump antagonist, versus omeprazole in patients with duodenal ulcer, we performed a randomized, double-blind, phase III, multicenter trial. Methods: Two hundred and twenty eight patients were randomized to 4 weeks of treatment with either revaprazan 200mg or omeprazole 20mg once daily. Primary efficacy parameter was complete ulcer healing by endoscopy and secondary parameter was the improvement in the severity of daytime and nighttime pain. Results: Healing rates at 4 weeks (intention-to-treat analysis) were 91.7% with revaprazan 200mg and 94.4% and 92.3%, respectively. There was no significant difference in healing rate between two groups (p = 0.3). Conclusions: Revaprazan 200mg was as effective and well tolerated as omeprazole 20mg in patients with duodenal ulcer. Grant support: This work was supported by grants from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PG6-01GN14-0007).

II-O-7-87
Pharmacological Profile of Ramosetron, a Novel Therapeutic Agent for IBS T. Hirata, T. Funatsu, Y. Kato, M. Nakata, M. Sasamata
Pharmacology Research Labs., Drug Discovery Research, Astellas Pharma Inc.

It is well known that serotonin (5-HT) plays important roles in the pathogenesis of irritable bowel syndrome (IBS). Ramosetron (RAM), a potent and selective serotonin 5-HT3 receptor antagonist, is already available as a medication for gastrointestinal symptoms caused by tumor agents in Japan, and currently under development for diarrhea-predominant IBS. In this study, we examined the effects of RAM on the colonic function and abdominal pain using various animal models. Defecation was stimulated in rats and mice by stress-related factors such as corticosterone releasing factor (CRF) or 5-HT, and in rats by conditioned-fear stress (CFS) and restraint stress. RAM (0.3–100 μg/kg, p.o.) significantly suppressed these abnormal defecation, and these effects were 6.7–1,400 times more potent than those of other 5-HT3 receptor antagonists, alosetron and cilansetron, and anti-diarrheal agents, loperamide, based on comparison of the ED50 values. On the other hand, RAM did not inhibit the normal defecation and betanechol-induced defecation in dogs and rats. To clarify the mechanisms by which RAM inhibits the abnormal defecation, we examined the effects of RAM on the colonic propulsion and water transport in rats. RAM (10–100μg/kg, p.o.) significantly suppressed CFS-stimulated colonic propulsion. Similarly, RAM (3–30μg/kg, p.o.) significantly ameliorated CRF-induced abnormal water transport in colon. Moreover, we established restraint stress-induced colonic hyperalgiesia model in rats, and the effect of RAM was examined. RAM (0.3–3μg/kg, p.o.) significantly ameliorated the stress-induced decrease in the colon pain threshold while loperamide did not ameliorate this abnormal stress-induced decrease in colon pain threshold. These results indicate that RAM may produce beneficial effects on IBS symptoms in clinical practice.

II-O-7-88
Mitencinal (GM-611), a Motilin Receptor Agonist Accelerates Colonic Motility and Defecation in Dogs K. Ozaki1, H. Sudo1, H. Muramatsu1, Y. Taka1, K. Kame1, Z. Itoh, S. Omura1
1Pharmaceutical Research Department I, Chugai Pharmaceutical Co., Ltd., 2Gyuri University, 3The Kitasato Institute

Introduction: Mitencinal, a motilin receptor agonist, is an erythromycin-derived macrocyclic lactone that accelerates gastric emptying in dogs and humans. It remains unclear whether motility of the other alimentary tracts is accelerated by mitencinal. In this study, we investigated the actions of mitencinal on the lower gastrointestine (GI) along with the upper GI in dogs. Methods: The effect of oral administration of mitencinal (0.1–1mg/kg) on gastric and colonic motilities by force transducers was examined in fasted conscious dogs. In addition, the effect of mitencinal was examined in the presence of a motilin antagonist (GM-109, 1 and 10mg/kg, i.v. infusion) using dogs that showed sufficient increase of colonic motility with mitencinal (1mg/kg). Experiment 2: Dogs were fed at 9:00 am and mitencinal (0.3–3mg/kg) was orally administered at 10:00 am to only the dogs that had defecated within 1h after feeding in order to test the bias against spontaneous defecation. The time until the first defecation was measured for each dog that had been administered mitencinal. Statistical analysis was performed using the paired Dunnett’s multiple comparison test and the Trend test. Results: The gastric and colonic motilities accelerated with significant increase from mitencinal doses of 0.1 and 0.3mg/kg, respectively, and these acceleratory effects were significantly abolished in the presence of GM-109 in a dose-dependent manner. The latency period of defecation was substantially shortened without inducing diarrhea at the mitencinal dose of 3mg/kg (220 ± 17 min) compared to vehicle treatment (373 ± 21 min). Conclusion: Mitencinal accelerated both upper and lower GI motilities via the motilin receptor in dogs. The promotion of defecation might be reflected by the acceleration of colonic motility. This suggests that mitencinal could be a promising agent for treatment of GI motility disorders, including constipation.

II-O-7-89
Z-501 – A Novel Neurokinin-2 Receptor Antagonist – Inhibits Stress-Induced Defecation M. Yoshimura, N. Tsuzuki, K. Nagahama, Y. Matsunaga, T. Tanaka, K. Kawase, M. Murata, M. Nagasawa
Central Research Laboratories, ZERIA Pharmaceutical Co. Ltd., Kohnan-machi, Satlarna, Japan

Introduction: Z-501, a novel benzylamine derivative, is orally active tachykinin NK2 receptor antagonist that is in development for the treatment of irritable bowel syndrome. In nonclinical pharmacodynamic studies, Z-501 demonstrated high affinity for NK2 receptor and antagonistic activity for NK2 according to the three specific radio-labeled ligands and human receptor preparations. (2) Antagonistic activity for NK2 ligands, alosetron and cilansetron, and anti-diarrheal agents, loperamide, based on comparison of the Ki values. Methods: (1) Binding affinity for NK2 receptor and other receptor subtypes (NK1 and NK3) were examined using specific radio-labeled ligands and human receptor preparations. (2) Antagonistic activity for NK2 receptor was examined using isolated rabbit pulmonary arteries. (3) Rats were administered NKA (10μg/kg, i.p.), and the fecal pellets were collected 1h after the injection. Z-501, Saredutant and Alosetron hydrochloride were given orally 30min before administration of NKA. (4) Rats were restrained for 1h. The number of fecal pellets excreted during restraint stress was collected and weighed. Z-501 and Alosetron hydrochloride were given orally 30min before exposure to restraint stress. Results: (1) Binding affinity (Ks value) for NK2 receptors was 1.1 × 10–8 M. For NK1 and NK3 were 7.3 × 10–3 M and 2.9 × 10–7 M, respectively. (2) PA2 value from response of isolated rabbit pulmonary artery was calculated as 9.8 ± 0.12. (3) Z-501 (0.3, 1 and 3mg/kg), Saredutant (30mg/kg) and Alosetron hydrochloride (3mg/kg) inhibited NKA-induced increase in fecal pellet output. (4) Z-501 (0.3 and 3mg/kg) and alosetron hydrochloride (3mg/kg) inhibited restraint stress-induced increase in fecal pellet output. Conclusions: Z-501 is orally active NK2 receptor antagonist, and inhibits stress-induced defecation.
defecation in rats. NK2 antagonist may be useful for treatment of stress-related disorder, such as bowel habits in irritable bowel syndrome.

OPC-6535, a Novel Oral Thiazole Compound for Inflammatory Bowel Disease (IBD)
H. Nagamato
Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

Otsuka conducted a screening of thiazole derivatives for anti-inflammatory properties, with the suppression of O₃₋ production by human neutrophils. OPC-6535 was selected for further development based on its high potency against O₂⁻ production and its solubility and bioavailability. With intensive assessments, it was demonstrated that OPC-6535 was neither an O₂⁻ scavenger nor an NADPH Oxidase inhibitor, but inhibited O₂⁻ production by modifying the intracellular signaling pathway. In addition to its inhibitory effect on O₂⁻ production, OPC-6535 has inhibitory effects on other specific activities in leukocytes, including endothelial cell adhesion, and pro-inflammatory cytokine production. Collectively stated, OPC-6535 has multiple inhibitory action against pro-inflammatory leukocyte activation. The anti-inflammatory action of OPC-6535 in IBD has been assessed in rat models of colitis. In a TNBS-induced model, OPC-6535 at once-daily oral administration showed efficacy in preventing or reversing colonic ulceration and diarrhea associated with colitis. In addition, OPC-6535 significantly suppressed the colonic levels of infiltrating neutrophils, lipid peroxide, and TNF-α in this model, demonstrating that it has the same mode of action in an in vivo animal model as it has exhibited in vitro studies. In a DSS-induced model, OPC-6535 at the same regimen showed a significant suppression of symptoms (diarrhea and rectal bleeding), as well as reduction in colonic erosion area. A double-blind phase 2 study in patients with active ulcerative colitis using once-daily OPC-6535 oral doses proved the safety and the concept of OPC-6535, and at present, phase 3 studies in patients with active ulcerative colitis are ongoing in the US and EU. Based on this non-clinical and clinical data, OPC-6535 is expected to be an effective new type of treatment for IBD due to its unique mode of action.

Risk of reflux of the duodenal contents in gastroesophageal reflux in the Japanese patients, who may have different background from the Western patients, was studied.

Methods: Intragastric pH and bilirubin concentration was monitored using Bilitec 2000, in 43 patients with reflux symptoms and 10 normal volunteers. The ratio (% of duration of absobency being 0.15 or more and pH less than 4.0 to the observation period were obtained as holding time (HT) of bilirubin and acid, respectively. Severity of esophagitis was classified following Savary-Miller classification (S-M). Results: Esophagitis was found in 37 patients, consisting of 5 patients, 10, 13, and 9 of S-M grade 1, 2, 3, and 4, respectively. Both HT of volunteers were less than 5%. Bilirubin HT was more than 5% in 3 patients among 6 without esophagitis, but none for acid. Acid HT was less than 5% in 4 patients, 2, 2, and 2 of S-M grade 1, 2, 3, and 4, respectively. Bilirubin HT was less than 5% in 1 patient of S-M grade 2. Bilirubin HT of the patients of S-M grade 3 and 4 (50.9 ± 5.8%) was significantly higher than that of grade 1 and 2 (14.9 ± 2.9%) (p < 0.0001), but not for acid. In 32 patients bilirubin HT exceeded acid HT. Bilirubin HT did not correlate to acid HT in the patients. Conclusion: Reflux of the duodenal contents was found independently and exceeded acid reflux. We found a graded increase in reflux of the duodenal contents across the spectrum of severity of esophagitis.

In the last decade, several emerging topics have taken center stage in the field of gastroesophageal reflux disease. Development of new techniques, such as the wireless pH capsule and multichannel, intra-luminal impedance have provided an opportunity for new areas of research. The wireless pH capsule allows pH measurements over a long duration of time and thus can be used for better assessment of reflux-related symptoms and effect of therapy. The multichannel, intra-luminal impedance in combination with a pH sensor allows assessment of weakly acidic reflux and its contribution to PPI failure and extra-esophageal manifestations of GERD. New areas of GERD include the close relationship between increase in BMI and the prevalence of gastroesophageal reflux disease (GERD), however, patients have felt uncomfortable wearing the pH probe for long time. We evaluated 3-hour postprandial pH monitoring and comparison with proton pump inhibitor test (PPI test). Methods: We performed a retrospective study of patients who underwent 24h pH monitoring without anti-secretary drugs and 32 patients with heartburn occurring 2 days or more days per week were recruited. A positive study was defined as pH < 4 for more than 4% of the total recording time in the esophagus and the same criterion was applied to the 3-hour pH monitoring, for the first meal after pH recording. PPI test: A short course of PPI therapy (rabeprazole 20mg/day; 2–3 weeks) achieves improvement or remission of the symptoms. Results: We performed a retrospective study of patients who underwent 24h pH monitoring without anti-secretary drugs and 32 patients with heartburn occurring 2 days or more days per week were recruited. A positive study was defined as pH < 4 for more than 4% of the total recording time in the esophagus and the same criterion was applied to the 3-hour pH monitoring, for the first meal after pH recording. PPI test: A short course of PPI therapy (rabeprazole 20mg/day; 2–3 weeks) achieves improvement or remission of the symptoms. Results: Under the 24-hour pH monitoring, two groups were classified as 25 nonreflux patients (pH < 4% of the recording time) and 7 reflux patients (pH < 4 for >4% of the recording time). No patient, that had been defined as a reflux patient by the 24-hour test, was incorrectly labeled under the nonreflux category during the 3-hour postprandial study. One nonreflux patients was reclassified under the reflux category based on the 3-hour postprandial study. Conclusion: we have demonstrated that postprandial 3-hour pH monitoring is a specific and sensitive method for the diagnosing of patients who have a positive 24-hour pH monitoring. Postprandial reflux was concerned with PPI test positive.
thought to be a complication of GERD. However, the relationship between Helicobacter pylori infection, gastric acid secretion and GE junction adenocarcinoma has not yet been investigated in Japan. The aim of this study was to evaluate this relationship in the Japanese population. Methods: A total of 168 Japanese patients (RE alone: 80, short-segment BE [SSBE]: 16, long-segment BE [LSBE]: 20, GE junction adenocarcinoma: 12, distal early gastric cancer [EGC]: 40; male/female = 106/62; mean age, 61.5 years) and 80 Japanese control subjects who had no localized lesions in the upper gastrointestinal tract (male/female = 43/37; mean age 58.1) were enrolled for this study. The prevalence of H. pylori infection was determined by biopsy; the rapid urease test and measurement of the serum H. pylori IgG antibody. Gastric acid secretion was assessed by the endoscopic gastric test (EGT). RE was diagnosed according to the Los Angeles classification. Results: The prevalence of H. pylori infection in the patients with RE alone (30%) was significantly lower than that in controls (71.2%). There was also a tendency for the prevalence of H. pylori infection to be lower in patients with BE (18.7%; LSBE, 0%) as compared to that in patients with RE alone. On the other hand, the prevalence of H. pylori infection in patients with GE junction adenocarcinoma (58.3%) was significantly lower than that in patients with EGC (87.5%). It tended to be higher than that in patients with RE alone or BE. The mean EGT value in patients with RE alone (3.74±10 min) was significantly higher than that in control subjects (1.83). The mean EGT value in patients with BE (SSBE 4.74, LSBE 4.76) tended to be even higher than that in patients with RE alone. The mean EGT value in patients with GE junction adenocarcinoma (3.94) was significantly higher than that in control subjects (71.2%). There was also a tendency for the number of H. pylori infected patients to be higher in patients with RE alone than in patients with BE.

Conclusion: Preservation of gastric acid secretion may be important for the development of GE junction adenocarcinoma in Japanese people, irrespective of the H. pylori infection status.

II-O-8-95
High Prevalence of Impaired Salivary Function in GERD
Department of General Medicine and Emergency Care, 1Department of Radiology, 2Division of Gastroenterology and Hepatology, Toho University School of Medicine, Tokyo, Japan

Gastro-esophageal reflux disease (GERD) is associated with a decreased salivary flow as well as gastric acid production. Recent evidences prove the esophagoprotective ability of salivary secretions. This study aimed to investigate functional disorders of salivary glands in patients with GERD. Patients and Methods: Forty-four consecutive patients with GERD underwent salivary gland scintigraphy. GERD was diagnosed by endoscopy and gastro-esophageal reflux self-report questionnaires. Following intravenous injection of 180-200MBq 99mTc-pertechnetate, anterior sequential imaging was performed every minute for 40 min. At 20 min after injection of radiouclide, a lemon candy was administered introrally to stimulate salivary secretion. Regions of Interests (ROI) were selected on the individual submandibular and parotid glands, oral cavity, and thyroid gland. Time activity curves were drawn for each of these. Washout ratio (peak count before lemon candy administration-lowest count after administration/peak count before administration) was examined as a functional parameter. Results: The mean washout ratio was 55.8% in the right parotid gland, 59.8% in the left parotid gland, 61.6% in the right submandibular gland, and 65.3% in the left submandibular gland. If the washout ratio of less than 50% is defined as functional disorders of salivary glands, a decreased salivary secretion of four major salivary glands was found in 12 (27%), 4 (9%), 12 (27%), and 8 cases (18%), respectively. An earlier increase of oral cavity count, which may reflect spontaneous secretion from all four major salivary glands, was found in 20 cases (45%) with GERD. Overall, salivary function disorder of at least one major salivary glands was found in 36 patients (82%) with GERD. Conclusions: Salivary gland function was more frequently diminished than expected. We concluded that the presence of impaired salivary gland function was considered to be one of risk factors for developing RE.

II-O-8-96
Relationship between Reflux Esophagitis and Helicobacter pylori Infection in Systemic Scleroderma
Department of Internal Medicine and Gastrointestinal Endoscopy, Saga Medical School, Saga, Japan

Aim: Reports have shown that reflux esophagitis is observed in approximately 60% of patients with systemic sclerosis. Helicobacter pylori infection is thought to inhibit the development of reflux esophagitis, and this study examined the possible effects of H. pylori infection in reflux esophagitis secondary to systemic scleroderma. Subjects and Methods: Among patients who visited the outpatient clinic of our Department of Gastroenterology with a diagnosis of scleroderma between October 1996 and January 2006, 65 patients underwent endoscopy at our hospital. In the subjects examined we found 47 patients in whom the presence or absence of H. pylori infection was confirmed: nine males and 38 females varying from 24–84 years of age. Results: Of the 47 patients tested, 24 patients (51.1%) were found to have H. pylori infection. Of these 24 H. pylori-positive patients, reflux esophagitis was observed in five (according to the Los Angeles classification, three patients were defined as grade A, one patient as grade B, no patients as grade C, and one patient as grade D). Of 23 H. pylori-infection-negative patients, reflux esophagitis was observed in 14 patients (according to the LA classification, six patients were defined as grade A, four patients as grade B, three patients as grade C, and one patient as grade D). Significantly fewer H. pylori-infected patients had reflux esophagitis (p < 0.01). No association with reflux esophagitis was found in the disease pattern of systemic scleroderma, complications of malignant diseases, treatment of systemic sclerosis, or inflammatory findings.

Conclusion: H. pylori infection was observed in 51.1% of patients with systemic scleroderma. Reflux esophagitis was observed in significantly fewer H. pylori-positive patients with systemic scleroderma. It was concluded that H. pylori infection inhibits the development of reflux esophagitis in scleroderma.

II-O-9-97
Can the Endoscopic Mucosal Dissection (ESD) be Standardized in the West?
R. Soetikno
VA Palo Alto Health Care System, Stanford University School of Medicine

Dissertation of EMR and ESD techniques has the potential to help many patients with early gastric cancer throughout the world. However, the training that endoscopists encounter to learn EMR or ESD may be difficult to achieve. The risks for bleeding, which may be brisk, and perforation, can be significant in EMR or ESD. Endoscopists performing EMR and ESD must also be proficient in the management of these potential complications. We will describe our experience in the learning of EMR and ESD using harvested porcine organs and anesthetized porcine model, and review the literature.

II-O-9-98
The Usefulness and Limitation of Endoscopic Mucosal Resection Using the Incision and Dissection Methods for Early Gastric Cancer and Gastric Flat Adenoma in Korea
Department of Internal Medicine and Pathology, Institute for Digestive Research, Soon Chun Hyang University College of Medicine, Bucheon and Seoul, Korea

Introduction: Conventional endoscopic mucosal resection (EMR) technique is technically simple and convenient but with this procedure the size of specimen obtained from one-piece resection is very limited. En bloc resection is beneficial for accurate histopathological assessment of specimen of EMR. EMR using incision and dissection methods (EISD) made it possible to perform en bloc resections of larger early stage gastric neoplasm with a reduction in the recurrence rate. But, it takes long time to perform the procedure and high incidence of complication. Aims and Methods: The aim of this study was to evaluate the clinical usefulness and limitation of EISD. Between February 2001 and June 2005, EISD (n = 366) using various kind of knives were performed on 389 lesions of early neoplastic lesions of the stomach (M:F = 199:167, mean age: 61.09). En bloc resection, complete resection rate, recurrence and associated complication were evaluated. Results: (1) The en bloc resection and complete resection rate with EISD were 90.4%, 85% for lesions between 11 and 15mm, 93.5%, 91.9% between 16 and 20mm, 84%, 83% between 21 and 30mm, 83.1%, 78.5% 31 mm or more in size; (2) Recurrence rate was 2.1% (9/389) in EISD. (3) Major bleeding including oozing with EISD 8.9%. Perforation with EISD occurred in 2.4%. Conclusion: EISD was effective modality to perform high en bloc resection and complete resection rate in larger gastric flat adenoma and early gastric cancer. To avoid the complications such as perforation and death, it takes a lot of technical experiences.

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Expanded Indication of Endoscopic Submucosal Dissection (ESD) for Early Gastric Cancer (GC) – Clinical Outcomes of a Prospective Study

Department of Gastroenterology, Osaka National Hospital

Background: Endoscopic mucosal resection (EMR) is recognized as a minimally invasive treatment for superficial GC without metastasis. ESD is a new technique developed to obtain en bloc resection even for large and/or ulcerative lesions. The aim of this study was to assess efficacy and safety of ESD against some conditions of GC which are hardly associated with nodal metastasis, as shown in Japanese guideline of treatment for GC. Patients and Methods: We designed a prospective study in which ESD was applied in patients with differentiated-type GC up to 30 mm in diameter regardless of ulceration or above 30 mm without ulceration, but definite signs of submucosal invasion. According to final diagnosis after the ESD, GCs are divided as considered to have hardly metastasis, were followed up and otherwise were advised to be operated on additionally; differentiated-type mucosal GC above 30 mm in diameter without ulceration, and GC up to 30 mm with ulceration and/or minute submucosal invasion. The protocol was approved by the ethics committee of Osaka National Hospital, and consent to take part in the study was obtained from patients. Complete resection was defined if the lateral and basal margins were free of tumor pathologically. Followed-up endoscopic examinations are performed 1, 3, 6 months later, and then every 6 months. Results: Between November 2001 and November 2005, 251 lesions were enrolled. The average size of GC resected was 22 mm (range: 2-70 mm). Complete resection was achieved in 87%. No emergent surgery and no immediate mortality was noted. Perforation and bleeding were encountered in 8 and 9%, respectively. Bleeding happened more frequently in case of GC with ulceration (p < 0.05). Forty-two patients were recommended to have additional operation mainly because of massive submucosal invasion or lymphovascular involvement, and nodal metastasis was recognized in 3 of 12 patients who were actually operated on. Among the remaining 209 followed-up patients, 3 (1.5%) recurred locally during follow-up period of median 18 mos (range: 2-36), and they were successfully treated by repeated ESD. No distant metastasis was recognized in 3 of 12 patients who were actually operated on. Among the remaining patients, 290 patients were followed up over 1 year, and 3 (1%) recurred locally during follow-up period of median 18 mos (range: 2-36), and they were successfully treated by repeated ESD. No distant metastasis was noted in follow-up period. Conclusions: ESD achieves complete resection even in large or ulcerative GC, providing the precise histological assessment, but is associated with a high risk of bleeding and perforation. Further, the long-term prognosis should be investigated.

Role of Radio Frequency Technology in Interventional/Therapeutic GI Endoscopy (With Special Emphasis on Output Control in ESD)

M. Hagg
ERBE Elektromedizin GmbH, Germany

Among various therapeutic GI procedures, ESD is particularly demanding for highly efficient RF technology, and different process such as marking, circumferential incision, submucosal dissection and coagulation requires specific output characteristics. The latest attempt to address such demands by highly controlled RF technology will be discussed.

Laparoscopic Lymph Node Dissection after Endoscopic Submucosal Dissection: A Potential Treatment Strategy for Patients with Early-Stage Gastric Cancer

N. Abe, H. Takeuchi, T. Mori, M. Sugiyama, Y. Atomi
Department of Surgery, Kyorin University School of Medicine

Objective: We have introduced laparoscopic lymph node dissection (LLND) after endoscopic submucosal dissection (ESD) for patients with early-stage gastric cancers (EGCs) having potential risk of LNM. Herein we present the preliminary results of the combination of ESD and LLND. Methods: Between February 2002 and September 2005, ten patients with EGCs deviating from the EMR criterion underwent the combination of ESD and LLND. ESD was performed using an insulation-tipped diathermic knife. Lymph nodes, which were determined on the basis of the location of the primary tumor and lymphatic drainage of the stomach, were removed laparoscopically. Results: The ESD enabled one-piece resection without any complications. LLND was successfully performed without any intraoperative complications. Postoperatively (1POD), one patient developed gastric perforation resulting from ischemic change of the stomach, and needed emergent gastrectomy. The mean number of the dissected lymph nodes was 14 (range: 3-29). In 8 of the 10 patients, the dissected lymph nodes were free of cancer cells, and therefore, the combination of ESD and LLND was considered a definitive treatment. The remaining two patients were found to have LNM but chose not to undergo any additional surgery. During follow-ups, the patients’ previous quality of life restored without any tumor recurrence. Conclusions: The combination of ESD and LLND enables the complete resection of the primary tumor and the histologic determination of lymph node status. This combination treatment may obviate unnecessary gastrectomy without compromising curability for EGC patients having the potential risk of LNM. However, determination of the appropriate dissection area that considered gastric blood flow is an important problem to be solved in the near future.

The Diagnostic and Therapeutic Role of Endoscopic Ultrasound in Europe

S. Seewald
Department of Interdisciplinary Endoscopy, University Hospital Hamburg, Germany

Endoscopic ultrasound is a fascinating technology, which has tremendously influenced the diagnostic and therapeutic modality in the field of gastrointestinal (GI) endoscopy over the past 20 years. Originally, EUS has been mainly used for staging of gastrointestinal tumors. However, introduction of EUS-guided fine needle aspiration (FNA) was an important breakthrough which has enormously broadened the therapeutic spectrum of interventional endoscopy.

Diagnoses: Besides staging of GI tumors (esophagus, stomach, pancreas and rectum), EUS and EUS-FNA also play an important role in the staging of non small cell lung cancer (NSCLC). EUS-FNA is able to detect early distant (left adrenal and liver) as well as contralateral lymph node (LN) metastases. Transesophageal EUS is able to detect LN in the posterior mediastinum. The introduction of transbronchial EUS with the possibility of transbronchial needle aspiration (TBNA) guarantees complete staging of the anterior mediastinum as well as the paratracheal region. Thus, EUS has a great impact on patients’ further treatment strategies.

Therapy: Another important issue is the role of EUS in drainage of abdominal abscess and pancreatic pseudocyst. Due to the well accessibility of the subphrenic spaces, the left lobe of the liver, the parapankreatric area, abscesses in these areas can safely be drained endoscopically under EUS guidance after exclusion of vessel interposition by color Doppler. Also non-huling abscesses can now be safely drained. In the future EUS may play a role in EUS-guided injection of chemotherapeutic agents and genes for pancreatic atrophy, pancreatogastrostomy and gastroenteral anastomosis. These approaches are still experimental. New technology and improvement of the echoendoscopes will help to improve the clinical application of EUS. In conclusion, EUS has tremendously broadened the therapeutic spectrum of interventional endoscopy.
II-O-10-104
Diagnostic Utility of Endoscopic Ultrasound Guided Fine Needle Aspiration Biopsy for Gastric Submucosal Tumors
Department of Gastroenterology, Aichi Cancer Center Hospital

Background: Submucosal lesions are incidentally detected on barium meal study or endoscopy. The differential diagnosis of them includes a variety of neoplasms, inflammation, and extramural compression. Since conventional endoscopic biopsy is superficial, the diagnostic yield of submucosal tumors (SMTs) covered with normal mucosa remains low. Intraluminal biopsy is required for preventing dissemination and endoscopic ultrasound guided fine needle aspiration biopsy (EUS-FNAB) has reported as effective modality. Purpose: The purpose of this study is to evaluate the diagnostic yield of gastric SMTs using EUS-FNAB.

Patients and Methods: A total of 294 consecutive patients with gastric submucosal lesions were examined by EUS between 1997 and 2005. 94 out of 294 cases underwent EUS-FNAB. The materials obtained from the EUS-FNAB were stained with the rapid Romanowsky for cytological examination. Immunohistochemical staining was performed for the final diagnosis of GIST. Results: Of the 294 cases, 115 were finally diagnosed by EUS: extraluminal compressions due to adjacent viscera were found in 32 cases and normal in 23, intraluminal lesions diagnosed as heterogenic pancreas in 40, lipoma in 7 and cyst in 8. The remaining 179 out of 294 cases were diagnosed as SMTs and 94 cases underwent EUS-FNAB because of tumor size more than 2 cm, patient’s request or malignant findings of EUS. The majority of SMTs examined with EUS-FNAB was GIST (n = 49), followed by spindle cell tumor (n = 13), inflammatory tumor (n = 5), gastric cancer (n = 5), cyst (n = 2), myogenic tumor (n = 2), neurogenic tumor (n = 2), carcinoma (n = 1), Glioma (n = 1) and unknown (n = 10). The diagnostic yield of EUS and EUS-FNAB in gastric lesions was 39.1% and 89.4%, respectively. Conclusions: EUS-FNAB is highly diagnostic in gastric SMT, particularly in patients with failure of previous EUS or endoscopic forceps biopsy to obtain a final diagnosis.

II-O-10-105
A Newly Developed Endoscopic Needle Knife Using Bipolar Current for Large Colorectal Tumors
Y. Sano1, K. Fu1, H. Machida2, M. Hanafusa3, Y. Sato4
1Endoscopy, National Cancer Center Hospital East (NCCHE), Chiba, 2Department of Gastroenterology, Osaka City University Medical School, Osaka, 3Division of Diabetes, Digestive and Kidney Diseases, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, 4Endoscopy, National Cancer Center Hospital, Tokyo, Japan

Background and Aim: Recently, the use of endoscopic submucosal dissection (ESD) in the colon has been recommended for the treatment of large colorectal tumors. To minimize deeper tissue damage, we have developed a novel endoscopic electrosurgical knife (B-KnifeTM, Zeon Medical Inc., Japan) for the bipolar system. The aim of this study was to evaluate the effectiveness and the safety of the new knife.

Methods: The B-Knife has a diffusion electrode attached to the tip of its sheath and an active electrode attached to the knife on the end of thesheath. With a design in which high-frequency electricity flows from the knife to the sheath tip, the amount of high-frequency current sent from the knife tip to the muscle layer has been reduced. In initial clinical use of the B-Knife, en bloc resection was achieved in all 15 patients with large colorectal tumors.

Conclusions: The B-Knife has a diffusion electrode and monopolar current. In initial clinical use of the B-knife, en bloc resection was achieved in all cases (range: 20-65 mm) and the mean operating time was 43 min. Conclusion: Our preliminary results suggest that ESD using the B-Knife is reliable and safe for the complete resection of a selected large flat lesion in the colorectum.

II-O-10-106
Effectiveness and Complications of Endoscopic Submucosal Dissection for Large Colorectal Tumors
K. Hotta, T. Oyama, Y. Miyata, A. Tomori
Department of Gastroenterology, Saku Central Hospital

Background and Aim: We have developed a novel endoscopic submucosal dissection (ESD) using Hook knife for the attempt of large en-bloc resection. The aim of this study is to clarify effectiveness and complications of ESD for colorectal tumors. Methods: A total of 37 patients (37 lesions) who had large colorectal tumors underwent ESD during the period from June 2000 to July 2005. Clinicopathological characteristics of the lesions, complete en-bloc resection and complication such as perforation were evaluated retrospectively. Results: (1) Clinicopathological characteristics (n = 37), (a) Location: rectum 15, sigmoid colon 3, descending colon 1, transverse colon 6, ascending colon 8, cecum 4, (b) Morphoscopic type: IA18, IA2 12, IIa1 IIc6, (c) Mean tumor size(range): 28.6 (7–65) mm, (d) Pathological type: submucosal invasive cancer 2, intramucosal cancer 18, tubular adenoma 15, serrated adenoma 1, severe dysplasia associated ulcerative colitis 1, (2) Complete en bloc resection rate 89.2% (33/37), (3) Local recurrence 0% (0/38), (4) Rates of complications, (a) perforation: 10.8% (4/37), (b) late bleeding 0% (0/37), (5) Causes of perforation (n = 4): during submucosal dissection 100% (4/4) (Flex knife 2, Hook knife 2), reasons of perforation in detail: technical factor 75% (3/4) (blind operation 1), cutting toward proper muscle layer 1, unrecognized proper muscle layer 1, lesion’s factor 25% (1/4) (severe fibrous of submucosal layer) 1. Conclusions: We successfully achieved large en-bloc resection in all the location of the colon and rectum using this novel ESD method. All of perforation of ESD occurred in the process of submucosal dissection and most of them were caused from technical reason. Therefore, perforation may be reduced due to improve technique of the process of submucosal dissection.

II-O-10-107
Endoscopic Diagnosis of Early Gastric Cancer for Endoscopic Surgery: Usefulness of Narrow Band Imaging System, Compared with Conventional Endoscopy
Department of Gastroenterology, Osaka City University, Graduate School of Medicine

Background and Aims: The morphologic changes in microvascular structure were useful in the diagnosis of early gastric cancer. The NBI system (NBI) enables to clearly observe the microvascular structure of the superficial lesion. The aim of this study is to evaluate the usefulness of NBI for the diagnosis of early gastric cancer.

Patients and Methods: Twenty-two patients with 22 lesions underwent endoscopic examination for early gastric cancer. All lesions were investigated twice each by magnifying endoscopy with and without NBI, and recorded on digital video. Endoscopic evaluation focused on two findings which are specific for early gastric cancer, (1) irregular microvascular pattern (IMP), defined as irregularity of subepithelial microvascular structure, and (2) demarcation line (DL), defined as the border line between normal mucosa and cancerous lesion. Two endoscopists (one was well-experienced for NBI and magnifying endoscopy and another had no experience for both) assessed the records, and compared each other.

Results: For the IMP delineation, NBI endoscopy was superior to conventional endoscopy (p < 0.0001). In NBI endoscopy, IMP was visualized clearly and differentiated easily from normal venules. Despite of the DL detectability was similar (NBI: 87.9% vs. conventional: 81.0%), but NBI endoscopy was superior to conventional endoscopy for the DL delineation (p < 0.0001), resulting in the margin of the lesion being clear. With conventional endoscopy, well-experienced endoscopist was superior to not-experienced endoscopist in identifying the DL delineation, but it became to be similar with NBI endoscopy without significant difference.

Conclusions: NBI endoscopy visualized the surface and microvascular structure evident, therefore it might be useful for the diagnosis of early gastric cancer, independent of experience. To estimate the usefulness using the NBI system for the diagnosis of early gastric cancer, randomized control trials should be conducted in the future.

II-O-10-108
Preliminary Study of Intra-Small Intestinal Ultrasonography (ISIU) in the Diagnosis of Small Intestinal Diseases
Department of Gastroenterology, Rui Jin Hospital, Shanghai Jiao Tong University Medical College

Objective: The aim of this study is to evaluate the clinical value and feasibility of intrasmall intestinal ultrasonography (ISIU) in the diagnosis of small-intestinal diseases. Methods: From June 2004 to February 2005, 11 patients with obscure GI bleeding, chronic diarrhea and unknown abdominal pain were enrolled into the study. Patients ranged in age from 24-72 with an average age of 56. All patients suspected with small intestinal diseases underwent double-balloon enteroscopy (DBE). In addition ISIU was performed in all DBE positive subjects in the same procedure to determine the differentiations and characteristics of the lesions. Ultrasound system SP-702 and prototype catheter probe (Fujinon, Japan) were used in these procedures. Results: Of the 11 patients in the study, 9 positive lesions were macroscopically diagnosed by DBE and ISIU was successfully completed in 8 cases, with 20 min on average for each procedure. ISIU revealed 2 cases of small intestinal carcinoma presenting with heterogeneous hyperechoic area with disappearance and interruption of intestinal layers. 1 case of GIST originated from muscularis propria characterized by heterogeneous hyperechoic area with inner hyperechoic foci. 1 case
of polyp originated from mucosa layer presenting with homogeneous hypoechoic area; 1 case of lymph-vessel tumor characterized by homogeneous hypoechoic area with tubular structure in the lesion, 2 cases of early acute stage Crohn’s disease characterized by disappearance of intestinal vil- lus layer with total wall thickening. However, the intra-hamal ultrasonic image is not clear enough to detect mucosal vascular deformity. One failure case occurred because the catheter probe could not be pushed out of the accessory channel of DBE because the tip of the scope could not be straightened. The ISIU assessments were in accordance with surgical interventions and histo- logical biopsy results with a diagnostic accuracy of 77.8% (7/9) while it was 100% in combined with DBE. Conclusions: ISIU is an effective and safe procedure for assisting the diagnosis of small intestinal diseases and especially helps to define SMTs, Crohn diseases and tumor invad- ing. The successful rate and accuracy of the diagnosis depends on the position of the lesion, selection of the proper approach and the experience of the endoscopist. Key words: Intra-small intestinal ultrasonography, Double-balloon enteroscopy, Small intestinal disease.

Sponsored Symposium by TSUMURA & CO. II-O-11 Herbal Medicine Chairpersons: T. Takahashi, K. Haruma

II-O-11-109 Interaction between Brain-Gut Axis and Neural Transduction for Interdigestive Motility T. Takahashi Department of Surgery, Duke University, Durham, North Carolina, USA

1. Gastric MMC and motilin in dogs and humans. The migrating motor complex (MMC) is well characterized by the appearance of gastrointestinal (GI) contractions in the interdigestive phase. MMC consists of three phases; phase I (period of motor quiescence), phase II (period of irregular low amplitude contractions) and phase III (period of regular high amplitude contractions). The occurrence of gastric phase III is regulated by the cyclic increase of plasma motilin released from the duodenum in dogs and humans. Gastric and duodenal acidification suppresses motilin-induced phase III activity. The phase III cycle in the stomach is not always regular. In patients with duodenal ulcer, phase III activity is not observed in the stomach although the plasma motilin concentration is increased. The regularity of MMC cycle tends to be disturbed toward the time even in dogs. When intraduodenal pH becomes acid, there are no typical MMC observed even though plasma motilin levels are elevated. These suggest that luminal acid reduces the sensitivity to motilin. 2. Gastric MMC and ghrelin in rats. In conscious rats, cyclic changes of gastric motility are also observed, including a quiescence period (phase I-like contractions) followed by a grouping of strong contractions (phase III-like contractions) after a 24h fasting. In contrast to dogs, motilin does not cause any phase III-like contractions in rats. Ghrelin, a 28-amino acid peptide with structural resemblance to motilin, was recently isolated from the rat stomach as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R). Acylated ghrelin with O-ω-acylated serine in position 3 serves as an endogenous ligand for GHS-R. Peripheral administration of acylated ghrelin induces phase III-like fasting motor activity of the rat stomach. This suggests that ghrelin, not motilin, plays an important role to mediate gastric MMC in rats.

