Aspirin Can Elicit The Recurrence of Gastric Ulcer Induced with Acetic Acid in Rats

Guo-Zhong Wang^{1,2}, Guo-Ping Huang¹, Guo-Li Yin³, Gang Zhou³, Chun-Juan Guo¹, Chun-Gang Xie¹, Bing-Bing Jia¹ and Jin-Fu Wang¹

¹Institute of Cell Biology, College of Life Science, Zi Jin Gang Campus, Zhejiang University, Hangzhou,

²Institute of Medicine, Qiqihaer Medical College, Heilongjiang, ³Institute of Peptic Disease, College of Medical Science, Zi Jin Gang Campus, Zhejiang University

Key Words

Aspirin • Gastric ulcer • Mucosal blood flow • Ulcer healing • Cyclooxygenase

Abstract

This study was supported by grants of New Ideas Capability for Backbone Teachers in Universities of Heilongjiang and of Scientific Research foundation in Qiqihar Medical College. Background/ Aims: Ulcer recurrence and poor healing may be critically important to the development of serious gastrointestinal complications in patients with long-term non-steroid anti-inflammatory drugs (NSAIDs). The present study is to investigate the effects of aspirin on ulcer recurrence and healing quality and to explore the mechanism. Methods: Gastric ulcers were induced with acetic acid in rats; aspirin was administrated by gavage from day 25 to day 54 after ulcer induction. The gastric juice volume, pH, gastric mucus, gastric mucosal blood flow (GMBF) and prostaglandin E₂ (PGE₂) were measured. The mRNA transcription of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) were analyzed with RT-PCR and protein expression with Western blot. Results: The gastric juice volume was significantly

increased in aspirin group compared with those of fasting or saline control groups ($P < 0.01$); while the pH, mucus, GMBF and PGE₂ were significantly decreased in aspirin treated rats compared with those of other two groups ($P < 0.01$). COX-2, evaluated with mRNA and protein expression, was significantly augmented in aspirin group compared with others. The quality of ulcer healing (QOUH) in Aspirin group was poorer than that of fasting or saline control groups. Conclusions: Aspirin enhance the recurrence of gastric ulcer. The inhibition of cyclooxygenase, mucus secretion and mucosal blood flow may be involved. Aspirin also impair the quality of ulcer healing.

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a major cause of morbidity and mortality among users. Epidemiological data suggest that they increase the risk of ulcer complications and death by 3-10-fold. The pathogenesis of these serious events is unknown. NSAIDs

are one kind of the most frequently used drugs world wide [1], aspirin is one of them. Cross-sectional studies indicate that gastric or duodenal ulcers are present in 10-25% of patients on long-term NSAIDs [2]. Furthermore, continuing usage of NSAIDs often results in the recurrence of gastric ulcer [3].

Prostaglandin deficiency can impair the quality of ulcer healing (QOUH) [4]. Aspirin directly damages gastric mucosa [5], decreases prostaglandin synthesis [6, 7], inhibits ulcer contraction [8] and decreases gastric mucosal blood flow (GMBF) in the ulcer margin [9]. In the healed ulcer, the mucosal structure is in disorder, such as reduced thickness of regenerative mucosa, dilated gland tissue, reduced angiogenesis, and excessive hyperplasia of connective tissue [10-13]. Therefore, gastric acid, prostaglandin, COX, GMBF and QOUH may affect the ulcer relapse.

The recurrent mechanism of peptic ulcer is still not clear [14]. We therefore established the gastric ulcer model in rats with acetic acid induction. Our hypothesis was that continuous aspirin administration can cause ulcer recurrence in acetic acid induced ulcer model; the ulcer recurrence may be because of the increased aggressive factors and decreased protective factors due to aspirin usage. The monitored aggressive factors in this study include gastric secretion and its pH and protective factors include PGE₂, COX-1, COX-2 and GMBF. Furthermore, the relationship between QOUH and aspirin administration was also evaluated.

