Thymoquinone Protects the Spinal Cord Neurons from Degeneration by Enhancing GAP-43, Bcl-2 and Decreasing Bax Expression in a Sciatic Nerve Lesion Model

J. Eliwa, H. Al-Ali, S. Smitha, M.S. Rao

Department of Anatomy, Faculty of Medicine, Kuwait University, Kuwait

Introduction: Studies have established beneficial properties of thymoquinone (TQ), the active constituent of Nigella sativa seed oil. TQ has been shown to have anti-inflammatory and neuroprotective roles. The aim of the present experiment was to investigate the mechanisms of protection of spinal cord motor neurons by TQ in a sciatic nerve lesion model. Methods: The sciatic nerve was exposed on the right side in 4-month-old Wistar rats and transected at its point of entry to the thigh. The animals were divided into lesion only (LO, n = 6, treated with saline 1 ml/day i.p. for 2 weeks) and lesioned and treated with TQ (L + TQ, n = 6, treated with 10 mg/kg TQ i.p. for 2 weeks) groups. Age-matched normal control (n = 6) and sham control (n = 6) groups were also maintained. Serial frozen sections of spinal cord were stained with cresyl violet and immunostained with NeuN, GAP-43, Bcl-2 and Bax. Numbers of immunostained neurons in the dorsal and ventral horn regions of the spinal cord were quantified. Data were analyzed with one-way ANOVA. Results: TQ treatment rescued a large number of neurons both in the ventral and dorsal horn regions of the spinal cord from degeneration due to sciatic nerve lesion. Quantification of neurons stained with NeuN and cresyl violet showed a significant increase in the number of neurons in the anterior and posterior horns of the spinal cord in rats from the L + TQ group in comparison to the LO group (p < 0.001). Additionally, immunohistochemical examination of spinal cord sections revealed that TQ increased the expression of GAP-43 and Bcl-2 (p < 0.001) and reduced the expression of Bax (p < 0.001). Conclusions: We conclude that TQ has protective effects on the anterior horn motor neurons and posterior horn neurons after peripheral nerve lesion. It may be because of the antioxidant and the anti-apoptotic effects of TQ.

The Long-Lasting Impact of the Prenatal Immune Challenge on the Process of Remyelination

H. Al-Hashash, S. Rakhshani-Moghadam, S. Kalakh, A. Mouihate

Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait

Introduction: Early life stress has long-lasting effects on brain function and plasticity. However, the impact of prenatal stress on remyelination after a demyelination insult has not yet been explored. Therefore, we explored the long-lasting impact of prenatal immune stress on the process of remyelination during adulthood. Methods: At gestation day 12, pregnant Sprague-Dawley rats were injected with either sterile saline solution or lipopolysaccharide (LPS) solution (100 μg/kg i.p.). Two microliters of the gliotoxin ethidium bromide (EB, 0.04%) were stereotaxically injected into the corpora callosa of male adult offspring. Brains were collected 7 days after injection, a time corresponding to the peak of demyelination. Oligodendrocyte progenitor cells (OPCs) and mature oligo-
dendrocytes in the vicinity of the lesion were detected by immunofluorescence using NG2 and CC1 antibodies, respectively. Microglia were detected by immunofluorescent staining (Iba1). Microglial M1 and M2 types were monitored using antibodies against the inducible nitric oxide synthase and arginase-1, respectively. Western blotting was used to assess the activation of the nuclear factor κB (NF-κB) signaling pathway by measuring the levels of phosphorylated and total inhibitor IκB. These data strongly suggest that prenatal immune challenge has a long-lasting impact on the offspring’s response to the demyelination insult. This effect is manifested by an increase in the number of OPCs and mature oligodendrocytes at the lesion site. It is likely that this enhanced myelination is, at least in part, due to dampening of the NF-κB signaling pathway.