II-O-11-110 Mechanism for Action of Shakuyaku-Kanzoh-To-Induced Suppression in Guinea Pig Gastric and Ileal Smooth Muscle: Comparison with Scorpionamide Butylbromide S. Kurawasa Gastroenterology and Hepatology, Saitama Medical Center, Saitama Medical School The herbal medicine, Shakuyaku-kanzoh-to (TJ68) is a mixture of two popular herbs, Shakuyaku (Psoralea radix) and Kanzoh (Clerodendron trichotomum) and has been experimentally used for symptoms such as convulsion, spasm, or abdominal pain for years. The clinical efficacy of TJ-68 is partly similar to scorpionamide butylbromide (SB), which has adverse effects such as cardiac arrhythmia or glaucoma. TJ-68 has no these adverse effects. The mechanisms for action of TJ-68 to inhibit GI smooth muscle are not well known. Therefore we undertook the study to elucidate the mechanisms for action of TJ-68 compare with SB on guinea pig gastric or ileal smooth mus- cle. Methods: The muscle strips of guinea pig gastric or ileal muscle were suspended from iso- metric transducers and TJ-68 was applied from lower concentration. All drugs were dissolved in water. The muscle strips were washed each time. Two components of TJ-68 were applied separately as same fashion. Electric field stimulation (EFS) (40V, 10Hz, 0.5ms) was applied with or without TJ-68 or its components. Results: TJ-68 (1-10mg/ml) relaxed gastric muscle

II-O-11-111 Pharmacological Properties of Liu-Jun-Zi-Tang, a Traditional Herbal Medicine, for Gastric Motility in Various Rat Models K. Tominga, K. Higuchi, T. Kido1, M. Ochi, E. Sasaki, M. Shiba, T. Watanabe, Y. Fujivara, N. Oshtani, T. Arakawa Department of Gastroenterology, Osaka City University Graduate School of Medicine, Osaka, 1Central Research Laboratories, Tsumura & Co., Ibaraki, Japan Background and Aim: Impairment of gastric accommodation causes early satiety and abdominal discomfort due to food retention in stomach. Disorder of gastrointestinal coordina- tion originated from the proximal stomach is one of the critical factors for the pathogenesis of functional dyspepsia (FD). Liu-Jun-Zi-Tang (TJ-43), a traditional herbal medicine is well used in clinical practice for its pharmacological property for gastric motility is unknown. We examined the therapeutic mechanism(s) of TJ-43 on gastric motility in various emptying-delayed rat mod- els. Materials and Methods: We produced three types of gastric emptying-delaying models using male Wistar rats (9-week): 1) nitrergic oxide (NO) model with NO synthesis inhibitor, Nω-nitro-L-arginine (L-NNA: 5-20mg/kg, p.o.), 2) dopamine model (0.001-1mg/kg, i.p.), 3) serotonin model (0.001-1mg/kg, i.p.). We evaluated the effects of TJ-43 (125-500mg/kg, p.o.) on gastric motility in these emptying-delayed models. To assess the gastric emptying, we used the previously reported phenol red method. Results: We firstly confirmed the delay of gastric emptying (about 50%) at the respective optimum dose in all types of models compared to control (about 90%), and that their decrease was affected in a dose-dependent manner. Treatment with TJ-43 for the NO models could improve the emptying-delay in a dose-dependent manner due to both L-arginine and hesperidine that are proved components of TJ-43. Its maximum dose (500mg/kg) reversed the emptying-delay to control levels. TJ-43 could improve the emptying-delay in the serotonin model at the maximum dose but not for the dopamine model at any doses. Interestingly, 5-HT3 antagonist but not 5-HT4 agonist affected the emptying-delay in this model. These functions induced by not only ondansetron but also TJ-43 were inhibited by the pretreat- ment with atropine. Conclusion: TJ-43 had the therapeutic efficacy for the motility dys- function caused by NO-related conditions and could also modulate the gastric motility mediated by 5-HT3 but not 5-HT4 receptor.

II-O-11-112 Herbal Medicine Dai-Kenchu-To Increases Colonic Vascular Conductance via Calcitonin Gene-Related Peptide in Anesthetized Rats T. Kono, T. Koseki1, J. Ivamoto2, S. Kasai1 1Department of Surgery II, 2Division of Applied Physiology, School of Nursing, Asahikawa Medical College, Asahikawa, Japan Background and Aims: Dai-Kenchu-To (DKT) is a traditional Chinese (Kampo) herbal medicine and has been used as the treatment of paralytic ileus DKT may increment gas- trointestinal motility by an up-regulation of the calcitonin gene-related peptide (CGRP). CGRP is also the most powerful vasoactive substance. In the present study, we investigated whether DKT has any effect on the colonic blood flow (CBF) in rats. Methods: Experiments were performed on 50 fasted anesthetized Wistar rats. Systemic mean arterial blood pressure (MAP) and heart rate (HR) were recorded. Red blood cell flux in CBF was measured using laser Doppler flowmetry, and colonic vascular conductance (CVC) was calculated as the ratio of flux to MAP. We produced six types of emptying-delay models using male Wistar rats (9-week): 1) NO model with NO synthesis inhibitor, Nω-nitro-L-arginine (L-NNA: 5-20mg/kg, p.o.), 2) dopamine model (0.001-1mg/kg, i.p.), 3) serotonin model (0.001-1mg/kg, i.p.). We evaluated the effects of DKT on the colonic blood flow and blood pressure in these models. Results: CVC was significantly increased in all types of samples by DKT in a dose-dependent manner. Treatment with DKT (2.20%, respectively) in a dose-dependent manner, whereas the MAP and HR were not affected. CGRP (10−8 M) completely abolished the DKT-induced hyperemia, whereas [4-Cl-DPhe6, Leu17]-VIP, a VIP receptor blocker (10−8 M) and indomethacin partially attenuated the DKT-induced hyperemia. Spantide did not affect the hyperemia.

Oral Presentations

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A Novel Suppository Herbal Medicine for Refractory Proctitis of Ulcerative Colitis Patient

K. Fukunaga1, N. Hida1, Y. Ohda1, Y. Yokoyama1, H. Miwa2, Y. Fukuda1, T. Matsumoto1

1Division of Lower Gastroenterology, 2Division of Upper Gastroenterology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

Background and Aim: Xilei-san (XS), a traditional herbal medicine, has been given for acute inflammation patients in China, especially for upper GI diseases. Pharmacological mechanism of XS has never been fully elucidated, but significant efficiency of this herbal medication has been proven in previous reports. Currently, there are few therapeutic options available for proctitis of ulcerative colitis (UC) although its subjective symptoms, such as rectal bleeding and tenesmus, may cause to deteriorate quality of life (QOL) severely. We have developed an original suppository-XS (SXS) for rectal UC inflammation. In this study, we have aimed to evaluate long-term efficacy and safety of SXS by giving it for UC patients with refractory proctitis.

Patients and Method: A prospective clinical evaluation study of SXS, which contains 0.1 g of XS mixed and formed with Vaseline, was conducted on proctitis UC refractory to suppository steroids. Four patients were enrolled, and SXS has been given once a day together with their current medication regimen. The primary end-point of this evaluation was 180 days, and endoscopic evaluation has been performed before and after the study.

Results: All patients have been proven to improve significantly in both their subjective symptoms and endoscopic findings after starting daily SXS. A 32 yo woman has decided by herself to quit other medication, including 5-aminosalicylic acids, and she has been controlling her remission status successfully by SXS only for 12 months. There was no significant adverse effect has been observed during this study in all patients. Discussion: Significant efficiency of a novel SXS has been proven in UC patients with refractory proctitis. We have been recognized that XS has a potential to be an additional therapeutic options of UC patients without causing severe adverse effect since its anti-biotic and anti-ischemic characteristics has been reported previously might effect to UC colitis.

Celery Seed Extracts have Anti-Helicobacter as well as Gastroprotective and Anti-Inflammatory Activities

K.D. Rainsford, Y. Zhou, T. Smith, M. Clench, Z. Liu
Sheffield Hallam University, Sheffield, UK

Celery seed extracts (CSE) from a unique source in India has been found to have anti-inflammatory and gastroprotective properties (Butters et al. 2002; US Patent 6,352,728; Butters et al. US Patent 6,761,913; Whitehouse et al: Inflammopharmacology 2001;9:201–209). Additionally, anti-Helicobacter activity of the crude extracts has been observed in vitro (Rainsford & Liu, 2003; PCT/GB03/ 02244; patent pending). A therapeutic agent that has combination of anti-inflammatory/ analgesic as well as gastroprotective and anti-Helicobacter effects would appear to have considerable potential for therapeutic application in the treatment of rheumatic and other chronic inflammatory diseases especially when combined with conventional drugs (e.g. NSAIDs). Here we describe the mode of action of CSE components in vitro. CSE was selectively fractionated using organic solvents and HPLC. The fractions were bio-assayed against different strains of H. pylori using conventional culture methods. Broad anti-H. pylori activities were observed in a number of fractions and the most potent of these was purified and characterised by mass spectrometry, NMR and other spectroscopic methods. The mechanisms of effects are irreversible and are comparable with tetracycline. In conclusion, novel CSE extracts that have anti-inflammatory activity also exhibit dual potential to prevent ulcer disease either from NSAIDs or H. pylori.
I-P-1 Endoscopic Submucosal Dissection 1
Chairpersons: Y. Sano, N. Uedo

I-P-1-1 Healing of Gastric Ulcer after Endoscopic Submucosal Dissection and Ulcer-Related Symptoms According to the Duration of Omeprazole Treatment
H. Doyama, T. Omori, K. Jakemura, S. Yamada, H. Shimazaki
Gastroenterology, Ishikawa Prefectural Central Hospital

Background: Although it has recently been reported that treatment with omeprazole for endoscopic mucosal resection (EMR)-related ulcer for only 1 week is equivalent to 4 weeks of treatment, there is no consensus regarding the duration of treatment for endoscopic submucosal dissection (ESD)-related ulcer. The aim of this prospective study was to evaluate both healing of ESD-related ulcers and ulcer-related symptoms according to the duration of omeprazole treatment.

Methods: Ninety patients were randomly assigned, after ESD, to treatment with omeprazole (20mg/day) for 1 week (1-week group) or 4 weeks (4-week group). Forty-five patients were randomized in each group. The ulcer sizes were evaluated by gastroscopy 4 and 8 weeks after ESD. Each patient kept a daily diary of the ulcer-related symptoms during the 8 weeks after ESD.

Results: No significant differences were observed between the two groups in initial ulcer size (p = 0.32), ulcer size after 4 weeks (p = 0.12), or ulcer size after 8 weeks (p = 0.49). Ulcer-related symptoms from 2 to 4 weeks were noted by 17 patients (37.8%) in the 1-week group and 7 (15.6%) in the 4-week group, representing a significant difference between both groups (p = 0.032). No significant differences were observed in other periods (within 1 week: 33.3% vs. 46.7%, p = 0.28; from 5 to 8 weeks: 2.2% vs. 4.4%, p = 0.99).

Conclusions: The ulcer healing rate of the 1-week group was equivalent to that of the 4-week group. However, the ulcer-related symptom rate was significantly higher in the 1-week group than in the 4-week group. Treatment with omeprazole for 4 weeks should therefore be considered for ESD-induced ulcer.

I-P-1-2 A Case Report on Delayed Wound Healing of Artificial Gastric Ulcer after Endoscopic Mucosal Resection Caused by Chemoradiation for Esophageal Cancer
R. Nakamura1, M. Yoshida1, Y. Saikawa1, N. Wada1, T. Nakamura1, T. Kubota1, K. Kuma1, M. Kaitajima2
1Department of Surgery, School of Medicine, Keio University, 2Center for Comprehensive and Advanced Medicine, Keio University Hospital, Center for Diagnostic and Therapeutic Endoscopy, Keio University

Introduction: Chemotherapy has been reported to delay wound healing. However, its clinical implications have been rarely reported. Endoscopic mucosal resection (EMR) is established treatment for indicated gastric mucosal cancer. However, it is not established when EMR for gastric cancer should be done for the patient who undergo chemotherapy for duplicated, another cancer. We encountered a patient with delayed healing of ulcer caused by EMR of the stomach.

Case: The patient was a 74-years old woman. She underwent EMR for the treatment of early gastric cancer (U less type0-IIa T1(M)) during receiving chemoradiotherapy (5FU/CDDP/radiation) for the treatment of early esophageal cancer (U less type0-IIa T1(M), N0M0 stageI).

Discussion: We studied 123 ulcers in 117 patients with ESD. Results: There is no consensus regarding the duration of treatment for endoscopic submucosal dissection (ESD)-related ulcer. The aim of this prospective study was to evaluate both healing of ESD-related ulcers and ulcer-related symptoms according to the duration of omeprazole treatment. Conclusion: Healing of Gastric Ulcer after Endoscopic Submucosal Dissection and Ulcer-Related Symptoms According to the Duration of Omeprazole Treatment.

I-P-1-3 Randomized Prospective Evaluation of the Effectiveness of Ranitidine for Gastric Ulcers
1Department of Surgery, School of Medicine, Keio University

Patients and Methods: We studied 123 ulcers in 117 patients with ESD. Results: There is no consensus regarding the duration of treatment for endoscopic submucosal dissection (ESD)-related ulcer. The aim of this prospective study was to evaluate both healing of ESD-related ulcers and ulcer-related symptoms according to the duration of omeprazole treatment. Conclusion: Healing of Gastric Ulcer after Endoscopic Submucosal Dissection and Ulcer-Related Symptoms According to the Duration of Omeprazole Treatment.

I-P-1-4 Randomized Controlled Study to Evaluate Endoscopic Resection with a Ligation Device for Rectal Carcinoid Tumors
Department of Internal Medicine, Saga Medical School, Saga, Japan

Background: Rectal carcinoid tumors smaller than 10 mm can be resected with local excision using endoscopy. In order to remove rectal carcinoid tumors completely, we evaluated endoscopic mucosal resection with a ligation device in this pilot control randomized study. Methods: Fifteen patients were diagnosed with rectal carcinoid tumor (less than 10 mm) in our hospital from 1993 to 2002. There were 9 males and 6 females, with a mean age 61.5 year (range, 34–77). The patients complained no symptom of carcinoid syndrome. Fifteen patients were randomly divided into 2 groups: 7 carcinoid tumors were treated by conventional endoscopic resection, and 8 carcinoid tumors were treated by endoscopic resection using a ligation device. Results: All patients were resected by ESD in our hospital from May 2003. Patients of secondary hemorrhage were defined as those who needed blood transfusion and/or endoscopic hemostasis. Conclusion: Anti-coagulation and/or anti-platelet drugs increase the incidence of secondary hemorrhage from the post ESD ulcers. Prophylactic hemostasis may be effective to prevent secondary hemorrhage.

I-P-1-5 Randomized Prospective Evaluation of the Effectiveness of Ranitidine and Omeprazole for Ulcers after Endoscopic Mucosal Resection
K. Tahara1, S. Tanabe1, H. Takeuchi1, K. Saigenji1
1Department of Gastroenterology, The Kitasato Institute Medical Center Hospital, 2Department of Gastroenterology, Kitasato University East Hospital

Background: H2-receptor antagonists are widely used to prevent ulcers after endoscopic mucosal resection (EMR). We compared the effects of ranitidine and omeprazole for the prevention of ulcers after EMR. Methods: We studied 123 ulcers in 117 patients with...
gastric tumors who underwent EMR from June 1999 through October 2003. The patients were ran-
domly assigned to receive ranitidine or omeprazole. EMR was done by endoscopic aspiration
mucosectomy EAM or strip biopsy. Ranitidine was administered as an intravenous infusion 10mg
twice daily for 3 days, starting the day before EMR, followed by oral ranitidine 150mg twice daily
for 8 weeks. Omeprazole was administered as an oral dose of 20mg once daily for 8 days, start-
ing the day before EMR, from June 1999 through April 2001. >From January 2002 onward,
ranitidine was given as an intravenous infusion 20mg twice daily, starting 1 day before EMR, for
3 days, followed by oral ranitidine 20mg once daily for 8 weeks. Hematemia, hematocritia,
deroscopic findings (coagula, visible vessels), and hemostasis were observed. Endoscopic exami-
nations were done 1–2 days, 7 days, and 28–48 days after EMR. Results: The ranitidine group
comprised 57 patients and the omeprazole group 60. There was no hematemia or hematocritia
in either group. The proportion of patients with coagula in the stomach was significantly higher
in the ranitidine group (11 patients) than in the omeprazole group (2 patients, p < 0.05). Other vari-
ables did not differ significantly between the groups. Conclusion: omeprazole and ranitidine
are similar in terms of promoting hemostasis after EMR in patients with gastric tumors.

I-P-1-6

Study of Endoscopical Pattern of Regenerative Mucosa after EMR
M. Kataoka1, T. Kawau1, J. Miyazaki2, K. Yagi1, K. Kawakami1, T. Yamagishi1, S. Taira1, T. Ito1, F. Moriyasu1, T. Takagi2, T. Aoki2
1Endoscopy Center, Tokyo Medical University Hospital, 2Fourth Department of Internal Medicine, Tokyo Medical University, 3Third Department of Surgery, Tokyo Medical University

Background: It is difficult to discriminate recurrence of cancer and benign elevated
regenerative lesion after endoscopic mucosal resection (EMR) of gastric cancer. In this study we
investigated endoscopically patterns of regenerative lesion in 3–12 months after EMR.
Method: Subjects were 94 patients with treated EMR for gastric adenoma or gastric cancer. We
divided the regenerative lesion into 3 patterns with reference to form, color and surface
respectively. Results: Proportion ratio according to form were 25.5, 70.2 and 4.2% for ele-
vated pattern, flat pattern and depressed pattern respectively. Proportion ration according to
color were 65.9, 30.8 and 3.1% red color, same color, discoloration respectively. Proportion ratio
according to surface were 69.1, 23.4 and 7.4% for smooth, granular and nodular respectively. The
elevated patterns were recognized 79.1, 8.3 and 12.5% in the L area, the M area and the U area
respectively. Two elevated lesions of recurrence of gastric cancer were recognized in only the U
area. Conclusions: We should suspect recurrence of gastric cancer if the elevated regenera-
tive lesion is confirmed in the U area after EMR.

I-P-1-7

Helicobacter pylori Eradication Therapy does not Accelerate Ulcer Healing after Endoscopic Mucosal Resection
Department of Medicine and Molecular Science, Hiroshima University

Background and Aim: It remains unclear whether Helicobacter pylori (H. pylori) eradication therapy accelerates the healing of acute gastric ulcer after endoscopic mucosal resec-
tion (EMR) of gastric tumor. We examined the effect of H. pylori eradication therapy on ulcer healing after EMR. Methods: Twenty-six patients who underwent successful H. pylori eradi-
cation therapy before EMR were followed-up prospectively. Patients underwent endoscopic exami-
nation 1 or 2 months after EMR, during which the ulcer status and reduction rate were assessed.
The effect of H. pylori eradication on the quality of ulcer healing was also evaluated. Six patients
in whom with eradication therapy failed and 26 patients who underwent EMR without eradication
therapy served as control subjects. Results: Endoscopically, 18 (75%) ulcers out of 24 in the eradication group were at the healing stage 1 month after EMR. The ulcer reduction rates were
85.0 ± 2.6% and 96.9 ± 1.1% at 1 and 2 months after EMR, respectively. Ulcer stage and reduc-
tion rate did not differ significantly between the eradication group and control group. However, we
frequently observed a better quality of ulcer healing in the eradication group than in the control
groups (p < 0.01). Conclusion: H. pylori eradication therapy does not accelerate ulcer healing after EMR but may improve the quality of ulcer healing of gastric ulcer after EMR.

I-P-2-8

Endoscopic Submucosal Dissection Techniques for Early Gastric Cancer
Second Department of Internal Medicine, Osaka Medical College

Although the strip biopsy method is popular endoscopic mucosal resection technique (EMR)
for its convenience and reliability, it has limitations in resectable tumor size. Endoscopic submucosal
dissection techniques (ESD) using the diathermic needle knife or the insulated-tip diathermic knives
have been introduced to overcome this disadvantage, but they have high risks for bleeding and perforation. Our intent in this study was to investigate the usefulness of ESD for early gastric can-
cer comparison with the strip biopsy method and the educational system in our hospital.
Materials and Methods: Studies were carried out on 505 lesions in whom EMR was per-
formed, 385 lesions were treated with the strip biopsy and 120 with ESD. We investigated the en-
bloc resection rate, the complication rate of bleeding and perforation, and the learning curve of
ESD in our hospital. Results: (1) The en-bloc resection rate of the strip biopsy method was
54.8% and that of ESD was 88.3%. (2) Irrespective of tumor size and location, we could resect the
tumor with a much higher en-bloc resection rate. (3) The complication rate of bleeding and perfo-
ration was 0.8% in the strip biopsy method and 6.7% in ESD. (4) Training for ESD was acquired in
an informal setting (observation of actual procedures by videotapes, animal models) and a for-
mal setting (nothing can replace live demonstrations). Conclusion: Endoscopists who seek to
perform ESD should avail themselves of training and education in a formal or informal setting.
ESD is promising as a safe and reliable technique for the treatment of early gastric cancer.

I-P-2-9

Clinical Management of Gastric Adenoma Based on Endoscopic Mucosal Resection
The Third Department of Internal Medicine, Kyorin University School of Medicine

We performed endoscopic mucosal resection (EMR) for gastric adenoma positively. However,
there are several reports that adenoma is at low risk of malignant transformation. We
analyzed 66 lesions in 58 cases with gastric adenoma underwent EMR at our hospital, from 1999
to 2005. All cases initially diagnosed gastric adenoma by biopsy specimens. 18 lesions (27.3%)
in 16 cases were finally diagnosed as adenocarcinoma by pathohistology. All lesions were intra-
mucosal and well-differentiated adenocarcinoma. In regard to an occupation part, the upper part,
middle part and lower part was each 6, 39 and 56%. The median diameter of the lesion was
24.2mm. The size larger than 20mm was observed 50%. At endoscopic findings, the depressed
type lesion was observed 11.2%. The appearance redness was observed 5.6%. On the other hand,
48 lesions (72.7%) in 42 cases were finally diagnosed as gastric adenoma by pathohistology. In
regard to an occupation part, the upper part, middle part and lower part was each 6, 63 and 31%. The
median diameter of the lesion was 20.7mm. The size larger than 20mm was observed 41.7%. At
endoscopic findings, the depressed type lesion was observed 14.6%. The appearance redness
was observed 4.3%. Comparison of these results with those from carcinoma group showed no sig-
nificant difference. In conclusion, we recommend that it would be reasonable to positively per-
form EMR for correct diagnosis and treatment from the point of view of total biopsy.

I-P-2-10

Initial Experience of Endoscopic Submucosal Dissection for the Treatment of Early Gastric Cancer or Gastric Adenoma
Department of Gastroenterology, Higashi-Osaka City General Hospital

Endoscopic submucosal dissection (ESD) has been shown to be a very useful method of
endoscopic mucosal resection (EMR) for a one-piece resection. We had began ESD method using
11 knife in September 2004 and reviewed all cases with early gastric cancer or gastric ade-
noma who underwent ESD in our hospital from a beginner’s point of view. Eighteen cases under-
went ESD until January 2006 and one-piece resection was performed in 16 cases (88%). We
experienced complications in 3 cases. A pin-hole perforation occurred and could be closed with
clips in one case. Although an abdominal X-ray after ESD showed free air, the patient was
asymptomatic and recovered without medications. A severe stenosis of pyloric part developed
after the treatment in one patient. The perforation of the stenotic part occurred during the dilatation procedure with balloon catheter. Surgical operation was performed in this case. In one patient, we had to discontinue the ESD due to massive bleeding. Although two-third of the lesion could be resected by snaring, the remainder of the lesion was resected by distal gastrectomy. In the initial five cases and difficult cases, we performed this method with the experts of other hospital. The training and education from experts in the method is essential for the endoscopists who seek to perform submucosal resection easily and safely.

I-P-2-11
Endoscopic Mucosal Resection for Gastric Tumors: Historical Pilot Evaluation between Endoscopic Submucosal Dissection and Conventional Mucosal Resection
K. Watanabe, S. Ogata, K. Watanabe, T. Koyama, S. Tsunada, R. Inakiri, K. Fujiimoto
Departments of Internal Medicine, Saga Medical School, Saga Prefectural Hospital Koseien, Saga, Japan

Background: Endoscopic mucosal resection (EMR) has been applied widely. In Japan, endoscopic submucosal dissection (ESD) was recently developed for en bloc resection. We evaluated the clinical outcomes of ESD compared to conventional EMR methods. Methods: EMR of 245 gastric tumors was performed. Lesions were divided into two groups: conventional EMR was performed on lesions in group A between February 1999 and June 2001, and ESD was performed on group B between July 2001 and March 2004. Group B was further divided into sub-groups: sub-group B-1 underwent ESD between July 2001 and March 2003, and sub-group B-2 between April 2003 and March 2004. Results: With regard to lesions > 10 mm in size, en bloc resection rate and rate in completeness of resection of group B was significantly higher than that of group A (p < 0.01). Although the required time was longer in group B than A (p < 0.01), it was shorter in sub-group B-2 compared to B-1 (p < 0.05) with lesions ≤10 mm in size. The remnant ratio and perforation rate were not different between groups. Conclusions: ESD has been more popular in Japan since the end of 2000, and the required resection time was longer with ESD, this disadvantage might improve with experience.

I-P-2-12
Perforation of Endoscopic Submucosal Dissection for Early Gastric Cancer
C. Kusano, H. Hekaha, T. Gotoda, I. Odla, D. Saito
Endoscopy, National Cancer Center Hospital

Introduction: Endoscopic resection (ER) has become the standard treatment for early gastric cancer (EGC) in Japan. A newly developed technique of dissecting the submucosa directly during endoscopy, the so-called Endoscopic Submucosal Dissection (ESD), allows en-block resection of larger lesions. However, it requires advanced skill and competence, including the ability to manage complications. Recently, we achieved endoscopic sealing of perforations that complicated ESD by means of end-clip application. Consequently, we aimed to investigate whether endoscopically treated ESD-derived perforation relates to peritoneal dissemination of malignancy. Patients and Methods: We reviewed the medical records of 1,307 EGC lesions in 1,183 patients treated by ESD at National Cancer Center Hospital between 1999 and 2003. To clarify the potential risk of peritoneal dissemination of malignancy by perforation, we reviewed our patients for more than 2 years with the mean follow up period of 38.7 months (range: 0.4–81.3 months). Furthermore, we reviewed the results of the intraoperative cytological investigation whenever a complementary operation followed. Patients with intramucosal cancer were followed-up with upper GI endoscopy and those with submucosal invasive cancer (SM1) with gastroscopy plus EUS and CT scan. Results: Among the 1,307 cases, the rate of perforation was 4.0% (52 cases in 51 patients). With the exception of two patients who underwent emergency surgery and another one treated conservatively, the rest 49 cases in 48 patients were successfully dealt with endoscopically. The cytology of 16 patients who underwent complementary gastrectomy was negative. Similarly, during the follow up no evidence of peritoneal dissemination appeared. Conclusion: Perforation during ESD for EGC can be managed by endoscopic clip application without apparent risk of peritoneal dissemination.

I-P-2-13
A Case of Cronkhite-Canada Syndrome (CCS) with Early Gastric Cancer Improved Remarkably due to Endoscopic Submucosal Dissection (ESD) and Steroid Therapy
S. Minami1, Y. Satsagawa, Y. Ueno, T. Fujii, K. Katoaka1, T. Okuda2, S. Sira2, K. Tateno, N. Saijo1, Y. Nit1
1Department of gastroenterology, Rumoi City Hospital, Rumoi, 2Fourth Department of Internal medicine, Sapporo Medical University, Sapporo, Japan

A 62-year-old man noticed appetite loss, diarrhea and dyspepsia, and visited our hospital. Multiple polyps were shown by endoscopic examinations. Histopathological findings revealed juvenile type polyp and one of biopsy specimens taking from gastric polyps revealed adenocarcinoma. He had ectodermal changes. His family had no history of significant gastrointestinal disease. These characteristic manifestations suggested the diagnosis of Cronkhite-Canada syndrome with early gastric cancer. He was first treated with endoscopic submucosal dissection (ESD) for early gastric cancer. Resected specimen showed two cancerous lesions and these were curative resections. Adrenocortical steroid therapy for CCS was administered beginning with a dose of prednisolon 30mg following ESD. Polyposis and his symptoms were improved remarkably. Moreover, the dose of prednisolon was tapered after confirming his conditions, and now he has no medication and no recurrence of CCS and gastric cancer.

I-P-3 Neoplasm-Clinical

I-P-3-11
The Treatment of Carcinoid Syndrome with the Use of Octreotide before and after Surgery
A. Severettey, T. Dmity, K. Lali, G. Irina
HPB-Surgical Department, The Central Clinical Hospital#1 of the OAO ‘RZD’ (Russian Railways)

The carcinoid tumor is a rare kind of tumor. The diagnosis before surgery is difficult, especially when the main tumor is localized in small intestine and there are multiple liver metastases. Usually the diagnosis confirmation is very controversial (even using such new modalities as PET and Octreoscan). Sometimes the clinical data are much more important than other investigations. According to the basic data octreotide is very effective for such kind of tumors. The purpose of this study was to present the possibility of using of octreotide as diagnostic and treatment test in the course of carcinoid disease. In the course of year (2005) we treated 2 patients with carcinoid tumors located in the upper part of jejunum and in the lower part of ileum with the development of carcinoid syndrome (and multiple liver metastases). All conventional tests before surgery confirmed the presence of malignant tumors without primary origin. Because of suspicion of carcinoid syndrome we used high doses of octreotide for prophylactic treatment. We had got the decline of symptoms before surgery for both patients. These results confirmed the diagnosis of carcinoid, so during the surgery we searched the tumor along small intestine. The surgery confirmed our preoperative diagnosis and the presence of tumors in above mentioned part of intestine. After intestine resection (in both cases) we made cholecystectomy of liver tumor nodules and the implantation of catheter into hepatic artery. During postoperative period patients received high doses of octreotide and Sandostatin LAR. Carcinoid syndrome resolved completely and the lost of metastases in liver were reduced dramatically. Our results confirmed that the use of octreotide is an effective treatment of carcinoid tumors and carcinoid syndrome before and after surgery and could be used as diagnostic modalities for these patients.

I-P-3-14
Rapid Development of Diffuse Large B-Cell Lymphoma after Successful Eradication of Helicobacter pylori for Gastric MALT Lymphoma
M. Iwano1, N. Watanabe2, Y. Matsushima2, K. Oka3, T. Sakurai2, H. Inagaki1, K. Okazaki1
1Gastroenterology, Japan Baptist Hospital, 2Department of Gastroenterology and Hepatology, Graduate School of Medicine, 3Department of Pathology, Kyoto University, 4The Third Department of Internal Medicine, Kansai Medical University, Osaka, 5Department of Pathology, Nagoya City University Medical School, Nagoya, 6Oki clinic, Kyoto

Background and Aims: Low-grade marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type can transform into high-grade diffuse large B cell lymphoma

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(DLBCL). However, the clonal relation between MALT lymphoma and de novo DLBCL is still controversial. We encountered three cases of gastric MALT lymphoma that progressed to DLBCL immediately after successful eradication of H. pylori. The aim of our study is to describe 3 patients with low-grade H. pylori positive gastric MALT lymphoma rapidly progressing to DLBCL after successful eradication of H. pylori and to clarify the clonal relation between preceding MALT lymphoma and de novo DLBCL. Methods: All three gastric MALT lymphoma patients were positive for H. pylori, and eradication was successful in all the patients. Follow-up endoscopy shortly after the completion of successful H. pylori eradication showed newly developed DLBCL at the same site. Biopsy specimens were analyzed to clarify the characteristics of the MALT lymphomas and DLBCLs. Results: In all these MALT lymphomas, R-PCR and direct sequence analyses did not reveal any API2-MALT1 chimeric transcript, suggesting that MALT lymphomas of our cases did not obtain t(11;18)(q21;q21). Sequence analysis of the rearranged immunoglobulin heavy chain gene (CDR III) showed no clonal relation between preceding MALT lymphoma cells and de novo DLBCL cells at the same site. Conclusions: These findings question the scenario of direct clonal progression of a low-grade MALT lymphoma without t(11;18)(q21;q21) to a DLBCL and serve as a reminder of a risk of the progression of DLBCL with a distinct clonality immediately after the H. pylori eradication for low-grade MALT lymphoma.

I-P-3-16 Study of Chemotherapy for Malignant Lymphoma of the Stomach Y. Kagami, T. Kato, H. Tsurumi, N. Gojo, N. Kamineura, M. Shiraki, S. Kasahara, H. Fuku-shima, T. Yamada, H. Araki, M. Nagaki, H. Morisaki 1st Department of Internal Medicine, Gifu University School of Medicine Background: It is well recognized that CHOP (a combination of cyclo-xytem, adriamycin, vincristine, and prednisone) is the standard chemotherapy regimen for non-Hogikin lymphoma (NHL). Recently, administration of Rituximab (R), which is CD20 antibody, has been available for NHL-S and to clarify the clonal relation between preceding MALT lymphoma and de novo DLBCL. Methods: From January 1999 to December 2005, 78 patients with NHL, who were checked with gastrointestinal endoscopy, were admitted to our hospital. Twenty-two patients (28.2%) of them were endoscopically diagnosed with NHL-S. Among them, thirteen patients who were treated with chemotherapy were selected as subjects in this study. The study group was comprised of 8 males and 5 females, and their median age was 64. Their pathological findings were 10 diffuse large B cell lymphoma, 2 peripheral T-cell lymphoma, and 1 small lymphocytic lymphoma. Their clinical stages (CS) were one IE, two IIIA, two IIIB, three IVA, four IVB, and one IVE (the Ann Arbor classification). We selected basically CHOP chemotherapy regimens of 6 times for every patient. R was added to 10 patients. Results: In 12 cases (92.3%), after chemotherapy, endoscopic findings of NHL-S had almost disappeared. Chemotherapy regimens of 6 times for every patient. R was added to 10 patients. Conclusion: The study group was comprised of 8 males and 5 females, and their median age was 64. No complications were observed in the chemotherapy regimen. After chemotherapy, endoscopic findings of NHL-S had almost disappeared.

I-P-3-17 Cholangiocarcinoma in the Specialized Hepatic Clinic (Russia) A.N. Severstev, T. Dimitry, K. Lal, G. Irina HPB-Surgical Department, The Central Clinical Hospital#1 of the OAO RZD Russian Railways Background: Cholangiocarcinoma is the second on frequency occurred liver cancer. It is extremely difficult to diagnose this type of cancer (‘incidentioma’ of the liver). In Russia this cancer is extremely rare occurred. The purpose of the study is to estimate the clinical situation by this kind of liver cancer and to define the prospects in its treatment. For the period from December 2002 till December 2005 in the specialized department of our hospital 12 patients with the confirmed diagnosis cholangiocarcinoma were observed. In spite of the fact that bilio-vascular involvement was applied to 7 patients, surgery is executed to all patients. The volume of liver resection was individual (lobectomy till up to trisectorectomy), but obligatory condition was fulfilled (5 patients) or maximal (7 patients) removal of a tumoral tissue (cortiectrective surgery) with extended lymphodissection. Surgery, all tumours were greater in the sizes (at least up to 5.5 cm and more in diameter). All patients in the postoperative period received immuno- and chemotherapy and the hyperthermal intraoperative intraperitoneal chemotherapy. All the patients got over the surgery successfully. Postoperative mortality was 0%. The long-term results: In the group without bilobar process, the minimal long term observation has made 1 year, the maximal – up to 3 years. 3 patients had an advanced process. In the group with bilobar involvement, the minimal long time observation has made 4 months, the maximal is up to 2 years. Two in 7 patients have died (the period of supervision of 5 months and 1.1 year). The received preliminary results allow to approve, that new intraoperative techniques and oncologic methods allow to prolong substantially the life in this group of patients.

I-P-3-18 Effects of Rikkunshito, A Traditional Japanese Medicine, on the Delay of Gastric Emptying Induced by N(G)-Nitro-L-Arginine H. Kato, Y. Nakai, K. Yase, I. Sakakibara, M. Yuzuhara, S. Takeda, H. Sasaki 1, K. Tominaga 1, H. Iguchi 1, T. Arakawa 2 1Central Laboratory, Tsumura & Co., 2Department of Gastroenterology, Osaka City University Graduate School of Medicine Background and Aim: Rikkunshito (Luo-Jun-Zi-Tang), one of the traditional Japanese medicines, is widely prescribed for patients with chronic dysfunction of the gastrointestinal tract, including gastric flu,anxrea, nausea, and vomiting. These gastrointestinal symptoms are closely associated with delayed gastric emptying in patients with chronic dyspepsia. However, the mechanisms of rikkunshito involved in gastric emptying are not well understood. In this study, we evaluated the effects of rikkunshito and several of its ingredients on the delay of gastric emptying using an experimental model. Materials and Methods: To assay for the gastric emptying, we used the previously reported phenol red method (1 Pharmacol Sci, 2005). We produced the gastric emptying-delayed models using a nitric oxide (NO) synthase inhibitor, N(G)-nitro-L-arginine (L-NNA) in male SD rats. To identify the active ingredient of rikkunshito, the components were separated according to polarity, and the effects of respective fractions and ingredients on gastric emptying were evaluated. Results: 1) The decrease of the gastric emptying rate induced by L-NNA treatment was markedly, almost abolished by administration of rikkunshito (250 and 500mg/kg, p.o.) in a dose-dependent manner. 2) Significant effective components were found in the water and methanol fractions, but not in the 50% aqueous-methanol fraction. 3) Hesperidin (1-42mg/kg, p.o. contained in the methanol fraction and L-arginine (4.5mg/kg, p.o.) contained in the water fraction ameliorated the decrease in the gastric emptying rate induced by L-NNA treatment. Conclusions: These results suggest that rikkunshito ameliorated abnormalities of NO-mediated gastric functions such as delayed gastric emptying, and hesperidin and L-arginine were identified as two active ingredients contributing to the ability of rikkunshito to facilitate gastric emptying.


Background: Postoperative adhesions often occur of abdominal surgery, which was a major clinical problem. Daikenchuto (DKT) is a herbal medicine and is currently used as the treatment of postoperative intestinal obstructions in Japan. DKT consists of ginseng, zanthoxylum fruit, processed ginger and maltose syrup powder. The present study was investigated the effect of DKT on postoperative intestinal adhesions in rats. Methods: Postoperative intestinal adhesions were induced by dispersing of talc to the small intestine in rats. DKT, its constituents and active compounds were orally administrated once a day for a week. On the day following final administration, and animals were evaluated adhesion rate in the 7 days after surgery. Various antagonists were administered subcutaneously before test drugs. We evaluated the effect of DKT-induced response using isolated longitudinal muscle of guinea pig ileum by an isotonic technique. Results: DKT significantly prevented the formation of the intestinal adhesions. The preventive-adshesion action of DKT was inhibited by pretreatment with a TRPV1 antagonist, ruthenium red, or muscarinic receptor antagonist, atropine. Furthermore, DKT-inhibited reactions were inhibited by tetrodotoxin, atropine and capsazepine (TRPV1 antagonist). In constituents of DKT, zanthoxylum fruit and maltose syrup powder significantly prevented the formation of the intestinal adhesions. However, the other constituents did not markedly prevent the formation of the intestinal adhesions. Moreover, hydroxy sanshool (HS) of compound of zanthoxylum fruit pre- empted the formation of intestinal adhesions. In addition, the preventive-adshesion action of HS was inhibited by pretreatment with ruthenium red. Conclusion: DKT was suggested to show a preventive-adshesion action on postoperative adhesive intestinal obstruction, and this action was mediated by sensory and cholinergic nerves. Furthermore, HS was found as one of active compound of DKT, and this action was mediated by sensory nerve. DKT may be useful for the patients with postoperative intestinal obstructions.
I-P-4-20

Possible Roles of a Selective 5-HT4 Agonist in Improvement of the Experimental Gastric Emptying-Delay Induced by a Nitric Oxide Synthesis Inhibitor and the Clinical Symptoms of Patients with Functional Dyspepsia

M. Ochi, K. Tominaga, E. Sasaki, M. Shibata, T. Watanabe, Y. Fujikawa, O. Nishitani, K. Higuchi, T. Arakawa

Departments of Gastroenterology, Osaka City University Graduate School of Medicine

Gastric accommodation is mediated by nitric oxide (NO) and its disorder leads to the delay of gastric emptying and clinical symptoms such as abdominal fullness and discomfort in functional dyspepsia (FD) patients. It was reported that Liu-Jun-Zi-Tang ( TJ-43) improved the delayed gastric emptying associated with NO deletion. On the other hand, mosapride (Mos) has ability for an increase in gastric motility. However, the effects of Mos on the delayed gastric emptying associated with NO deletion were not elucidated. In this study, we examined a therapeutic efficacy of Mos not only for the delayed gastric emptying induced by NO synthesis inhibitor but also for clinical symptoms in FD patients. Methods: We produced gastric emptying-delay models using male Wistar rats induced by a NO synthesis inhibitor, NG-nitro-L-arginine (L- NNA: 10mg/kg, p.o.) and L-arginine (4.5mg/kg, p.o.) as a substrate of NO and a component of TJ-43, we assessed their efficacy for the delayed gastric emptying. We also clinically analyzed the therapeutic effects of Mos for 4 weeks-medication by the rat model and the degree of NO deletion. Results: Administration of Mos (0.3, 1, 3, 10mg/kg) TJ-43 (125-500mg/kg, p.o.) and L-arginine (4.5mg/kg, p.o.), a substrate of NO and a component of TJ-43, we assessed their efficacy for the delayed gastric emptying. We also clinically analyzed the therapeutic effects of Mos for 4 weeks by medication by the rat model and the degree of NO deletion in male Wistar rats induced by a NO synthesis inhibitor, NG-nitro-L-arginine (L- NNA: 10mg/kg, p.o.) and L-arginine (4.5mg/kg, p.o.), a substrate of NO and a component of TJ-43, we assessed their efficacy for the delayed gastric emptying. After the administration of Mos (0.3, 1, 3, 10mg/kg), TJ-43 (125-500mg/kg, p.o.) and L-arginine (4.5mg/kg, p.o.), a substrate of NO and a component of TJ-43, we assessed their efficacy for the delayed gastric emptying. We also clinically analyzed the therapeutic effects of Mos for 4 weeks by medication by the rat model and the degree of NO deletion in male Wistar rats induced by a NO synthesis inhibitor. Results: Administration of L-NNA decreased the gastric emptying rate from 73 to 51%. L-arginine and TJ-43 reversed the delayed emptying to the control levels. Interestingly, Mos could also improve in a dose-dependent manner to the control levels. As well as TJ-43, Mos showed a therapeutic efficacy for the delayed gastric emptying, followed by improvement of the subjective clinical symptoms in FD patients. Conclusion: Mosapride, a selective 5-HT4 agonist, was suggested to be effective for the delayed gastric emptying associated with NO deletion, which is mimic for the clinical feature of FD.