Materials and Methods

Ulcer induction, aspirin induced recurrence and morphological observation

Male Wistar rats weighing 180-200 g were used in this study. Rats were fasted 24 hours before experiment with free access to drinking water. Experiments were divided into two steps: Firstly, gastric ulcer was induced according to the method previously reported [15]. Some of the rats with ulcers were killed on the test day (day 3, 15 or day 25) and ulcer area was measured with the method described previously [16]. Secondly, on day 25, the remains were randomly assigned to the following three groups: Fasting control group: rats were fasted for 5 hours per day from day 25 to day 54 and then rats were killed on day 55 to collect samples; Saline control group: fasting and sample collection procedure was the same as fasting control group, saline (4ml/kg/day) was given by gavage at the point of 4 hours fasting and continued for another hour fasting from day 25 to day 54; Aspirin group, all of the procedures are the same as saline control group the only difference was to substitute aspirin (40 mg/ kg/day, Sigma Chemical, St Louis, Missouri, USA) for saline.

Histology

Rat stomach was exposed. After observation of gastric ulcer, the specimen were collected, fixed and cut along gastric long axis and stained with haematoxylin and eosins (H-E). The slides were examined under microscope (Olympus, Tokyo, Japan).

Gastric secretion

In order to determine gastric secretion, the pylorus was ligated for 4 hours and then cardia part was also ligated, finally the stomach was cannulated to collect gastric contents which were centrifuged at 3,500 rpm/min for 10 min, the supernatant volume and pH (pH meter, PHS-25, Shanghai, China) were measured.

Gastric mucus, gastric mucosal blood flow (GMBF) and mucosal PGE₂

Gastric mucus was determined according to the previous method [17] with a slight modification. Briefly, stomach was cut along lesser curvature. Stomach was inside out, immersed in 20 ml Alcian blue buffer (8mg/100ml, buffer: 0.2M Na₂HPO₄, 0.1M citric acid, pH=5.8) and incubated for 2 hours. Then, 6ml of incubation liquid were collected and centrifuged at 3000 rpm/min for 10 min. Optical density (OD) of the supernatant was determined at 615 nm wavelength. Result was expressed as a conjunctive dye quantity/20ml Alcian blue staining solution (mg/20ml).

GMBF was measured using hydrogen gas clearance technique [18]. The clearance curve of tissue hydrogen was used to calculate the volume of blood flow (ml/min/100g).

To determine PGE₂, ulcer area was scraped with glass slides, weighed and put in a tube containing 100% ethanol plus 0.1 M indomethacin [19]. Then, gastric mucosa was homogenized and centrifuged for 10 min at 12,000 rpm/min and 4°C. PGE₂ content in supernatant was determined with enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA), following manufacturer's instruction. PGE₂ concentration was expressed as ng /g tissue.

COX-1 and COX-2 mRNA expression quantified by RT-PCR

Total RNA from rat gastric ulcer tissue was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. First single-stranded cDNA was generated with 5 µg total RNA using ReverttAid™ First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). Primer sequences of cyclooxygenase-1, cyclooxygenase-2 and β-actin (as an internal control) are as following: cyclooxygenase-1, forward 5'-AGC CCC TCA TTC ACC CAT CAT TT-3', reverse 5'-CAG GGA CGC CTG TTC TAC GG-3'; cyclooxygenase-2, forward 5'-ACA ACA TTC CTT CCT TC-3', reverse 5'-CCT TAT TTC CTT TCA CAC C-3'. β-actin, forward 5'-GAT CTT GAT CTT CAT GGT GCT AGG-3', reverse 5'-TTG TAA CCA ACT GGG ACG ATA TGG-3'; Briefly, PCR initial reaction mixture contained 2 µl of RT product in 25-µl reaction volume. Mixture was amplified in a DNA thermal cycler (Perkin-Elmer, Norwalk, CT). In each PCR cycle, heat denaturation was set at 94°C for 1min, primer annealing at 60°C for 45 s, and polymerization at

Fig. 1. Macroscopic appearance of ulcer at different times. A: day 3; B: day 15; C: day 25. D: fasting control group on day 55; E: saline control group on day 55; F: Aspirin group on day 55: shows relapsed ulcer, bleeding points on mucosa and linear bleeding on ulcer margins. Vertical arrows show ulcers and the transverse arrows show the healed ulcer.

Fig. 2. Microscopic appearance of gastric ulcer at different times. H-E stain, original magnification: $\times 100$. A: normal mucosa. B: day 25, dilated glands. C and D: partially recovered dilated glands from fasting control and saline control on day 55; E: thinner mucosa by ulcer margins from aspirin treated group on day 55. F: mucosa from aspirin group without the ulcer on day 55.