**Best Postgraduate Awards**

1. Graduate MSc (Basic Science)

   **Investigating the Desensitization and Internalization of the Incretin Receptors**

   G. Shaaban, M.A. Oriowo, S. Al-Sabah
   Department of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University, Kuwait

   **Introduction:** Incretins – the gut hormones glucose-dependent insulino-motropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) – function to potentiate insulin secretion in a glucose-dependent manner. This makes their respective receptors (GIPR and GLP-1R) attractive targets in the treatment of type 2 diabetes mellitus (T2DM). However, in T2DM there is an almost complete lack of response to GIP whereas this is not true for GLP-1R. In both cases, washing cells following preincubation resulted in a rapid loss of response to a second stimulation for both GIPR and GLP-1R. In the absence of second stimulation. Receptor internalization following agonist stimulation was monitored using an indirect ELISA-based method. Preincubation of cells with agonist resulted in a rapid loss of response to a second stimulation for both GIPR and GLP-1R. In both cases, washing cells following preincubation failed to bring cAMP levels back to basal. Taking this into account, two rates of desensitization were calculated for GLP-1R: ‘apparent’ ($t_0 = 19.3$ min) and ‘net’ ($t_{50} = 3.0$ min). GIPR desensitization was too rapid to accurately calculate rates. Incubation of cells with GLP-1 resulted in a time-dependent loss of GLP-1R cell surface expression ($t_{50} = 2.1$ min). However, GIP did not appear to induce internalization of GIPR.

   **Conclusions:** There appear to be fundamental differences in the regulation of GIPR and GLP-1R when expressed in HEK-293 cells. This may have relevance in the development of new incretin-based therapies. **Funding Agency:** The College of Graduate Studies, Kuwait University, and the Research Sector, Kuwait University, grant No. YM 08/13. The authors would also like to acknowledge the Research Core Facility, Kuwait University (General Facility KU Project No. SRUL02/13) for use of their tissue culture facility.

2. **Mn(III)N-Alkylpyridylporphyrins Increase Vitamin C Anticancer Activity through Generation of Reactive Oxygen Species**

   B. Bader, L. Benov, J. Craik
   Department of Biochemistry, Faculty of Medicine, Kuwait University, Kuwait

   **Introduction:** Ascorbate administration has shown promising results in the treatment of cancer. Mn(III)N-alkylpyridylporphyrins (MnPs) are redox active superoxide dismutase mimetics with potential therapeutic applications. It is known that cancer cells accumulate porphyrins and show aberrant redox homeostasis. MnPs taken up by cancer cells can produce reactive oxygen species (ROS) by redox-cycling with ascorbate, thus augmenting its cytotoxicity. This study investigates how structural modifications of MnPs affect their anticancer activity. **Methods:** Rates of MnP catalyzed ascorbate oxidation, which corresponds to ROS generation, and MnP uptakes were determined spectrophotometrically. Subcellular distributions of metalloporphyrins were investigated by confocal microscopy. MTT and SRB assays were used to quantify the combined effect of MnPs and ascorbate on human breast cancer PII/MDA and nontumorigenic breast epithelial HBL100 cell lines. **Results:** The hydrophilic meta- and ortho-isomers MnTE-2-PyP and MnTE-3-PyP produced the highest rate of ascorbate oxidation and oxygen consumption. A combination of 5 μM MnTE-2-PyP and 1 mM ascorbate had the highest cytotoxicity in PII, MDA and HBL100 cell lines and gave the greatest cell proliferation inhibition in PII cells. The lipophilic MnTnHexOE-2-PyP and MnTnOct-2-PyP showed the greatest cellular accumulation in cells but displayed lower cytotoxicity than MnTE-2-PyP and MnTE-3-PyP which had lower cellular uptakes. Addition of catalase prevented cytotoxicity. HBL100 cells were as susceptible to MnP + ascorbate as PII cells. **Conclusions:** Ascorbate (vitamin C) and MnP redox cycle to generate cytotoxic ROS, mainly $\text{H}_2\text{O}_2$. The anticancer efficiency of MnPs correlated with their ability to oxidize ascorbate while differences in uptake and subcellular distribution had a minor effect. This suggests that the main factor determining the anticancer activity of MnPs is their redox potential. **Funding Agency:** Supported by the College of Graduate Studies, Kuwait University, Kuwait (YM04/14).
2. Graduate Resident

1 Estradiol Dampens the Recruitment of Oligodendrocyte Precursor Cells to the Site of Brain Inflammation: Role of Cyclooxygenase-2
B. Dawi, S. Kalakh, A. Mouihate
Department of Physiology, Faculty of Medicine, Kuwait