I-P-4-21

Contractile and Relaxant Effects of Capsaicin via Vanilloid Receptor TRPV1 in Isolated Mouse Lower Gastrointestinal Tract and their Modulation by Neonatal Maternal Separation Stress

S. Hori, K. Matsumoto, K. Tashima

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Josai International University, Sogane, Chiba, Japan

The role of afferent nerve expressing transient receptor potential vanilloid subtype 1 (TRPV1) in lower gastrointestinal tract remains to be fully elucidated. In the present study, we investigated effects of capsaicin, a TRPV1 receptor agonist, on smooth muscle tension in isolated mouse colon and rectum. The rectum and distal, transverse and proximal colon were surgically isolated from male, ddY mice. The longitudinal smooth muscle tension was isotonically measured using Magnus apparatus. In rectum and distal colon, capsaicin induced transient relaxation followed by transient contraction. Subsequently long lasting contraction appeared. Meanwhile, in transverse and proximal colon, only transient contraction was observed after application of capsaicin. The reactivity to capsaicin in rectum and distal colon is more sensitive than that in transverse and proximal colon. The neurotransmitter blocker tetradotoxin and the TRPV1 receptor antagonist indo-3-merferon almost abolished the capsaicin-induced transient relaxation in rectum and distal colon, and the transient contraction observed in all parts. Moreover, the muscarinic receptor antagonist atropine abolished the transient contraction in rectum and distal colon, but markedly, but not completely, inhibited that in transverse and proximal colon. These results suggest that TRPV1-expressed sensory nerves facilitate lower gastrointestinal motility through release of acetylcholine and/or other neurotransmitters. In addition, the reactivity to capsaicin is different among regions of the lower intestinal tract: rectum and distal colon is more sensitive to capsaicin. Next, influence of maternal separation stress on capsaicin-induced motility was studied in lower gastrointestinal tract. Neonatal maternal separation slightly increased the capsaicin-induced transient relaxation and contraction in rectum and distal colon, but did not affect the transient contraction in transverse and proximal colon. It is speculated that stress enhances reactivity or number of TRPV1 in lower gastrointestinal tract.

I-P-4-22

The Presentation and the Organization of Stomach-Duodenum-Colon Adaptive Cytoprotection in Rat


Department of Pharmacology, School of Medicine, University of Zagreb

We define adaptive cytoprotection in the whole GI tract. With adaptive cytoprotection appeared, and lesion attenuated challenged were stomach, duodenum or colon, in various combinations, with initial and/or final challenge throughout two weeks period. In rat, the specific challenges-mild/strong irritants were 25 or 96% ethanol i.e., 1mL/rat (stomach), cysteine 40mg or 400mg/kg s.c. (duodenum), or intrarectally (colon). For prostaglandin relation known in Robert’s cytoprotection and adaptive cytoprotection, indomethacin (1mg/kg s.c.) was given simultaneously with second challenge. Results: Preloading mild and strong irritant protocol within the same part of GI tract, adaptive cytoprotection presents in stomach-stomach ( i.e., 1h–14 days), duodenum-duodenum (i.e., 2h–14 days), while not in colon-colon. With mild and strong irritant protocols that affect the different parts of GI tract to generate adaptive cytoprotection, cross-react stomach-duodenum, duodenum-stomach (1h–14 days, or 2h–14 days), stomach-colon, duodenum-colon (both 2–24 h), but not colon-stomach or colon-duodenum. This is fully antagonized with indomethacin. Conclusion: Evidenced for days-weeks, this is a new defensive phenomenon.

I-P-4-23

Novel Action of Gastric Proton Pump Inhibitor on the Suppression of Helicobacter pylori-Induced Angiogenesis

Y-N. Na, S. J. Suk, M.S. Kawk, D-J. Lim, J.A. Lee, M.H. Kim, M. Ye, K-B. Hahn

Genome Research Center Iat Gastroenterology, Ajou University Medical Center

Background: Though the activation of mitogen activated protein kinases (MAPKs) by Helicobacter pylori (H. pylori) infection is associated with induction of host angiogenesis, which might contribute to H. pylori-associated gastric carcinogenesis, the strategy for its prevention has not been identified. Since we previously reported a strong inhibitory action of gastric proton pump inhibitor (PPI) on MAPK ERK1/2 phosphorylation, we investigated whether PPI could suppress the H. pylori-induced angiogenesis via inhibiting MAPK ERK1/2. Methods: To address the relationship between H. pylori infection and angiogenesis, comparative analysis of density of CD 34+ blood vessel was performed in tissues obtained from 20 H. pylori-positive gastritis and 18 H. pylori-negative gastritis. Expression of HIF-1a and VEGF were tested by RT-PCR and secretion of IL-8 and VEGF was measured with ELISA. To evaluate the direct effect of H. pylori infection on the tubular formation of human endothelial HUVEC cells, in vitro angiogenesis assay was employed. Activation of MAPK and NF-kB was detected by immunoblotting. Results: H. pylori-positive gastritis (409 ± 4.4) showed a higher density of CD34+ blood vessel than H. pylori-negative gastritis (7.2 ± 0.8), which was well correlated with the expression of HIF-1α. Conditioned media from H. pylori-infected gastric epithelial cells directly induced a tubular formation of HUVEC cells and the increase of in vitro angiogenesis was suppressed by PPI treatment. Infection of H. pylori significantly up-regulated expression of HIF-1α and VEGF in gastric epithelial cells and the expression of proangiogenic factors was mediated by MAPK activation and partially responsible for NF-kB activation. PPI effectively inhibited the phosphorylation of MAPK ERK1/2 that is a principal signal for H. pylori-induced angiogenesis. Conclusions: The fact that PPI could down regulate H. pylori-induced angiogenesis shed light on that anti-angiogenic treatment using PPI could be a promising protective therapeutic approach. H. pylori-associated gastric carcinogenesis. Grant support: This work was supported by grants from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PG6-01GN14-0007).

I-P-4-24

The Effect of an N-3-Fatty Acid Rich Diet (RACOL®) on the Expression of Cytokines and Epithelial Cell Proliferation in TNBS-Colitis Rat Model

K. Yokoyama, N. Nakajima, Y. Hori, R. Nishiyama, Y. Akai, T. Watanabe, T. Ohnita, A. Hwasaki, Y. Arakawa

Department of Gastroenterology & Hepatology, Nihon University School of Medicine, Tokyo, Japan

Inflammatory bowel disease (IBD) is a chronic disease of the digestive tract, with complicated and multifactorial etiology. An exaggerated intestinal immune response to otherwise innocuous stimuli plays a key role in the pathophysiology of this intestinal disorder. Nutritional intervention is an important therapy for patients with IBD. But the ratio of n-3 and n-6 fatty acids on the modulation of intestinal inflammation is not clear. Interleukin-16 (IL-16) is a pleiotropic cytokine secreted mainly by CD8 T cells the properties of CD8 T cells the properties of CD8 T cells may suggest that it may be involved in pathophysiological process of chronic inflammatory diseases. Aim: To determine the effect
of an n-3 fatty acid-rich diet (Racol®) on the expressions of cytokines and mucosal integrity in trinitrobenzene sulfonic acid (TNBS) induced rat colitis. Methods: 10 week male Wistar rats were divided into 4 groups. 1) Normal laboratory diet, 2) Racol diet only, 3) TNBS induced colitis with normal laboratory diet and 4) TNBS induced colitis with Racol diet. From day 2, rats were fed 90% of diet of Racol and/or normal laboratory diet, and sacrificed on day 8 and 15. One hour before sacrifice, 0.2 mg/g/rat of BrdU was injected ip. The expressions of BrdU, TNF-α, IFN-γ and IL-16 were studied by the ABC. Apoptosis was assessed by the TUNEL method. Macroscopic and histological findings were classified by the Morris method. Results: TNBS application increased colonic epithelial cell proliferation, but nutrition by Racol reduced this stimulation. Number of apoptosis cells was also increased TNBS application, but nutrition of Racol reduced number of apoptosis cells, which was increased by TNBS treatment. The expressions of cytokines were increased in TNBS-colitis rats, but reduced by the Racol. Conclusions: The effect of nutrition by n-3 fatty acid-rich diet, Racol, is an important treatment through inhibition of inflammatory mediators, and colonic hyper-proliferation.

I-P-5-25
Heme Oxygenase Regulates Mucosal Repair in Dextran Sodium Sulfate-Induced Colitis in Mice

T. Takagi1, Y. Haido1, T. Suzuki1, K. Tosa1, H. Tsukao1, H. Kajikawa2, H. Mizushima2, K. Kamada3, T. Okuda3, O. Handa3, S. Kokura1, T. Ichikawa1, N. Yoshida1, T. Yosihikawa1,2,3

Biomedical Safety Science,1 Medical Proteomics,2 Inflammation and Immunology,3 Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Background: The heme oxygenase-1 (HO-1) has been shown to mediate antioxidant, anti-inflammatory, and anti-apoptotic functions as well as the regulation of vascular tone in several experimental models. Recently it has been also described that HO-1 plays a cytoprotective role via the expression of various growth factors in the process of inflammation. The present study investigated the possible role of HO-1 in the regulation of repair process using a dextran sodium sulfate (DSS)-induced colitis in mice. Materials and Methods: Male BALB/c mice, 7–8 weeks old, received the administration DSS for 7 days followed by distilled water for the next 7 days. A disease activity index (DAI) was determined on a daily basis for each animal, and consists of a calculated score based on changes in body weight, stool consistency, and intestinal bleeding. We assessed intestinal cell proliferation by use of 5-bromo-2′-deoxyuridine (BrdU). The expression of mucosal repair-related genes mRNA (EGF, HGF, VEGF) were determined by Real-time PCR at different points after DSS induction. Moreover we evaluated the enhancement by treatment of an HO-1 inhibitor, zinc protoporphyrin IX (ZnPP 25 mg/kg ip daily) the healing stage from colitis. Results: The administration with ZnPP during the healing stage reversed the decrease in DAI score. BrdU labeling studies showed a pronounced inhibition of colonic epithelial cell proliferation during ZnPP treatment. EGF, HGF and VEGF expression were remarkably increased, however, the administration with ZnPP inhibited the increase of their expression. Conclusion: These results indicate that HO-1 plays an important role in the intestinal mucosal repair. These effects probably result from the regulation of mucosal repair-related genes in intestinal tissues.

I-P-5-26
Hyperthermia Ameliorates TNBS-Induced Experimental Colitis in Rats: A Role of Heat Shock Protein

S. Kokura1, T. Okuda3, N. Nakabe2, N. Sakamoto2, Y. Isozaki1, T. Hattori1, S. Adachi1, O. Handa1, T. Takagi2, Y. Haido1, N. Yoshida1, T. Yosihikawa4

Biomedi cal Safety Science,1 Inflammation and Immunology,2 Molecular Gastroenterology and Hepatology,3 Medical Proteomics, Kyoto Prefectural University of Medicine

Background and Aim: Hyperthermia is known to protect against cellular injury through the expression of heat shock proteins (HSPs). In this study, the therapeutic effects of hyperthermia on experimental colitis induced by 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) in the rat were evaluated. Methods: Male Wistar rats weighing 200 g were given a single intracolonic injection of TNBS (30 mg/rat, dissolved in 50% ethanol). Hyperthermia was induced in anesthetized rats by placement in a temperature-controlled water bath. Hyperthermia to a core temperature of 42 degrees for 20 minutes was followed by passive cooling. We started the hyperthermic treatment on the next day of enema. The severity of colitis was evaluated pathologically, and consisted of a calculated score based on changes in body weight, stool consistency, and ulcer size on day 10. The healing rate of the colitis at the proximal colon was more rapid than that at the middle colon. Conclusions: There was a regional difference on the healing process of the colitis. The middle colon, where there is a difference in the healing process of the colitis, the middle colon exhibited higher level of PGE2 generation, ulcer size was the largest among these three regions, and healing rate was slower as compared with that at the proximal colon. These findings suggest that higher level of PGE2 generation needs to maintain the colonic mucosal integrity. Therefore, if the integrity of colonic mucosa was disrupted, it may be hard to enhance the healing of colitis.

I-P-5-27
Regional Difference in Healing Process of Colitis Induced by 2, 4, 6-Trinitrobenzene Sulfonic Acid in Rats

M. Uchida1, O. Mogami1, K. Shizuku1, M. Katsused2

1 Food Science Institute, Meiji Dairies Corp., Kanagawa, 2 NHO National Saigata Hospital, Division of Gastroenterology, Niigata, Japan

Background and Aims: 2, 4, 6-Trinitrobenzene sulfonic acid (TNBS) has been used to induce colitis by injecting its ethanol solution from the anus. The authors improved the method to induce colitis and made possible to induce the colitis of the same size at appropriate region of the colon. This study aimed to investigate the regional differences on the healing process of colitis in rats. Methods: Colitis was induced at the proximal, middle or distal colon as follows: The colon was clamped by a pair of forces at a clamped portion, in which 35% ethanol solution of TNBS (0.1M solution) was injected. Prostaglandin E2 (PGE2) generation in the normal colonic mucosa was measured. Healing process was observed till day 31. Results: The sizes of the colitis on day 10 of the proximal, middle and distal colon were 10.3 ± 3.9, 61.8 ± 15.4 and 31.0 ± 8.9 mm2, respectively, and PGE2 generations 3.69 ± 0.64, 32.2 ± 4.95 and 20.1 ± 11.0 ng/mg tissue, respectively. Significant positive correlation was observed between prostaglandin E2 generations and ulcer sizes on day 10. The healing rate of the colitis at the proximal colon was more rapid than that at the middle colon. Conclusions: There was a regional difference on the healing process of the colitis. The middle colon of higher level of PGE2 generation, ulcer size was the largest among these three regions, and healing rate was slower as compared with that at the proximal colon. These findings suggest that higher level of PGE2 generation needs to maintain the colonic mucosal integrity. Therefore, if the integrity of colonic mucosa was disrupted, it may be hard to enhance the healing of colitis.

I-P-5-28
Prebiotic of Milk Whey Culture with Pripionibacterium Freudenleichi ET-3, Accelerates Healing of 2, 4, 6-Trinitrobenzene Sulfonic Acid-Induced Colitis in Rats

M. Uchida1, O. Mogami1, K. Matsueda2

1 Food Science Institute, Meiji Dairies Corp., Kanagawa, 2 NHO National Saigata Hospital, Division of Gastroenterology, Niigata, Japan

Background and Aims: Inflammatory bowel disease (IBD) has been increasing. Recently, many probiotics or prebiotics have been developed for the treatment of IBD. Milk whey culture with Pripionibacterium Freudenleichi ET-3 culture (milk whey culture) is a functional food to improve constipation by proliferating our own bifidobacteria in the colon. The present study aimed to evaluate the effect of milk whey culture on TNBS-induced colitis. Methods: Male Sprague-Dawley rats were used in this study. Colitis was induced as follows: The middle colon was punctured with ring forces (i.d. 8 mm), and 0.2 ml of 35% ethanol solution containing final 0.1M TNBS was injected into the luminal site of the clamped portion with the injection tube with needle. After 2 min, the colon was returned into the abdominal cavity and the incision was sutured. On and after day 2, milk whey culture or milk whey medium was administered orally twice a day for 9 days. Control rats were administered distilled water. On day 10, rats were sacrificed and ulcer index (length × width) was measured. Milk whey culture contains the short chain fatty acid such as propionic acid and acetic acid. Then, the effects of these short chain fatty acids were also evaluated. Results: In the control group, ulcer index was 49.7 ± 9.7 mm2. Milk whey culture significantly accelerated the healing of colitis and ulcer index was 20.9 ± 3.3 mm2, but the culture medium did not and ulcer index was 69.5 ± 22.3 mm2. Sodium propionate also accelerated the healing of colitis (27.8 ± 3.0 vs. 53.8 ± 6.1 mm2, p < 0.01). Conclusions: From above results, it was found that milk whey culture may be a useful prebiotic for the therapy of inflammatory bowel disease and propionic acid may be one of the active substances for accelerating the healing of the colitis.
I-P-5-29
Acceleration by Nitric Oxide (NO)/Inducible NO Synthase (iNOS) on Healing of Dextran Sulfate Sodium-Induced Colonic Lesions in Rats
Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yarnashina, Kyoto, Japan

Background and Aims: NO synthase (NOS) exists in two isoforms, constitutively expressed NOS (cNOS) and inducible NOS (iNOS). We previously reported that NO production endogenously plays a dual role in the development of rat colitis model induced by dextran sulfate sodium (DSS), depending on the NOS isoform; a protective effect by cNOS and proinflammatory effect of iNOS. In the present study, we examined the effects of various NO inhibitors on the healing of DSS-induced rat colitis. Methods: Experimental colitis was induced in male Wistar rats by feeding the rats for 6 days with 2.5% DSS in drinking water. After DSS treatment, the animals were fed normally with tap water for subsequent 7 days. L-NAME (a non-selective NOS inhibitor) or aminoguanidine (a relatively selective iNOS inhibitor) was given p.o. twice daily for 6 days, starting from the termination of DSS treatment. Results: DSS treatment caused severe lesions in the colon with an increase in MPO activity and TBARS as well as a decrease in body weight gain and colon length. These lesions healed gradually after discontinuation of DSS treatment. Daily administration of L-NAME or aminoguanidine significantly delayed the healing of colonic lesions. The gene expression of eNOS was constantly observed in the colonic mucosa while that of iNOS mRNA was up-regulated by DSS treatment. Likewise, the expression of bFGF in the colonic mucosa by DSS treatment. Both L-NAME and aminoguanidine significantly suppressed the increase in nitrite plus nitrate (NO metabolites) content but had no effect on the expression of basic fibroblast growth factor (bFGF). Conclusion: These results suggest that endogenous NO produced by iNOS plays an important role in the healing of DSS-induced colonic lesions. It is assumed that the healing promoting action of NO is accounted for by effects other than modulation of the bFGF expression.

I-P-5-30
Regulation of Intestinal Inflammation by Immune-Suppressive Ligand for C-Type Lectin
Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine

Background and Aims: Inappropriate regulation of Toll-like receptors (TLRs) is suggested to have a role in the pathogenesis of inflammatory bowel disease (IBD). Recently, ligation of C-type lectins, e.g., mannanose receptor (MR) and Dendritic Cell-Specific Intercellular Adhesion Molecule-3-Grabbing Nonintegrin (DC-SIGN) have been shown to downregulate TLR-mediated cytokine production. In the present study, we investigated the role of C-type lectins in the mouse model of IBD. Methods: Peritoneal macrophages from C57BL/6 mice were cultured in vitro with lipopolysaccharide (LPS, a ligand for TLR4) for 24 h and were further cultured with lipopolysaccharide (LPS, a ligand for TLR4) for 24 h. The culture supernatants were collected and were analyzed for TNF-α and IL-10 production. Inflammation was induced by administration of 3% dextran sodium sulphate (DSS) in drinking water in C57BL/6 mice and these mice were treated by intraperitoneal administration of LAM started 1 day prior to DSS administration. Production of TNF-α and IL-10 in the spleen and colonic lamina propria was analyzed. Results: LPS induced TNF-α production from peritoneal macrophages in vitro and the addition of LAM in the culture significantly suppressed TNF-α production and upregulated IL-10 production. In the DSS colitis model, administration of LAM induced IL-10 production in both the splenic and colonic lamina propria. In addition, TNF-α production was significantly suppressed by the treatment of LAM. Administration of LAM significantly improved macroscopic and histologic feature of colitis when compared with PBS-treated mice. Conclusions: Colonic injury symptoms—significantly decreased the inflamed area to total colon area ratio; and tended to reduce the colon weight to colon length ratio. Furthermore, the inflammatory response was evaluated by measuring myeloperoxidase (MPO) activity, malondialdehyde (MDA) and glutathione (GSH) levels. Green tea lowered the MPO activity, but not significantly ameliorated the MDA and GSH levels. In conclusion, green tea consumption reduces oxidative stress and tissue damage in experimentally induced colitis and may therefore serve as a preventive agent in inflammatory bowel diseases. This work is supported by research grants from The University of Hong Kong. Koo MWL, Cho CH. Pharmacological effect of green tea on the gastrointestinal system. Eur J Pharmacol 2004;500:177–185.

I-P-6-31
The Effects of Green Tea Catechins on DNBS-Induced Ulcerative Colitis in Mice
Department of Pharmacology, Faculty of Medicine, The University of Hong Kong

Ulcerative colitis is a worldwide, chronic, inflammatory disease with unknown aetiology. Multifactoral pathogenesis of the disease includes environmental and dietary factors. Green tea drinking is common in Asia, and its beneficial effects on gastrointestinal disorders have been widely reported (1). In this study the effects of green tea administration on chemically induced acute ulcerative colitis have been investigated. ICR female mice of 25-30g were pretreated either with 1%, 1.5%, 2% (w/v) green tea leaves solutions, or equivalent amount of tea polyphenol solutions for a period of 3 weeks. The consumption of chew, fluid and body weight of animals were monitored daily. After the pretreatment period, acute colitis was induced by intracolonic administration of 4mg dinitrobenzene sulphonylic acid (DNBS) dissolved in 100μl of 50% (v/v) ethanol. After 24h, animals were sacrificed and the colon was removed for examination. The results of macroscopic observations on colon tissues showed that green tea administration alleviated colon injury symptoms—significantly decreased the inflamed area to total colon area ratio; and tended to reduce the colon weight to colon length ratio. Furthermore, the inflammatory response was evaluated by measuring myeloperoxidase (MPO) activity, malondaldehyde (MDA) and glutathione (GSH) levels. Green tea lowered the MPO activity, but not significantly ameliorated the MDA and GSH levels. In conclusion, green tea consumption reduces oxidative stress and tissue damage in experimentally induced colitis and may therefore serve as a preventive agent in inflammatory bowel diseases. This work is supported by research grants from The University of Hong Kong. Koo MWL, Cho CH. Pharmacological effect of green tea on the gastrointestinal system. Eur J Pharmacol 2004;500:177–185.

I-P-6-32
A Herbal Medicine Orengedokuto Prevents NSAID-Induced Enteropathy
N. Muru1, M. Fukutake1, M. Yamamoto1, S. Izuka1, S. Imamura1, N. Tsujiya2, A. Ishige3, K. Watanabe1, Y. Kase1, S. Takeda1
Tsumura Research Institute, Tsumura & Co., Ibaraki, 1Department of Kampo Medicine, Keio University School of Medicine, Tokyo, Japan.

Background and Aims: Orengedokuto (OGT, Huan-Lian-Jie-Du-Tang in Chinese), one of the Japanese traditional herbal medicines (the Kampo medicines), has been used for the therapies of various diseases including gastritis, gastric ulcers and melena. In the animal model it has been known that OGT is effective for the enteritis induced by dextran sulfate sodium or trinitrobenzene sulphonionic acid. On the other hand, there are serious gastrointestinal complications of nonsteroidal anti-inflammatory drugs (NSAID) that are widely used for analgesic and anti-inflammatory properties. Selective COX-2 inhibitors are improving gastric tolerability, but the bowel damage remains the major limitation for their use. Hence, in the present investigation we examined whether OGT is effective to the animal model of NSAID-induced enteritis. Methods: BALB/c mice have injected indometacin (at 20mg/kg body weight once a day for 2 days) to induce the enteral lethal. OGT and the extract of its constituent herbs were administered at 25% into the diet. The morphological studies and the quantitation of intestinal prostaglandin E2 (PGE2) levels were performed. Results: Indomethacin-injection caused the enteritis and melena to mice and as a result the mice died. OGT-administration had protected enteritis and melena as a result the mice had survived. The mice administered with some OGT-constituent herbs, Oren (Coptidis thizoma), Obaku (Phellodendri cortex) and Ogon (Scutellarie radix) also had survived. Indometacin-injection reduced PGE2 levels in small intestinal mucosa and muscularis propria, and OGT significantly inhibited the reduction of mucosal PGE2. OGT also increased mucosal PGE2 levels by itself without indomethacin-injection. Conclusions: OGT and its constituent herbs significantly ameliorate the lethality, intestinal lesions, and mucosal PGE2-reduction induced by indomethacin, presumably via modulating the production of PGE2. OGT may be useful for the treatment of a variety of intestinal disorders including NSAID-induced enteropathy.

I-P-6-33
Macrophage Inhibition by Recombinant LXRα Agonist as a Novel Therapy for Inflammatory Bowel Diseases
T. Naka, T. Sato, T. Hibi
Division of Gastroenterology, Department of Internal Medicine, Keio University School of Medicine

Background and Aims: Liver X receptor α (LXRα) is one of the nuclear receptors and known to play an established role in lipid metabolism. It is highly expressed in macrophages and monocytes and has revealed its cross-talk with macrophage inflammatory pathways, but its involvement in inflammatory bowel diseases (IBD) remains unknown. In this study we examined...
Regulation of Toll-like Receptor-2 by Cyclooxygenase-2 on Intestinal Epithelial Cells

Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine

Background and Aim: Expression of cyclooxygenase (COX)-2 is upregulated on intestinal epithelial cells (IECs) of active inflammatory bowel disease (IBD). Toll-like receptors (TLRs) are also expressed on IECs and play an essential role on the innate immune system. In particular, TLR2, TLR4 and TLR5 are reported to contribute to the pathogenesis of IBD. We examined whether COX-2 regulates the TLR expression and TLR-mediated signaling pathways.

Methods: Expression of TLR mRNA were determined by quantitative RT-PCR in two intestinal cell lines, Caco-2 which express low baseline level of COX-2 and HT-29 which express large amount of COX-2. These cells were modified to overexpress or inhibit the production of COX-2. These cells were then stimulated with Pam3CSK4, a selective ligand for TLR2, and production of interleukin(IL)-8 in the culture supernatant was measured.

Results: Production of IL-8 by stimulation of TLR2 was increased by the presence of PGE2. Ligation of TLR2 by Pam3CSK4 in Caco-2-COX-2 cells which express high TLR2 expression was significantly decreased when COX-2 was knocked down by RNAi in these cells. Production of IL-8 by stimulation of TLR2 was increased by the presence of PGE2.

Conclusion: These results suggest that COX-2 and PGE2 regulate the expression of TLR2 and its downstream IL-8 production in the IECs. Controlling the TLR2 pathway by COX-2 or PGE2 may be of therapeutic benefit for IBD.

I-P-7-34
Upper GI Lesion in Crohn
T. Kosaka
Department of Internal Medicine, Hyogo College of Medicine

Not only the large and small intestine, but in an upper gastrointestinal tract (a stomach/the duodenum) is also injured in Crohn’s disease (CD). It is reported that several characteristic upper GI lesions are useful for an initial diagnosis, but relevance between CD are still unsure.

Methods: We investigated an upper GI lesion of CD patients and inquired relation with general condition, concomitant medicine, and Helicobacter pylori infection. Until October, 2005, 45 CD patients (36.6 ± 9.5 years old) were performed GI scope and reviewed presence of a stomach and duodenal lesion.

Results: Gastro-duodenal lesion was seen in all examples, as well as the small and large intestinal lesion for CD. The merger lesion was an erosion, edema. In addition, specific lesion of CD such as longitudinal ulcer, cobble stone appearance and bamboo joints-shaped appearance were seen in 40% of them. The HP infection of CD was a low rate, but duodenal lesions were frequent.

Discussion: It seemed that inflammatory response of CD contributed to the upper GI lesion. In CD, it is thought that the atrophy of fundus is rare, because of hyperacidity. As for this, opportunities of HP infection may decrease by hyperacidity conditions since their childhood.

I-P-7-36
An Elemental Diet may be Useful in Improving Long-Term Convalescence in Crohn’s Disease
Department of Gastroenterology, Nagoya University Graduate School of Medicine

Purpose: The advent of infliximab has brought a change in the treatment of Crohn’s disease. Although nutritional therapy is no longer considered an effective treatment for adult Crohn’s disease in Western countries, it still plays an important role in Japan where it is thought to be at least partly responsible for the lower cumulative operation rate in this country, possibly owing to a constant promotive effect on the induction and maintenance of remission. Here we investigated the relationship between nutritional therapy and the avoidance of surgery.

Methods: A total of 153 patients with Crohn’s disease who visited our hospital between July, 1999 to July, 2005 were enrolled. The relationship between caloric content of elemental diets and surgery as an endpoint in these patients was examined using Cox regression analysis. Cumulative non-operation rates were calculated by the Kaplan-Meier method. Statistical significance was determined by the Log-rank test. Results: Among patients without jejunoileal involvement, the cumulative non-operation rate did not differ among those receiving an elemental diet of less or more than 900kcal/day. Among those with jejunoileal involvement, in contrast, those taking an elemental diet of more than 900kcal/day showed a statistically significant improvement in cumulative non-operation rate. Results were similar for Cox regression analysis and the Kaplan-Meier method.

Conclusions: The use of an elemental diet of more than 900kcal/day may be effective in avoiding surgery in patients with jejunoileal lesions. This diet may be useful in improving long-term convalescence in these patients.
I-P-7-38
Alendronate Prevents Loss of Bone Mineral Density in Crohn Disease Induced by Corticosteroid Therapy
T. Sugawa
Internal Medicine, Shiga University of Medical Science
There were few reports about relationship between Japanese patients with Crohn disease (CD) and loss of bone mineral density (BMD), and about influences of steroids therapy. We investiga-
ted whether steroids therapy for the CD patients influence BMD and the alendronate is effec-
tive for loss of BMD. We recruited 49 outpatients with CD in remission. The BMD of the whole
body, the lumbar spine, and proximal femoral neck was measured at baseline and at 12 months
by DEXA. There are mainly two groups about steroids treatment. One is the steroid-use of 13
patients, the other is the steroid-dependent user or ex-users of 36 patients. Among steroid-depen-
dent group, mean dose of prednisolone was 1.774mg/year and 4.9mg per day. The BMD value
before alendronate administration could be measured in 15 patients with CD. Although the dura-
tion of the disease was not related to T score, the amount of total steroids was negatively corre-
lated with T score. Estimated dose of total steroid below -1.0 and - 2.5 of T score were 9,900 mg
and 21,400 mg respectively in whole body, 5,300 mg and 17,800 mg in the lumbar spine, 9,200 mg
and 23,500 mg in the proximal femoral neck. The changes of BMD after a year among CD
patients taking minetoretene or alendronate were measured. The mean value of BMD in menet-
t纶ne group did not alter in the 3 lesions. However, the same value in alendronate group
increased in the whole body, 4.5% in the lumbar spine, 3.4% in the proximal femoral neck. We conclude that the BMD in CD patients had been lost depending the total amount of steroids, and oral administration of alendronate improved the loss of BMD.

I-P-7-39
Significance of MRI Study for Detection of Asymptomatic Osteonecrosis of the Femoral Head in Patients with Inflammatory Bowel Disease with Long-Term Corticosteroids Administration
N. Kamata, N. Oshitani, H. Yamagami, K. Watanabe, S. Nakamura, Y. Fujivara, K. Higuchi, T. Arakawa
Department of Gastroenterology, Osaka City University Graduate School of Medicine, Department of Gastroenterology, Rinku General Medical Center, Osaka, Japan
Osteonecrosis of the femoral head is increasing and approximately 3,000 new cases are diagnosed in Japan each year. More than 40% of these patients are associated with corticos-
teroids treatments, however, the mechanism has not been well understood. Recently it is reported that MRI study is useful to diagnose early osteonecrotic changes before the patient complains symptoms at the very early stage of osteonecrosis. It is possible to diagnose the osteonecrosis with MRI study earlier than plain X-ray. In this study, we report the utility of MRI study for the detection in the early stage, and the relationship between the osteonecrosis of the femoral head and corticosteroids treatment. Methods: We researched 18 patients with Inflammatory Bowel Disease (IBD) (Ulcerative colitis: 14, Crohn’s disease: 2, Intestinal Behcet’s disease: 1, Simple ulcer: 1). who are dependent on the long-term corticosteroids treatment. MRI study with the age, duration of the disease, cumulative corticosteroids dose, whether they had pulse therapy or not, and relationship between osteonecrosis and bone mineral density (BMD) are studied. Result: Osteonecrosis of the femoral head was detected in 3 patients with MRI study as a characteristic ‘crecent sign’ including 2 asymptomatic patients. In addition, they were taking more than 18,000 mg of cumulative prednisolone and over 5 years duration of corticosteroids treatment. They were taking more than 50 mg of maximum daily prednisolone dosage over 14 days dura-
tion of corticosteroids treatment. Only one case had taken a steroid pulse therapy, the others had not. Osteoporosis seemed to be irrelevant to osteonecrosis. Conclusion: MRI study is useful for the early detection of osteonecrosis of the femoral head in the corticosteroids depen-
dent patients. Short term high dose treatment increases the risk of osteonecrosis.

I-P-7-40
Efficacy of Clinical Response to Leukocytapheresis in Patients with Corticosteroid Refractory or Dependent Ulcerative Colitis
K. Watanabe, N. Oshitani, N. Kamata, H. Yamagami, K. Higuchi, T. Arakawa
Department of Gastroenterology, Osaka City University Graduate School of Medicine
Ulcerative colitis (UC) is empirically treated with conventional medications. Use of these drugs is associated with side effects, which add to the disease complications. Two systems are available for depleting excess and activated PBL, GCAP (Adacolumn), which selectively depletes granulocytes and monocytes together with a small fraction of lymphocytes and LCAP (Cellecoha), which non-selectively depletes all leucocytes populations. We have investigated the efficacy of GCAP and LCAP, especially in corticosteroid refractory and dependent cases.

Methods: Thirty-four patients, age 19-52 years and duration of UC 1-12 years were treated. Twenty-seven had total colitis, 7 had left-sided colitis, 24 were drug refractory and 10 were steroid-depen-
dent. Each patient received one or two GCAP (n = 21) or LCAP (n = 13) sessions/week for 5 weeks, with colonoscopy performed before and after each session. Efficacy was evaluated by measuring changes in clinical activity index after each session. The predicator factor we evaluated was endoscopic findings, defined by Truelove Activity Index, with a decrease of >10 points or <13 points as marked improvement and 9 to 4 points as an improvement. A marked improvement was when all ulcers healed; an improvement was when some ulcers healed to scars but open ulcers remained. Results: Twenty-five patients (73.5%) had a clinical response and most steroid-
dependent patients or patients who had steroid side effects were successfully treated. Thirteen of 24 steroid-refractory patients had a clinical response and 18 of 25 patients who were on steroids tapered their steroid dose. Endoscopic improvements were seen in 14 of 16 (87.5%) patients with shallow ulcers or erosions throughout the colon and 10 of 13 with deep ulcers, but 2 with mas-
ive ulceration of the whole mucosa did not respond. Conclusion: GCAP or LCAP is valu-
able for refractory or steroid-dependent UC, most patients can reduce steroid dose or be spared of additional medication.
leukocytosis (LCAP), a non-pharmacological modality for UC, these parameters were compared immediately before and after LCAP. Results: Five out of 17 patients were categorized to SG, and a significant correlation proven between EI and EMBF (y = 6.125 x – 26.19; r² = 0.801, p < 0.05). LCAP was induced to 4 out of 5 patients in SG. Their vascular permeability improved has improved in the CS evaluations performed after an hour of LCAP and the average of EMBF has been proven to elevate significantly after LCAP (29.3 ± 11.5 to 44.2 ± 16.4; p < 0.002, n = 40). EI scores have not been proven to change significantly during LCAP. Discussion: We have proven a strong correlation between EMBF and EI in the patients of SG. For mild to moderate UC, we may need more detailed EI scale. We have proven that the mucosal blood flow has been increased rapidly after LCAP. EMBF in SG treated with LCAP could be a good predictive marker for response. Furthermore, improvement of EMBF might be one of the therapeutic mechanisms of LCAP by restoring micro blood circulation. EMBF has been suggested as a unique adjunct parameter to evaluate UC inflammation rapidly and accurately during CS.

I-P-8-43
Pit Pattern Diagnosis in Ulcerative Colitis Associated Neoplastic Lesions
N. Hida1, K. Watanabe1, K. Horii2, H. Ikeuchi3, T. Matsumoto4
1Department of Gastroenterology, Osaka City University Graduate School of Medicine, Osaka, Japan; 2Division of Upper Gastroenterology, Department of Internal Medicine, 3Department of Surgery, 4Division of Lower Gastroenterology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

Background: Few previous studies have shown that magnifying endoscopy is a useful tool for detection of neoplastic lesions in patients with longstanding ulcerative colitis (UC). Further investigation of the magnifying view of UC associated neoplasia is required to elucidate its natural history. We examined 12 cases of UC surgically resected and found that pathological material of UC associated neoplastic lesions with methylene blue-aided stereomicroscopy. The mucosal crypt patterns of focal lesions suspicious for neoplasia and their surrounding mucosa were classified according to the Kudo pit pattern classification and were compared with their corresponding histopathology. Results: Fifteen lesions of low-grade dysplasia (LGD), 3 lesions of high-grade dysplasia (HGD), and 5 lesions of invasive cancer were detected from 12 patients. Macroscopically, 77.8% (14/18) of intraepithelial neoplasias were identified as elevated lesions and 4 of which were flat lesions. Nine of 15 lesions of LGD showed type III and IV pit pattern, 2 showed non-neoplastic type I pit pattern, and 2 lesions showed unclassified pits. All cases of HGD had dysplastic pit pattern (type III or IV). Three of 5 lesions of invasive neoplasia showed type V pits, and 2 showed type IV pits. Most of the surrounding flat non-neoplastic mucosa showed type I pit pattern. However, in three cases, lesions showing type III and IV pits were non-neoplastic mucosa with active inflammation and regenerative changes. The overall sensitivity of a differentiation of neoplastic from non-neoplastic lesions by pit pattern diagnosis was 81% with specificity of 80%. Conclusions: Neoplastic pit patterns corresponded well to UC associated neoplastic lesions. Our data provides further evidence to support magnifying chromoendoscopy as a useful tool for improving endoscopic detection of neoplastic lesions in patients with longstanding UC. However, non-neoplastic pits in some lesions of LGD and neoplastic pit patterns in some inflammatory or regenerative lesions will become a subject of discussion.