72°C for 1 min. The cycle number for β -actin was 30, for COX-1 and COX-2 were 35. PCR product was electrophoresed in 1.5% agarose gels containing 0.2 μ g/ml of ethidium bromide. The density of COX-1 or COX-2 is expressed in arbitrary densitometry units normalized to β -actin expression.

Tissues collected in gastric ulcer location were homogenized in lysis buffer containing 50 mM Tris-HCl (pH 7.4), 1% Nonidet (NP40), 150 mM NaCl and protease inhibitor cocktail, then, centrifuged at 12,000 $\times$ g for 20 min. the

supernatant was collected. Equal amounts (50 μ g) of the denatured proteins per lane were loaded and separated on sodium dodecyl sulfate-10% polyacrylamide gels by electrophoresis. Proteins were then transferred to PVDF membrane (BioRad Laboratories, Hercules, CA) by electroblotting. Blots were blocked at least one hour at room temperature with 0.4% I-Block (Tropix, Bedford, MA) in TBS-Tween-20 buffer (137 mmol NaCl, 20 mmol Tris-HCl, pH 7.4, 0.1% Tween-20). The membranes were washed with TBS-T (3

Fig. 3. Effects of aspirin on gastric secretion. A: gastric juice; B: pH of gastric juice. Data are expressed as mean $\pm$ S.D. $n=6-8$. ** $p<0.01$ compared with fasting and saline control groups.

times, 10 min each) and then incubated at room temperature for one hour with highly specific mouse monoclonal anti-COX-1 antibody (1:500, Santa Cruz, CA) or goat polyclonal anti-COX-2 antibody (1:200, Santa Cruz, CA) or mouse monoclonal anti- β -actin antibody (Sigma-Aldrich, St. Louis, MO). After 3 washes, the membranes were incubated with horseradish peroxidase-linked rabbit anti-mouse or rabbit anti-goat immunoglobulin (1:2000). Blots were detected with chemiluminescence (Applied Biosystems, Bedford, MA). The relative expression of COX-1 and COX-2 protein in different groups was quantified by computerized optical densitometric scanning of the images and β -actin band was used as internal control.

Evaluation of QOUH

QOUH was evaluated under light microscope. The inner diameter of gland cavity $>8\ \mu\text{m}$ (about 3 times of normal gland) was considered as dilated glands which were counted in regenerative mucosa. Result was expressed as dilated gland number/HP.

Statistical analysis

Results are expressed as mean $\pm$ SD. SPSS (Version 11.0) software was used. For analysis among groups, one-way ANOVA with least significant difference (LSD) or rank sum test with Mann-Whitney. The significance level was set at $P < 0.05$.

Fig. 4. Effects of aspirin on gastric mucus; ϕ gastric mucosal blood flow and gastric mucosal PGE₂. A: gastric mucus, B: gastric mucosal blood flow, C: gastric mucosal PGE₂. Data was expressed as mean $\pm$ S.D. $n=6-8$. ** $p<0.01$ compared with fasting and saline control groups.

Results

Ulcer size and morphologic features

Ulcer area was $27 \pm 1.07\ \text{mm}^2$, $3.13 \pm 1.13\ \text{mm}^2$ and $0 \pm 0\ \text{mm}^2$ respectively on day 3, day 15 and day 25 following ulcer induction (Fig 1A, Fig 1B and Fig 1C). Figure 1 showed us that 25 days later after acetic acid application, induced ulcer healed completely, however, the area contacted directly with acetic acid was pale. Continuous experiment demonstrated that on day 45, 2 rats showed ulcer relapse. This number increased to 5

Fig. 5. Representative RT-PCR and western blot photograph of COX-1 and COX-2. A: COX-1 and COX-2 mRNA transcription (Lane 1: day 15, Lane 2: fasting control group on day 55, Lane 3: saline control group on day 55, Lane 4: aspirin group on day 55). B: densitometry analysis for COX-1 and COX-2 mRNA, Data was expressed as mean $\pm$ S.D. $n=8$, ** $p<0.01$ compared with fasting and saline control groups. C: protein expression of COX-1 and COX-1 (Lane 1: day 15, Lane 2: fasting control group on day 55, Lane 3: saline control group on day 55, Lane 4: aspirin group on day 55).