I-P-8-44
DNA Hypermethylation in Colorectal Polyps and Inflammatory Bowel Disease
O. Maeda, T. Ando, O. Watanabe, K. Ishiguro, N. Ohmiya, Y. Niwa, H. Goto
Department of Gastroenterology, Nagoya University Graduate School of Medicine

Backgrounds: Epigenetic changes including DNA hypermethylation have been reported to be found in gastrointestinal inflammation and tumors. Aims: To evaluate methylation profile of colorectal mucosa of inflammatory bowel disease and colorectal polyps. Materials and Methods: We obtained 27 biopsy specimens (13 rectal mucosa, 7 colonic mucosa, 1 ileal mucosa and 6 adenomas) from 15 patients including 7 ulcerative colitis and 2 Crohn’s disease. We extracted genomic DNA from biopsy specimens of ulcerative colitis. Colonic and rectal samples of adenomas and colorectal polyps. We performed bisulfite modification and methylation specific PCR using primer pairs for Vimentin exon 1, DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) promoter and Estrogen receptor (ER). The Ethics Committee of Nagoya University Hospital approved the study, and all patients gave their informed consent for inclusion before-hand. Results: Vimentin was methylated in four biopsy specimens and three (75%) of them were from adenoma. MGMT methylation was detected in two specimens and three (75%) of them were from adenoma, and two of them were both Vimentin and MGMT methylated. ER methylation was detected in 11 patients (65%) including 6 ulcerative colitis, 1 Crohn’s disease and 2 adenomas. Conclusion: These results suggest that hypermethylation of Vimentin and MGMT are specific for adenoma, and ER methylation is for ulcerative colitis. Methylation status of these genes may relate with the mechanism of chronic inflammation and colorectal neoplas.

I-P-8-45
Dysplasia-Colitic Cancer Sequence in Ulcerative Colitis is Frequently Associated with Microsatellite Instability Pathway
O. Maeda, K. Fujita, N. Kudo, K. Mades, K. Hirakawa
Department of Surgical Oncology, Osaka City University Graduate School of Medicine, Osaka, Japan

Purpose: Colitic cancers (colorectal cancer complicating ulcerative colitis) arise in chronic inflamed epithelium, placing them in a continuum including epithelial inflammation, dysplasia, and invasive carcinoma. Colitic cancers show characteristic clinicopathologic features, setting them apart from sporadic colorectal cancer. To understand the carcinogenesis process of colitic cancer, genetic alterations of dysplasia-cancer sequence were analyzed in ulcerative colitis (UC). Patients and Methods: DNA was obtained from colonic mucosal specimens from 57 UC patients. According to histologic findings of epithelium by the Riddell classification, these 57 cases included 11 with cancer, 15 with non-neoplastic mucosa showing ulcerative colitis negative for dysplasia, 15 of which were flat lesions. Nine of 15 lesions of LGD showed type IIIL or IV pit pattern, grade dysplasia (HGD), and 5 lesions of invasive cancer were detected from 12 patients. We have proven a strong correlation between EMBF and EI in the patients of SG. For mild to moderate UC, we may need more detailed EI scale. We have proven that the mucosal blood flow has been increased rapidly after LCAP. EMBF in SG treated with LCAP could be a good predictive marker for response. Furthermore, improvement of EMBF might be one of the therapeutic mechanisms of LCAP by restoring micro blood circulation. EMBF has been suggested as a unique adjunct parameter to evaluate UC inflammation rapidly and accurately during CS.

I-P-8-46
Expression of GRAIL, E3 Ubiquitin Ligase for Induction of T Cell Energy, is Decreased in Active Ulcerative Colitis
Department of Gastroenterology and Hepatology, Osaka University

Background and Aims: Insufficient tolerance against antigens in the mucosal immune system is considered as a factor that causes inflammatory bowel disease. GRAIL (gene related to anergy induced in lymphocyte), an E3 ubiquitin ligase, plays an important role in T cell anergy and the following immune tolerance. The aim of this study was to investigate the level of GRAIL expression in the peripheral CD4+ T cell of ulcerative colitis (UC) patients. Methods: Peripheral mononuclear cells were obtained from four active UC patients and seven healthy volunteers. These cells were obtained by centrifugal leukocytapheresis or sampling of peripheral blood. CD4+ T cells were separated using magnet cell sorter from these cells. Expression of GRAIL mRNA in CD4+ T cells was quantified by real time PCR. The expression of GRAIL was followed up in one active UC patient who was treated by centrifugal leukocytapheresis repeated weekly for three times. Results: The GRAIL expression in CD4+ T cells was decreased in active UC patients (average 4.3±0.9 arbitrary unit, GRAIL-bta-actin) as compared to that of healthy volunteers (average 11.9±0.8 arbitrary unit). In the patient treated with the repeated leukocytapheresis, the GRAIL expression was low before the therapy (3.1±0.2 arbitrary unit). However, his GRAIL level increased to 14.0±0.2 arbitrary unit after the initial intervention, and remained high during the following therapy. This patient was promptly introduced into clinical remission during the leukocytapheresis. Conclusion: Decrease of GRAIL expression in CD4+ T cells in active UC patients leads to difficulty to induce immune tolerance, and may result in UC activation. The GRAIL expression in CD4+ T cells seems to reflect mucosal tolerance in human samples. These results warrant further prospective study examining potential usefulness of GRAIL in patients with UC.

I-P-9-Mucosal Injury 1
Chairpersons: S. Tsuji, H. Satoh

I-P-9-47
A Selective Adenosine A2A Receptor Agonist, ATL-146e, Ameliorates Aspirin-Induced Gastric Lesions in Rats
M. Odashima, M. Otaka, M. Jin, K. Komatsu, I. Wada, T. Matsuhashi, Y. Horikawa, N. Hatakeyama, J. Dyake, R. Oho, J. Linden, S. Watanabe
Department of Gastroenterology, Akita University

Background: Activation of adenosine A2A receptors reduces the production of various proinflammatory cytokines and suppresses neutrophil activation. Non-steroidal anti-inflammatory

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drugs such as aspirin induce gastric mucosal lesions. As pathogenesis of aspirin-induced gastric mucosal lesion, the contribution of activation of inflammatory cells and proinflammatory cytokine production plays critical roles. A specific adenosine A2A receptor agonist (ATL-146e) has been known as potent inhibitors of inflammation by increasing of intracellular cyclic AMP in leukocytes. The aim of present study was to determine whether ATL-146e can ameliorate aspirin-induced gastric mucosal lesion in rats, and the agent can inhibit the increase in neutrophil accumulation and the production of proinflammatory cytokines. Materials and Methods: Gastric lesions were produced by administration of aspirin (200 mg/kg) and HCl (0.15 N, 8.0 ml/kg). ATL-146e was injected 30 min before the aspirin administration. Tissue myeloperoxidase (MPO) concentration in gastric mucosa was measured as an index of neutrophils infiltration. The gastric mucosal concentration of tumor necrosis factor-α (TNF-α) was determined by ELISA. Also, we examined the effect of ATL-146e on tissue prostaglandin E2 production. Results: The intragastric administration of aspirin induced multiple hemorrhagic erosions in rat gastric mucosa. The total length of gastric erosions (ulcer index) in control rats was 21.6 ± 3.23 mm and was reduced by 96% to 3.1 ± 0.83 mm by pretreatment with 5.0 mg/kg ATL-146e. (p < 0.001). The gastric content of MPO and TNF-α were all increased after aspirin administration and reduced to near normal levels by ATL-146e. Gastric mucosal PGE2 concentration was not affected by intraperitoneal injection of ATL-146e, decreased by aspirin only given p.o. Supplementation of PGE2 prevented the expression of COX-2 in the stomach following p.o. or s.c. administration of indomethacin but had no effect on the COX-2 expression seen after p.o. administration of aspirin. The COX-2 expression induced by indomethacin was also inhibited by atropine, while omeprazole had no effect. In addition, SC-560 (a selective COX-2 inhibitor) but not rofecoxib (a selective COX-2 inhibitor) caused a decrease in PGE2; content and an hypermortality as well as COX-2 expression, without induction of any damage. Conclusion: These results suggest that the expression of COX-2 in the stomach after NSAID treatment may be due to the mucosal irritative stimuli caused by the physico-chemical properties of drug (aspirin, p.o.) or the abnormal stomach contraction due to COX-1 inhibition (other NSAIDs), rather than being a result of PG deficiency. Luminal acid does not play a role in the up-regulation of COX-2 in the stomach following NSAIDs, inhibition of COX-1.
lesions through the suppression of acid secretion. However, there is no information about the role of pepsin in the pathogenesis of I/R-induced gastric injury. In the present study, we focused on changes in pepsin secretion during I/R-induced conditions and investigated the role of pepsin in the development of gastric I/R injury. Methods: Male SD rats were used after 18 h fasting. Under urethane anesthesia, the pylorus was ligated, the celiac artery clamped, and 1 ml of 100 mM HCl was instilled in the stomach. Then, reperfusion was established 15 min later by removal of the clamp and after a 2 h reperfusion period. During I/R-induced conditions, both pepsin output and luminal acid loss were measured; the former was determined by the modified Anson method, while the latter was measured in presence of omeprazole. Results: I/R induced hemorrhagic injury in the gastric mucosa in the presence of HCl. The development of these lesions was significantly prevented by atropine, but not omeprazole or cimetidine. Treatment with a selective pepsin inhibitor also significantly reduced the severity of I/R-induced gastric lesions, while that of pepsin markedly aggravated these lesions. Pepsin secretion was significantly increased following I/R, and these changes were in parallel with the increase in luminal acid loss. In addition, the increased pepsin output during I/R-induced conditions was significantly inhibited by atropine but not cimetidine or omeprazole, while pepsin significantly inhibited the pepsin activity. Conclusion: These results suggest that 1) pepsin plays a pivotal role in the pathogenesis of I/R-induced gastric lesions, and 2) pepsin secretion is increased during I/R-induced conditions, the process being in association with acid back-diffusion and mediated through a cholinergic pathway.

I-P-9-52
Gastroprotective Action of Probiotic Lactobacillus GG on Ethanol-Induced Gastric Lesions
Pharmacology, The University of Hong Kong

The gastric mucosa is frequently exposed to different exogenous and endogenous ulcerative agents. Alcoholism is one of the risk factors for the development of mucosal damage to the stomach. This study was aimed to assess if a probiotic strain Lactobacillus GG (LGG) is capable of protecting the gastric mucosa from damage induced by ethanol applied intragastrically. Male Sprague-Dawley rats were intragastrically gavaged with 10% (w/v) of LGG for three consecutive days before subjected to 60% ethanol (v/v) to induce gastric mucosal lesions. We demonstrated the protective action of LGG on ethanol-induced gastric mucosal lesions. The LGG pre-treated group showed 45% smaller gastric lesion area than the control. This effect was strongly associated with the significant increase in mucous-secreting layer in the LGG-treated group. By TUNEL staining, LGG also prevented cellular apoptosis. However, heat shock protein 70 (HSP 70), which was increased during I/R-induced conditions and mediated through a cholinergic pathway.

I-P-9-53
Aggravation by Selective Serotonin Re-uptake Inhibitors (SSRI) of Antral Ulcers Induced by Indomethacin in Rats: Effects of Mucosal Protective Drugs
A. Tanaka, N. Isami, Y. Ikahira, R. Hatazawa, K. Takeuchi
Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto, Japan

Recent clinical studies suggested a risk of gastric adverse reaction on the concomitant use of selective serotonin re-uptake inhibitors (SSRI) with nonsteroidal anti-inflammatory drugs (NSAID). In the present study, we investigated the adverse effect of SSRI on gastric antral ulcers induced by indomethacin in rats and further investigated the effects of several antispasmodic drugs on the occurrence of antral ulcers induced by NSAID and SSRI. Male SD rats fasted for 24 h were reared for 1 h, and then administered indomethacin (30 mg/kg) SC 1 h after refeeding and killed 6 h later. Indomethacin caused antral ulcers in reared rats. Paroxetine dose-dependently worsened the severity of antral ulcers in response to indomethacin, despite provoking by itself no damage; the original ulcers induced by indomethacin were mostly non-hemorrhagic while they became hemorrhagic by co-administration of paroxetine. This effect of paroxetine was mimicked by fluvoxamine. In conclusion, these results suggest that SSRI exert a harmful influence on the antral mucosa when given with NSAID, resulting in aggravation of antral ulcers, and 5-HT3 receptors may be involved in the aggravating action of SSRI. Rebamipide is useful for preventing such adverse effects in the antral mucosa caused by the combined administration of NSAID and SSRI, and this action may be partly accounted for by the scavenging action of superoxide radicals.

I-P-10 Mucosal Injury 2
Chairpersons: H. Matsui, M. Yoshida

I-P-10-54
Restoration of Heat Shock Protein70 Suppresses Gastric Mucosal iNOS Expression Induced by Helicobacter pylori
D.J. Lim, Y.M. Na, S.J. Suk, M.S. Kawk, J.A. Lee, M.H. Kim, M. Yeo, K.B. Hahn
Ajou University Medical Center, Ajou University Medical Center

Heat shock proteins (HSPs) are crucial for the maintenance of cell integrity during normal cell growth as well as during pathophysiological conditions. While functioning mainly as molecular chaperones, heat-shock proteins also appear to be involved in diverse biological activities, such as apoptosis, carcinogenesis, and cytoprotection from cytotoxic damage. Infection with Helicobacter pylori (H. pylori) causes inflammation in the gastric mucosa, leading to gastritis, gastric ulcers, duodenal ulcer disease, and even gastric cancer, but the role of HSPs in H. pylori-associated gastropathy is not known. Using two-dimensional electrophoretic analysis, we have observed significant shifts in HSP profiles after H. pylori infection in KRM-1 cells. We therefore evaluated the effects of treatments that induce HSPs on H. pylori-induced iNOS expression. We found that H. pylori infection significantly attenuated the expression of HSP70, whereas exposure of cells to non-cytotoxic heat shock or gastrin/nerve growth factor (NGF) restored HSP70 expression, as well as suppressing the expression of iNOS, a major cause of H. pylori-induced gastric tissue damage. Our results suggest that induction of HSP70 confers cytoprotection against H. pylori infection by inhibiting the expression of iNOS. In conclusion, these results provide important insights into the flux in HSPs profiles in response to H. pylori infection and highlight the cytoprotective role of HSP70 in H. pylori infection. Grant support: This work was supported by grants from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PFG-01GN14-0007).

I-P-10-55
Angiotensin AT1 Receptor Blockers Suppress Ischemia-Reperfusion-Induced Gastric Injury by Inhibition of NADH/NADPH Oxidase Activity in Rats
A. Nakagiri, M. Sunamoto
Pharmacotherapeutics, Doshisha Women

In the oxidative damage, NADH/NADPH oxidase (NADH/NADPH ox) is the enzyme which produces hydroxyl-free radical, source of HO· and OR· in peroxidation of molecules. It has been demonstrated that angiotensin II enhances NADH/NADPH ox activity in cultured vascular muscle cells. Subsequently NADH/NADPH ox is demonstrated to be existent in the other cells such as endothelial cells, fibroblasts, and myocytes. It has been reported that the oxidative stress or infection of H. pylori caused the cell damage in the gastric mucosa. In the present study, we examined the effects of AT1 receptor blockers (ARBs) and the role of NADPH ox on I/R-induced gastric injury in rats. Male SD rats were used after 18 h fasting. Under urethane anesthesia, a rat stomach was mounted in an ex-vivo chamber, applied with 100 mM HCl, and a catheter connected to a 5 ml syringe was passed through a left femoral vein. After the operation, blood was collected to the syringe at 4 ml, and 30 min later reperfusion. ARB (losartan, candesartan or valsartan) or diphenylene iodonium (DPI; NADPH ox inhibitor) was given i.v. 10 min before reperfusion. The combination of 30 min ischemia and 90 min reperfusion produced hemorrhagic injury in rat stomachs. The injury was dose-dependently attenuated by pretreatment of each ARB. In addition, NADPH ox activity in the gastric tissues was increased within 10 min from reperfusion start and this increase was attenuated by pretreatment of ARB as well as DPI with a concomitant inhibition in I/R-induced gastric injury. These results demonstrated that the pretreatment of ARB and inhibition of NADH/NADPH ox activity suppressed I/R-induced gastric injury, which strongly suggests involvement of AT1 receptor and the NADPH ox system in the pathogenic mechanism of gastric I/R injury. AT1 receptor antagonists could be of therapeutic significance in the gastrointestinal mucosal damage.
I-P-10-56
Dual Action of Nitric Oxide (NO) in Ischemia/Reperfusion-Induced Gastric Mucosal Injury in Mice.
Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto, Japan

Background and Aims: Nitric oxide (NO) plays an important role in maintaining of the mucosal integrity of the stomach. NO synthase (NOS) exists in two isoforms, eNOS expressed constitutively in various tissues, including the stomach, and iNOS expressed in few tissues but rapidly induced in response to cytokines. Recent results showed that NO is involved in the pathogenesis of gastric injury induced by ischemia-reperfusion (IR), yet the relative role of NOS isoforms remains unexplored. In the present study, we investigated the roles of NO/NOS isoforms in the pathogenesis of I/R-induced injury in mouse stomach.

Materials: Male C57BL/6 (WT) mice were used after 18h fasting. Under urethane anesthesia, the celiac artery was clamped (ischemia) for 30min, then reperfusion was achieved for 60min through removal of the clamp, and the stomach was examined for lesions.

Methods: I/R produced hemorrhagic injury in the gastric mucosa of WT mice. The severity of injury was significantly increased by pretreatment with L-NAME (a non-selective NOS inhibitor) and this effect was significantly antagonized by co-administration of L-arginine. Pretreatment of 1,400 W (a selective iNOS inhibitor) also significantly prevented the I/R-induced gastric injury. The expression of endothelial NOs mRNA in the mucosa remained unchanged under normal and I/R conditions while the NO expression was markedly up-regulated in I/R with an increase in the mucosal nitrate plus nitrite (NO metabolites) content. The increased NO production during I/R was completely attenuated by L-NAME and partially mitigated by 1,400 W. Acid secretion showed no difference among the groups.

Conclusion: These results suggest that endogenous NO plays a dual action in the pathogenesis of I/R-induced gastric lesion; NO derives from eNOS is protective while NO derived from iNOS is pro-ulcerogenic in the stomach during I/R-induced conditions.

I-P-10-57
Role of Free Radical and Mucus in Rat Experimental Model of Gastritis with Ammonia
Second Department of Internal Medicine, Osaka Medical College

There are reports that Helicobacter pylori (H. pylori) damages mucin and undermines gastric mucosal integrity. On the other hand, there are reports that mucins act as radical scavengers in gastric mucosa. In this study, we used the experimental model of gastritis with ammonia and assessed the effects of ammonia on the gastric mucosa and gastric mucus, and whether free radicals are involved in the induction of gastric mucosal lesions. In addition, we evaluated the effect of novel anti-ulcer agents (teprenone, tetraprenylacetone) on free radicals and gastric mucins.

Materials and Methods: Male Wistar rats were used and sacrificed 6 weeks after the beginning of the study. Gastric mucosal injury was induced by giving 0.1% ammonia water ad libitum for 6weeks. Group A: Water was offered ad libitum. Group B: 0.1% ammonia water was offered ad libitum for 6 hours. Group C: 0.1% ammonia water was offered ad libitum for 6 hours and 200 mg/kg/day of teprenone was administrated orally for 6 weeks. The tissue sections of removed stomach were prepared to measure the thickness of gastric mucosa and to stain superficial mucus by PAS and deep mucus by Concanavalin A. The quantity of each positive mucus were measured as the area ratio (%). Tissue level of malondialdehyde (MDA), glutathione (GSH) and protein content (protease) in the frozen gastric mucosa were determined.

Results: Gastric mucosal atrophy was noted after the administration of ammonia water, but was not induced in the rats given teprenone despite the administration of ammonia water. On the other hand, MPO activity and LPO activity were significantly elevated after the administration of ammonia water. The elevation in MPO and LPO activity after the administration of ammonia water was significantly inhibited with teprenone.

Conclusion: It was suggested that a significant elevation in MPO and LPO activity is associated with gastric mucosal atrophy by long-term administration of ammonia water, and that the anti-ulcer agent teprenone preserves the intra-mucosal gastric mucous and inhibits the gastric mucosal atrophy without the elevation in MPO and LPO activity.

I-P-10-58
Hypertensive Gastric Mucosa: Roles of Nitric Oxide and NF-κB
T. Akahoshi, Y. Maseha, M.K. Jones
1Department of surgery, Kyushu medical center, 2University of California, Irvine, California, 3Department of Surgery and Science, Graduate School of Medical Science, Kyushu University

Poral hypertension (PHT) is associated with increased susceptibility of the gastric mucosa to injury by a variety of factors including non-steroidal anti-inflammatory drugs (NSAIDs) that non-selectively inhibit both isoforms of cyclooxygenase (COX-1 and -2). PHT gastric mucosa also has excessive nitric oxide (NO) production that contributes to the general increased susceptibility to injury. Using a rat model of PHT, we studied whether selective COX inhibition, which does not damage normal (normotensive) gastric mucosa, is sufficient to cause PHT gastric damage and, if so, whether and how excessive NO is involved. Indomethacin, a non-selective NSAID, caused 2.4-fold more gastric injury to PHT vs. normotensive sham operated (SO) control rats. Neither NS-398 nor celecoxib, selective COX-2 inhibitors, caused gastric damage in either SO or PHT rats. SC-560, a selective COX-1 inhibitor, did not cause gastric damage in SO rats but dose-dependently caused gastric damage in PHT rats. There was a compensatory increase in COX-2 expression and activity in SC-560-treated SO rats but not SC-560-treated PHT rats. Partial inhibition of NO production restored gastric COX-2 expression and activity levels in SC-560-treated PHT rats to those of SC-560-treated SO rats, by a mechanism consistent with induction of NF-κB, and significantly reduced gastric damage. These studies indicate that, in contrast to normotensive gastric mucosa, inhibition of COX-1 alone is sufficient to cause PHT gastric damage as a result of excessive NO that prevents the induction of NF-κB and the compensatory increase in COX-2.

I-P-10-59
Essential Role of Bile Acids for COX-2 and iNOS Expressions as well as Lesion Formation in Rat Small Intestine Following Administration of Indomethacin
Y. Takahira, A. Tanaka, K. Takeuchi
Department of Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto, Japan

Background and Aims: Nonsteroidal anti-inflammatory drugs (NSAIDs) cause hemorrhagic lesions in the small intestine. Numbers of elements are involved in the pathogenesis of these lesions. A recent study demonstrated the up-regulation of cyclooxygenase (COX)-2 expression in this tissue after indomethacin and suggested that this event is a key to NSAID-induced intestinal damage. However, the role of bile acids in the induction of these lesions remains unexplored.

In the present study, we investigated the role of bile acids in the intestinal ulcerogenic response to indomethacin, together with the relation to the up-regulation of COX-2 and iNOS expression.

Methods: Male SD rats were used. The animals were given indomethacin (10mg/kg) s.c. and killed various intervals after indomethacin administration. Bile duct ligation or cholylthymolexine (300–1,000mg/kg, p.o.), a bile acid sequestant, was performed 30min before or 3h after indomethacin administration. Enterobacterial counts were determined 24h after indomethacin administration. The expression of mRNAs in the small intestine was examined by RT-PCR.

Results: Indomethacin caused hemorrhagic lesions. Indomethacin increased enterobacterial counts in the mucosa and up-regulated the expression of both COX-2 and iNOS in the small intestine. Cholestyamine, given 30min before, significantly prevented the development of these lesions, whereas this agent, given 3h after indomethacin, failed to affect the intestinal ulcerogenic response. Similar results were obtained by bile duct ligation; the ligation 30min before prevented the occurrence of intestinal lesions. In addition, cholestyamine given 30min before prevented bacterial invasion in the mucosa and inhibited the up-regulation of COX-2 and iNOS following the indomethacin administration.

Conclusion: These results suggest that 1) the presence of bile acids in the small intestine is essential for the occurrence of NSAID-induced intestinal damage, and 2) bile acids facilitates bacteria invasion in the mucosa probably through the disrupting action, contributing to the up-regulation of COX-2 and iNOS expression.

I-P-10-60
Ascorbic Acid Deficiency Aggravates Stress-Induced Gastric Mucosal Lesions in Genetically Scorbutive ODS Rats
Y. Ohita, S. Chiba, Y. Imai, Y. Kamiya, T. Asawara, A. Atsugaya
Departments of 1Chemistry and 2Internal Medicine, School of Medicine, 3Graduate School of Health Sciences, Fujita Health University, 4Department of Clinical Biochemistry, Fujita Health University College, Toyoake, 5Department of Nutrition, Faculty of Wellness, Chukyo Women’s University, Otsu, Japan

Purpose: We have reported that gastric mucosal ascorbic acid (AA) level decreases with the development of gastric mucosal lesions induced by water immersion restraint stress (WIRS) in Wistar rats able to synthesize AA and that AA administration prevents this gastric mucosal lesion development by attenuating oxidative damage in the gastric mucosa. Thus, AA seems to play an important role in WIRS-induced gastric mucosal lesion development. However, the role of AA during the induction gastric mucosal lesions in animals unable to synthesize AA is still unclear. Therefore, we examined how AA deficiency affects WIRS-induced gastric mucosal lesion development in genetically scorbutive ODS rats.

Methods: Five-week-old male ODS rats were given AA-free diet and drinking water with and without AA (1g) for 2 weeks. Then, AA-deficient and sufficient ODS rats were subjected to WIRS in a water bath (23°C) for 3 and 6h. Gastric mucosal lesion index, AA, vitamin E (VE), non-protein SH (NPSH), and lipid peroxide (LPO) were determined.

Results: Gastric mucosal AA content in the AA-deficient group was 12% of that in the AA-sufficient group. Gastric mucosal VE and NPSH contents were decreased slightly at 6h of WIRS, while that content in the AA-sufficient group decreased about 40%. AA-deficient group showed more severe gastric mucosal lesions than the AA-sufficient group at 3 and 6h of WIRS. Gastric mucosal AA content in the AA-deficient group decreased slightly at 6h of WIRS, while that content in the AA-sufficient group decreased at 3 and 6h of WIRS. The AA-deficient group had decreased gastric mucosal MPO and LPO activity.
I-P-11 Mucosal Defense
Chairpersons: M.D. Basson, M. Otaka

I-P-11-61
Expression and Localization of MFG-E8 in Mice Gut
Department of Gastroenterology and Histopathology, Shimane University school of Medicine

Background: Milk fat globule-EGF-factor 8 (MFG-E8) is a secreted glycoprotein that promotes phagocytic engulfment of apoptotic cells by macrophages. Impaired phagocytosis of apoptotic cells in MFG-E8−/− mice leads to the development of organ specific lupus like autoimmune disease. In the gastrointestinal tract, MFG-E8 is prerequisite for phagocytic clearance of large number of infiltrating apoptotic cells and thus plays role against inflammation.

Aims and Objectives: This study was aimed to check the expression profile of MFG-E8 in mouse gut tissues and isolated cells considering its importance in the gut tissue homeostasis and maintenance.

Materials and Methods: Eight week old BALB/c mice digestive tract was segmented into glandular stomach, proximal and distal small intestine, cecum and distal colon. Gut epithelial cells and macrophages were isolated and sorted by collagenase A and Mac-1 micro beads respectively. MFG-E8 mRNA and protein from the tissue parts and isolated cells was detected by northern blot and western blot assay. Tissue specific MFG-E8 localization was revealed by immunohistochemistry.

Results: Almost same level of MFG-E8 expression was found in all the studied tissue samples. Our finding of MFG-E8 expression in the isolated gut epithelial cells indicated that its expression was not solely confined to the macrophages and dendritic cells as reported in several studies. There was no considerable variation in the localization pattern of MFG-E8 in various gut tissues of mice.

Conclusion: In normal mice gut, MFG-E8 was found to be ubiquitously expressed with slightly varied levels and this was due to the presence of varied number of cell population in different compartments of gut.

I-P-11-62
Mitogen-Activated Protein Kinase Pathway Partly Regulated Differentiation of Gastric Surface Mucous Cells with Air Liquid Interface
Department of Internal Medicine and Gastrointestinal Endoscopy, Saga Medical School, Saga, Japan

This study aimed to examine a role of mitogen-activated protein (MAP) kinase pathway on gastric surface epithelium using our cell culture model in that differentiation is promoted in GSM06 cells with air liquid interface. A double-dish culture system of mouse gastric surface mucous cell line GSM06 in Ham’s F12 medium supplemented with 10% fetal calf serum and 50 &micro;g/ml gentamicin at 37°C for 5 days. Indomethacin, SC-560 (COX-1 inhibitor), rofecoxib (COX-2 inhibitor) was given p.o., while L-NAME (NOS inhibitor) or aminoguanidine (a selective iNOS inhibitor) was given s.c. twice 24h and 3h before the termination of indomethacin treatment.

Results: The treatment with indomethacin produced minimal damage in the stomach, with increase in myeloperoxidase (MPO) activity. The damage was significantly promoted by l-NNAME and L-NAME treatment. Air liquid interface treatment significantly reduced the MPO activity by the either SC-560 or rofecoxib, although the effect of SC-560 was less pronounced.

Conclusion: Results suggest that not only endogenous PGs derived from both COX-1 and COX-2 but also NO produced by both eNOS and iNOS are involved in the mucosal defense of the inflamed stomach, partly by decreasing acid secretion and neutrophil migration.

I-P-11-64
Cytoprotection Mechanisms of Retinoids in Rats, in Healthy Human Mucosa and in Patients with Different Gastrointestinal Disorders
G. Lezzi, D. Andriès, R. Gérard
First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

Background: Retinoids prevent the chemical-produced gastric mucosal damage with-out presence of any inhibition of gastric acid secretion (‘nutritional cytoprotection’). Aims: to give a survey of the cytoprotective properties and modification of enzymes and substrates participated in the natural antioxidant system (catalase, glutathione peroxidase, superoxide dismutase, red, glutathione, malondialdehyde).

Materials and Methods: The physical, biochemical and clinical observations were carried out in rats (n = 900–1,000), healthy human subjects (n = 40) and in patients with different gastrointestinal disorders (n = 431).

Results: 1) Cytoprotective effects of retinoids depend on: a) Intact gastric gland, b) Intact adrenals, c) Glucocorticoid supplementation after surgical adrenalectomy. 2) Consequent biochemical changes (reduced inhibition of ATP transformed in ADP both together a dose-dependent increase of ATP in cAMP) in GI mucosa; e) Partly their scavenger properties and modification of enzymes and substrates participated in the natural antioxidant system (catalase, glutathione peroxidase, superoxide dismutase, red, glutathione, malondialdehyde).

Conclusion: 1) Experimental and human observations clearly proved the GI cytoprotective effects of retinoids. 2) The clinical manifestations of retinoid’s GI cytoprotection are very wide (acute and chronic inflammations, ulcer, precancerous states and cancers); 3) There is a correlation between the a) Scavenger properties of retinoids vs. intact gastric gland; b) scavenging properties vs. intact gastric gland; c) GI mucosal protective effects of retinoids vs. biochemical changes in GI mucosa. Grant: RET-II-08/2005.

I-P-11-65
Expression and Localization of Heme-Oxygenase (HO)-1 and its Protective Role in Acute Gastric Mucosal Lesion
K. Ueda1, T. Ueyama2, Y. Shimizu1, T. Ito1, Y. Tsuura1, M. Ichinose1
1Second Department of Internal Medicine, Wakayama Medical University, 2Department Anat. and Cell Biol., Wakayama Medical University

Introduction: HO-1 is the inducible isomer of heme oxygenase which together with the constitutive isomer, HO-2, catalyzes the first and rate-limiting step in heme degradation. Heme, released from proliferated erythrocytes is a pro-oxidant. Considering that by-products of heme catabolism, biliverdin, carbon monoxide and Fe2+ have been shown to be cytoprotective. At present, the role of HO-1 in the digestive system was not fully understood. In this study, the expression and localization of HO-1 during the course of acute gastric mucosal lesion (AGML) was investigated.

Method: AGML was induced by intragastric administration of 0.6 N HCl (0.4ml/100 g) in male rats. Expression of HO-1 was analyzed chronologically by RTPCR, in situ hybridization (ISH) and immunohistochemistry, respectively. In addition, mucosal lesion was assessed histologically following the treatment with HO-1 inhibitor, zincproporphyrin (ZP). Results: At 15 min after administration of HCl, bloody clot and linear hemorrhage were present on the surface of the damaged mucosa. They reached a maximal level by 1 h and

Background: Role of Endogenous Prostaglandins and Nitric Oxide in Mucosal Defense

I-P-11-63
Defense of Inflamed Rat Stomach Following Iodoacetamide Treatment
H. Nishio, S. Terashima, Y. Hayashi, K. Takeuchi
Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Miasaag, Yashima, Kyoto, Japan

Background and Aims: The roles of prostaglandins (PGs) and nitric oxide (NO) play an important role in the production of mucosal defense of the inflamed stomach. They reached a maximal level by 1h and
remained at the same severity until 6h. At 24h, gastric mucosa appeared almost normal. The levels of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. ISH of HO-1 mRNA began to increase at 90min, and showed 15-folds elevation at 3h. 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I-P-12-70
Achievement of Accelerated Healing and Resistance to Recurrence in Rat Model of Acid-Induced Gastric Ulcer with Gastroprotectant
Y-M. Na, S.J. Suk, M.S. Kawk, D.J. Lim, J.A. Lee, M.H. Kim, M. Yeo, K-B. Hahn
Genome Research Center for Gastroenterology, Ajou University Medical Center

Background: Quality of ulcer healing (QOUH) is defined as ideal ulcer healing presenting with the fine granular ulcer scar, high functional restoration and the resistance to ulcer recurrence. The aim of the study was either to compare QOUH between anti-secretory agents and gastroprotectants or to explore its underlying molecular mechanisms in rat gastric ulcer model. Methods: Serial injection of acetic acid for gastric ulcerogenesis and intraperitoneal injection of recombinant IL-1b for recurring scared ulcer was done in SD rats (200–250g). The 72 rats were divided into three groups according to treatment; no further treatment (Group I, no treatment, that is, naturally healed), acid suppressant (Group II, PPI treated for 8 weeks) and gastroprotogistant (Group III, DA-9601 for 8 weeks) and maintained up to 28 weeks after acetic acid injections. Results: In 58.3% (7/12) of group III rats at four weeks after gastric ulcerogenesis, active stage of gastric ulcer was converted to healing stage, but 16.7% (2/12) among group II rats and none among group I rats was in healing stage of gastric ulcer at the same time point, for which significant levels of EGF and pS2/TFF1 and abundant amounts of neutral mucin were contributive. Intrapерitoneal injections of IL-1b (200 μg/kg) at 28 weeks after acetic acid injection led to 100% of ulcer recurrence in group I and 75.0% in group II, but only 16.7% in group III rats showed ulcer recurrence (p < 0.001). Significantly attenuated levels of inflammatory cytokines like IL-2, TNF-α, COX-2, nitrotyrosine and efficient remodeling of regenerated gastric glands led to resistance to ulcer recurrence in group III. Conclusion: The gastroprotectant is prerequisite for achieving QOUH since they could accelerate ulcer healing and impose resistance to ulcer recurrence with regenerating and anti-inflammatory activities.

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I-P-12-71
Candida Albicans Infection is Involved in the Process of Duodenal Ulcer Perforation
1Department of Emergency and Critical Care Medicine, 2Department of Surgery, 3Department of Pathology, 4Center for Diagnostic and Therapeutic Endoscopy, 5Division of Comprehensive and Advanced Medicine, Keio University School of Medicine, Tokyo, 6First Department of Surgery, Iwate Medical University, Iwate, Japan

Introduction: The mechanisms of ulcer formation were well studied, but the mechanisms of perforation have not been elucidated. Candida is positive in about 40% of the culture of acid resistant bacteria taken from patients with gastric or duodenal ulcer perforation, but its importance has not been investigated. This study was performed to examine whether Candida infection is involved in the process of ulcer perforation. Materials and Methods: Male Wistar rats weighing 220-240g were anesthetized with diethyl ether, and administered cymastatin 28 mg/kg every 3 rounds for 4h. Candida albicans (C. albicans 19,002 Fujisawa, kindly provided by Fujisawa Pharmaceutical Co., Osaka, Japan) 108 in 0.5 ml saline were administered before, 1h after, 24h after and 48h after cymastatin (n = 17) or saline (n = 15) administration. Results: Perforations of duodenal ulcers were observed in 16 out of 17 rats (94.1%) in the Candida group. On the other hand, 4 out of 15 cases (26.7%) had perforated duodenal ulcers in the control group (p < 0.01). The area of duodenal ulcers in the Candida group was 163.55 ± 132.27mm² and that in the control group was 66.10 ± 81.59mm² (p < 0.05). The survival rate of the Candida group was significantly worse than that in the control group (p < 0.05). In the Candida group, C. albicans colonized at ulcer base after stained with hematoxylin-eosin, periodic acid-Schiff reagent and by Grocott.

I-P-12-72
Effect of Dopamine on the Healing of Acid-Induced Gastric Ulcers in Rats
K. Nishikawa, K. Armagase, H. Shinaiishi, K. Takeuchi
Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasaiga, Yamashina, Kyoto, Japan

Dopamine, a neurotransmitter in the brain, is known to be also present in the stomach and has been implicated in the regulation of several gastric functions. Recent study has shown that dopamine has the potent antinflammatory activity in gastric cancer through suppression of growth factor expression. Since growth factors also play a crucial role in the mechanism of gastric ulcer healing, it is possible that dopamine has a deleterious influence on the healing. However, there is no information about the influence of dopamine on the healing of the pre-existing gastric ulcers. In the present study, we examined the effect of dopamine on the healing of gastric ulcers in rats. Methods: Gastric ulcers were induced in Male SD rats by serosal application of acetic acid. Dopamine was given s.c. twice daily for 7 days, starting from 3 days after ulcer induction. In other case, dopamine filled osmotic pump was implanted into the dorsal subcutaneous space in the rats and maintained for 7 days. After 7 days, the ulcerated area (mm²) was measured under a dissecting microscope. Gene expression of growth factors such as basic fibroblast growth factors (bFGF) and vascular endothelial growth factor (VEGF) was determined by RT-PCR. Results: Dopamine given s.c. at 1-30 mg/kg twice daily for 7 days have no effect on the healing of gastric ulcers, as determined by macroscopically or histologically. The expression of both bFGF and VEGF was markedly up-regulated in the ulcerated mucosa, but these expressions were not affected by dopamine. Similar results were obtained when dopamine was infused continuously at a rate of 10 μg/kg/h for 7 days. Conclusion: These results suggest that dopamine, although reportedly known to have a potent antinflammatory activity in gastric cancer, does not cause any influence on the healing of the pre-existing gastric ulcers in rats.

I-P-12-73
Gastric Pentadecapeptide BPC 157 Heals Gastrocutaneous Fistula in Rats
Department of Pharmacology, School of Medicine, University of Zagreb

For gastrocutaneous (GC) fistula anaesthetized rats were subjected to laparotomy and gastrotomy, the open defect through the stomach (3 mm diameter) fixed by two stitches to front abdominal wall getting full communication between the lumen of the stomach and the skin defect (3 mm diameter). Therapy (stable gastric pentadecapeptide BPC-157 (in inflammatory bowel disease-PL-10, PLD-116, PL-14736, Pliva, Croatia, heals external and internal wounds) compared with placebo, BPC 157 in inflammatory bowel disease-PL-10, PLD-116, PL-14736, Pliva, Croatia, heals external and internal wounds) was performed (3mm) compared with anticholinergics, H2-blockers, and PPIs) was given intraperitonally (50g), first application 30min following surgery, last 24h before sacrifice (at 1, 2, 3, 7, 14, 21 days postoperatively). Results: Pentadecapeptide BPC 157 (10 μg, 10ng, 10pg) strongly improves both skin and stomach mucosa healing, and closure of fistulas since the earliest period, macro-micro-scopically, and functionally (fistula does not leak upon volume application). Contrary, atropine (10 mg), cimetidine (50mg), omeprazole (50mg) improve firstly skin healing, and then stomach mucosal healing, but regularly fail to affect fistula leaking and bursting strength. Conclusion: Pentadecapeptide BPC 157 could solve complex healing of GC fistula.

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Likewise, with respect to virtually no toxicity in clinical studies, these findings could be likely inhibition of all events related to abdominal aorta anastomosis is along with this pentadecapeptide BPC 157 (dissolved in saline, 0/ml, 2pg/ml, 2ng/ml, 2\(\mu\)g/ml) was given, and the assessment micro-scopically (program SIFORM, VAMITECH-Software Company, Croatia) was 24h thereafter, and compared with the values obtained in corresponding healthy rats. Results: Regularly, following abdominal aorta anastomosis controls present with thrombus formed at the anastomotic site, and almost no blood flow in blood vessel with apparently narrow diameter. Contrasting with the findings in thrombotic controls, gastric pentadecapeptide BPC 157 (dissolved in saline, 0/ml, 2ng/ml, 2\(\mu\)g/ml) was given, and TJ functions in the presence or absence of acid. Background and Aims: The recent reports demonstrated that angiotensin II receptor blockade (ARB) could protect against progression of fibrosis in liver and pancreas or promote wound healing, however, the role of angiotensin II receptor in ulcer healing following gastrointestinal mucosal injury remains unknown. The aim of the present study was to determine the role of angiotensin II receptor in healing stage of gastric ulcers. Methods: We examined 1) expression and localization of ZO-1 in human esophageal epithelial cells. 2) Effect of acidified medium on ZO-1 and TJ functions. 3) Whether the epidermal growth factor (EGF) and prostaglandin E2 (PGE2) affect ZO-1 expression and TJ functions in the presence or absence of acid. Methods: Human cultured esophageal epithelial cells (TE-1 cells) were incubated with acidified medium (pH 2.0–6.0) for several periods. To examine effect of EGF and PGE2 on ZO-1 expression and TJ functions of esophageal epithelial cells, EGF or PGE2 was added to the cells 24h before acid exposure. Results: 1) Localization of ZO-1 by confocal microscopy. 2) TJ functions by transepithelial resistance (TER) and paracellular flux using epithelial voltohmmeter or FITC-conjugated dextran. 3) TJ functions by transepithelial resistance (TER) and paracellular flux using epithelial voltohmmeter or FITC-conjugated dextran. Background: The aim of the present study was to determine the role of angiotensin II receptor in healing stage of gastric ulcers. Methods: We examined 1) expression and localization of ZO-1 in human esophageal epithelial cells. 2) Effect of acidified medium on ZO-1 and TJ functions. 3) Whether the epidermal growth factor (EGF) and prostaglandin E2 (PGE2) affect ZO-1 expression and TJ functions in the presence or absence of acid. 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Background and Aims: The mechanism of transformation to intestinal metaplasia in Barrett’s oesophagus has not been clarified. We investigated the effects of various bile acids on the expression of the caudal-related homeobox gene Cdx2 in cultured oesophageal squamous epithelial cells. In addition, morphological and histochemical changes of squamous cells to intestinal epithelial cells were studied in response to bile acid-induced expression of Cdx2.

Methods: A rat model of Barrett’s oesophagus was created by anastomosing the oesophagus and jejunum, and Cdx2 expression was investigated by immunohistochemistry. Further, the response of various bile acids on Cdx2 gene expression was studied in human colon epithelial cell lines CaCo-2 and HT-29, as well as cultured rat oesophageal squamous epithelial cells using a Cdx2 promoter luciferase assay. In addition, primary cultured oesophageal squamous epithelial cells were transfected with Cdx2 expression vectors and their possible transformation to intestinal type epithelial cells was investigated.

Results: Esophageo-jejunal anastomosis formed intestinal goblet cell metaplasia in rat oesophageal specimens and metaplastic epithelia strongly expressed Cdx2. When the effects of 11 kinds of bile acids on Cdx2 gene expression were examined, only cholic acid (CA) and dehydrocholic acid dose-dependently increased the Cdx2 promoter activity and Cdx2 protein production in CaCo-2 and HT-29, and cultured rat oesophageal keratinocytes. Results from the mutation analysis of Cdx2 promoter suggested that 2 NF-kB binding sites are responsible for the bile acid-induced activation of the Cdx2 promoter. When bile acids were measured in the oesophageal refluxate of the rats with experimental Barrett’s oesophagus, the concentration of CA was found to be consistent with the experimental dose that augmented Cdx2 expression in vitro. Further, transfection of the Cdx2 expression vector in cultured rat oesophageal keratinocytes induced production of intestinal type mucin, MUC2, in cells that expressed Cdx2.

Conclusions: We found that CA activates Cdx2 promoter via NF-kB and stimulates the production of Cdx2 protein in oesophageal keratinocytes with production of intestinal type mucin. This may be one of the mechanisms of metaplasia in Barrett’s oesophagus.

I-P-13-77
Prolonged Esophagitis in Rat: Stable Gastric Pentadecapeptide BPC 157 (PLD116, PL14736, Pliva) Relation to Critical Period for Threatening Outcome and Failure of Lower Esophageal and Pyloric Sphincter
I. Petrovic, I. Dobric, P. Diviv, D. Shejbal, L. Batelja, L. Bricic, A. Boban Blagac, N. Kokic, A. Tonkic, S. Mise, T. Badicic, M. Staresinic, T. Anic, S. Seiwert, P. Skiric School of Medicine, University of Zagreb

Aim: We focus whether the critical period for threatening outcome in prolonged esophagitis and relation with prolonged failure of lower esophageal sphincter (LES) and pyloric sphincter (PS) could be modified with a stable gastric pentadecapeptide BPC 157, promising in inflammatory bowel disease (PLD116, PL14736, Pliva). It attenuates esophago-jejunal anastomosis-esophagitis (J Gastroenterol 2005;40:781–790). Where the localization of tight-junctional protein is reported to be altered from the cell membrane to the cytoplasm (J Gastroenterol 2005;40:781–790).

Methods: The procedure induces prominent esophagitis, LES- and PS-malfunction (one tube inserted into the LES, and the other tube into PS, for 1 week, then both artlessly removed), gastroduodenal contents reflux. Now, considerably extending esophagitis period, BPC 157 (10 µg/kg) i.p. once 5 min before assessment.

Results: (i) Sphincter pressure. Esophagitis-rats present a marked LES- and PS-pressure (cm H2O) (median) 50 vs. control 55, 60 vs. control 65, 70 vs. control 75; (ii) Esophagitis-attenuation. BPC 157 0.9% NaCl 10 µg/kg i.p. once daily or in drinking water. Results: (i) Sphincter pressure. Esophagitis-rats present a marked LES- and PS-pressure (cm H2O) (median) 50 vs. control 55, 60 vs. control 65, 70 vs. control 75; (ii) Esophagitis-attenuation. BPC 157 0.9% NaCl 10 µg/kg i.p. once daily or in drinking water.

Conclusion: Application (1 ml) into stomach 5 min before assessment 0.9% NaCl 10 µg/kg NaCl 0.9% BPC 157 0.9% BPC 157 50 55 60 65 70 75 Application (1 ml) into stomach 5 min before assessment 0.9% NaCl 10 µg/kg NaCl 0.9% BPC 157 0.9% BPC 157 50 55 60 65 70 75

I-P-13-78
Bile Acids Directly Augment Caudal-Related Homeobox Gene Cdx2 Expression in Oesophageal Keratinocytes in Barrett’s Epithelium
H. Kazumori, S. Ishihara, M.A.K. Rumi, Y. Kadokawa, Y. Kinoshita
Department of Gastroenterology and Hepatology, Shimane University, School of Medicine, Izumo, Japan

Background and Aims: There is an increasing evidence that esophageal mucosal oxidative stress was involved in the pathogenesis of reflux esophagitis (Gut 2001;49:364–371). The present study was designed to investigate the role of oxidative stress in the rat model of chronic gastroesophageal acid reflux. Materials and Methods: Male Sprague-Dawley rats weighing 200–250 g were used for the study. Chronic acid reflux esophagitis was induced by ligating the tracheal region between the stomach and the tracheal region to enhance reflux of gastric acid into the esophagus, and wrapping the pylorus with a piece of 18 Fr Nelaton catheter (Scand J Gastroenterol 1999;34:948–953). Esophagus and stomach in rats were examined 1 and 2 weeks after the operation. The intraluminal pH level in esophagus and stomach was measured. Immunohistochimistry against 4-hydroxy-2-nonenal (HNE)-modified protein, which is a major lipid peroxidation product, was performed in esophageal tissues. Result: In rats with chronic reflux esophagitis, macroscopic thickening of the esophageal mucosa and microscopic hypertrophy of the esophageal epithelium and remarkable elongation of lamina propria papillae into the epithelium were observed. The esophageal luminal pH decreased significantly compared with that in the controls. Immunohistochemical staining of HNE-modified protein was shown in the cytoplasm in the spinous layers of esophageal stratified squamous epithelium and was significantly enhanced in rats with esophagitis compared with that in rats with sham operation. Conclusion: In rat model of chronic acid reflux esophagitis, cytoplasmic lipid peroxidation was induced in the spinous layers of esophageal stratified squamous epithelium, where the localization of tight-junctional protein is reported to be altered from the cell membrane to the cytoplasm (J Gastroenterol 2005;40:781–790).

I-P-13-79
Esophageal Mucosal Oxidative Stress in Rats with Chronic Acid Reflux
E. Wasaki1, H. Suzuki1, T. Masaoka2, Y. Minegishi3, Y. Nishizawa4, T. Hibi4
1Department of Internal Medicine, School of Medicine, 2Department of Emergency Medicine, School of Medicine, Keio University, Tokyo, Japan

The mechanism of transformation to intestinal metaplasia in Barrett’s oesophagus has not been clarified. We investigated the effects of various bile acids on the expression of the caudal-related homeobox gene Cdx2 in cultured oesophageal squamous epithelial cells. In addition, morphological and histochemical changes of squamous cells to intestinal epithelial cells were studied in response to bile acid-induced expression of Cdx2.

Methods: A rat model of Barrett’s oesophagus was created by anastomosing the oesophagus and jejunum, and Cdx2 expression was investigated by immunohistochemistry. Further, the response of various bile acids on Cdx2 gene expression was studied in human colon epithelial cell lines CaCo-2 and HT-29, as well as cultured rat oesophageal squamous epithelial cells using a Cdx2 promoter luciferase assay. In addition, primary cultured oesophageal squamous epithelial cells were transfected with Cdx2 expression vectors and their possible transformation to intestinal type epithelial cells was investigated.

Results: Esophago-jejunal anastomosis formed intestinal goblet cell metaplasia in rat oesophageal specimens and metaplastic epithelia strongly expressed Cdx2. When the effects of 11 kinds of bile acids on Cdx2 gene expression were examined, only cholic acid (CA) and dehydrocholic acid dose-dependently increased the Cdx2 promoter activity and Cdx2 protein production in CaCo-2 and HT-29, and cultured rat oesophageal keratinocytes. Results from the mutation analysis of Cdx2 promoter suggested that 2 NF-kB binding sites are responsible for the bile acid-induced activation of the Cdx2 promoter. When bile acids were measured in the oesophageal refluxate of the rats with experimental Barrett’s oesophagus, the concentration of CA was found to be consistent with the experimental dose that augmented Cdx2 expression in vitro. Further, transfection of the Cdx2 expression vector in cultured rat oesophageal keratinocytes induced production of intestinal type mucin, MUC2, in cells that expressed Cdx2.

Conclusions: We found that CA activates Cdx2 promoter via NF-kB and stimulates the production of Cdx2 protein in oesophageal keratinocytes with production of intestinal type mucin. This may be one of the mechanisms of metaplasia in Barrett’s oesophagus.

I-P-13-80
Role of Cyclooxygenase-2 and Microsomal Prostaglandin E Synthase-1 in Barrett’s Oesophagus
Department of Gastroenterology, Osaka City University, Graduate School of Medicine

Background: Although Prostaglandin E2 (PGE2), cyclooxygenase-2 (COX-2), and microsomal PGES synthesis-1 (mPGES-1) are known to play roles in various inflammatory

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events, their roles in the pathogenesis of gastroesophageal reflux disease are unknown. **Aims:** We examined the dynamics of COX and PGES, and PG2 synthesis in rat acid reflux esophagitis and the effects of COX-2 inhibitors on the severity of esophagitis. **Methods:** Acid reflux esophagitis was induced by ligating the transitional region between the forestomach and the glandular portion and wrapping the duodenum near the pylorus. Rats were killed on day 3 (acute phase) or day 21 (chronic phase) after induction of esophagitis. Esophageal proliferation was assessed by PCNA staining. Protein and mRNA expressions were determined by western blotting or immunohistochemistry and real-time RT-PCR. **Results:** Expression of COX-2 and mPGES-1 was markedly increased in esophagitis, while the modest changes in COX-1, iPGES, and mPGES-2 expression were observed. COX-2 and mPGES-1 were co-localized in epithelial cells of the basal layer, as well as inflammatory and mesenchymal cells in the lamina propria and submucosa. COX-2 inhibitors significantly reduced the severity of chronic esophagitis but did not affect acute esophageal lesions. COX-2 inhibitors significantly inhibited increase in PGE2 synthesis in esophageal lesions on both days 3 and 21. Epithelial proliferation was significantly increased in the basal layer on day 21. **Conclusion:** PGE2 derived from COX-2 and mPGES-1 plays a significant role in the pathogenesis of chronic acid reflux esophagitis, and possibly in basal hyperplasia and persistent inflammatory cell infiltration.

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**I-P-13-81**

**Effect of Ecabet Sodium on Esophageal Lesion Induced by the Reflux of Gastric Juice in Rats**

K. Okuyama, N. Saito, E. Kame, T. Noto, M. Nagasaki
Pharmacology Research Laboratories, Tarabe Seiyaku Co., Ltd., Toda, Japan

We investigated the effect of ecabet sodium (ecabet) on rat acute esophageal lesion induced by the reflux of gastric juice. The reflux of gastric juice was induced by the ligation of both pylorus and forestomach. One hour after the ligation, the amount of esophageal mucus markedly decreased prior to the formation of hemorrhagic lesion, which was observed from 3h after the ligation. Intragastric injection of ecabet (20mg/kg) prevented the decrease of mucus, and it (3, 10 and 30mg/kg) dose-dependently inhibited both formation of lesion and pepsin activity of gastric juice. Intraduodenal injection of omeprazole (10mg/kg) and ranitidine (100mg/kg) but neither rebamipide nor terbiphenol (both 100mg/kg) inhibited the lesion formation. Omeprazole and ranitidine also decreased acid concentration of gastric juice. The combination of ecabet with omeprazole or ranitidine showed additive effect on the lesion formation without affecting their acid concentration in the duodenal contents. The combination of ecabet with omeprazole or ranitidine decreased prior to the formation of hemorrhagic lesion, which was observed from 3h after the ligation.

**Conclusion:** Ecabet sodium (ecabet) could protect the esophageal mucosa against the gastric juice reflux in vivo.

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**I-P-14 Neoplasm-Basic 1**

**I-P-14-82**

**Estrogen Modulates the Expression of COX-2 in the Esophageal Cancer Cells**

Y. Goto, H. Fuji, Y. Hayashi, K. Asahi, H. Eguchi, S. Kawanoh
Department of Clinical Laboratory Science, Osaka University Graduate School of Medicine, Suita, Japan

**Background and Aims:** Cigarette smoking has been implicated as a major risk factor in gastrointestinal cancers. Furthermore, the incidence of GI cancer in women is less than those in men. On the other hand, the up-regulation of cyclooxygenase-2 (COX-2) is recognized to play an important role in the development/progression of cancer. Therefore, in this study, we investigated the effect of estrogen on COX-2 expression induced by the cigarette water extract in the human esophageal cancer cell line (SC450). **Materials and Methods:** Two cigarettes (Mild Seven) were vigorously mixed by Vortex in 30ml of petrol red free RPMI-1640 medium with 10% FBS treated with dextran/charcoal and incubated for 24h in the room temperature. SC450 cells derived from human esophageal squamous cell cancer (1 x 10^5 per well) were seeded using RPMI-1640 medium with 10% FBS. Thereafter, cells were cultured with 10% cigarette water extract and/or 17β-estradiol (10μM) in petrol red free RPMI-1640 medium with 10% FBS treated with dextran/charcoal. COX-2 expression was investigated by Western blotting analysis and PGE2 production was measured by ELISA kit. The expressions of estrogen α and β receptor (ER) in SC450 cells were analyzed by RT-PCR. **Result:** COX-2 expression was obviously up-regulated by 6 folds after the addition of 10% cigarette water extract. On the other hand, 17β-estradiol decreased COX-2 expression by 35% and PGE2 production by 65% when compared to those without 17β-estradiol. Furthermore, the increased COX-2 expression and PGE2 production by cigarette water extract was suppressed by 37% and 30% with 17β-estradiol co-treatment. SC450 cells expressed ER β but not ER α in RNA level. **Conclusion:** 17β-estradiol suppressed COX-2 expression and PGE2 production up-regulated by cigarette water extract. The results suggested that estrogen may affect the development and/or progression of GI cancer via COX-2 expression. This work was supported by the grant from the Smoking Research Foundation in Japan Tabacco Co.

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**I-P-14-83**

**Bile Salts Induce COX-2 Expression in Barrett Adenocarcinoma Cells via Stimulation of NF-κB**

P.C. Konturek, G. Burnat, E.G. Hahn
First Department of Medicine, University Erlangen-Nuremberg, Erlangen, Germany

**Background:** Bile salts play an important pathogenic role in the development of Barrett adenocarcinoma (BA). However, the precise role of different bile salts in this process is still unknown. The aim of the present study was to compare the effects of two different bile salts, deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) on the expression of COX-2, CDX-2 and DNA repair enzymes (MUTYH, OGG1) in the Barrett epithelial cancer cells (OE-19). **Methods:** OE-19 cells were incubated with DCA or UDCA (100μM or 300μM at pH = 7.0) over 12h. To investigate the involvement of NFκB, in separate experiments the cells were incubated with DCA in the presence of proteasome inhibitor (MG-132). Cell survival was analyzed by MTT assay and apoptosis rate by FACS analysis. After incubation of OE-19 cells with bile salts, the expression of mRNA of COX-2, DNA repair enzymes (MUTYH, OGG1) and c-Rel like homedox transcription factor CDX2 was measured by quantitative RT-PCR. **Results:** DCA caused a stronger reduction in cell survival of OE-19 cells than UDCA due to increased apoptosis rate. In addition, DCA stimulated directly the translocation of NFκB p65 (active form) in the nuclei of OE-19 cells. DCA caused stronger than UDCA stimulation of the COX-2 mRNA expression in these cells and this effect was significantly attenuated by the addition of inhibitor of NFκB activity (proteasome inhibitor MG-132). DCA caused stronger downregulation of mRNA for DNA repair enzymes MUTYH and OGG1 than UDCA. In contrast, UDCA induced stronger CDX-2 mRNA expression than DCA in OE-19 cells. **Conclusion:** Bile salts are involved in the carcinogenesis of Barrett adenocarcinoma via inhibition of DNA repair enzymes and induction of COX-2 and this last effect is at least partly mediated by NFκB. UDCA promotes upregulation of CDX-2 modulating development of Barrett carcinoma.

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**I-P-14-84**

**Role of ERαs in Cell Growth and Chemoresistance of Gastric Cancer Cells**

E. Kubota1, H. Kataoka1, M. Sasaki1, S. Tanida1, T. Oshima1, N. Ogasaوار1, T. Wada1, T. Yamada1, Y. Mon1, F. Fujita1, T. Shimura1, K. Asai1, T. Joh1
1Department of Internal Medicine and Bioregulation, 2Department of Molecular Neurobiology, Nagoya City University Graduate School of Medical Sciences

**Background and Aims:** ERαs are well known to be expressed in ES cells. ERαs is constitutively active without any mutations and plays a crucial role in the tumor-like growth properties of ES cells. The aim of the current study is to investigate the expression and the roles of ERαs in human gastric cancer cells. **Materials and Methods:** We examined the expression of ERαs protein in human gastric cancer tissue and nine gastric cancer cell lines using mouse cDNA microarrays. **Results:** We further investigated the possible target genes of ERαs using mouse cDNA microarrays. **Results:** ERαs was expressed in human gastric cancer tissue and all gastric cancer cell lines. Overexpression of ERαs did not appear to increase cell proliferation but was found to induce phosphorylation of Akt and ERαs transfectants were significantly more resistant to CPT-11 than untransfected parent lines (survival rate: control 23.0% vs. ERαs transfectants 67.4%, p < 0.001), but not to 5-Fluorouracil, Cisplatin, or Paclitaxel. The ERαs target genes were identified using microarray analysis. **Conclusion:** The ERαstargets were identified in gastric cancer cells and this effect was significantly attenuated by the addition of inhibitor of NFκB activity (proteasome inhibitor MG-132). DCA caused stronger downregulation of mRNA for DNA repair enzymes MUTYH and OGG1 than UDCA. In contrast, UDCA induced stronger CDX-2 mRNA expression than DCA in OE-19 cells. **Conclusion:** Bile salts are involved in the carcinogenesis of Barrett adenocarcinoma via inhibition of DNA repair enzymes and induction of COX-2 and this last effect is at least partly mediated by NFκB. UDCA promotes upregulation of CDX-2 modulating development of Barrett carcinoma.
Aquired Tumor-Genesis of an N-Methyl-N'-Nitro-N-Nitosoguanidine (MNNG)-Induced Transformed Gastric Mucosal Epithelial Cell-Line
O. Shimokawa, H. Matsui, Y. Nagano, T. Manjima, Y. Matsuizaki, A. Nakahara, I. Hyodo, H. Majima
Graduate School for Comprehensive Human Sciences, Department of Gastroenterology, University of Tsukuba, 1Kagoshima University, Graduate School of Medical and Dental Sciences

Background and Aim: N-methyl-N'-nitro-N-nitosoguanidine (MNNG) treatment induces gastric cancer in animal model. DNA methylation and oxidative stresses derived from MNNG are reported to involve in carcinogenesis. We aimed to establish in an in vitro model of MNNG-induced cancer. Methods: RGM-1, a rat gastric mucosa-derived cell line, was incubated in a medium containing MNNG for 28 days. Cells were first examined morphologically. In order to examine the tumor genesis, cells were incubated within soft agar, and were injected subcutaneously into the thighs of nude mice. In order to investigate cellular migration ability, RKG-1 cells were incubated in a 12 well plate, above which mounted 44 μm pore filters. The number of cells migrated above each filter were counted. DNA mutation in p53 and ras were examined by DNA sequence, and RNA extract of the cells were examined by RNA micro-array.

Results: MNNG-treated RGM-1 cells showed morphological changes with reproducibility. We named this transformed cells as RKG-1. RKG-1 proliferated in soft agar, and subcutaneously injected RKG-1 formed tumors in mice thigh. RKG-1 cells migrated above the filters. p53 and ras point mutations were confirmed. RNA micro-array analysis demonstrated mRNA which involved anti-oxidant response enzymes showed increased expression. We propose that RGM-1/RKG-RGM system is a desired in vitro model of MNNG-induced gastric cancer.

Conclusions: We established a MNNG-induced transformed cell line, RKG-1, from a gastric mucosa-derived cell line in vitro. RKG-1 demonstrated tumor genesis, migration ability and p53/ras mutations. We propose that RGM-1/RKG-1 system is a desired in vitro model of gastric cancer. The expression of MDM2 on mucosa can be a mediator for gastric cancer.

The Expression of MDM2 on Helicobacter pylori Infected Intestinal Metaplasia and Gastric Cancer
Department of Gastroenterology and Hepatology, 1Department of Pathology, Nihon University School of Medicine, 2Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, USA

Gastric cancers arise through a multistep process from chronic gastritis and intestinal metaplasia and finally invasive carcinoma. Long-term infection with Helicobacter pylori (H. pylori) is one of the causatives for gastric cancer. MDM2, an oncoprotein, is overexpressed in several human tumors. Increased expression levels of MDM2 inactivate the apoptotic and cell cycle arrest function of p53. Interleukin-16 (IL-16) is a pleiotropic cytokine secreted mainly by CD8 T cells. The properties of IL-16 suggest that it may be involved in pathophysiological processes of chronic inflammatory diseases. Aim: We investigated the expression of MDM2 in intestinal metaplasia and gastric cancer as well as the effect of H. pylori infection and IL-16 on epithelial cell proliferation and MDM2 expression in gastric cells in vitro. Methods: Gastric biopsies were classified by histological findings as chronic gastritis, intestinal metaplasia, gastric cancer with H. pylori infection and uninfected chronic gastritis. AGS cells were incubated with a combination of IL-16 and H. pylori. Gastric epithelial cell proliferation was studied by BrdU uptake. The expressions of MDM2 were studied by ABC, ELISA and RT-PCR. Results: IL-16 expression was detected in H. pylori infected gastric mucosa. There was no significant difference on the expression of MDM2 between the chronic gastritis with and without H. pylori infection. The MDM2 expression was higher in intestinal metaplasia and gastric cancer than in chronic gastritis with H. pylori infection. IL-16 administration increased the BrdU uptake in H. pylori infected cells. MDM2 protein levels were increased by IL-16 administration more than in H. pylori infected AGS cells. MDM2 mRNA expression was increased by H. pylori infection, but decreased by IL-16 subsequently. Conclusions: The expression of MDM2 in long-term H. pylori infected gastric mucosa may indicate a risk for carcinogenesis. IL-16 secretion in H. pylori infected mucosa is one of the factors for gastric cancer. The expression of MDM2 on mucosa can be a mediator for gastric cancer.

I-P-15 Helicobacter pylori- Basic 1
Chairpersons: A. Shiotani, I. Imoto

I-P-15-88
H. pylori Infection Up-Regulated TLR4 and MD-2, and IL-8 Production in the Human Gastric Mucosa
K. Iwata, H.Y. Fu, Y. Hayashi, H. Umemura, H. Eguchi, S. Kawano
1Department of Clinical Laboratory Science, School of Allied Health Sciences, Faculty of Medicine, Osaka University, 2Department of Gastroenterology, Osaka Kaisei Hospital

Background and Aim: Toll-like receptor 4 (TLR4) is a main receptor for lipopolysaccharide (LPS) of Gram-negative bacteria. However, it is still unclear about the effect of LPS and H. pylori infection on Toll-like receptors. We aimed to evaluate the expression levels of TLR4 and its downstream molecule in gastric mucosa. Materials and Methods: Biopsy samples from antrum and corpus mucosa were obtained from 45 patients with chronic gastritis infected with H. pylori. The mucosa was classified into 3 groups: patients with H. pylori infection (H. pylori Group 1), patients with H. pylori infection but negative for H. pylori IgG (IgG Ab, RUT and/or histology) (H. pylori Group 2) and patients without H. pylori infection (H. pylori Group 3). TLR4, MD-2 and IL-8 mRNA expression were analyzed by RT-PCR. Results: TLR4, MD-2 and IL-8 mRNA expression were lower in patients with H. pylori infection than that without H. pylori infection. In H. pylori infected patients, TLR4, MD-2 and IL-8 mRNA expressions in the antral mucosa were significantly less than those of the corpus mucosa. The immunohistochemical study revealed that TLR4 was expressed in the gastric epithelial cells of the antral and corpus mucosa in patients with and without H. pylori infection. In H. pylori infected patients, TLR4, MD-2 and IL-8 mRNA expressions in the antral mucosa were significantly less than those of the corpus mucosa. The other hand, in H. pylori (-) patients, TLR4, MD-2 and IL-8 mRNA expressions were not significantly different between the antral and corpus mucosa in patients with and without H. pylori infection.

Background and Aim: Endoscopic treatment is established as a treatment for patients with early gastric cancer. It is very important to find predictive factors of multiple gastric cancers after endoscopic resection. To find out genetic markers to identify high risk patients for multiple early gastric cancers after treatment with endoscopic mucosal resection, we analyzed the MSI status. Materials and Methods: Biopsy samples from antrum and corpus mucosa were obtained from 45 patients with H. pylori infection. TLR4, MD-2 and IL-8 mRNA expression were lower in patients with H. pylori infection than that without H. pylori infection. Increased expression levels of TLR4 in patients with H. pylori infection and uninfected chronic gastritis. AGS cells were incubated with a combination of IL-16 and H. pylori. Gastric epithelial cell proliferation was studied by BrdU uptake. The expressions of MDM2 were studied by ABC, ELISA and RT-PCR. Results: IL-16 expression was detected in H. pylori infected gastric mucosa. There was no significant difference on the expression of MDM2 between the chronic gastritis with and without H. pylori infection. The MDM2 expression was higher in intestinal metaplasia and gastric cancer than in chronic gastritis with H. pylori infection. IL-16 administration increased the BrdU uptake in H. pylori infected cells. MDM2 protein levels were increased by IL-16 administration more than in H. pylori infected AGS cells. MDM2 mRNA expression was increased by H. pylori infection, but decreased by IL-16 subsequently. Conclusions: The expression of MDM2 in long-term H. pylori infected gastric mucosa may indicate a risk for carcinogenesis. IL-16 secretion in H. pylori infected mucosa is one of the factors for gastric cancer. The expression of MDM2 on mucosa can be a mediator for gastric cancer.

I-P-14-85
Genetic Markers to Identify High Risk Patients for Multiple Early Gastric Cancers After Treatment with Endoscopic Mucosal Resection
M. Fukuda, K. Hijuchi, H. Yokozaki, M. Shiibue, Y. Watanabe, T. Tominaga, Y. Fujivara, N. Oshitani, T. Arakawa
Department of Gastroenterology, Graduate School of Medicine, Osaka City University

Background: Endoscopic treatment is established as a treatment for patients with early gastric cancer. It is very important to find predictive factors of multiple gastric cancers after endoscopic resection. To find out genetic markers to identify high risk patients for multiple early gastric cancers after treatment with endoscopic mucosal resection, we analyzed the MSI status.
The Effects of PGE2 Receptor Antagonist and COX-2 Inhibitor on Helicobacter pylori - Induced Urokinase-Type Plasminogen Activator Expression in the Gastric Cancer Cells

J. Jeon
Fifth Department of Internal Medicine, Tokyo Medical University

**Aim:** It has been reported that a relationship exists between high expression of urokinase-type plasminogen activator (uPA) system and poor prognosis in the patients with cancers such as gastric cancer, colon cancer, and breast cancer. We have demonstrated that Helicobacter pylori (Hp) induces the expressions of uPA and its receptor (uPAR) in vitro. It has been already demonstrated that Hp induces the expression of cyclo-oxygenase (COX)-2 and the production of prostaglandin (PG)E2 in the gastric cancer cells. Furthermore, the involvement of COX-PG pathway in the uPA system has been suggested. We investigated the effects of PGE2 receptor antagonist and COX-2 inhibitor on Hp-induced urokinase-type plasminogen activator expression in the gastric cancer cells to clarify the mechanism of expression of the uPA system.

**Method:** Gastric cancer cells (cell line: MKN45) were co-cultured with Hp standard strain (NCTC11637). Specific induction of uPA and uPAR mRNA was examined by reverse transcription and polymerase chain reaction (RT-PCR) amplification. To evaluate the involvement of COX-PG pathway in the Hp-induced uPA, uPAR expression, we examined the effects of NS398 and AH6809, PGE2 receptor antagonist, on the induction of uPA, uPAR. Results: The gastric cancer cells expressed little uPA and uPAR mRNA under the un-stimulated condition and the expression level increased (uPA mRNA; 12-fold: MKN45) (uPAR mRNA; 3-fold: MKN45) significantly with Hp NCTC11637 strain stimulation. The gastric cancer cells secreted uPA antigen into the culture medium, and the amount of uPA antigen increased dramatically with Hp NCTC11637. The Hp-induced uPA mRNA expression was strongly down-regulated by pretreatment of the cancer cells with NS398 and AH6809. Conclusion: Our results indicated the possibility that COX-PG pathway is involved in Hp-associated uPA induction. Furthermore, these results indicated that COX-2 inhibitor or PGE2 receptor antagonist may inhibit angiogenesis and tumor invasion via suppression of the uPA system.

**I-P-15-90**

Analysis of the SHP-2 Binding Site of Helicobacter pylori CagA Protein in Korean

Division of Gastroenterology, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

**Background and Aims:** There are two major CagA; i) the East Asian type (A-B-D of EPIYA motifs) and ii) the Western type (A-B-C of EPIYA motifs). Here we analyzed the amino acid sequences of the SHP-2 binding site of CagA gene, and investigated whether there is any relationship between the diversities of CagA and the disease outcome in Korea. We also examined the relationship between cagA and vacA alleles. Methods: The 62 clinical isolates of H. pylori from 14 patients with gastric cancer and 48 patients with chronic gastritis were studied. DNA sequencing of CagA SHP-2 binding site was performed. Parts of the vacA s- and m-regions were amplified using appropriate primers. Results: Most of Korean H. pylori strains showed A-B-D motifs, and only one strain showed A-B-B-D motifs, which are the East Asian type. All strains contained s1 in the signal sequence region and m1 in the middle region of vacA. The predominant vacA genotype in Korea was s1c/m1 (43/62, 69%). We also demonstrated that the predominant East Asian type CagA-positive strains had vacA s1c/m1 genotype in Korea. Conclusions: The high frequency of the East Asian type CagA among Korean H. pylori strains would be involved in increasing the risk of gastric cancer in Korean populations.

**I-P-15-91**

Emergence of Ciprofloxacin Resistance in Helicobacter pylori Isolated from Korea: Mutational Changes in gyrA and other Genetic Loci

Division of Gastroenterology, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

**Background and Aims:** Failures of proton pump inhibitor-based triple therapies for eradication of H. pylori are frequently due to resistance to either clarithromycin or metronidazole. Thus, other antibacterial drugs, such as quinolones, are required for effective H. pylori therapy. In this study, we analyzed the rate of primary resistance of ciprofloxacin-resistant H. pylori and studied the mechanisms of ciprofloxacin resistance. Methods: H. pylori strains were obtained from gastric biopsy specimens of 64 consecutive H. pylori-positive patients at Asan Medical Center (AMC) in Seoul. Genomic DNA of ciprofloxacin-resistant strains was extracted and used for PCR and DNA sequencing to determine the mutations in gyrA. To establish that the mutations in gyrA and gyrB caused ciprofloxacin resistance, the PCR products were used to transform a ciprofloxacin-resistant strain of H. pylori to a resistant phenotype. Results: In one Korean Hospital (AMC) the rate of resistance of H. pylori to ciprofloxacin was 10.9% (7 of 64 isolates) in 2004. High-level resistance was mainly observed in mutant of gyrA, but not gyrB. Isolates showing low-level resistance displayed no mutations in gyrA or gyrB. Ciprofloxacin-resistant strains of H. pylori could be transformed to ciprofloxacin resistance by using the amplified fragment of gyrA and gyrB from resistant strains as donor DNA. Transformation by the amplified fragment of gyrB with double mutations in Asp484Glu and Arg484Lys from a ciprofloxacin-resistant strain showed that the gyrB mutations are not associated with ciprofloxacin resistance in H. pylori. Conclusions: The rate of ciprofloxacin-resistant H. pylori was 10.9% at AMC, Seoul, Korea. The mechanisms of ciprofloxacin resistance in H. pylori are not only mutational changes in gyrA but also in other, as yet unidentified, genetic loci.
II-P-1 Endoscopic Submucosal Dissection 2

Chairpersons: M.Y. Su, T. Michida

II-P-1-95

How Can We Overcome the Difficult Cases in Early Gastric Cancer Treatment with ESD Method?: Challenging New Technique with Double Scope ESD

M. Toyoda¹, Y. Morita², I. Miki, T. Mitani, T. Tanaka, N. Torio, D. Shirasaka, T. Tamura¹, M. Yoshida, H. Kutsurni, N. Aoyama¹, T. Azumaa³

¹Department of Digestive, Digestive and Kidney, ²Department of Frontier Medical Science in Gastroenterology, ³International Center for Medical Research and Treatment, ⁴Department of Endoscopy, Kobe University Graduate School of Medicine

Background: Endoscopic submucosal dissection (ESD) method has enabled en bloc resection of early gastric cancer (EGC) regardless of tumor size. However, ESD requires a long procedure time and a high technical skill in holding endoscopy at appropriate location and handling fluently with a precise motion. Conventional ESD method with single scope (S-ESD) is often difficult to keep a clear view for which to dissect submucosal layer easily and detect vessels before bleeding; however, S-ESD sometimes brings about severe complications such as bleeding or perforation, especially in the case of quite a large lesion, difficult location, and severe fibrosis.

Aim: In order to achieve a safer ESD treatment, we developed double scope ESD (D-ESD).

Method: Three consecutive patients with EGC were enrolled, whose lesions have some difficulties due to the following reasons: 1) Large tumor. 2) Tumor has ulcerative change. 3) Difficult visualization of the submucosal layer makes submucosal incision easily and safely, even though submucosal fibrosis exists. We report a case of early gastric carcinoma resected en bloc using IT knife, although complete circumferential mucosal incision all around the lesion cannot be performed because of submucosal fibrosis. A 78-year-old man with a history of peptic ulcer disease came to our hospital because of early gastric adenocarcinoma located in the lesser curvature of the gastric antrum. After possible treatments including surgery are discussed and informed consent was obtained, ESD using IT knife was performed. After marking around the lesion and lifting the mucosa by submucosal injection, circumferential mucosal incision around the lesion using IT knife was tried to perform. The distal mucosa incision (oral side) was first performed, followed by the proximal mucosal incision (anal side) under retroflexed endoscopic position. Complete mucosal incision around the lesion was performed because of submucosal fibrosis; however, complete mucosal incision around the lesion was difficult because of the strict submucosal fibrosis due to the previous ulcer scar. Therefore, before completing circumferential mucosal incision all around the lesion, I started submucosal incision from the distal area. The submucosal incision was continued until the lesion was almost excised except only the proximal area where mucosal incision was not performed because of the submucosal fibrosis. Finally we performed the remaining mucosal incision by moving the IT knife from the submucosal layer side to the mucosal side and completed en-bloc resection (43 mm × 45 mm). Histopathological analysis of the resected lesion showed this treatment curative. In summary, this case shows that ESD using IT knife is an attractive approach for en-bloc resection of mucosally-limited differentiated adenocarcinoma with submucosal fibrosis.

II-P-1-98

Novel Endoscopic Hemostasis Technique by Using Hemostatic Forceps and an Endoscope Equipped with a Water-Jet System

Y. Morik1, M. Fujishiro1, N. Kukushima1, S. Kodashima1, A. Tateshi2, M. Okamoto3, Y. Yama4, K. Ogura1, H. Watabe1, T. Matsui1, M. Ichino2, M. Omata1

1Department of Gastroenterology, The University of Tokyo, Tokyo, 2Second Department of Internal Medicine, Kagawa Medical University, Kagawa, Japan

Bleeding gastroduodenal ulcer is one of life-threatening emergency diseases and achievement of endoscopic hemostasis is a major clue to result in successful outcomes. In addition to available hemostatic methods, we developed a novel endoscopic hemostatic technique by using hemostatic forceps (HD2423W, Pentax) that effectively coagulate only a targeted vessel and an endoscope equipped with a water-jet system (EG-2931-SA-P2, Pentax) which is very useful to identify the bleeding vessel in a pool of blood.