Fig. 6. Effects of aspirin on gland dilation. Data was expressed as mean $\pm$ S.D. $n=8$. ** $p<0.01$ compared with fasting and saline control groups.

after 10 more days continuous aspirin treatment ($n=8$); furthermore, there were bleeding points on the surface of mucosa (Fig 1F). There is no ulcer recurrence in other two groups (Table 1). Moreover, the pale area decreased on day 55 in these animals (Fig 1D, Fig 1E).

On day 25, although the ulcer was healed, the glands in acetic acid attacked area were dilated (Fig 2B). On day 55, there are still dilated glands in ulcer area in fasting (Fig 2C) and saline control groups (Fig 2D). In Aspirin group, 5 out of 8 rats with ulcer recurrence had a thin mucosa at ulcer margin (Fig 2E), while there was no difference between aspirin treated but ulcer (Fig. 2F, $n=3$) rats and other animals (Fig 2A).

The effects of aspirin on aggressive and protective factors

The gastric juice volume in aspirin group was significantly higher than those of fasting and saline control groups on day 55 ($P<0.01$, Fig 3A), while the pH value of gastric juice in aspirin group was significantly lower than that of other two groups ($P<0.01$, Fig 3B).

Gastric mucus, GMBF and mucosal PGE_2 concentration were all significantly decreased in aspirin group compared with other two groups ($P<0.01$, Fig 4A, Fig 4B and Fig 4C).

The effect of aspirin on COX-1 and COX-2 expression

Neither COX-1 mRNA transcription nor protein expression differed among all three groups. However, COX-2 mRNA transcription and protein expression were

	35th day	45th day	55th day
Fasting control group	0 ± 0	0 ± 0	0 ± 0
Saline control group	0 ± 0	0 ± 0	0 ± 0
Aspirin group	0 ± 0	0.56 ± 1.12	3.95 ± 3.64**

Table 1. The ulcer area at different times in Fasting control group, Saline control group and Aspirin group. Data are expressed as mean±S.D. n=8 rats.

** $p < 0.01$ versus Fasting control group and Saline control group.

significantly increased in aspirin group compared with other two groups ($P < 0.01$, Fig 5A, Fig 5B, Fig 5C).

The effect of aspirin on QOUH

The number of dilated glands in aspirin treated group was significantly increased compared with fasting or saline control group ($P < 0.01$, Fig 6).

Discussion

Previous studies showed that aspirin induced gastric ulcer and delayed its healing [20-23]. The present study showed that aspirin cause ulcer recurrence in healed ulcer area. Aspirin decreases GMBF and synthesis of gastric mucus and PGE_2 , increases gastric acid secretion, affects the quality of ulcer healing, and all of these factors together result in the ulcer recurrence.

Acetic acid ulcer model resembles human ulcers in terms of both pathological features and healing process [24]. In the present study, the ulcerated area contracted gradually and ulcers healed completely in 25 days (Fig 1A, Fig 1B and Fig 1C). This study is consistent with the previous one [15]. On day 45 and 55, the healed ulcer area showed recurrence in aspirin group. In comparison, there is no ulcer relapse in fasting and saline control groups (Table.1). These results indicated that it is aspirin that caused the ulcer recurrence. It is well documented that gastric ulcers induced by acetic acid injection (0.05ml of 20%) into the submucosa can show up again on day 91 [25]. The end point of this study is 55 days, so it remains to be clarified whether ulcer relapse occur in fasting or saline control group after 55 day.

The treatment of peptic ulcer has been based mainly on inhibiting the secretion of gastric acid [26]. The present study showed that gastric juice was significantly higher in aspirin group than that in fasting or saline control group.

While the pH value in aspirin group was significantly lower than that in other two groups. All of these results suggested that gastric acid was a pivotal factor in ulcer relapse.

Gastric mucus covers the surface of gastric epithelium and forms a layer of mucus barrier. This barrier constitutes the first line of defense that protects gastric mucosa from its own gastric juice. In the present study, the gastric mucus in aspirin group was significantly lower than those in other two groups. This result indicates that aspirin reduces the production of gastric mucus. Our result is consisted with other studies [27-29] and the gastric mucus deficiency may be involved in ulcer relapse in aspirin group. GMBF is also one of the important protective factors [30]. GMBF provides gastric epithelial cells with bicarbonate which neutralizes the acid diffused back to epithelial and thus maintains pH gradient in mucus layer [31, 32]. GMBF also provides oxygen and nutrient substances to gastric mucosa to support mucosal renewal. An adequate blood flow is important for the production of mucus and bicarbonate [33]. Classic NSAIDs can reduce GMBF [15]. Our study showed that GMBF was significantly lower in aspirin group than those in other two groups. Therefore, GMBF reduction is also a factor for ulcer recurrence. Another protective factor is PGE_2 . PGE_2 is one of the major prostanoids in gastric tissue [34]. PGE_2 can inhibit gastric acid secretion [6], our result demonstrated that PGE_2 was significantly decline in aspirin treated group. Thus, PGE_2 might indirectly take part in ulcer relapse via acid secretion.