Methods: Twenty-six consecutive patients with a bleeding gastroduodenal ulcer between January 2005 and December 2005 were enrolled in this study. Above endoscopic technique was inserted in these patients and bleeding vessels or visible non-bleeding vessels on the ulcer base were first treated by hemostatic forceps (soft coagulation: 50–80 W, ICC200, ERBE). Before applying hemostatic forceps, HSE was injected in case of...
severe bleeding or a large vessel. If bleeding could not be controlled with hemostatic forceps after several attempts, endoclips were additionally used. Achievement of temporary hemostasis and rebleeding were investigated. Results: Among 26 bleeding ulcers, 15 (58%) were stopped with hemostatic forceps alone; 3 (12%) were with HSE plus hemostatic forceps, 8 (31%) were with rescues of endoclips. Rebleeding was occurred in 1 (3.8%) treated with hemostatic forceps alone at 4 days after hemostasis. Conclusions: Combination of hemostatic forceps and an endoscope equipped with a water-jet system is a promising hemostatic technique for bleeding gastrointestinal ulcers. However, further improvements of technical skills or methodologies are necessary because additional rescues of endoclips were needed in a third of the enrolled patients.

II-P-1-99 Novel Biodegradable Stents for Benign Esophageal Strictures Following Endoscopic Submucosal Dissection
Department of Gastroenterology, Shiga University of Medical Science

The application of metallic stents for benign stenosis is limited due to long-term complications. Here, we report the results of the implantation of a novel biodegradable poly-L-lactic acid (PLLA) esophageal stent in 2 patients with benign esophageal stenosis after endoscopic submucosal dissection (ESD). Case 1: a 64-year-old man received ESD for an early squamous esophageal cancer in the middle esophagus. The mucosal defect was seven eighths of the circumference, and the distal margin of the resection scar formed the stenosis. After balloon dilatation, the PLLA esophageal stent was endoscopically placed. For 6 months, he did not experience any symptoms of re-stenosis. Case 2: a 62-year-old man developed an early squamous esophageal cancer in the middle esophagus. The lesion was resected by ESD, and the mucosal defect was 78% of the circumference. The resection scar formed the stenosis, and the PLLA esophageal stent was endoscopically placed. He did not experience any symptoms of re-stenosis for 6 months. In conclusion, the PLLA esophageal stent provides a new possibility for the management of benign esophageal strictures after ESD. Due to the biodegradable features of this stent, longer-term studies are necessary to investigate the relationship between the expected disappearance of the stent and the patency of the stenosis.

II-P-1-100 Differentiation Between Depressed-Type Early Gastric Cancer from Benign Depressed Lesion with Magnifying Narrow Band Imaging
Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Disease

Backgrounds: Differentiation of small depressed-type early gastric cancer (SegC) from benign small depressed lesion (ulcer or erosion: BDL) by conventional endoscopy is often difficult and biopsies are required. The narrow band imaging system in combination with magnifying endoscopy (NBI-ME) yields contrasted images of capillaries or microvessels and surface architecture of the mucosal lesions. Therefore, NBI-ME images could make a histological diagnosis without biopsy.

Aim and Methods: An aim of this study is to examine whether NBI-ME images can differentiate SegCs from BDLs. A total of 29 SegCs in 27 patients and 22 BDLs in 19 patients were collected and their NBI-ME images were analyzed. According to our early studies, difference indexes were determined as followed; difference in gland pattern (A), difference in gland size (B), disappearance of surface architecture (C), presence of demarcation line (D). All examinations were digitally recorded or videotaped, and prevalence of each finding was compared between SegCs and BDLs.

Results: The NBI-ME indexes B, C and D were significantly frequently found in SegCs for 6 months. In conclusion, the PLLA esophageal stent provides a new possibility for the management of benign esophageal strictures after ESD. Due to the biodegradable features of this stent, longer-term studies are necessary to investigate the relationship between the expected disappearance of the stent and the patency of the stenosis.

II-P-2 New Trend in GI Endoscopy
Chairpersons: K.N. Shim, E. Urnegaki

II-P-2-101 Study on Japanese Patients with Obstructive Gastrointestinal Bleeding Based on the Combined Use of Capsule Endoscopy and Double-Balloon Endoscopy
The Third Department of Internal Medicine, Kyorin University School of Medicine

Background: Recently, the development of capsule endoscopy (CE) and double-balloon endoscopy (DBE) has made diagnosis and treatment on the disease of small-intestinal disorders more effective. Aim: The aim of this study was to evaluate the utility of CE and DBE for obscure gastrointestinal bleeding (OGB). Patients and Methods: The subjects were 46 patients for obscure bleeding (mean age: 54 ± 17 years, 22 males and 24 females) who underwent treatment in our hospital between March 2004 and October 2005. In principle, we performed CE at first and then DBE on the basis of the diagnosis of CE. CE was performed in all patients. DBE examinations were performed in 15 patients. As the approach method of DBE, we choosed ‘oral approach’ or ‘anal approach’, which depend on the presumable source of the abnormal small bowel located by CE. Results: The rate of concomitant disease of the case of OGB at our hospital was 52%. The details of concomitant disease are as follows: Liver cirrhosis (6 cases), Chronic renal failure (3 cases), no concomitant disease (24 cases). Median hemoglobin was 9.9 ± 2.0 g/dL, 41% of patients had required transfusion. CE detected significant diagnosis in the OGB to 26 patients (60%) out of 46 patients: 5 cases of Angiodysplasia, 5 cases of Ulcer, 10 cases of erosion, 6 cases of active bleeding, one case of esophageal varix. The rate of significant diagnosis by DBE was 62%. The significant diagnosis were Angiodysplasia (5 cases), Ulcer (3 cases). With regard to treatment, endoscopic treatment was performed in 5 patients (argon plasma coagulation and clipping). 1 case, argon plasma coagulation: 1 case, clipping: 3 cases) for Angiodysplasia.

Conclusion: Performing CE before DBE was useful not only for getting the information of small bowel disease, but also for choosing approach method of DBE as well as for diagnosis and treatment of OGB.

II-P-2-102 Double-Balloon Enteroscopy in the Diagnosis and Management of the Patients with Obstructive Gastrointestinal Bleeding
C.M. Hsu, M.-Y. Su, C.-T. Chiu, P.-C. Chen
Department of Gastroenterology and Hepatology, Chang-Gung Memorial Hospital, Linkou Medical Center and Chang-Gung University, Taipei, Taiwan

Background: Investigating and treating in patients with obscure gastrointestinal bleeding is a clinical challenge. Most of the lesions responsible for the origin of the obscure gastrointestinal bleeding are located in the small bowel. Double-balloon endoscopy (DBE) is a novel method in the exploration of the small intestine with therapeutic potential. The study evaluated DBE in patients with obscure gastrointestinal bleeding. Patients and Methods: From October 2003 to January 2006, a total of 20 patients (6 men, 14 women; mean age 55.2 years) with obscure gastrointestinal bleeding were enrolled. CE detected significant diagnosis in the OGIB to 26 patients (60%) out of 46 patients: 5 cases of Angiodysplasia, 5 cases of Ulcer, 10 cases of erosion, 6 cases of active bleeding, one case of esophageal varix. The rate of significant diagnosis by DBE is 62%. The significant diagnosis were Angiodysplasia (5 cases), Ulcer (3 cases). With regard to treatment, endoscopic treatment was performed in 5 patients (argon plasma coagulation and clipping). 1 case, argon plasma coagulation: 1 case, clipping: 3 cases) for Angiodysplasia.

Conclusion: Performing CE before DBE was useful not only for getting the information of small bowel disease, but also for choosing approach method of DBE as well as for diagnosis and treatment of OGB.

II-P-2-103 Double-Balloon Endoscopy for the Treatment of Small Intestinal Diseases
Department of Medicine, Division of Gastroenterology, Nippon Medical School

Endoscopic observation and treatment of the small bowel has been limited by its inaccessibility. Double-balloon endoscopy (DBE) enables endoscopic scrutiny and treatment of the entire small bowel and is now used for the diagnosis and treatment of obscure gastrointestinal bleeding.
small bowel: with intervention capabilities that allow for targeted biopsies, electrocoagulation, clip placement, endoscopic polypectomy, balloon dilation, stent placement, and the retrieval of foreign bodies. This method allows for timely endoscopic treatment of disorders heretofore requiring surgical intervention. In this study we evaluated the efficacy of the DBE system for the endoscopic retrieval of diverse diseases of the small bowel.

**Results:** A total of 144 patients underwent 221 DBE examinations between June 2003 and January 2006 at Nippon Medical School Hospital, Tokyo, Japan. In all, 19 patients were successfully treated in the process of endoscopic examination. Electrocoagulation or clipping for hemostasis at the bleeding source in the small bowel was conducted in 11 patients. Regarding gastrointestinal strictures, endoscopic balloon dilation was performed in 3 patients and in 2 patients, a stent was safely placed across the stricture. The DBE could then easily pass through the lesion after dilation, and clinical symptoms of ileus improved in all patients. A foreign body (a video capsule endoscope) trapped by ileal stenosis was successfully retrieved with net forceps in 2 patients. Endoscopic polypectomy was performed in one patient. All these treatments were beyond the capability of the conventional scope, but were made possible by this innovative system, which greatly improved access to previously inaccessible areas of the small intestine. We encountered no complications during and after any of the procedures.

**Conclusions:** DBE is being proven to be a safe and valuable method, with high diagnostic yield offering a wide range of therapeutic capabilities. Our results suggest that DBE can dramatically increase the range of endoscopic treatment and may in certain cases preclude unnecessary surgical intervention.

**Background:**

In Japan, percutaneous endoscopic gastrostomy (PEG) is an essential procedure for aged patients in general physicians due to its usefulness and its overall safety. To date, however, a number of complications and medical accidents have been reported.

**Patients and Methods:**

PEG was performed in a total of 58 patients between October 1997 and September 2005. The subjects were aged 8 to 89 years. Diseases of the subjects included cerebral degenerative disease (n = 11), malignant tumor (n = 8), including encephalopathy, poor post-operative general condition (n = 8), pediatric diseases (n = 6), cerebrovascular diseases (n = 4) and others (n = 17). A PEG kit purchased from Nippon Sherwood Medical Industries Ltd. was used. In all the patients, the ‘pull technique’ was performed for the placement of PEG.

**Results:** The most common post-operative complication consisted of local infections in 11 patients (22%) (stoma infection, subcutaneous abscess). Buried bumper occurred in three patients (6%), and cutaneous ulcer in one patient (2%). One patient required surgical reconstruction of a PEG stoma. Culture of exudate from the wound in the 11 patients with local infections revealed methicillin-resistant Staphylococcus aureus in five patients and Pseudomonas aeruginosa in 4 patients. Since a switch to an infection-preventive kit in May 2003, local infections markedly decreased. Complications after PEG placement were more commonly observed in elderly patients, and no complications were observed in pediatric patients.

**Conclusions:** The most common complication with PEG placement is local infection. While PEG devices do require further improvement, if we are to minimize complications in the short term, it is critical that we work to establish a safer and simpler universally applicable procedure using devices commercially available at present.

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**II-P-2-104**

**Percutaneous Endoscopic Gastrostomy at the University Hospital in Japan**


Department of Internal Medicine and Gastrointestinal Endoscopy, Saga Medical School, Saga, Japan

In Japan, percutaneous endoscopic gastrostomy (PEG) is an essential procedure for aged patients in general physicians due to its usefulness and its overall safety. To date, however, a number of complications and medical accidents have been reported.

**Patients and Methods:**

PEG was performed in a total of 58 patients between October 1997 and September 2005. The subjects were aged 8 to 89 years. Diseases of the subjects included cerebral degenerative disease (n = 11), malignant tumor (n = 8), including encephalopathy, poor post-operative general condition (n = 8), pediatric diseases (n = 6), cerebrovascular diseases (n = 4) and others (n = 17). A PEG kit purchased from Nippon Sherwood Medical Industries Ltd. was used. In all the patients, the ‘pull technique’ was performed for the placement of PEG.

**Results:** The most common post-operative complication consisted of local infections in 11 patients (22%) (stoma infection, subcutaneous abscess). Buried bumper occurred in three patients (6%), and cutaneous ulcer in one patient (2%). One patient required surgical reconstruction of a PEG stoma. Culture of exudate from the wound in the 11 patients with local infections revealed methicillin-resistant Staphylococcus aureus in five patients and Pseudomonas aeruginosa in 4 patients. Since a switch to an infection-preventive kit in May 2003, local infections markedly decreased. Complications after PEG placement were more commonly observed in elderly patients, and no complications were observed in pediatric patients.

**Conclusions:** The most common complication with PEG placement is local infection. While PEG devices do require further improvement, if we are to minimize complications in the short term, it is critical that we work to establish a safer and simpler universally applicable procedure using devices commercially available at present.
II-P-3-108
Prevalence of Extraesophageal Manifestations in Japanese Patients with GERD
T. Sugawa, Y. Fujisawa, K. Higuchi, T. Arakawa
Department of Gastroenterology, Osaka City University Graduate School of Medicine

Background: Gastroesophageal reflux disease (GERD) is associated with a variety of extraesophageal manifestations including pulmonary and ear, nose and throat (ENT) symptoms. Although many studies are performed in Western countries, there are few studies in Japan. Aims: We examined prevalence of chronic cough, and ENT symptoms such as sore throat and throat discomfort, BMI, a drinking habit, a smoking habit, endoscopic severity, the presence of hiatus hernia, gastric mucosal atrophy, and typical GERD symptoms such as heartburn and acid regurgitation. Results: Throat symptoms were found in 68 cases (49%) and chronic cough in 46 cases (33%). Thirty cases (22%) had both symptoms. Presence of throat symptoms in NERD group (54%) was significantly higher than in EE group (45%). No difference was no difference in the prevalence of chronic cough between the two groups. Prevalence of chronic cough and throat symptoms was low (19, 33%) in patients with severe EE such as LA grade C and D. Acid suppressive therapy was effective in about 50% of GERD patients for chronic cough and throat symptoms. Conclusion: Prevalence of chronic cough and throat symptoms in Japanese GERD patients was 33 and 49% respectively, and about half of these cases were healed by acid suppressive therapy.

II-P-3-109
Effect of High Dose PPI (Rabeprazole) on Laryngopharyngeal Reflux Disease: A Prospective, Double Blind, Randomized Controlled Study
S. Gonishchandh1, A. Suramethakul1, P. Isirikul2, S. Saengpanich2
1Department of Internal Medicine, 2Department of Otolaryngology, Chulalongkorn University, Bangkok, Thailand

The role of high dose PPI in laryngopharyngeal reflux disease has not been well established. We performed a randomized, placebo-controlled, double blind, single center study to evaluate the effect of high dose PPI in patients with chronic laryngopharyngeal reflux symptoms. Methods: Thirty patients (8M, age 45 ± 2 years, mean ± SEM) with suspected of having laryngopharyngeal reflux disease were recruited in this study. We performed laryngeal and pharynx endoscopy at baseline and at the end of treatment. Posture, activity, alcohol consumption, and smoking status were recorded. Baseline laryngopharyngeal reflux symptoms including, hoarseness, laryngitis, sore throat, burning throat, and choking were noted. Results: The symptoms score at baseline was 4.0 ± 1.2, significantly improved compared to P (8.7 ± 1.3, p < 0.05). Baseline symptom scores for patients with positive pH test in R and P group were similar (11.4 ± 1.9 vs. 14.2 ± 1.9, p > 0.05). R significantly improved the symptom scores compared to P at the end of the 12th week (2.9 ± 1.2 vs. 9.7 ± 1.7, p < 0.01) in patients with positive pH test and 12 patients with negative pH tests. Baseline symptom score at both baseline (14.5 ± 3.4 vs. 12.3 ± 1.7, p > 0.05) and the 12th week (6.0 ± 3.4 vs. 7.2 ± 2.4, p > 0.05). Reflux findings scores (RFS) evaluated by video endoscopies were improved similarly in both R and P (p > 0.05). There was no significant different of RFS improvements after R or P irrespective of the pH results (p > 0.05). Conclusions: High dose rabeprazole is superior to placebo for relieving ENT symptoms in patients with laryngopharyngeal reflux disease especially in patients with positive pH tests, but not in patients with negative pH tests. The dual channels, 24-hour esophageal pH monitoring is helpful for identifying patients who will have benefits from high dose PPI therapy.

II-P-3-110
Low-Dose of Lansoprazole after Breakfast and Before Dinner Can Equally Inhibit Gastric Acid Secretion
M. Nishi, K. Adachi, T. Azaumi, K. Koshino, K. Furuta, Y. Kanozaka
Department of Gastroenterology and hepatology, Shimane university

Background and Aim: Proton pump inhibitor is mainly administered before dinner. However, it was not fully investigated whether the degree of gastric acid inhibition by PPI is different in pre-meal dosing or post-meal dosing. Therefore, this study was performed to clarify whether the inhibitory effect of PPI is highest before meal or after meal. Subjects and Methods: Ten healthy male volunteers (mean age 42.5 years) were enrolled in this study after obtaining their written informed consents. All subjects were examined by ambulatory intra gastric 24-hour pH monitoring on three conditions: i) without medication, ii) 7th day of administration of at a dose of lansoprazole 15mg, 30 min after breakfast, and iii) 8th day of administration of lansoprazole at a dose of 15 mg, 60 min before dinner. Each pH monitoring study was performed at random order and all pH monitoring studies were performed by using standard meals. The data of percentage of intragastric pH <4.0 were analyzed during 24h, nighttime (22:30-6:30) and daytime periods. Results: The percentage time of intragastric pH <4.0 was significantly lower during the administration of lansoprazole in both of the two dosing regimens than that of without medication. There was no significant difference in the percentage time of pH <4.0 during 24h, daytime and nighttime between the administration after breakfast and before dinner (44.6 ± 54.4, 43.1 ± 46.7 and 47.9 vs. 41.8). The percentage time of intragastric pH <4.0 in 3 H. pylori-positive subjects were significantly higher than that in 7 H. pylori-negative subjects in all pH monitoring study. There was no significant difference in inhibition of gastric acid secretion between the two lansoprazole dosing regimes in both H. pylori-positive and H. pylori-negative subjects. Conclusions: The timing of administration of low-dose lansoprazole did not significantly influence on the inhibitory effect of gastric acid secretion.

II-P-3-111
Gastroesophageal Reflux Disease (GERD) Associated Osteoporosis
S. Mue, A. Tanok, A. Funada, M. Tilio, V.P. Pisac, I. Julesc, S. Swierth, P. Skiric
Department of Pharmacology, School of Medicine, University of Zagreb

The pathophysiology of bone loss associated with gastrointestinal diseases has not been clearly defined. In addition to previous relation to inflammatory bowel disease or post-gastrectomy status, we report an association with gastroesophageal reflux disease (GERD). In this study 131 subjects were randomly assigned, 62 with endoscopically determined GERD patients (35 female, 27 male), and 69 patients with normal endoscopy findings examined because of degenerative rheumatic disorders that need NSAIDs therapy (32 female, 37 male). Patients had not different ages GERD (min/med/max: 34/65/84), rheumatic patients (min/med/max: 30/53/82), high GERD (min/med/max: 146/166/186) cm, rheumatic patients (min/med/max: 151/165/190), weight GERD (min/med/max: 47/72/117) kg, rheumatic patients (min/med/max: 48/76/107), menarche GERD (min/med/max: 12/14/19) years, rheumatic patients (min/med/max: 11/14/18), menopause GERD (min/med/max: 35/49/57) years, rheumatic patients (min/med/max: 38/49/55) years. Lumbosacral spine and left hip were used for densitometry analysis (≥1 normol, 1.0–2.5 osteopenia, ≤2.5 osteoporosis). The following values were obtained: lumbosacral spine: GERD (min/med/max: −4.6/−3.2/1.3), rheumatic patients (min/med/max: −3.0/−0.2/0.6) (p < 0.0001), left hip: GERD (min/med/max: −2.9/−0.9/2.8), rheumatic patients (min/med/max: −2.5/−2.2/2.2) (p < 0.003). Frequency of osteoporosis: rheumatic patients: left hip 22%, lumbosacral spine 10.1%; GERD: left hip 43.5% (p < 0.008), lumbosacral spine 22.6% (p < 0.05). Sex relation in GERD-patients: lumbosacral spine: male (min/med/max: −2.7/−0.6/1.4), female (min/med/max: −2.9/−1.1/1.1) (p < 0.028). No special difference was noted in nutrition, physical activity, alcohol consumption, as well as neither of patients received any therapy for osteoporosis. Conclusion: These data indicate an indicative association between GERD and osteoporosis, and their relation remain to be further determined.

II-P-3-112
Reconstructive Procedures Effects on Duodenogastroesophageal Reflux after Distal Gastroctomy
K. Fukuhara, S. Lee, T. Kishida, S. Nishizawa, H. Iwasaki, H. Osumi
Department of Gastroenterological Surgery, Osaka City University Graduate School of Medicine

Introduction: After distal gastroctomy, bile reflux into the gastric remnant can be occurred depending on the reconstructive procedures. The reflux of duodenal juice into the gastric remnant and esophagus has been reported to deeply correlate with the occurrence of gastritis, esophagitis, gastric cancer or esophageal cancer. In this study, we investigated which

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reconstructive procedure is effective in preventing bile reflux. **Materials and Method:** Ninety-two patients who had distal gastrectomy for cancer of the stomach in recent 3 years were subjected. The patients were classified into 3 groups; Group A: 29 patients resected by Roux-Y method, Group B: 41 patients resected by Billroth I method, Group C: 22 patients reconstructed by Billroth II method. Distal gastrectomy was performed with the same method with radical lymphadenectomy and was curative in all of the patients. By spectrophotometry using Bilitec 2000 (Systec Medical AB, Stockholm, Sweden), bilirubin concentration at 5 cm oral and anal to the esophagogastroduodenal junction was monitored for 24h. Bile reflux was judged to be present when absorbance was 0.15 or more, the ratio of the duration of bile reflux to the total observation period was obtained to determine % time of bile reflux. **Result:** In group A, % time in the esophagus was 1.6% and 26.6% in the stomach. In group, % time in the esophagus was 9.6% and 47.3% in the stomach. In Group C, percentage time in the esophagus was 31.8% and 79.2% in the stomach. There was significant difference in percentage of time among 3 groups. **Conclusion:** Roux-Y reconstruction was superior to Billroth I or II reconstructions with respect to preventing bile reflux into the esophagus and the gastric remnant. Roux-Y reconstruction was thought to be effective on preventing remnant gastritis, reflux esophagitis, oropharyngeal reflux, and reflux esophagitis.

II-P-4 Neoplasm-Basic 2

**Chairpersons:** H. Kataoka, M. Yashiro

**II-P-4 Neoplasm-Basic 2**

**Analysis of Genomic DNA Mutations of Toll – Like Receptors in Patients with Gastric Cancers**

T. Ohara¹, T. Morishita¹, J. Kasanuki²

¹Department of Internal Medicine, Tokyo Dental College, ²Department of Gastroenterology, Tokyo Women’s Medical University, Yachiyo Medical Center, Chiba, Japan

**Objective:** To date, there have been no reports on the relationship between mutations in toll-like receptors (TLRs) and the onset of cancers. To determine whether a relationship existed, we investigated TLR2, TLR4, radioprotective (RP) 105, TLR6, and TLR9 for possible mutations in their genomic DNAs in 48 patients with gastric cancers or gastric adenomas and 10 healthy volunteers. **Subjects and Methods:** The experimental group consisted of 22 patients with well-differentiated, and 20 with poorly-differentiated, adenocarcinomas, the latter group including 10 with signet ring cell carcinomas. The remaining 6 had gastric adenomas. Ten healthy volunteers with no family history of malignant disease served as controls. DNA was extracted from peripheral blood and subjected to electrophoresis using PCR oligonucleotide primers. Following electrophoresis by PCR, the gels were analyzed for DNA sequences. **Results:** None of the healthy volunteers, patients with gastric adenomas, or those with well-differentiated gastric adenocarcinomas showed mutations. However, 8 of the 20 with poorly-differentiated gastric adenocarcinomas showed heterozygosity at the 153th position of the amino acid sequence of TLR4, with a mutation from threonine (Thr) to alanine (Ala) being found at this site. Analysis of the entire available amino acid sequence of TLR4 revealed that this mutation occurred at a leucine–serine repeat corresponding to one of its extracellular components. **Conclusion:** About 20 types of amino acid have been reported, but only 3 – serine, Thr, and tyrosine – contain a hydroxyl group within their structure. For a protein phosphorylation reaction to take place, an amino acid must contain a hydroxyl group within its structure. This suggests a disturbance in the protein phosphorylation reaction of TLR4, and that this disturbance is related to the development of poorly-differentiated gastric adenocarcinomas.
II-P-4-117  
Cigarette Smoke Extracts Increase the Proliferation of Esophageal Cancer Cells  
Department of Pharmacology, The University of Hong Kong

It is well known that cigarette smoking can promote the risk of various human cancers, including esophageal cancer. A large body of genetic and biochemical evidence shows that the biosynthetic pathway of prostaglandin E2 (PGE2) may play an important role in tumorigenesis. The correlation between the arachidonic acid metabolism and tumorigenesis is suggested to be related to the potential anti-tumor action of non-steroidal anti-inflammatory drugs (NSAIDs). Long-term use of NSAIDs in thymus patients is related to a reduced risk of various human cancers, including esophageal cancer. However, there is rare report to explore the role of PGE2 and prostaglandin E2 receptors in the initiation and development of esophageal cancer. PGE2 mediates its effects, in part, through G protein-coupled PGE2 receptors, designated EP1, EP2, EP3 and EP4. Therefore, we examined the effects of cigarette smoke extracts on the proliferation of esophageal squamous cancer cell line, EC109, by 3H-thymidine incorporation experiment and cell cycle by using flow cytometry, as well as the expression pattern of prostaglandin E2 receptor subtypes by reverse transcription-polymerase chain reaction. Results indicated that cigarette smoke extracts (10, 40 and 100μg/ml) from ethanol and chloroform significantly increased the EC109 cell proliferation in a dose-dependent manner, and they mimicked the effects of 10% serum that increased the S and G2/M populations. Furthermore, the RTPCR experiment showed that all the EP receptors were expressed in EC109 cells. Further studies are needed to explore which EP receptor(s) is/are involved in the effects of cigarette smoke extracts to promote proliferation, and its signal transduction pathway in EC109 cells related to EP receptor(s). The work was supported by research grants from the University of Hong Kong and the Hong Kong Research Grants Council.

II-P-4-118  
c-kit, K-ras, H-ras and β-catenin Gene Mutation of Gastrointestinal Stromal Tumor  
R. Ashida1, N. Ogasawara2, A. Sawaki2, K. Yamazaki2, T. Mizoshita2, T. Tsukamoto3, K. Tominaga2, K. Higuchi1, M. Tatematsu4, T. Arakawa1  
1Department of Gastroenterology, Graduate school of Medicine, Osaka City University, Osaka, 2Department of Gastroenterology, Aichi Cancer Center Hospital, Division of Oncological Pathology, Aichi Cancer Center Research Institute, Nagoya, Japan

Background: A few studies have investigated the genetic mutations associated with gastrointestinal stromal tumor (GIST) except for c-kit gene. Mutations in the ras and β-catenin genes were common genetic abnormality in human malignant tumors such as colon and pancreatic cancer. Method: Eighteen cases of GIST were evaluated for the mutation of the c-kit, ras (K-ras and H-ras), and β-catenin genes. DNA was extracted from the formaldehyde-fixed paraffinized sections of GIST and analyzed by using PCR and direct sequencing techniques. Immunoreactive expressions of β-catenin and MIB-1 were histologically investigated. Results c-kit mutations were confirmed in 15 of the 18 patients (83.3%). Among these cases, a mutation in exon 13 was detected in one case (5.6%), whose clinical prognosis was extremely poor. In the other 14 patients, a mutation in exon 11 was detected, but there was no significant correlation with the clinical prognosis. No mutations were found in the K-ras gene. With regard to the H-ras gene, a missense mutation (Gly-Asp) in codon 15 and a mutation in codon 27 were seen in one and three patients, respectively. With regard to β-catenin, a missense mutation was seen in codon 32, 34, or 49, but no correlation with prognosis was identified (p = 0.837). Immunoreactive expression of β-catenin was found in the cytoplasm but not in the nuclei of each section in cases including the cases with the mutation of β-catenin gene. There was no correlation of labeling indices (LI) between MIB-1 immunostaining and β-catenin immunostaining, and the mutation status of H-ras and β-catenin genes. On the other hand, high MIB-1 LI (more than 5%) significantly correlated with poor prognosis. Conclusion: H-ras and β-catenin gene mutation did not seem a major prognostic factor. However, our results suggest that β-catenin pathway may be involved in the development and progression of GIST because β-catenin expression was observed in all cases and genetic mutations were suggested to assist its activation.

II-P-4-119  
Evaluation of Mucin Expression Patterns in Gastric Borderline (Group III) Lesions  
H. Minematsu, Y. Saito, R. Kakinoki, A. Andoh, R. Kushima, Y. Fujiyama  
Department of Gastroenterology, Shiga University of Medical Science

Background: Recommendation for diagnosis and treatment of gastric borderline (group III) lesions remains controversial. We examined the mucin expression patterns in endoscopically-rected and forceps-biopsy samples. Methods: Sixty-three gastric lesions were histopathologically diagnosed as group III based on endoscopic forceps biopsy. All of these patients underwent endoscopic resection, and were divided into group A (final diagnosis an adenocarcinoma) and group B (final diagnosis as an adenoma). Immunostaining for MUC2, 5AC, 6 and CD10 were performed. The mucin phenotype classification was determined according to the criteria by Tsukashita et al. Similar evaluation was performed in 28 forceps-biopsy samples. Results: The percentages of a complete gastric phenotype (positive for MUC5AC and MUC6) plus a mixed phenotype (gastric predominant type) were significantly higher in group A (58.0%) than in group B (21.8%) (p < 0.05). The percentage of a complete intestinal phenotype (positive for MUC2 and CD10) was significantly higher in group B (68.8%) than in group A (19.4%) (p < 0.05). Similar results were also observed in 28 samples of forceps-biopsy, which were patho-histologically diagnosed as group III lesion. The percentage of a Ki-67 diffuse-immunostaining pattern was significantly higher in group A than in group B (p < 0.05). The positive ratio of p53 expression was 29.2% in group A and 4.3% in group B respectively, and was significantly higher in group A (p < 0.05). Conclusion: Determination of immunostaining for a mucin phenotype in forceps-biopsy samples may be helpful for a diagnosis of gastric borderline (group III) lesions.

II-P-5-120  
Effects of the Anti-VEGFR2 Monoclonal Antibody on Dextran Sodium Sulphate-Induced Colitis in Mice  
T. Ando1, T. Ohshima2, M. Sasaki3, K. Kataoka4, T. Joh5, B. Pytovsk6, J.S. Alexander7  
1Department of Internal Medicine and Bioregulation, Nagoya City University Graduate School of Medical Sciences, Japan; 2InClone Systems, 3LSU Health Sciences Center, Shreveport, LA, USA

Aim: Inflammatory bowel diseases have been associated with disturbances in vascular permeability and angiogenesis, which regulated by the endothelial intercellular junctions. We evaluated the effects of monoclonal antibodies to VEGFR on colitis induced by dextran sodium sulphate (DSS) in mice. Method: Colitis was induced by feeding mice 3% DSS for 7 days. Anti-VEGFR 1, 2, 3 antibody or IgG was injected intraperitoneally in mice daily from day 0 to day 6. We measured the body weight and the disease activity index (DAI) (diarrhea, bloody stool, body weight changes). On day 7 the mice were sacrificed and colitis was evaluated histologically at the proximal and distal colon. Result: Administration of anti-VEGFR2 antibody significantly prevented decreasing body weight and showed the better DAI scoring as compared to other antibodies. DAI was decreased in anti-VEGFR2 antibody treatment mice compared to other antibodies. Also, anti-VEGFR3 antibody reduced colonic injury at the distal colon, not proximal. On the other hand, anti-VEGFR1 or VEGFR3 antibodies did not protect the colonic injury at both colons. Conclusion: In the present study, we demonstrated that anti-VEGFR2 antibody significantly ameliorates DSS-induced colitis, suggesting that VEGFR2 may be useful for control of inflammatory bowel diseases. Our findings might indicate that angiogenesis has an important role on the progression in inflammatory bowel disease.

II-P-5-121  
Loss of Transgelin in Repeated Bouts of Ulcerative Colitis-Induced Colon Carcinogenesis  
J.S. Song, Y.-M. Na, M.S. Kawk, J.D. Lim, J.A. Lee, M.H. Kim, M. Yeo, K.-B. Hahm  
Genome Research Center for Gastroenterology, Ajou University Medical Center

Background: CD44 expression is associated with the progression of colorectal cancer. Transgelin (TG) is associated with the progression of colorectal polyps. Western blot and immunohistochemistry analysis. Down expression of transgelin was also noted in colon tumor. Subsequent 10 days. Colorectal tumors developed in 22 out of 24 repeated colitis-induced mice (91.6%) and the tumor multiplicity was 1.72 per a tumor-bearing mouse. Comparative 2DE analysis showed 38 protein spots differentially expressed in colon tumor compared to normal colon. Twenty seven proteins including GRPM4, HSC70, enolase, prohibitin, and transtelin were identified. Transtelin out of 27 identified spots significantly reduced in mouse colon tumor documented by Western blot and immunohistochemistry analysis. Down expression of transtelin was also noted in neoplastic human colon compared to abundant expression in adjacent non-tumorous tissues. These results suggest that loss of transtelin could be a candidate for biomarker of repeated inflammation.

Ulcerative colitis (UC)-associated cancer develops from dysplasia lesion caused by chronic inflammation. However, the specific mechanistic link between chronic inflammation and carcinogenesis in colon has not been integrated into molecular understanding. In this study, we established experimental animal model of human UC and to identify proteins involved in development of UC-associated colorectal cancer, proteomics based on 2-dimensional electrophoresis and MALDI-TOF MS was employed. Five mice C57BL/6 mice were exposed to 15 cycles of dextran sodium sulfate (DSS) that each cycle comprised of 0.7% DSS for a week followed by distilled water for the subsequent 10 days. Colorectal tumors developed in 22 out of 24 repeated colitis-induced mice (91.6%) and the tumor multiplicity was 1.72 per a tumor-bearing mouse. Comparative 2DE analysis showed 38 protein spots differentially expressed in colon tumor compared to normal colon. Twenty seven proteins including GRPM4, HSC70, enolase, prohibitin, and transtelin were identified. Transtelin out of 27 identified spots significantly reduced in mouse colon tumor documented by Western blot and immunohistochemistry analysis. Down expression of transtelin was also noted in neoplastic human colon compared to abundant expression in adjacent non-tumorous tissues. These results suggest that loss of transtelin could be a candidate for biomarker of repeated UC-associated colon cancer. Grant support: This work was supported by grants from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-P010-Pg6-01uth0007).

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II-P-5-122

Anti-Inflammatory Effects Induced by Bone Marrow-Derived Mesenchymal Stem Cells for Dextran Sulfate Sodium-Induced Colitis in Rats

Department of Gastroenterology, Osaka City University, Graduate School of Medicine

**Background:** Bone marrow-derived cells including mesenchymal stem cells (MSCs) have therapeutic effects for clinical human and experimental animal colitis. Its detailed mechanism(s) may be partly mediated by mucosal regeneration, since MSCs have potential for differentiation to several parts of cells. But MSCs were thought to have other functions such as anti-inflammation as well as mucosal regeneration, because anti-inflammatory system is involved in the repair of colitis. We examined the therapeutic efficacy and anti-inflammatory effects of bone marrow-derived MSCs for dextran sulfate sodium (DSS)-induced acute colitis in rats.

**Materials and Methods:** Experimental colitis was induced by 3% DSS drinking water for 7 days in inbred male Lewis rats. Bone marrow was extruded and then the mononuclear cells were isolated and cultured. On day 0, 2, and 4, MSCs were injected via tail vein. We checked the volumes of food and water intake, stool condition, and body weight every day. On day 7, total colon was excised and each colonic mRNA expression of inflammatory cytokine such as TNF-a, IL-1b, IL-10, and COX2 was measured by real time RT-PCR.

**Results:** Optimal dose of DSS was confirmed at 4%. MSC treatment improved the bloody stool and body weight loss, and significantly inhibited the shortening of colon length. At the rectum of MSC-treated rats, expressions of local inflammatory cytokines such as TNF-a and IL-1b were markedly decreased to about 40 and 15%. Local COX2 expression was also suppressed to 15%.

**Conclusions:** These findings suggested that MSC could have the therapeutic efficacy for inflammatory colitis via anti-inflammatory mechanisms.

II-P-5-124

**Rebamipide Promotes Functional and Morphological Epithelial Barrier Formation in Cultured Human Colon Epithelial Cell Line, T84**

Tokushima Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan

**Background and Aims:** Rebamipide is an anti-ulcerative and anti-inflammatory agent having mucoprotective, radical-scavenging, anti-inflammatory, and wound-healing activities. Recently, Makiyama et al. (2000) reported the potential therapeutic effects of a rebamipide enema on ulcerative colitis patients, although the modes of action remained to be elucidated. In the present study, we examined the effects of rebamipide on epithelial cell sheet formation in cultured human colon cancer cell line (T84) monolayers.

**Methods:** T84 cells were cultured in DMEM/Ham's F-12 medium (1:1) supplemented with 5% (vol/vol) newborn calf serum, and seeded at a density of 5.0 105 cells/cm2 in 12 mm diameter, 3 mm pore size transwell-COL inserts (Corning Inc.) in 12-well plates. After 3-day incubation at 37°C in a 5% CO2 atmosphere, the seeded cells were treated with rebamipide, 5-aminosalicylic acid (5-ASA), or control. Vehicle. At 3-day intervals, trans-epithelial electrical resistance (TEER) was measured as a marker of barrier formation using a MILLICELL-ERS voltohmmeter (Millipore Co.).

**Results:** T84 cell sheets showed a gradual increase in TEER over the culture period, and TEER attained to 78 ± 141 ohms x cm2 (mean ± SD) on day 18 in the control group. At 1mM, rebamipide accelerated the generation of TEER, which attained to 150% of control, whereas 5-ASA did not accelerate but rather suppressed it. T84 cells grew and proliferated to 4 times the initially seeded counts in accordance with the generation of TEER on day 18. Interestingly, rebamipide at 1mM halved the cell counts on day 18 in spite of accelerating TEER generation. At 10mM, 5-ASA actually suppressed cell growth concomitantly with the suppression of TEER generation. The T84 cell sheets treated with 1mM rebamipide were almost mono-layers, whereas the control sheets were mostly multi-layers. The cell sheets treated with 10mM 5-ASA showed the formation of sparse cell layers with many intercellular gaps.

**Conclusions:** It was suggested that rebamipide accelerated epithelial barrier formation in T84 cells.
Association between IL-18 Gene Promoter Polymorphisms and Inflammatory Bowel Disease in a Japanese Population

T. Takagawa1, K. Tamura2, N. Gakeda1, T. Tomita1, Y. Ohda2, K. Fukunaga3, N. Hida1, K. Ohtsuki1, K. Hori1, T. Kosaka1, Y. Fukuda1, H. Inoue1, Y. Yamamura1, H. Miwa1, T. Matsumoto1

1Division of Lower Gastroenterology, Department of Internal Medicine, 2Laboratory of Hereditary Tumor, Institute for Advanced Medical Sciences, 3Division of Upper Gastroenterology, Department of Internal Medicine

Department of Surgery, Hyogo College of Medicine, Nishinomiya, Japan

Interleukin-18 (IL-18) is a pleiotropic cytokine to induce the production of IFN-γ and also to regulate Th2 cytokines. Recently, the association studies between IL-18 gene promoter polymorphisms and several Th1 or Th2-mediated inflammatory diseases were reported. In inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn’s disease (CD), recent evidence suggests that IL-18 is involved in the pathogenesis. Therefore, we investigated IL-18 gene promoter polymorphisms at −607C/A and −137G/C in this study. Allele, genotype and haplotype frequencies were determined in 205 Japanese UC patients, 210 CD patients and 212 control patients with active and remission phase of UC and control group were measured by ELISA. In conclusion, IL-18 promoter polymorphisms may not be associated with disease susceptibility, but related to the extent of disease in UC.

The pathogen of ulcerative colitis (UC) is remained unavailed. Analysis of the local phenomenon of UC, is one of the most important things. We already reported the presence of high proliferative CD19+CD20-, CD138+ plasma-lineage cells in ulcer base of UC. This time, we attempted to clarify the relationship between the high proliferative plasma-lineage cells and the mucosal change and appendix of UC. Intestinal specimens obtained during surgery for 25 patients with UC and 20 Crohn’s disease (CrD) were used for immunohistochemical staining, immunofluorescent staining and Immunoelectron microscopy with various lymphocyte markers and Ki-67, a proliferation related antigen, as previously described (Saki et al. Lab Invest 1996). In the inflammatory mucosa and appendix of UC, there were abundant Ki-67+CD19+, CD20-, CD138+ cells. Although the labeling index of Ki-67 in CD19+cells was positively correlated with the clinical activity in the inflammatory mucosa of UC, that was not in appendix of UC. In appendix of UC, the labeling indexes of Ki-67 were high in spite of the clinical activity. The presence of high proliferative CD19+, CD20-, CD138+ plasma-lineage cells in the inflammatory mucosa and appendix of UC show the strong relation between the high proliferative plasma-lineage cells and the of UC. And the presence of those cells in any statement in appendix of UC may involve the continuous disease statement.

The significance of IP-10 in the pathogenesis of ulcerative colitis

A. Noguchi1, K. Watanabe1, T. Yukawa1, T. Suekane1, K. Aomatsu2, N. Kamata, H. Yamagami1, K. Higuchi1, T. Arakawa1

1Department of Gastroenterology, Osaka City University Graduate School of Medicine, 2Department of Obstetrics and Gynecology, Osaka City University Graduate School of Medicine

Background: Although edopathogenesis of ulcerative colitis (UC) remains unclear, interleukin-18 (IL-18) is finally recognized as one of the important cytokine in the pathogenesis of UC. Therefore, we investigated the role of IP-10 in the pathogenesis of UC.

Methods: Serum IP-10 levels in patients with active and remission phase of UC and control group were measured by ELISA. In active UC patients who received GCAP (G1-adacumol), pre- and post-GCAP IP-10 levels were measured, and examined lipopolysaccharide stimulated IP-10 production of peripheral blood cells by whole blood culture. Results: Serum IP-10 levels in patients with active UC was significantly higher than that in patients with inactive UC and controls. After GCAP, serum IP-10 levels were significantly decreased compared with before GCAP. IP-10 production by peripheral blood mononuclear cells was also significantly decreased after GCAP.

Conclusion: The findings of the present study suggest that UC is coupled with Th1 responses induced by over-produced IL-18, and GCAP down modulate IP-10 producing capacity of leukocytes from active UC patients. The mechanism which upregulate IP-10 may be an important issue to be clarified in the pathogenesis of UC.
II-P-7.131

Effects of Polymorphisms of Pro-Inflammatory and Anti-Inflammatory Cytokines on the Development of Peptic Ulcer and Gastric Cancer
M. Sugimoto, T. Furuta1, N. Shirai, M. Ikuma, A. Hishida
Hamamatsu University School of Medicine, First Department of Medicine, 1Hamamatsu University School of Medicine, Center for Clinical Research

Backgrounds and Aims: In Western countries, pro-inflammatory and anti-inflammatory cytokine genes polymorphisms are associated with the development of gastric cancer and duodenal ulcer. The aim of the present study was to clarify the association between the several pro-inflammatory cytokine and anti-inflammatory cytokine polymorphisms and the susceptibility to peptic ulcer diseases and gastric cancer in Japan. Methods: We determined IL-1β-51/31, IL-4Rα, TNF-A-857/-863/-1031 and IL-10-1082/-819/-592 genotypes in Helicobacter pylori-positive patients with gastritis only (n = 164), gastric ulcers (n = 110), duodenal ulcers (n = 94), or gastric cancers (n = 105), and H. pylori-negative controls (n = 172). Results: TNF-A-857 T (odd ratio [OR]: 1.826, 95% confidence interval [CI]: 1.097–3.039), TNF-A-863 A (OR: 1.788, 95% CI: 1.079–2.905) and TNF-A-1031 C (OR: 1.912, 95% CI: 1.152–3.171) alleles carriage were associated with increased risk for gastric ulcer development. The ORs of TNF-A-857 T, TNF-A-863 A and TNF-A-1031 C alleles carriage for gastric cancer were 1.863 (95% CI: 1.113–3.107) and 2.074 (95% CI: 1.244–3.457), respectively. There was no relationship between the development of H. pylori-related diseases and polymorphisms of IL-1β-51/31 and TNF-A-308. The simultaneous carriage of three different high-producer alleles of TNF-A-857/-863/-1031 significantly increased the risk of gastric ulcer (OR: 6.57, 95% CI: 2.34–18.40) and gastric cancer (OR: 5.20, 95% CI: 1.83–14.78). Conclusions: Polymorphisms in TNF-A rather than IL-1B are associated with increased risk for gastric ulcers and gastric cancer in Japan. The simultaneous carriage of more than one high-producer allele of TNF-A further increased the risks for gastric ulcer and cancer.

II-P-7.132

Eradication Rates after Randomized Treatment in the Region with High Prevalence of Helicobacter pylori Infection
A. Makarenko
Pirmans Sergey Therapy, No. 2, Vitebsk State Medical University

The aim of this study was to estimate the efficacy of different variants of therapy regimen of eradication in patients with Helicobacter pylori (HP) associated peptic ulcer in the region with high prevalence of the infection. Patients and Methods: A total of 220 patients with HP-associated peptic ulcer were randomized to receive omeprazole 20 mg, clarithromycin 500 mg and amoxicillin 1,000 mg b.i.d. for 7 days (OCA7), for 14 days (OCA14), the same but omeprazole 40 mg b.i.d. for 7 days (OCA7), the same but rabeprazole 20 mg b.i.d., (RC7), colloidal bismuth subcutirate 240 mg, amoxicillin 1,000 and furazolidone 200 mg b.i.d. for 7 days (BA7); this protocol was stopped prematurely, the same but with omeprazole 20 mg b.i.d. (OB7), the same for 14 days (OB14). The results of eradication were estimated in accordance with RUT, data of PCR method and histology examinations in 2 months after treatment and the results of reinfection – in one year after treatment. Results: Accoding to the results of the RUT the per protocol (PP) eradication rates were 88.9% for the OCA7, 96.2% for the OCA14, 92.9% for the OOC7A, 83.3% for the RC7, 12.5% for the BAF7, 35.7% for the OB7; 81.3% for the OB14 groups respectively. According to the results of the RUT and histology/PCR the PP eradication rates were dramatically lower. If the results of eradication according to RUT and histology/PCR methods were used, one-year reinfection rate was only 3.8%. Conclusion: According to RUT data the efficacy of different variants of triple classic eradication therapy in the population with high prevalence of HP infection makes up about 83.3%–96.2% (RUT).

II-P-7.133

Impact of Changes in H. pylori Infection Rate and Upper Gastrointestinal Endoscopic Finding in Japan
T. Kawai1, M. Katsaka1, I. Miyazaki2, K. Yagi1, K. Kawakami1, T. Yamasghis1, S. Taira1, T. Ito1, F. Moriyasu1, Y. Takagi1, T. Aoki1
1Endoscopy Center, Tokyo Medical University Hospital, 2Fourth Department of Internal Medicine, 3Third Department of Internal Medicine, Tokyo Medical University

Background: Environmental changes are now predicted to bring about a rapid decline in the prevalence of H. pylori infection, and subsequent changes in endoscopic findings. In this study, we examined the relationship between H. pylori infection and endoscopic findings. Subjects and Methods: The subjects were consecutive 419 patients who underwent routine health checks between July 2003 and April 2004 as well as H. pylori serology and gastroscopy, including endoscopic evaluation of gastric mucosal atrophy. Their average age was 39.2 ± 8.4 years (range 22–58). The diagnosis of H. pylori infection was conducted using the serum IgG anti-HP antibody assay method. Endoscopic diagnoses available were reflux esophagitis (RE), fundic polyps (FP), hyperplastic polypos (HP), gastric xanthoma (GX), gastric ulcer (GU), and duodenal ulcer (DU). Results: The overall H. pylori infection rate was 33.7%. Infection rates by age group were: 20–29 15.7%, 30–39 28.0%, 40–49 34.3%, and 50–59 69.6%. The proportions of the endoscopic diagnoses RE, FP, HP, GX, GU and DU were 23.4, 8.6, 0, 0, 1.1 and 1.4, respectively, in H. pylori (+) subjects. In the H. pylori (−) subjects, these were 11.4, 0, 4.3, 5.0, 13.4 and 21.9. Conclusions: H. pylori infection rates were considerably reduced in comparison to previous reports. Clear differences were also seen in the endoscopic findings according to the presence of H. pylori infection. These results suggest future changes in endoscopic findings are likely as the prevalence of H. pylori infection declines.

II-P-7.134

Influence of Changes Helicobacter pylori Eradication Therapy on Decision of the Pepsinogen Test Method Following in Japan
K. Yagi1, T. Kawai, M. Katsaka1, T. Yamasghis1, I. Miyazaki2, K. Kawakami1, F. Moriyasu1, T. Aoki1, K. Murak1, M. Sasat2, K. Mi1
1Endoscopy Center, Tokyo Medical University Hospital, 2Fourth Department of Internal Medicine, 3Third Department of Surgery, Tokyo Medical University, 4Department of Pathology, Tokyo Medical University Hospital, 5Department of Pathogenic Microbiology, Tokyo University of Pharmacy and Life Science, 6Division of Gastroenterology and Hepatology, Internal Medicine, School of Medicine of Toho University (Orinomi)

Aims: The pepsinogen (PG) test method is used in Japan for screening for gastric cancer. Serum PG levels are reported to change following Helicobacter pylori (H. pylori) eradication therapy. In this study, we investigated the changes in decision of PG test method following H. pylori eradication. Methods: The subjects were 120 H. pylori consecutive positive patients with upper gastrointestinal endoscopic findings who underwent H. pylori eradication therapy. In this study, we investigated the changes in decision of PG test method following H. pylori eradication. Results: The overall eradication rates were 73.3% (intention-to-treat) and 78.5% (per protocol). Prior to eradication therapy, the PG test was positive in 57 subjects and negative in 63. Followin eradication therapy, the decision of PG test converted from positive to negative, despite an absence of improvement in the histological findings of atrophy and intestinal metaplasia, in 80.4% (37/46) of cases of successful eradication, and in 0% (0/6) of cases of eradication failure. Conclusions: These results suggest that the decision of PG test method should be used for the definitive confirmation of the success or failure of H. pylori eradication therapy.

II-P-7.135

Helicobacter pylori Infection and Non-Steroidal Anti-Inflammatory Drug Usage in Bleeding Ulcers
Department of Internal Medicine, Saga Medical School, Saga, Japan

H. pylori infection and NSAIDs are well-known major causes of peptic ulcers. This study aimed to characterize the features of bleeding peptic ulcers in Japan. Patients and Methods: This prospective study evaluated 116 patients revealed to have bleeding peptic ulcers from January 2000 to December 2002. We studied the characteristics of these patients. Results: Among the 116 patients, 88 (75.9%) patients had H. pylori infection. Seventy (63.0%) patients were positive for H. pylori with no history of NSAIDs usage (Group A), and 18 (15.5%) were positive for H. pylori with a history of NSAIDs usage (Group B). Among the H. pylori-negative patients, 15 (12.9%) were associated with NSAIDs usage (Group C). Thirteen (11.2%) patients had no H. pylori infection or history of NSAIDs usage (Group D). Among the 33 patients with a history of NSAIDs usage, 11 were on-demand NSAIDs users, 14 took daily low-dose aspirin. The patients in groups B and C were significantly older than those in groups A and D, and had more frequent coexisting co-morbid diseases compared to group A. In group D, 11 had atrophic changes by endoscopic examination, suggesting that they had a past H. pylori infection and the atrophic changes remained at the time of bleeding. Many of the patients in group D had a serious co-morbid disease. Compared with healthy control subjects, the concentrations of both phosphatidylcholine and phosphatidylethanolamine were significantly decreased in the antral gastric mucosa in all patient groups. Conclusions: The contribution of NSAIDs usage to bleeding ulcers was 28.4%, and low-dose aspirin or on-demand NSAIDs usage could be causes of bleeding ulcers. There were only 2 (1.7%) confirmed cases of non-H. pylori non-NSAIDs ulcers.
Il-P-8 Peptic Ulcer

Chairpersons: Y. Mizokami, T. Arisawa

Il-P-8-136 Upper Gastrointestinal Bleeding in NSAIDs Users and Helicobacter pylori Infection in Thailand
R. U. Visitchayanont, V. Mahachak 1
1Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Pathumthani, 2Gastroenterology Unit, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand

Background and Aims: Upper gastrointestinal bleeding (UGIB) is an important emergency situation with a high incidence and carries significant morbidity and mortality. Helicobacter pylori (H. pylori) infection and nonsteroidal anti-inflammatory drugs (NSAIDs) remain the major factors of peptic ulcers and bleeding complication. This match case-control study was designed to evaluate association between H. pylori and UGIB in NSAIDs users. Methods: Clinical information, endoscopic findings and H. pylori infection were collected between January 2001 and April 2002. NSAIDs use was defined as consumption of NSAIDs or aspirin for at least 3 days period preceding the episode of the bleeding. The UGIB was defined as overt bleeding (hematemesis, positive nasogastric aspirate, and melena) or fall in baseline hematocrit ≥5 points within 24h of admission. UGIB was defined as endoscopy by breaking mucosa >3 mm in diameter. H. pylori status assessed by rapid urease test, culture or serology and patients were regarded as H. pylori-positive if at least one of the examinations gave a positive result. Results: Total of 154 NSAIDs users (71 men and 83 women, mean age of 60.9 years) were evaluated in this study including 89 patients (57.8%) with UGIB and 65 patients (42.2%) with both dyspeptic symptom. In the bleeding group, gastric ulcer (62.9%) was the most frequent cause of bleeding followed by duodenal ulcer (34.9%), erosive gastritis (23.6%). H. pylori infection was found in 65/89 (73.0%) of bleeding group and 16/65 (24.6%) in dyspeptic group. The multivariable model suggested that the probability of UGIB event increased with H. pylori infection (OR = 4.2, p < 0.01, 95% CI = 1.5–12.0) and multiple NSAIDs use (OR = 8.1, p < 0.01, 95% CI = 1.9–34.9). Summary: H. pylori infection was significantly higher in patients with NSAIDs use of increased risk of non-variceal UGIB. H. pylori should be eradicated to reduce the risk of non-variceal UGIB in NSAIDs users.

Il-P-8-137 Upper Gastrointestinal Disorders Induced by Non-Steroidal Anti-Inflammatory Drug
First Department of Internal Medicine, Iwate Medical University

Aim: We examined the characteristics of upper gastrointestinal disorders induced by non-steroidal anti-inflammatory drug (NSAID). Methodology: The questionnaire investigation was performed over a five year period. Results: A study was performed on 354 patients (161 men and 193 women, mean age of 60.9 years, with mean duration of 30 years) who developed NSAID-associated upper GI disorders: 21 patients had AGML, 212 had gastric ulcer, 63 had duodenal ulcer, 17 had gastrointestinal ulcers and 41 other cases. About 75% of patients received NSAID for orthopedic conditions. Sixty percent of gastric disorders induced by NSAID affected the antrum or angulus of the stomach. The incidence of disorders of the gastric antrum was significantly higher in women than in men whilst the incidence of disorders on the gastric angulus was significantly higher in men (p < 0.05). The proportion of patients with abdominal pain was significantly lower in patients over 65 years old than in those under 65 years old, and the proportion of patients with hematemesis or melena was significantly higher in patients over 80 years old than in those under 80 years old (p < 0.05). The time taken to achieve the healing stage was significantly longer in patients with greater than 3 months NSAID ingestion compared to patients that had received NSAID for less than 3 months (p < 0.05). Conclusions: Patients 65 years old and over with continuous NSAID use had asymptomatic ulcers, and patients 80 years old and over had hemorrhagic ulcers.

Il-P-8-138 The Preventive Effect by Regular or Half-Dose, Not High-Dose Histamine-H2 Receptor Antagonists for NSAID-Associated Peptic Ulcers in Japanese Patients with Rheumatoid Arthritis
Department of Medicine, Division of Gastroenterology, Nippon Medical School

Background and Aims: It is established in Europe and USA that proton pump inhibitors (PPIs), prostaglandin E1 analog (PG), and high-dose histamine H2 receptor antagonists (H2RA) effectively prevent NSAID-caused gastric mucosal injuries. However, strategies to prevent NSAID-related gastric injuries have yet to be elucidated in Japan where the elderly have a lower gastric acidity and the dosage of NSAID is relatively low. Therefore we investigated the relationship between anti-ulcer medications and NSAID-related peptic ulcer in Japanese patients with rheumatoid arthritis (RA) receiving long-term NSAID treatment. Methods: 176 patients with RA treated over a long-term with NSAID were enrolled. Their demographic data were recorded and laboratory data were measured. Endoscopy was performed to assess the prevalence of peptic ulcer. To verify the ulcer-preventive efficacy of anti-ulcer agents in patients with PU induced by long-term use of NSAID, the demographic factors and prevalence of PU were compared according to the use of anti-ulcer agents in this survey. For anti-ulcer agents, the remaining 176 patients were divided into those not taking anti-ulcer agents (non-medication group); those taking mucosal protective agents (mucosal protective group), regular or half-dose H2RA (H2RA group), PPI or PG (PPI-PG group). Results: No significant difference was seen among any two groups when comparing age, sex, RU history, PSL usage, NSAID using condition (variety and dosage) and H. pylori infection rate. Thirty-eight (21.6%) of 176 RA patients had peptic ulcer, including 31 with gastric ulcer, 6 with duodenal ulcer and 1 with gastric-duodenal ulcer. The prevalence of PU in the H2RA group or PPI-PG group was significantly low compared to the mucosal protective group (p < 0.05, respectively). On the other hand, no significant difference was seen between any other groups. Conclusions: In Japan, regular or half-dose of H2RA might be effective for preventing peptic ulcer induced by long-term NSAID treatment.

Il-P-8-139 Low Prevalence of Peptic Ulcer at Endoscopy in Two Decades in Bangladesh
Department of Gastroenterology, Bangabandhu Sheikh Mujib Medical University, Consultant Gastroenterologist, Popular Center, Dhaka, Bangladesh

Introduction: The prevalence of Peptic ulcer is gradually declining. This effect could be due to eradication of H. pylori and by treatment with powerful anti-ulcer drugs. The prevalence of Peptic ulcer was very high in eighties in Bangladesh. The point prevalence of Peptic ulcer was around 15%. It was the reason why we examined the prevalence of Peptic ulcer in Bangladeshi subjects now. Aims and Methods: The aim of the study was to assess the data of Upper GI endoscopic findings of Bangladesh subjects from January 2004 to September 2005. We designed retrospective study in patients who underwent endoscopies in 1984 and 1985. We examined 1,743 patients having endoscopy in 2004-2005 and 1,695 patients having endoscopy in 1984-1985. We included those who had only Peptic ulcer in the form Duodenal ulcer, benign Gastric ulcer includ- ing pre pyloric ulcer, Gastric outlet obstruction due to Peptic ulcer. Results: Upper GI endoscopy were performed in 1,743 patients in 2004 & 2005 of which Duodenal ulcer, benign Gastric ulcer were found in 175 (10%) and 66 (4%) patients respectively. Normal findings at endoscopy were found in 1,341 (77%) subjects. Retrospective analysis of data of endoscopic findings in 1984 and 1985 showed Duodenal ulcer and benign Gastric ulcer in 646 (38%) and 145 (9%) patients respectively. Normal findings at endoscopy were found in 447 (26%) subjects. Overall reduction of prevalence of Peptic ulcer was from 791 (47%) in 1984–1985 to 241 (14%) in 2004–2005 (p < 0.005). Conclusion: The decrease in prevalence of Peptic ulcer was observed in patients having Upper GI endoscopy from 47% in 1984–1985 to 14% in 2004–2005, which is statistically significant. This decrease in prevalence of Peptic ulcer may be due to eradication of H. pylori and treatment and maintenance by anti-ulcer drugs.

Il-P-8-140 Location of Peptic Ulcers is Related to Extent of Atrophy Assessed by Kimura Takemoto Classification – An Insight in Contrast to the Muscle Bundles of the Stomach
1Department of Surgery, 2Center for Diagnostic and Therapeutic Endoscopy, Keio University School of Medicine, 3Center for Comprehensive and Advanced Medicine, Keio University Hospital, 4Department of Emergency and Critical Care Medicine, 5Department of Pathology, Keio University School of Medicine, Tokyo, Japan

It is accepted that duodenal ulcers are related to gastritis confined to the antrum and gastric ulcers are related to gastritis extended to the body of the stomach. However, the relationship between the location of ulcers and the Kimura Takemoto classification has not been examined. We analyze the data in contrast to distribution of the muscle bundles of the stomach reported in the dual control mechanism. Methods: Endoscopic findings of 487 patients were classified according to the Kimura-Takemoto classification and location of ulcers was examined. Results: The incidences of Helicobacter pylori (HP) infection according to the Kimura-Takemoto classification were as follows: C-0; 8.8%, C-1; 31.0%, C-2; 77.2%, C-3; 78.4%, O-1; 74.1%, O-2; 74.1%, O-3; 57.7%. Statistical analysis showed that HP infection was associated with
In the process of peptic ulcer formation, excess acidity and H. pylori infection play important roles in the disease deterioration. Moreover, we reported the substantial contribution of fungus infection to the pathophysiology in perforated duodenal ulcer. Here we investigated the breakdown of extracellular matrix (ECM) and inflammatory cells infiltrated in ulcer tissue. In the ulcerated specimen, we observed increased expression of matrix metalloproteinase-1 (MMP-1), which seems to be produced mainly by granulocytes and fibroblasts in the tissue. Eosinophils infiltration was prominent in the ulcer tissue of some cases infected with fungus, which suggest eosinophils may secrete some factors such as major basic protein (MBP) or MMPs attacking not only fungus but also normal architecture of gastric wall. In clinical settings, we investigated wound healing process in a suggestive case of perforated gastric ulcer treated with omipental patch surgery. Early gastric cancer was found by upper gastrointestinal (GI) endoscopy after the emergency operation. One month later, curative resection for gastric cancer including perforated ulcer was done and specimen was histologically evaluated in order to clarify the role of omenetal in ulcer healing. We used immunostaining for TGF-β1 to detect BM-derived cells (TgN(α-CAG-EF1α)LacZ) and observed intense staining in the endothelial cells of omental vesicles. These results suggest omental patch procedure may be beneficial as a source of cytokines related to ulcer healing.

**II-P-9 Wound Repair 2**

**Chairpersons:** M. Tsuji, M. Sasaki

**II-P-9-142**

**Involvement of Bone Marrow-Derived Cells in Gastric Ulcer Healing in Mice**


1Department of Gastroenterology and Hepatology, 2Clinical Laboratory Sciences, Osaka University Graduate School of Medicine

**Background and Aim:** Bone marrow (BM) has been found to be a source of multi-potent stem cells that could distribute into various organs including gut. In this study, we examined a possible contribution of BM-derived cells in peptic ulcer healing. Methods: Wild-type C57BL/6 mice were irradiated to 10 Gy to ablate their BM and transplanted retro-orbital with 4 × 10^6 BM cells, prepared from BM of GFP-transgenic mice (TgN(α-CAG-EF1α)LacZ). Chronic gastric ulcers were produced in mice by topical application of acetic acid to the serosal surface of the stomach at 4 weeks after bone marrow transplantation. The animals were sacrificed at 2 weeks following ulcer induction. The origin and phenotypes of gastric myofibroblasts were examined by confocal microscopy and double-staining immunofluorescent immunohistochemistry for GFP, vimentin, cytokeratin, α-smooth muscle actin (α-SMA), CD45, F4/80, CD31. Results: BM-derived cells homing to the ulcer site expressed α-SMA (fibroblast/myofibroblast marker) and the morphology of these cells is spindle shape. And these cells did not express CD45-positive leukocyte and F4/80-positive macrophages. Our results show that BM-derived cells may function as a source of cytokines related to gastric ulcer healing.

**II-P-9-143**

**The Role of Bone Marrow-derived Cells in the Regeneration of Damaged Colonic Epithelium in Mice**


1Department of Clinical Laboratory Science, School of Allied Health Sciences, Faculty of Medicine, Osaka University, 2Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Saita, Osaka, Japan

**Background and Aim:** It was reported that Bone Marrow-derived Cells (BMDCs) differentiate into gastrointestinal epithelial cells in humans. Many researchers have reported the role of BMDCs in the regeneration of damaged gastrointestinal mucosa. However, the precise role of BMDCs during the regeneration of damaged gastrointestinal mucosa remains unknown. In this study, we examined the role of BMDCs in the regeneration of damaged colonic epithelium. Materials and Methods: lethally irradiated wild-type female mice (C57BL/6) were rescued by a bone marrow transplant from male green fluorescent protein (GFP)-transgenic mice (TgN(α-CAG-EF1α)LacZ) as donors. Chronic colitis were induced in this chimera mice by drinking water containing 3% dextran sodium sulfate (DSS) for 5 days at 4-6 weeks after bone marrow transplantation. On day 5, they were switched to regular drinking water. The animals were sacrificed on day 25. The phenotypes of BMDCs in colonic mucosa were examined by confocal microscopy and double-staining immunofluorescent immunohistochemistry for GFP, vimentin, cytokeratin, α-smooth muscle actin (α-SMA), CD45, F4/80, Ki-67, CD31. Results: GFP-positive, BMDCs were found in the vimentin-positive colonic interstitial cells, but not in the cytokeratin-positive epithelial cells. These cells express α-SMA (fibroblast/myofibroblast marker) and the morphology of these cells is spindle shape. And these cells did not express CD45-positive leukocyte and F4/80-positive macrophages. We could not detect BMDCs Ki-67-positive proliferating cells and CD31-positive endothelial cells. Conclusion: Our data show that BMDCs transdifferentiate into intestinal myofibroblasts in the regeneration of damaged colonic mucosa.

**II-P-9-144**

**Stable Antiulcer Gastric Pentadecapeptide BPC 157: The Relevance of Muscle Healing for Better Ulcer Healing**

P. Siksic, S. Seiwerth, B. Turlovic, S. Mise, T. Anic, R. Rucman, M. Petek

Department of Pharmacology, School of Medicine, University of Zagreb

Recently, stable antiulcer gastric pentadecapeptide BPC 157 (GEPPPPKGKDADLV, M.W. 1419) (PL-10, PLD-116, PL 14736 Pila, Croatia), in trials for inflammatory bowel disease, and wound therapy, no toxicity reported) given following complete transection of rat quadriceps muscle induces muscle healing promptly and then it maintains the healing along with full function restoration. The relevance of this muscle healing was challenged with respect to better ulcer healing, in the rat models with sphincter dysfunction or extensive muscle damage. It (sphincter lower esophageal (LES), pyloric (PS)) pressure. Esophagitis (tubes into sphincters for 1 week)-rats presents a marked sphincter dysfunction, constantly lessened LES- and PS-pressure. For both, LES and PS, BPC 157 (a) promptly increases the decreased values till the level in healthy, then, (b) maintains pressure preserved at the healthy level during 12-20 months period, while (c) in normal rats, it increases LES-, but decreases PS-pressure. (ii) Circular gastrootomy induces large flat areas without any fold, particular for injured stomach and muscle damage. BPC 157 improved folds presentation to nearly 100%, with desmin immunoreactive cell clusters (muscle regeneration) penetrating the granulation tissue, and stronger immunoreactivity in vessel walls surrounding transplantation. On day 5, they were switched to regular drinking water. The animals were sacrificed on day 25. The phenotypes of BMDCs in colonic mucosa were examined by confocal microscopy and double-staining immunofluorescent immunohistochemistry for GFP, vimentin, cytokeratin, α-smooth muscle actin (α-SMA), CD45, F4/80, Ki-67, CD31. Results: GFP-positive, BMDCs were found in the vimentin-positive colonic interstitial cells, but not in the cyto-keratin-positive epithelial cells. These cells express α-SMA (fibroblast/myofibroblast marker) and the morphology of these cells is spindle shape. And these cells did not express CD45-positive leukocyte and F4/80-positive macrophages. We could not detect BMDCs Ki-67-positive proliferating cells and CD31-positive endothelial cells. Conclusion: Our data show that BMDCs transdifferentiate into intestinal myofibroblasts in the regeneration of damaged colonic mucosa.

**II-P-9-145**

**Stable Gastric Pentadecapeptide BPC 157 in Trials for Inflammatory Bowel Disease (PL-10, PLD-116, Pila, Croatia). Heals Ileoleval – Anatomosis and Counteracts Corticosteroid-Negative Effect in Rat**

T. Vukic, B. Radic, R. Klicek, L. Broc, S. Seiwerth, P. Siksic

Department of Pharmacology, School of Medicine, University of Zagreb

Ileoleval anastomosis healing (after 1, 2, 3, 4, 5, 6, 7, 14 days) was assessed in rats in normal and impaired conditions: (i) adhesions (0-7 (neighboring loops, stomach, liver packed)), loop diameters, anastomosis arcade vessels, (ii) leak induction (the time (sec), the volume (ml) (through spraying-perfusion pump system 1 ml/10 sec) and the pressure (mmHg) catheter (BD Carefll F31 200mm, Becton Dickinson, USA) connected with chamber (BD Gabarit PMSET 1D-FXX, Becton Dickinson, USA) and monitor Sireuct 732 (Siemens, Germany) at 10 cm proximal to anastomosis), and (iii) microscopy. Treatment was once daily (first after surgery, last the Kimura Takemoto classification. Incidences of ulcers in the stomach, duodenum or both were highest in C-2 and -3. In stomachs with C-2 and -3 atopy, the borders of atopy cross the medial oblique muscle bundle and the border circular muscle bundle which form the lesser curvature and the angle. Incidences of gastric ulcers in C-2 and -3 were also highest at lesser curvature. Whereas, in stomachs with C-0 and -1, the border of atopy does not cross the medial oblique muscle bundle nor the border circular muscle bundle. The incidences of gastric ulcers in C-0 and -1 were low. Moreover, most of ulcers developed in O-1 in -2 were located at the anterior or posterior walls where the borders of atopy cross the medial oblique muscle bundle and the muscle bundle of incisura major superior muscle. Conclusion: It is suggested that IFP infection makes the extent of atrophy larger and atrophic border cross the medial oblique or the border circular muscle bundle, and result in ulcer formation along lesser curvature.
at 24 h before sacrifice), saline, BPC 157 (10 µg, 10 mg, 100 µg/kg i.p.) and/or 6-β-methylprednisolone (1 mg/kg i.p.). Results: BPC 157 clearly improves all parameters of anastomotic wound healing. Moreover, the low dose of pentadecapeptide BPC 157 without effect by itself, became effective combined with corticosteroids treatment that adversely affects healing of anastomoses in the rat. Conclusion: In inflammatory bowel disease (PL-10, PLD-116, Pliva, Croatia) pentadecapeptide BPC 157 is valuable after resection for anastomosis healing.

II-P-146
Circular Gastrostomy in Rat: A New Healing Model. Stable Gastric Pentadecapeptide BPC 157, Atropine, Cimetidine, Omeprazole
I. Zoricic, M. Sevec, B. Radić, A. Jakšić, L. Broć, T. Anić, S. Solomon, P. Sikiric
Department of Pharmacology, School of Medicine, University of Zagreb

Like gastric folds for stomach integrity, large flat areas without any fold could be particular for injured stomach and muscle damage. Circular gastrostomy was at 1 cm bellow rat cardia, only 5 mm at small curvature remained intact. Therapy (mg/kg) (gastric pentadecapeptide BPC 157 (in IBD, PLED-116, Pliva, Croatia) (0.01), cimetidine (50), atropine (10), omeprazole (50)) was i.p. once daily, first immediately after surgery, last at 24 h before sacrifice (at 1 h, 2h, 6h, 24h, 5 days, 7 days, 14 days). Results: Largely flattened stomach and only 40% area with thin gastric folds are along with poor healing of transected muscle, grossly and microscopically. Also, desmin immuno-histochemistry/muscle regeneration shows sharp demarcation of positive fibers on the muscle-granulation tissue border with only very scant immunoreactive fibers in vessel walls in the granulation tissue. All agents improved folds presentation, but only BPC 157 approaches to nearly 100%, with desmin immunoreactive cell clusters (muscle regeneration) penetrating the granulation tissue, and stronger immunoreactivity in vessel walls throughout the granulation tissue. Conclusion: BPC 157 is valuable for major stomach resection.

II-P-147
The Effects of Candida Albicans on Cysteamine-Induced Duodenal Ulcer at Phase of Healing in Rats
L. Jin1, M. Yoshida1, T. Nakamura1, H. Ishikawa2, G. Wakabayashi3, M. Tanabe3, S. Kawachi4, Y. Saiakawa1, K. Kameyama1, L. Kurnia1, T. Kubota1, K. Saro1, M. Kito1
1Department of Surgery, 2Department of Emergencies and Critical Care Medicine, First Department of Surgery, 3Department of Pathology, 4Center for Diagnostic and Therapeutic Endoscopy, Division of Comprehensive and Advanced Medicine, Iwate University School of Medicine

Background and Purpose: The low curability of Candida positive ulcers was reported as a result of clinical analysis (Morishita T et al: Acta Gastroent Latinoamer 1993:23:223–229). We examined the duodenal ulcer 7 days after cysteamine administration in order to investigate the effects of Candida albicans (C. albicans) on duodenal ulcer at phase of healing. Methods: In this study, male Wistar rats (220–250 g) were divided into the Candida group and the saline group. Rats were administrated cysteamine (350 mg/kg body weight) three times intragastrically every 4 h. The rats in the Candida group were administrated C. albicans 48 h after the first administration of cysteamine. In the control group, the rats were administrated saline. The rats were sacrificed one week after the first administration of cysteamine. The stomach was removed and opened along the greater curvature. The gastroduodenal ulcer was observed and the size of gastroduodenal ulcer was measured. The stomach was fixed in 10% formalin for histological examination revealed the presence of neither the hyphal formation nor the yeast of C. albicans in the C. albicans group. There was no significant difference between the vehicle group and the Candida group. The area of the duodenal ulcers was also smaller in the Candida group as compared to that in the vehicle group (p < 0.05). In addition, the survival rate of the rats was significantly higher in the micafungin group than in the vehicle group (p < 0.05). In the vehicle group, the ulcer base was found to be colonized by C. albicans, surrounded by marked granulocytic infiltration. However, histological examination revealed the presence of neither hyphal formation nor the yeast of C. albicans in the Candida group. Conclusion: It was shown in the present study that intravenous injection of micafungin counteracts the aggravation of cysteamine-induced duodenal ulcers caused by C. albicans in rats. This data may support the concept of Candida as an aggravating factor of peptic ulcers.

II-P-148
The Effects of Micafungin on the Severity of Duodenal Ulcers Induced by Cysteamine and Candida Albicans Administration in Rats
1Department of Surgery, 2Department of Emergency and Critical Care Medicine, 3Division of Diagnostic Pathology, 4Center for Diagnostic and Therapeutic Endoscopy, 5Center for Comprehensive and Advanced Medicine, Keio University School of Medicine, 6First Department of Surgery, Iwaste University School of Medicine

Introduction: C. albicans is often positive in the culture of ascites from the patients with upper gastrointestinal ulcer perforation, but its importance has not been investigated well. We previously proved that Candida infection aggravates duodenal ulcer perforation experimentally. We investigated whether or not micafungin, an anti-fungal drug, administrated intra-venously has the potential to attenuate the severity of duodenal ulceration induced by intra-gastric administration of cysteamine, duodenal ulcerogen, and Candida albicans. Materials and Methods: Cysteamine was administered thrice on day 1 to Male Wistar rats. C. albicans was administrated at a density of 108 in 0.5 ml of saline 1 h before, and 12 and 24 h after the first administration of cysteamine. Autopsies were performed 72 h after cysteamine administration for assessment of the size and depth of gastroduodenal ulcers. Micafungin at the dose of 10 mg/kg (the micafungin group and the vehicle group (n = 22)), or saline (the vehicle group (n = 24)) were administrated 1, 2, 4, 8 h after the administration of cysteamine. Results: In the present study that the infection of C. albicans not only promote the process of duodenal ulcer perforation but also the delayed healing process of duodenal ulcer induced by cysteamine in rats. We investigated whether or not micafungin, an anti-fungal drug, administrated in a venously has the potential to attenuate the severity of duodenal ulceration induced by intra-gastric administration of cysteamine, duodenal ulcerogen, and Candida albicans.

II-P-149
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Materials and Methods: Cysteamine was administered thrice on day 1 to Male Wistar rats. C. albicans was administrated at a density of 108 in 0.5 ml of saline 1 h before, and 12 and 24 h after the first administration of cysteamine. Autopsies were performed 72 h after cysteamine administration for assessment of the size and depth of gastroduodenal ulcers. Micafungin at the dose of 10 mg/kg (the micafungin group and the vehicle group (n = 22)), or saline (the vehicle group (n = 24)) were administrated 1, 2, 4, 8 h after the administration of cysteamine. Results: In the present study that the infection of C. albicans not only promote the process of duodenal ulcer perforation but also the delayed healing process of duodenal ulcer induced by cysteamine in rats. We investigated whether or not micafungin, an anti-fungal drug, administrated in a venously has the potential to attenuate the severity of duodenal ulceration induced by intra-gastric administration of cysteamine, duodenal ulcerogen, and Candida albicans.
II-P-10-151
HGF Treatment Reduces Mucosal Inflammation and Apoptosis on Rat TNBS and DSS Models
Y. Ohida, K. Hosoi, T. Torii, N. Hido, K. Fukunaga, Y. Jinno, T. Kosa, Y. Fukuda, H. Misawa, T. Matsumoto
Division of Gastroenterology, Department of Internal Medicine, Hyogo College of Medicine

Background: Although hepatocyte growth factor (HGF) was originally identified as a hepatotropic factor, it could function as an epithelial growth factor. We examined the therapeutic effects of HGF on rat inflammatory bowel disease (IBD) models. Methods: We used two different colitis models induced by trinitrobenzenesulfonic acid (TNBS) and dextran sulfate sodium (DSS). Recombinant human HGF was continuously administered at a dose of 50 μg/day using an intraperitoneal implanted pump for 7 days. The effects of HGF were assessed by macroscopic observation, colonic length shortening, histological damage score, epithelial mitotic rate, mucosal myeloperoxidase (MPO) activity, immunohistochemical labeling indices of epithelial cell proliferation (bromodeoxyuridine (BrdU), Ki-67, proliferating cell nuclear antigen (PCNA)), apoptotic cell count, and the pro-inflammatory cytokine levels such as TNF-α and IFN-γ. Results: Ulcerative area, histological damage scores, MPO activities, and apoptotic cell counts in the HGF-administered group were significantly lower than those in the control group in both models. Body weight loss and colonic length shortening in the HGF-administered group were significantly lower than those in the control group in DSS model. Moreover, the levels of TNF-α and IFN-γ in the HGF-administered group were lower than those in the control group. There were no significant differences of labeling indices of epithelial cell proliferation. Interestingly, mitotic rates in the HGF group were significantly lower than those in the control group in TNBS model and showed a tendency to be lower in DSS model. We then examined the epithelial localization of the HGF receptor c-met and identified it on the surface epithelia, where apoptosis was observed, but did not find in the proliferative zone. Conclusion: These results suggest that HGF exhibits the therapeutic effects via the anti-inflammatory reactions and anti-apoptotic function rather than epithelial cell proliferation for these IBD models.