Cyclooxygenase (COX), the key enzyme for synthesis of prostaglandins, exists in three isoforms COX-1, COX-2 and COX-3. COX-1 is a constitutive enzyme that expressed in gastrointestinal tract and has been suggested to maintain mucosal integrity through continuous generation of prostaglandins. COX-2 is an induced enzyme that expressed in large quantities during

inflammation [35]. Administration of aspirin was accompanied by the suppression of COX-1 and COX-2 activity in the gastric mucosa [36]. Inhibition of prostaglandin synthesis probably weakened the function of the gastric mucosal defense [37]. The present study showed that COX-1 mRNA and protein were expressed in ulcer area on day 15 after ulcer induction as well as in the following three groups on day 55. COX-2, the induced isoform, its mRNA and protein was detectable in fasting and saline control groups on day 55, COX-2 mRNA transcription and protein expression were significantly induced in ulcer area tissue on day 15 after ulcer induction and on day 55 in aspirin group. It indicated that the increased expressions of COX-2 during ulceration and ulcer recurrence. These results indicated that aspirin can reduce the activities of COX-1 and COX-2 and thus, reduce PGE₂ concentration in mucosa, aspirin did not prohibit COX-1 and COX-2 synthesis.

Gastric cellular epithelia and tight junction among gastric cellular epithelia form a mucosal barrier. This mucosal barrier constitutes the second line of defense that protects the gastric mucosa from harmful factors. QOUH plays an important role in ulcer relapse. The deformed gastric mucosa weakens barrier function. The quantity of dilated glands was significantly increased in aspirin group compared with fasting and saline control ones, while the mucosal structure in no-recurrence animals was similar to that of the normal mucosa (Fig 2F, Fig 2A). This indicates that aspirin can result in poor QOUH. In addition, aspirin elevates plasma proinflammatory cytokines such as TNF- α , which leads to leukocyte

infiltration in the gastric mucosa [6] and thus, induces gastric ulcer relapse [38].

In summary, the mechanism that aspirin causes ulcer recurrence may be: aspirin inhibit COX-1 and COX-2 activities, the consequence of this inhibition is to decrease PGE₂ synthesis, PGE₂ deficiency results in the increase of gastric acid secretion, decrease of GMBF and gastric mucus synthesis. The imbalanced protective and aggressive factors finally resulted in poor quality of ulcer healing and ulcer relapse.

Abbreviations

COX (Cyclooxygenase); GMBF (Gastric mucosal blood flow); mRNA (Messenger ribonucleic acid); NSAIDs (Non-steroidal anti-inflammatory drugs); PGE₂ (Prostaglandin E₂); QOUH (Quality of ulcer healing); RT-PCR (Reverse transcription polymerase chain reaction).

Acknowledgements

The authors are very grateful for the technical support provided by Professors Jie Yan in Medical School of Zhejiang University. The authors are also very grateful for Xiuling Wang researcher's administrative all around assistance in Medical School of University of Calgary, Canada. Dr. Janet Passman from America, the foreign teacher of Qiqihar Medical College, critically read the manuscript.

References

- 1 Villegas I, La Casa C, de la Lastra CA, Motilva V, Herreras JM, Martin MJ: Mucosal damage induced by preferential COX-1 and COX-2 inhibitors: role of prostaglandins and inflammatory response. *Life Sci* 2004;74:873-884.
- 2 Tibble J, Sigthorsson G, Caldwell C, Palmer RH, Bjarnason I: Effects of NSAIDs on cryoprobe-induced gastric ulcer healing in rats. *Aliment Pharmacol Ther* 2001;15:2001-2008.
- 3 Hull MA, Knifton A, Filipowicz B, Brough JL, Vautier G, Hawkey CJ: Healing with basic fibroblast growth factor is associated with reduced indomethacin induced relapse in a human model of gastric ulceration. *Gut* 1997;40:204-210.
- 4 Arakawa T, Watanabe T, Fukuda T, Higuchi K, Fujiwara Y, Kobayashi K, Tarnawski A: Ulcer recurrence: cytokines and inflammatory response-dependent process. *Dig Dis Sci* 1998;43:61S-66S.
- 5 Kauffman G: Aspirin-induced gastric mucosal injury: lessons learned from animal models. *Gastroenterology* 1989;96:606-614.
- 6 Pawlik T, Konturek PC, Konturek JW, Konturek SJ, Brzozowski T, Czesnikiewicz M, Plonka M, Bielanski W, Areny H: Impact of *Helicobacter pylori* and nonsteroidal anti-inflammatory drugs on gastric ulcerogenesis in experimental animals and in humans. *Eur J Pharmacol* 2002;449:1-15.