II-P-10-152
Reg I Knockout Mice Reveal its Role in Regulation of Cell Growth that is required in Generation and Maintenance of the Villous Structure of Small Intestine
T. Ozawa, K. Kadowaki, H. Fujikawa, H. Kazumori, S. Ishihara, Y. Kinosita
Department of Gastroenterology and Hepatology, Shimane University, School of Medicine

Background: Regenerating gene product 1 (Reg I) is a growth factor involved in pancreatic and gastric regeneration. We recently created Reg I transgenic mice to demonstrate that Reg I has an activity to direct the differentiation of the gastric stem cells into chief cell and parietal cell lineages as well as the activity to stimulate growth. On the other hand, mouse Reg I mRNA is expressed in the gastrointestinal tract at the highest levels in the small intestine, suggesting a critical role in the tissue. Method: To clarify the role of Reg I protein in the small intestine, we investigated the location of the Reg I-expressing cells and determined its temporal expression pattern during embryogenesis. We also analyzed the small intestinal tissue of Reg I knockout mice for histological changes and the state of cell proliferation and migration. Results: In the wild-type mice, immunohistochemistry localized Reg I protein expression in absorptive cells located in the lower half of the intestinal villi. Reg I expression was undetectable up to embryonic day 13, when the fetal intestine still lacks villus structure, however, it dramatically increased at embryonic day 15 and 17 as the fetal intestinal villi form. In the small intestine of the adult Reg I knockout mice, the morphology of the absorptive cells was aberrantly round-shaped. Further examination by electron microscopy revealed a strikingly loose connective tissue of the basement membrane. Anti-c-met (HGF receptor) staining and anti-Ki-67 staining demonstrated the marked decrease in the number of proliferating cells in the small intestinal mucosa of the knockout mice. The cell migration speed visualized by one shot labeling of BrdU was significantly slower in the knockout mice. These phenotypes of Reg I knockout mice emerged in accordance with the temporal pattern of Reg I expression, at embryonic day 17. Conclusion: Reg I was considered to be a regulator of cell growth that is required to generate and maintain the villous structure of the small intestine.

II-P-10-153
Role of Pineal Gland and Endogenous Prostaglandins in Gastroprotection and Ulcer Healing Activity of Melatonin and its Precursor, L-Tryptophan
Department of Physiology, Jagiellonian University Medical College, Cracow, Poland, 1Department of Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany

Melatonin, a major hormone of pineal gland, exhibits protectoceptive and ulcer healing activities but the role of pineal gland, an important source of melatonin, in these actions remains controversial. We compared the effects of i.g. or i.c.v. melatonin and its precursor, L-tryptophan in rats with or without removed pineal gland (pinealectomy) on gastric lesions induced by water immersion restraint stress (WRS) and healing of gastric ulcers (acetic acid, ulcer area ≈ 28 mm2). At 3.5 h after the end of WRS or 9 days after ulcer induction, gastric lesions and ulcers were measured by planimetry, gastric blood flow (GBF) was determined by H2-gas clearance technique and plasma melatonin and gastrin levels were measured by specific RIA. Biopsy mucosal samples were taken for determination of expression of mRNA for COX-1 and COX-2 by RT-PCR. Melatonin (1.25-10 mg/kg i.g. or 1.25-10 mg/kg i.c.v.) dose-dependently attenuated the WRS-induced gastric damage and accelerated ulcer healing while raising GBF, plasma melatonin and gastrin levels and mucosal generation of PGE2. Pinealectomy, which suppressed plasma melatonin levels, aggravated the gastric lesions induced by WRS and significantly delayed ulcer healing. Indomethacin and rofecoxib, which diminished PGE2 biosynthesis by 80% and 95%, respectively, or luzindole, an antagonist of Mel2 receptors, significantly attenuated melatonin- and L-tryptophan-induced protection and the rise in the GBF. COX-1 mRNA was detected by RT-PCR in the intact and melatonin-treated gastric mucosa, while COX-2 mRNA appeared in melatonin-treated animals. We conclude that 1) exogenous melatonin and its precursor, L-tryptophan, attenuate WRS-induced gastric lesions via interaction with MT2 receptors and due to an enhancement of gastric microcirculation, probably mediated by PGE2 derived from COX-2 overexpression and activity, and 2) the pineal gland plays an important role in the limitation of WRS-induced gastric lesions and acceleration of ulcer healing via releasing melatonin, which exerts gastroprotective and ulcer healing actions.

II-P-10-154
Leptin Promotes Angiogenesis via Induction of Proangiogenic Growth Factors During Gastric Ulcer Healing
T. Tanigawa, T. Watanabe, E. Sasaki, M. Shibata, K. Tominaga, Y. Fujisawa, N. Oishi, K. Higuchi, T. Arakawa
Department of Gastroenterology, Osaka City University Graduate School of Medicine

Background and Aim: Leptin, a key mediator of food intake and energy metabolism, has been shown to be involved in cutaneous wound healing. In this study we investigated the role of leptin in healing of acetic acid-induced gastric ulcer using leptin-deficient mice. Methods: In C57BL/6J wild-type and leptin-deficient mice (ob/ob mice), gastric ulcers were produced by focal ischaemic application of acetic acid. Mouse recombinant leptin (0.5 μg/g BW) or vehicle (normal saline) was given intraperitoneally twice daily to ob/ob mice from 3 days after ulceration for up to 7 days. Stomach was removed and ulcer size was measured by day 7 of treatment. Expression of mRNA for leptin, leptin receptor, vascular endothelial growth factor (VEGF) and basic-fibroblast growth factor (bFGF) in ulcerous tissue and normal mucosa were assessed by real-time RT-PCR. Localization of leptin, leptin receptor, and VEGF was evaluated by immunohistochemistry. Results: In wild-type mice, ulceration increased mRNAs for leptin receptor, VEGF, and bFGF, whereas it did not change mRNA level of leptin. The levels of mRNAs for VEGF and bFGF in ulcerous tissue were lower in ob/ob mice than in wild-type mice. In ob/ob mice, restoration of leptin reversed the delayed expression of gastric ulcer healing, accompanied by upregulation of mRNA expression of VEGF and bFGF. The number of microvessels in the ulcer bed identified by immunohistochemical staining of CD31 (an endothelial cell marker) was reduced. Genetic disruption of leptin resulted in delay of ulcer healing. In wild-type mice, ulceration increased mRNAs for leptin receptor, VEGF, and bFGF, whereas it did not change mRNA level of leptin. The levels of mRNAs for VEGF and bFGF in ulcerous tissue were lower in ob/ob mice than in wild-type mice. In ob/ob mice, restoration of leptin reversed the delayed expression of gastric ulcer healing, accompanied by upregulation of mRNA expression of VEGF and bFGF. The number of microvessels in the ulcer bed was smaller in ob/ob mice than in wild-type mice. These results suggest that leptin contributes to gastric ulcer healing via upregulation of proangiogenic growth factors.
In the present study, we investigated the effects of subtype selective EP agonists and antagonists on the healing of gastric ulcers and investigated the possible mechanism involved in this action. Gastric ulcers were produced in male SD rats by thermocauterization under ether anesthesia. Various COX inhibitors were given s.c. once daily for 10 days starting 3 days after ulceration. Various EP agonists were given s.c., with or without indomethacin. Gastric fibroblasts were isolated from rat stomachs. Ulcer healing was significantly delayed by daily administration of indomethacin and rofecoxib (a selective COX-2 inhibitor), but not by SC-560 (a selective COX-1 inhibitor). Mucosal PGE2 content was markedly increased after ulceration, and this response was significantly suppressed by administration of rofecoxib but not SC-560. The delayed healing caused by administration of indomethacin was significantly reversed by co-administration of 11-deoxy PGE2 (a EP3/EP4 agonist), but not by others including EP1, EP2 and EP3 agonists. Daily administration of compound X (a EP4 agonist), also significantly delayed the healing of gastric ulcers in rats and mice, as effectively as rofecoxib did. Furthermore, the expression of VEGF protein was up-regulated in the ulcerated mucosa. The intensity of VEGF expression in the ulcerated mucosa was further enhanced by AE1-329 (a EP4 agonist), and suppressed by indomethacin, rofecoxib and the compound X, but not SC-560. On the other hand, the expression of VEGF in gastric fibroblasts was also increased by the incubation with PGE2, and AE1-329, and these changes were inhibited by the pre-incubation with the compound X. These results demonstrated that COX-2/PGE2 plays an important role in the healing process of gastric ulcers, and further suggested that this action of PGE2 is mediated by the activation of EP4 receptors through stimulation of VEGF expression in the gastric fibroblasts.

**II-P-11-155**

**Prostaglandins/Cyclooxygenase (COX) 2 Contribute to Healing of Gastric Ulcers through Up-Regulation of VEGF via EP4 Receptors**

R. Hatada, A. Tanaka, Y. Tashima, M. Tanigami, K. Takeuchi

Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yarnashina, Kyoto, Japan

In the present study, we investigated the effects of subtype selective EP agonists and antagonists on the healing of gastric ulcers and investigated the possible mechanism involved in this action. Gastric ulcers were produced in male SD rats by thermocauterization under ether anesthesia. Various COX inhibitors were given s.c. once daily for 10 days starting 3 days after ulceration. Various EP agonists were given s.c., with or without indomethacin. Gastric fibroblasts were isolated from rat stomachs. Ulcer healing was significantly delayed by daily administration of indomethacin and rofecoxib (a selective COX-2 inhibitor), but not by SC-560 (a selective COX-1 inhibitor). Mucosal PGE2 content was markedly increased after ulceration, and this response was significantly suppressed by administration of rofecoxib but not SC-560. The delayed healing caused by administration of indomethacin was significantly reversed by co-administration of 11-deoxy PGE2 (a EP3/EP4 agonist), but not by others including EP1, EP2 and EP3 agonists. Daily administration of compound X (a EP4 agonist), also significantly delayed the healing of gastric ulcers in rats and mice, as effectively as rofecoxib did. Furthermore, the expression of VEGF protein was up-regulated in the ulcerated mucosa. The intensity of VEGF expression in the ulcerated mucosa was further enhanced by AE1-329 (a EP4 agonist), and suppressed by indomethacin, rofecoxib and the compound X, but not SC-560. On the other hand, the expression of VEGF in gastric fibroblasts was also increased by the incubation with PGE2, and AE1-329, and these changes were inhibited by the pre-incubation with the compound X. These results demonstrated that COX-2/PGE2 plays an important role in the healing process of gastric ulcers, and further suggested that this action of PGE2 is mediated by the activation of EP4 receptors through stimulation of VEGF expression in the gastric fibroblasts.
II-P-11-160
Epithelial Carbonic Anhydrases Facilitate Disposal of Luminal Acid as CO₂ in Rat Duodenum
M. Mazumor, Y. Aibas, J. Meyerowitz, C.T. Supuran, Ph. Guth, E. Engel, J.D. Kaufitz Department of Medicine, West LA VA Medical Center, CURF-UCLA Med Sch, Brentwood Biomed Res Inst, Los Angeles, USA, Università di Firenze, Firenze, Italy

In the duodenum, the neutralization of H⁺ with secreted HCO₃⁻ generates CO₂, >500 Torr, which dissipates in the jejunum, implying that the duodenum absorbs almost all secreted gastric acid and HCO₃⁻. We hypothesized that duodenal acid disposal is facilitated by ecao isoforms. We examined the effect of novel cell-impermeant, specific ecao inhibitors on CO₂ diffusion into the duodenum. Rat duodenum were perfused with different pH and pCO₂ solutions with or without or cell-impermeant CA inhibitors (CAI, 0.1 μM), or permeant methanolamine (MTZ, 1 mM). Flow-through pH and pCO₂ electrodes simultaneously and continuously measured perfusate and effluent pH and pCO₂. Portal venous (PV) pCO₂ and pH were analyzed at the end of the luminal challenge. Furthermore, ¹³C-labeled high CO₂ solution was perfused to measure PV ¹³C/¹²C. Luminal perfusion with high CO₂ solution (pCO₂ 260 Torr) increased net CO₂ loss from the perfusate compared with controls (pH 6.4, pCO₂ 0) accompanied by PV acidification and pCO₂ increase. Impermeant CAI abolished perfusate net CO₂ loss, but increased HCO₃⁻ secretion, whereas all CAI inhibited PV acidification and pCO₂ increase. Net CO₂ loss during perfusion of 30% CO₂ solution (pH 6.5) was delayed by impermeant CAI and abolished by MTZ. Perfused ¹³CO₂ was increased in PV 10 min after the challenge with PV pH decrease, inhibited by all CAI and HCO₃⁻ loss from the perfusate were accompanied by increases of PV H⁺ and tracer CO₂, but unchanged PV total CO₂ consistent with transmucosal net H⁺ and tracer CO₂ movement, inhibited by impermeant CAI. The duodenum absorbs luminal acid by neutralization with secreted HCO₃⁻ to CO₂, absorption of CO₂, cytosolic conversion of CO₂ to H⁺ and transport of cellular H⁺ to the subepithelium and to the PV. Thus, the duodenum, like the red cell, absorbs H⁺ as CO₂ with CA-mediated interconversion between CO₂ and HCO₃⁻.

II-P-11-161
Immunohistological Analysis of Distribution of the Histamine H3 Receptor in the Gastric Mucosal Tissue of the Rat
M. Abe¹, H. Ajioka¹, S. Kitano¹, M. Nami¹, T. Kinmoto¹, S. Hori², T. Oka¹
¹TAINO Pharmaceutical Co., Ltd., Pharmaceutical Research Laboratory, 7Josai International University, Faculty of Pharmaceutical Sciences, Department of Medical Pharmacy

Objective: The histamine H3 receptor (H3-R) has been reported to be present in the enterochromaffin-like (ECL) cells in the stomach, but precise distribution and function have been little elucidated. Immunohistological analysis of H3-R in the stomach has been considered difficult, in this study, we tried to elucidate the distribution of H3-R in the stomach by using immunohistological technique. Method: We obtained stomach tissue sample from over-night-fasted Ctrl CO (SD) male rats (8-week-old). We used the modified method described by Shishido et al. for fixation and staining. The prepared sections were stained with hematoxylin-eosin (HE) and immunostained with anti-histamine antibody, anti-H₁, K⁺/ATPase antibody, and anti-H₁-R antibody to examine the tissue morphology of the stomach. Results and Discussion: ECL cells in the fundic glands stained well by histamine staining. We then used a new approach and fixed the tissue briefly by perfusion with it 4% paraformaldehyde solution supplemented with 10% saturated picric acid. We also tried immersion fixation briefly with 4% paraformaldehyde and 80% ethanol, and then embedded the sample in paraffin for 24 h. The perfusion method allowed good immunostaining for H₁-R. H₁-R immunostaining-positive sites were also identified in the vicinity of the parietal cells. In addition to shishido’s report that is, existence of H₁-R in ECL cells, it was appeared that H₁-R exists in parietal cells from our studies. From our results, it was appeared that H₁-R in the parietal cells plays same important a role in acid secretion.

II-P-11-162
Stimulatory Effect of Sparkling Water on Gastric and Duodenal HCO₃⁻ Secretion in Rats
Y. Sasaki, E. Abaharu, Y. Nagumo, F. Ito, K. Takeuchi
Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasaig, Yarashina, Kyoto, Japan

Background and Aim: Recent study demonstrated that luminal CO₂ diffused in the duodenal epithelial cells, followed by HCO₃⁻ secretion, and the latter process is regulated by carbonic anhydrase and dependent on a functioning basolateral Na⁺/H⁺ exchanger and endogenous prostaglandins (PGs). However, it remains unknown whether the same occurs in the stomach. In the present study, we examined the effect of sparkling water on HCO₃⁻ secretion in the stomach and duodenum and investigated the factors involved in this response. Methods: Under urethane anesthesia, a chambered stomach or a duodenal loop was perfused with saline, and the HCO₃⁻ secretion was measured at pH 7.0 using a pH-stat method and by adding 2 mM HCl. Results: The mucosal perfusion with sparkling water (pH 4.5 and 7.5 g/CO₂) markedly increased the secretion of HCO₃⁻ in both the stomach and duodenum. The HCO₃⁻ response in the duodenum was partly inhibited by indomethacin, acetazolamide or sensory deafferentation, and totally abolished by co-administration of indomethacin and acetazolamide. In contrast, the HCO₃⁻ stimulatory action on the stomach was totally inhibited by acetazolamide and partially mitigated by indomethacin but not by sensory deafferentation. Moreover, 5(N,N-dimethyl)amirolide, an inhibitor of NHE1 and NHE2, partially mitigated the HCO₃⁻ response induced in the duodenum but not the stomach by sparkling water. Similar results were obtained by Coca-cola (pH 2.5, 7.1 g/CO₂), although the HCO₃⁻ stimulatory effect of Coca-cola was significantly suppressed by indomethacin in both the stomach and duodenum. Conclusion: These results suggest that sparkling water induces HCO₃⁻ secretion in both the stomach and duodenum, but the mechanism differs between these two tissues; the response in the latter is mainly due to the response is mediated by both endogenous prostaglandins and capsaicin-sensitive afferent neurons, probably related to the acidic pH of the solution, in addition to the intracellular supply of HCO₃⁻.

II-P-12 Gastrointestinal Motility and Functional Disorder-clinical
Chairpersons: T. Mine, M. Kusano

II-P-12-163
The Reduction of Gastric Motility and Secretion with the use of Octreotide for the Patients with Cystogastrectomy (Jurasz Operation)
A. Shvetsev, D. Tshuder
HPB-Surgical Department, The Central Clinical Hospital/1 of the OAO RZD (Russian Railways)

Nowadays cystogastrectomy (Jurasz operation) is carried out very seldom, but sometimes it is the unique one for the patients with pancreatic cysts. The reason to reduce the number of this surgery is the high rate of postoperative complications. The most often occurring complications are abscess formation (inside of cysts) and the bleeding from the anastomosis. These complications are interconnected. The improvement of the safety of this surgery will increase the number of these (much easier) surgeries. According to the basic data octreotide reduces the upper GI-motility and secretion. The purpose of this study is to assess the use of octreotide for the patients with Jurasz operation. From December 2003 till December 2005 4 patients with pancreatic cysts had Jurasz operation. During postoperative period all patients were treated with octreotide (7 postoperative days, 100 mg/kg 3 times/day s.c. plus 500 mg/day i.v. bolus). All patients had nano-gastric-cystic intubations postoperatively (7 days). The restoration of bowel peristalsis was registered on the fifth postoperative day (clinically and US). The gastric volume (through naso-cysto-gastric tube) was 500 ml/day. As a result, no postoperative complications were noted. The absence of cystic cavity was confirmed by the X-ray films. All patients were discharged on the seventh postoperative day. The long term results (30 days and 45 days after surgery) demonstrated the absence of pancreatic cysts, upper GI-motility disorders, and other upper GI malfunctions. These results show that the use of octreotide in postoperative period could reduce the number of postoperative complications for the patients with Jurasz operation and hospital stay. The reason of these good results is the reduction of gastric motility and secretion.
II-P-12-164  
Study to Ascertain Whether Omeprazole (Omepral(R)) has an Add-on Effect or Modifying Effect for Epigastric and Thoracic Symptoms  
T. Suzuki,1, T. Konagaya,2, H. Imamura,1, H. Kaneko1, H. Ohira1, N. Kanasu1, A. Shinoda1, H. Imamura4, Y. Ikeda4  
1Division of Gastroenterology, Tokyo Metropolitan Police Hospital, 2Tokyo Kosei Nenkin Hospital  

Purpose: To ascertain whether Omepral is effective or not for the treatment of patients with epigastric and thoracic symptoms. Subjects: Forty one patients with epigastric and thoracic symptoms. At the time of the start of the study, all of the subjects exhibited symptoms despite doses of stomach medicine. Methods: All of the subjects were graded for degree of epigastric and thoracic symptoms and whether they suffered from sleep disturbance or not. From the same day subjects were told to take continuous doses of Omepral (20mg/day), make a record of any changes in symptoms, and report back to the hospital after 2 weeks. Subjects who had previously received doses of H2 blockers or another proton pump inhibitor (PPI) changed to a regime of doses of Omepral, while subjects taking doses of mucosal membrane protective agents were given add-on doses of Omepral. In this study, whether any difference in effect with regard to pain and discomfort in different regions of the upper abdomen and chest (5 locations) was also investigated. Results: (1) Previous done details: H2 blockier Group 19, H2 blockier + mucosal membrane protective agent Group 11, mucosal membrane protective agent Group 7, PPI other than Omepral Group 4 subjects. (2) Changes in symptoms: for heartburn, a significant improvement in symptoms was observed after 1 week of administration. For the 3 symptoms of pain, reflux symptoms and discomfort, a significant improvement was observed the first day after administration. (3) Symptom evaluation by location: No significant change in scores was observed between locations for the evaluation of pain and discomfort. (4) An improvement of sleep conditions was observed due to doses of Omepral. Conclusions: This study indicated that add-on effect and modifying effect of Omepral clearly improved epigastric and thoracic symptoms.

II-P-12-165  
Study of the Relationship between Functional Dyspepsia and Mild Depression Using the SRQ-D  
Y. Soyama, T. Suzuki, S. Nishimura, H. Saegusa, M. Hirano  
Division of Gastroenterology, Tokyo Metropolitan Police Hospital  

Background: It has been presumed that Functional dyspepsia is strongly associated with psychological factors. Therefore there is considerable overlap of not only epigastric symptoms but also non-specific symptoms like depression or sleep disturbance in patients with FD. However, in fact, how much of a causal relationship there is between FD and emotional factors is still in question. Methods and Subjects: Between 2003 and 2005, 129 Japanese FD patients who visited our hospital answered the Self-Rating Questionnaire for Depression (SRQ-D) as well as a questionnaire on their sleep. We scored the SRQ-D on a basis of 54 points and diagnosed patients with scores of 16 and over as having mild depression. In the same way, we scored the SRQ-D as well as a questionnaire on their sleep. We scored the SRQ-D on a basis of 54 points and diagnosed patients with scores of 16 and over as having mild depression. In the same way, we scored the SRQ-D as well as a questionnaire on their sleep. We scored the SRQ-D on a basis of 54 points and diagnosed patients with scores of 16 and over as having mild depression. We also selected patients with both dyspepsia and depression. Results: In this study it was suspected that 36 of the 129 patients had a correlation between depression and depression. In this study it was suspected that 36 of the 129 patients had a correlation between depression and depression. Conclusions: In this study it was suggested that 36 patients had correlation between the SRQ-D and FD. This study suggested that 36 patients had correlation between the SRQ-D and FD. Furthermore, clinical characteristics could not explain the mild depression and sleep disturbance in FD as no significant statistical differences were observed.

II-P-12-166  
Effects of Psychological Stress on Rectal Hyperalgesia and Brain Blood Flow in Patients with Irritable Bowel Syndrome  
T. Kanaeza1, T. Konagaya1, H. Imamura1, H. Kaneko1, H. Ohira1, N. Kanasu1, A. Shinoda1, S. Fukuyama2  
1Department of Gastroenterology, Aichi Medical University, 2Graduate School of Environmental Studies, Nagoya University, 2Chubu Medical Center for Prolonged Traumatic Brain Dysfunction, Kizawa Memorial Hospital  

Brain image studies have demonstrated that the difference of brain regions activated during continuous mental arithmetic stress, was assigned during 4min, changes in brain blood flow were evaluated using H215O-water positron emission tomography 3) Rectal pain and stress level with VAS were recorded before and after the respective stress load. Results: 1) Average pain threshold level was significant lower in IBS (14.4mmHg) than that in healthy volunteers (26.3mmHg). 2) A significant increase in blood flow was observed in insula at rectal stress alone, in derostral prefrontal cortex and lingual gyrus at additional psychological stress in IBS as compared with that in healthy volunteers. In contrast, that was observed in cerebellum and pons under mixed stress load in healthy volunteers. 3) Physical stress level at rectal stress alone and rectal pain level at additional psychological stress were significant higher in IBS. Conclusions: In IBS patients, rectal pain threshold level was significant lower, and rectal distention-induced activation in insula area was apparent. An additional psychological stress enhanced rectal pain with a different change in brain activation area as compared with that in healthy volunteers. Deviated brain-gut interaction in patient with IBS may be one of the important pathophysiologies of this syndrome.

II-P-12-167  
Correlation of Plasma Ghrelin Levels and Delayed Gastric Emptying Time in FD Patients with Loss of Emptiness Feeling  
Third Department of Internal Medicine, Nippon Medical School  

Background and Aims: Since functional dyspepsia (FD) seems to be caused by multifactorials such as gastric motility, depression, and visceral hypersensitivity, its clinical symptoms are complex and resistant to cure. Gastric emptying has been reported to be delayed in some FD patients. However, the involvement of gut hormones and local cytokines in gastric emptying has yet to be elucidated in these FD patients. Thus, we measured gastric emptying time and plasma ghrelin levels, one of the motility factors released into the peripheral circulation from the stomach, and examined the relationship between these data and symptom patterns in FD patients. Methods: Forty seven patients with FD and 30 healthy controls were enrolled according to Rome II criteria after endoscopy for this study. 47 FD patients were separated into three groups: subjects with symptoms of dysmotility-like dyspepsia (dysmotility group), ulcer-like dyspepsia (ulcer-like group) and unspecified dyspepsia (unspecified group). Gastric motility was evaluated by measuring gastric emptying time using 13C-acetate breath test, and partly assessed by abdominal ultrasound. Upper abdominal symptoms including pain, nausea, fullness, discomfort, and emptiness were assessed by questionnaire scores. Scores from the Self-rating Questionnaire for Depression (SRQ-D) were also used to evaluate depression. Plasma ghrelin levels were measured using ELISA method in patients with FD. Results: Gastric emptying time in the dysmotility group (33% of FD) was significantly delayed compared to the healthy control group (58.6± 3.6 vs. 46.2± 0.6 min, p< 0.05), while emptying time in the ulcer-like (14% of FD) and unspecified (53% of FD) groups did not differ significantly from healthy controls. In contrast, SRQ-D scores for the ulcer-like and unspecified groups (11.7± 2.5 and 11.7± 2.5, respectively p< 0.05), were significantly higher than healthy controls, while scores for the dysmotility group did not differ from that of the healthy control group. One of the symptoms of FD patients with delayed gastric emptying time was characterized as loss of emptiness feeling compared to patients with normal gastric emptying time (3 ± 1.5 vs. 8.9 ± 1.3, p< 0.05). Moreover, loss of depression did not differ between FD patients with normal and delayed gastric emptying. Furthermore, levels of ghrelin phenotypes, acylated and desacylated ghrelin, decreased significantly in patients with delayed gastric emptying time compared with those in patients with normal gastric emptying time. Conclusion: Our results suggest that decreased plasma ghrelin levels might be involved in delayed gastric emptying time and loss of emptiness feeling in FD patients.

II-P-12-168  
Effects of Red Chili on Rectal Sensation in Healthy Humans: The Mechanism of Actions  
S. Gonlachanvit1, P. Fongkam2, S. Wittayalertpanya2  
1Department of Internal Medicine, GI Motility Research Unit, 2Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand  

Background: Patients with functional bowel disorders often complain fecal urgency or rectal burning symptom after red chili ingestions. However, the effects of red chili on rectal sensation has not been well explored. Aim: To determine the effects of red chili on rectal sensation in humans that have not been well explored. Methods: Fourteen healthy humans (9 M, age 38 ± 10year) underwent 3 barostat studies under 3 conditions; 1) the oral placebo with intrarectal NSS (15ml), 2) the oral chili with intrarectal placebo, 3) the oral chili with intrarectal NSS. Results: For the 3 symptoms of pain, rectal burning symptom after red chili ingestions. However, the effects of red chili on rectal sensation has not been well explored. Aim: To determine the effects of red chili on rectal sensation in humans that have not been well explored. Methods: Fourteen healthy humans (9 M, age 38 ± 10year) underwent 3 barostat studies under 3 conditions; 1) the oral placebo with intrarectal NSS (15ml), 2) the oral chili with intrarectal placebo, 3) the oral chili with intrarectal NSS. Our results suggest that decreased plasma ghrelin levels might be involved in delayed gastric emptying time and loss of emptiness feeling in FD patients.
intrarectal NSS, and 3) the oral chili with intrarectal lidocaine viscous (2%, 15ml). Slow ramp and rapid phasic distensions of ascending method of limits' protocols were performed using an electronic barostat and a catheter with a 500 ml polyethylene bag. Rectal perception thresholds and compliances were compared. Results: Red chili ingestion significantly increased rectal threshold for first sensation of urgency, moderate urgency, and severe urgency during slow ramp distensions (21 ± 1 vs. 17 ± 1, 27 ± 1 vs. 23 ± 1, and 35 ± 1 vs. 31 ± 1 mmHg, respectively, p < 0.01) and rapid phasic distensions (22 ± 1 vs. 18 ± 1, 31 ± 1 vs. 24 ± 1, and 45 ± 1 vs. 38 ± 1 mmHg, respectively, p < 0.01) compared to placebo without significant effect on rectal compliance (p > 0.05). Geumnetrin and intrarectal lidocaine significantly reversed these effects (p < 0.05).

Conclusion: Red chili ingestion increases rectal perception in response to slow ramp and rapid phasic distensions, which are reversed by intrarectal lidocaine and geumnetrin. These results suggest that red chili increase rectal perception by its action onafferent receptors in the rectal mucosa, which mediates via 5HT3 pathways.

**II-P-13 Helicobacter pylori- Basic 2**
Chairpersons: T. Azuma, K. Murakami

**II-P-13-169 Helicobacter pylori Infection Increases Reactive Oxygen Production in Peripheral Blood**
Biochemistry and Molecular Pathology, *Gastroenterology, Osaka City University Medical School*

**Background:** Helicobacter pylori (H. pylori) infection causes various diseases in the gastrointestinal tract. Some reports have already been reported that reactive oxygen species (ROS) production was increased in local gastric mucosa. Recent studies have suggested that H. pylori infection may also cause other systemic diseases, including hematological disorders, skin and cardiovascular injury, by unknown mechanisms. We hypothesized that ROS contribute to the etiology of these systemic diseases other than gastrointestinal disorders in H. pylori infection.

**Methods:** Production of ROS was determined in peripheral blood samples from 86 patients (34 H. pylori-negative and 52 H. pylori-positive subjects) using a highly sensitive chemiluminescence probe [8-amin-5-chloro-7-phenylpyridazin-4(4H)-pyridazine-1 and 4 (2H, 3H) dione] (L-012).

**Results:** ROS production was significantly higher in individuals with H. pylori infection than in those without such infection (non-H. pylori-infected: 1.76 ± 1.26 kcpm; H. pylori-infected: 3.98 ± 3.21 kcpm, p < 0.005). Enhanced production of ROS was decreased significantly after eradication of H. pylori (Paired t-test: p = 0.01). No correlation was found between the extent of ROS production and sex, age, smoking status, alcohol ingestion, use of medications, or serum level of C-reactive protein.

**Conclusion:** These findings suggest the possibility of involvement of enhanced ROS production in circulating blood in the etiology of various systemic diseases in patients with H. pylori infection.

**II-P-13-170 Alterations in Gastric Mucous Cell Kinetics. Part of the Defense System Against H. pylori Infection in the Regenerating Zone**
A. Tanaka1, H. Ota2, T. Katsuya3, M. Hayama2, K. Tokunaga1, H. Ishida1, S. Takahashi1
1The Third Department of Internal Medicine, Kyorin University School of Medicine, Tokyo, 2Department of Biomedical Laboratories Science, School of Health Sciences, Shinshu University, 3Department of Laboratory Medicine, Shinshu University School of Medicine, Matsumoto, Japan

**Background:** Gastric mucosa has two types of mucous cells: surface mucous cells (SMCs) and gland mucous cells (GMCS). H. pylori do not attach to GMCS and mucins from GMCS inhibit the movement of H. pylori in the gastric mucous gel layer. H. pylori increase the cell kinetics of gastric epithelial cells. Aim: To examine the effect of H. pylori infection on gastric epithelial cell kinetics from the viewpoint of the phenotype of gastric mucous cells.

**Methods:** The subjects included 10 infected volunteers and 20 H. pylori infected patients before and after eradication. Formalin-fixed and paraffin-embedded tissue sections were examined by a sequential staining consisting of Ki-67, HIK1083, and periodic acid oxidation-thionine Schiff reaction (PA-TS) Ki-67 labeling indices (LIs) and phenotype of proliferating gastric mucous cells were analyzed. Results: SMCs, GMCS and proliferating cells were simultaneously visualized in histological specimens using PA-TS/HK1083/Ki-67 triple staining: SMCs were stained blue with PA-TS, GMCS were stained red with HK1083, and nuclei of proliferating cells stained brown with Ki-67. Proliferating SMCs were distributed in upper part of gastric foveola and proliferating GMCS were localized in the lower part of gastric foveola around the generating zone. Ki-67 LIs were higher in H. pylori infected patients than in volunteers (p < 0.001). GMC-Ki-67/GC ratio was higher in H. pylori infected patients than in volunteers (p < 0.001). After the eradication of H. pylori, the increased Ki-67 LIs and the GMC-Ki-67/GC ratio in gastric mucosa of H. pylori-infected patients decreased to almost normal levels.

**Conclusion:** H. pylori increased cell kinetics of gastric mucous cells and increased ratios of proliferating GMCS to proliferating gastric epithelial cells. The increase of GMCs in the gastric generating zone of H. pylori infected patients may act as a part of the defense system against H. pylori infection in the generating zone.
II-P-13-173
Cytotoxin-Associated Gene Product A of Helicobacter pylori-Induced Cellular Response in Rat Gastric Epithelial Cell

O. HANDA1*, Y. NAITO1, Y. ISHI1, H. NASU1, S. ADECHI1, T. TAKAY1, S. KOKURA1, N. YOSHI1DA1, H. MATSU1TA2, T. AZUMA1, H. YU1FU1, S. KAWANO4, T. YOSHINOKAWA1
1Department of Biomedical Safety Science, Kyoto Prefectural University of Medicine, Kyoto, 2Tsukuba University, Ibaraki, 3Kobe University, Kobe, 4Osaka University, Osaka, Japan

Background: Cytotoxin-associated gene A (CagA)-positive Helicobacter pylori (H. pylori) has been shown to increase the risk of gastritis or gastric ulcer. However, it is not clear whether CagA protein induces gastric or gastric ulcer. Aim: In this study, we investigated the effect of CagA of H. pylori on the cellular response in rat gastric epithelial cell. Materials and Methods: We used CagA gene- and a Tet-off system-transfected rat gastric epithelial cell (RGM-1), in which CagA expression could be controlled by tetracycline. CagA production in the cell was detected by western blotting and laser scanning confocal microscopy (LSCM). ROS production in RGM-1 was assessed by redox sensitive fluorescent dye, RedoxSensor, and mitochondria selective fluorescent dye, MitoTracker, under LSCM and a fluorometer. Since ROS production in RGM-1 was assessed by redox sensitive fluorescent dye, RedoxSensor, and cell (RGM-1), in which CagA expression could be controlled by tetracycline. CagA production in the cell was detected by western blotting and laser scanning confocal microscopy (LSCM). ROS production in RGM-1 was assessed by redox sensitive fluorescent dye, RedoxSensor, and mitochondria selective fluorescent dye, MitoTracker, under LSCM and a fluorometer. Since nuclear factor kappa B (NFκB), a redox sensitive transcription factor, has been shown to play a key role in the pathogenesis of H. pylori-associated disease through the production of various cytokines, we also investigated the effect of CagA on NFκB nuclear translocation and subsequent production of cytokine-induced neutrophil chemotactrant-1 (CINC-1) by RGM-1. Cell growth was evaluated by doubling time and the number of proliferating cell nuclear antigen (PCNA) -positive cell. Results: CagA expression was detected after removal of tetracycline in a time dependent manner. This was accompanied with significant ROS production by mitochondria in RGM-1. Although CagA did not induce NFκB translocation into the nucleus and CINC-1 production by RGM-1, it increased cell growth of RGM-1. Conclusion: CagA itself might increase the cell growth of normal gastric epithelial cell through mitochondria- derived ROS-mediated mechanism and thus participate in the gastric carcinogenesis.

II-P-13-174
Molecular Mechanisms Underlying Inflammatory vs. Defensive Responses Elicited by Helicobacter pylori

J.S. Lee1, Y.-M. Niu2, S.-J. Suk3, M.S. Kwak4, D.J. Lim5, J.A. Lee5, M.H. Kim5, M. Yeo1, K.-B. Hahm6
1Genome Research Center for Gastroenterology, Ajou University Medical Center, Suwon, 2Institute of Bioscience and Biotechnology, Korea Research Institute of Ships, 3Department of Biochemistry, College of Medicine, University of Ulsan, 4Department of Preventive Medicine and Public Health, Inje University College of Medicine, 5Department of Biomedical Safety Science, Kyoto Prefectural University of Medicine, 6Department of Medicine, College of Medicine, Catholic University of Korea

Multiple lines of evidence suggest that the Helicobacter pylori are one of the primary causes of gastritis and peptic ulcer diseases, which are provoked by oxidative stress and inflammation. More than 50% of the world’s population is infected by this bacterium. The H. pylori-induced oxidative stress has been implicated in the pathogenesis and progression of gastric cancer. Our body has an intrinsic ability to fight against oxidative stress. A wide array of phase II detoxifying or antioxidant enzymes constitute a fundamental cellular defense system against oxidative insults. In the present study, we investigated molecular mechanisms responsible for H. pylori-induced inflammation in human gastric cancer (AGS) cells. H. pylori induced inflammatory response via up-regulation of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), and both the secretion and protein expression of interleukin-8 (IL-8) in AGS cells. The nuclear translocation and subsequent DNA binding of nuclear factor-kappaB (NFκB), a eukaryotic transcription factor known to regulate aforementioned pro-inflammatory enzymes and mediators, were increased after H. pylori treatment. Similarly, the DNA binding of another eukaryotic transcription factor activator protein-1 (AP-1) was induced by H. pylori treatment. In addition, c-Jun that is the major component of AP-1 was detected by super shift assay, and the level of phospho-c-Jun was found to be increased. H. pylori infection accelerated the DNA binding of another transcription factor CREB. In another experiment, H. pylori-infected AGS cells exhibited increased phosphorylation of upstream kinases, ERK. However, long term administration of H. pylori resulted in the activation of proliferating cell nuclear antigens (PCNA) and terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) in the C57BL6 mice, suggesting H. pylori to create a balance between proliferation and the apoptotic process in the stomach. This coordinated balancing phenomena led us to propose the cellular adaptive survival response and/or a defense mechanism against H. pylori-infection. One of the major cellular antioxidants/detoxifying enzymes is hemoxygenase-1 (HO-1). The comparison of HO-1 levels in the several gastric cell lines before and after H. pylori treatment showed that HO-1 mRNA and protein levels were most evident in the AGS cells. The immunofluorescence staining, using a FITC-conjugate HO-1 antibody, revealed the up-regulation of HO-1 protein in the cytoplasm after H. pylori treatment. In contrast, the expression of heat shock protein 70 (HSP70), a molecular chaperone, was rapidly diminished. However, there were no remarkable changes in the expression of another antioxidant enzyme MnSOD. The H. pylori treatment enhanced ARE binding and nuclear translocation of NFκB, thereby upregulating HO-1. Taken together, H. pylori treatment can induce the proinflammatory response through up-regulation of COX-2, while it can provoke adaptive/defensive response by activating antioxidant enzymes, such as HO-1, thereby protecting cells and tissues against subsequent prooxidative and pro-inflammatory insults. Grant support: This work was supported by grants from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PG6-01GN14-0007).
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