- 7 Wang JY, Yamasaki S, Takeuchi K, Okabe S: Delayed healing of acetic acid-induced gastric ulcers in rats by indomethacin. *Gastroenterology* 1989;96:393-402.
- 8 Penney AG, Andrews FJ, O'Brien PE: Effects of misoprostol on delayed ulcer healing induced by aspirin. *Dig Dis Sci* 1994;39:934-939.
- 9 Hirose H, Takeuchi K, Okabe S: Effect of indomethacin on gastric mucosal blood flow around acetic acid-induced gastric ulcers in rats. *Gastroenterology* 1991;100:1259-1265.
- 10 Wallace JL: Nonsteroidal anti-inflammatory drugs and the gastrointestinal tract. Mechanisms of protection and healing: current knowledge and future research. *Am J Med* 2001;110:19S-23S.
- 11 Tarnawski A, Douglass TG, Stachura J, Krause WJ: Quality of gastric ulcer healing: histological and ultrastructural assessment. *Aliment Pharmacol Ther* 1991;5:79-90.
- 12 Tarnawski A, Stachura J, Krause WJ, Douglass TG, Gergely H: Quality of gastric ulcer healing: a new, emerging concept. *J Clin Gastroenterol* 1991;13:S42-47.
- 13 Tarnawski A, Hollander D, Krause WJ, Dabros W, Stachura J, Gergely H: "Healed" experimental gastric ulcers remain histologically and ultrastructurally abnormal. *J Clin Gastroenterol* 1990;12:S139-147.
- 14 Watanabe T, Higuchi K, Tominaga K, Fujiwara Y, Arakawa T: Acid regulates inflammatory response in a rat model of induction of gastric ulcer recurrence by interleukin 1 β . *Gut* 2001;48:774-781.
- 15 Brzozowski T, Konturek PC, Konturek SJ, Sliwowski Z, Pajdo R, Drozdowicz D, Ptak A, Hahn EG: Classic NSAID and selective cyclooxygenase (COX)-1 and COX-2 inhibitors in healing of chronic gastric ulcers. *Microsc Res Tech* 2001;53:343-353.
- 16 Ma L, Wang WP, Chow JY, Yuen ST, Cho CH: Reduction of EGF is associated with the delay of ulcer healing by cigarette smoking. *Am J Physiol Gastrointest Liver Physiol* 2000;278:G10-G17.
- 17 Piper DW, Whitecross D, Leonard P, Clarke A: Alcian blue binding properties of gastric juice. *Gastroenterology* 1970;59:534-538.
- 18 Brzozowski T, Konturek PC, Konturek SJ, Ernst H, Stachura J, Hahn EG: Gastric adaptation to injury by repeated doses of aspirin strengthens mucosal defence against subsequent exposure to various strong irritants in rats. *Gut* 1995;37:749-757.
- 19 Futaki N, Arai I, Hamasaka Y, Takahashi S, Higuchi S, Otomo S: Selective inhibition of NS-398 on prostanoïd production in inflamed tissue in rat carrageenan-air-pouch inflammation. *J Pharm Pharmacol*. 1993;45:753 -755.
- 20 Hawkey CJ: Nonsteroidal anti-inflammatory drug gastropathy. *Gastroenterology* 2000;119:521-535.
- 21 Hawkey CJ, Naesdal J, Wilson I, Langstrom G, Swannell AJ, Peacock RA, Yeomans ND: Relative contribution of mucosal injury and *Helicobacter pylori* in the development of gastroduodenal lesions in patients taking non-steroidal anti-inflammatory drugs. *Gut* 2002;51:336-343.
- 22 Slomiany BL, Piotrowski J, Slomiany A: Up-regulation of gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide by aspirin but not indomethacin. *J Endotoxin Res* 2001;7:203-209.
- 23 Konturek PC, Brzozowski T, Kwiecień S, Drozdowicz D, Harsch IA, Meixner H, Stachura J, Hahn EG, Konturek SJ: Effect of *Helicobacter pylori* on delay in ulcer healing induced by aspirin in rats. *Eur J Pharmacol* 2002;451:191-202.
- 24 Okabe S, Amagase K: An overview of acetic acid ulcer models-the history and state of the art of peptic ulcer research. *Biol Pharm Bull* 2005;28:1321-1341.
- 25 Uchida M, Kawano O, Misaki N, Irino O: Healing process of acetic acid-induced gastric ulcer and gastric mucosal prostaglandin E generation level in rats. *Jpn J Pharmacol* 1989;50:366-368.
- 26 Warzecha Z, Dembinski A, Brzozowski T, Ceranowicz P, Pajdo R, Niemiec J, Drozdowicz D, Mitis-Musiol M, Konturek SJ: Gastroprotective effect of histamine and acid secretion on ammonia-induced gastric lesions in rats. *Scand J Gastroenterol* 2000;35:916-924.
- 27 Sarosiek J, Mizuta K, Slomiany A, Slomiany BL: Effect of acetylsalicylic acid on gastric mucin viscosity, permeability to hydrogen ion, and susceptibility to pepsin. *Biochem Pharmacol* 1986;35:4291-4295.
- 28 Rainsford KD, Whitehouse MW: Biochemical gastroprotection from acute ulceration induced by aspirin and related drugs. *Biochem Pharmacol* 1980;29:1281-1289.
- 29 Menguy R, Desbaillets L: Role of inhibition of gastric mucous secretion in the phenomenon of gastric mucosal injury by indomethacin. *Am J Dig Dis* 1967;12:862-866.
- 30 Phillipson M, Henriksnas J, Holstad M, Sandler S, Holm L: Inducible nitric oxide synthase is involved in acid-induced gastric hyperemia in rats and mice. *Am J Physiol Gastrointest Liver Physiol* 2003;285:G154-G162.
- 31 Phillipson M, Atuma C, Henriksnas J, Holm L: The importance of mucus layers and bicarbonate transport in preservation of gastric juxtamucosal pH. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G211-G219.
- 32 Synnerstad I, Johansson M, Nylander O, Holm L: Intraluminal acid and gastric mucosal integrity: the importance of blood-borne bicarbonate. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G121- G129.
- 33 Bjorne H H, Petersson J, Phillipson M, Weitzberg E, Holm L, Lundberg JO: Nitrite in saliva increases gastric mucosal blood flow and mucus thickness. *J Clin Invest* 2004;113:106-114.
- 34 Sun WH, Tsuji S, Tsujii M, Gunawan ES, Kawai N, Kimura A, Kakiuchi Y, Yasumaru M, Iijima H, Okuda Y, Sasaki Y, Hori M, Kawano S: Induction of cyclooxygenase-2 in rat gastric mucosa by rebamipide, a mucoprotective agent. *J Pharmacol Exp Ther* 2000;295:447-452.
- 35 Halter F, Tarnawski AS, Schmassmann A, Peskar BM: Cyclooxygenase 2-implications on maintenance of gastric mucosal integrity and ulcer healing: controversial issues and perspectives. *Gut* 2001;49:443-453.
- 36 Brzozowski T: Experimental production of peptic ulcer, gastric damage and cancer models and their use in pathophysiological studies and pharmacological treatment-Polish achievements. *J Physiol Pharmacol* 2003;54:99-126.
- 37 Chan FK, Leung WK: Peptic-ulcer disease. *Lancet* 2002;360:933-941.
- 38 Watanabe T, Higuchi K, Hamaguchi M, Shiba M, Tominaga K, Fujiwara Y, Matsumoto T, Arakawa T: Monocyte chemotactic protein-1 regulates leukocyte recruitment during gastric ulcer recurrence induced by tumor necrosis factor- α . *Am J Physiol Gastrointest Liver Physiol* 2004;287:G919-G928.