Introduction: Conflicting findings have been reported on the immunomodulatory actions of 17β-estradiol in the central nervous system. We have previously shown that 17β-estradiol exacerbates lipopolysaccharide (LPS)-induced cyclooxygenase-2 (COX-2) in the brain striatal region of ovariectomized (OVX) rats. Owing to the role of COX-2-produced prostaglandins in myelination, we investigated the effect of 17β-estradiol on oligodendrocyte precursor cells (OPCs) and mature oligodendrocytes during brain inflammation. Methods: OVX adult Sprague-Dawley female rats received intrastratial injections of 2 μl of LPS solution (500 ng/μl) using a stereotaxic apparatus. They were subsequently given either 17β-estradiol (100 μg/kg s.c.) or subcutaneous sesame oil in conjunction with either the selective COX-2 inhibitor celecoxib (2.5 mg/kg i.p.) or intraperitoneal dimethyl sulfoxide. The brains were collected on day 3 after LPS injection, fixed and processed for immunofluorescent staining. Primary antibodies against OPCs and mature oligodendroglia, NG2 and CC1, respectively, were used to detect the levels of these cells in the vicinity of the inflammatory lesion. Results: LPS injection into the striatum of OVX rats increased the number of OPCs, but not that of mature oligodendroglia, in the vicinity of the inflammatory lesion. Such a cellular increase was significantly attenuated when OVX rats were given 17β-estradiol. Interestingly, the coadministration of celecoxib with 17β-estradiol restored the number of these myelinating cells in the vicinity of the lesion site. Conclusions: These preliminary results strongly suggest that 17β-estradiol reduces the recruitment of OPCs to the site of inflammation. These modulatory effects are likely mediated through a COX-2-dependent mechanism.

2 Follicular Lymphoma in situ with Hyaline Vascular Castleman Disease-Like Features: A Case Report
A. Al Taleb, A. Ali, M. El-Kabbany
Kuwait Cancer Control Center, Kuwait

Background: Hyaline vascular Castleman disease (HV-CD) is a benign lymphoid hyperplasia characterized by hyperplastic follicles with atrophic germinal centers rich in hyaline deposits and vascular proliferation. Several B-cell lymphomas should be considered including follicular lymphoma before a diagnosis of CD is rendered. Case Report: A 36-year-old lady presented with a 3-day history of epigastric pain and nausea. Physical examination was unremarkable. Initial laboratory investigations were within normal limits. Upper gastrointestinal endoscopy revealed multiple small gastric polyps. Ultrasound of the abdomen and pelvis revealed multiple hypoechoic solid mesenteric lymph nodes. CT scan of the abdomen and pelvis showed multiple enlarged lymph nodes in the mesentery and retroperitoneum. PET-CT imaging demonstrated large hypermetabolic lymph nodes in the para-aortic, mesenteric, retroperitoneal and right inguinal and femoral nodes. Other laboratory results including lactate dehydrogenase, and viral studies for human immunodeficiency virus, hepatitis B and C virus and human herpesvirus-8 were negative. Right inguinal lymph node biopsy for histopathological examination showed focal localization of monotonous groups of small cells that displayed strong staining for BCL-2 protein and CD10 in occasional germinal centers that otherwise appeared reactive. The background nodal tissue showed HV-CD-like changes. A diagnosis of in situ follicular lymphoma on a background of CD-like changes was established. Bone marrow biopsy showed paratrabeular atypical lymphoid aggregates. Fluorescence in situ hybridization analysis showed positivity for t(14;18) translocation involving the IGH/BCL2 genes. Also, the monoclonal population of B cells was detected by PCR. Conclusion: HV-CD has been associated with several B-cell lymphomas. However, there is no case reported showing the association between in situ follicular lymphomas and HV-CD. To our knowledge this is the first reported case. We highlight the clinicopathological features of the case with a multifaceted approach.

3 Low-Grade Appendiceal Mucinous Neoplasm: Eight Cases from Mubarak Al-Kabeer Hospital
J. Salmeen, R. Ali
Kuwait Institute of Medical Specialization, Mubarak Al-Kabeer Hospital, Kuwait

Introduction: Low-grade appendiceal mucinous neoplasm (LAMN) is a rare tumor of low malignant potential often discovered intraoperatively or during pathological assessment of appendectomy specimens. It may give rise to pseudomyxoma peritonei (PMP), a slow-growing but potentially fatal tumor. We have recently encountered 4 appendectomies with incidental LAMN, which has prompted a retrospective search in the pathology files of Mubarak Al-Kabeer Hospital to explore the extent to which LAMN has been reported. Methods: We searched a total of 10,236 appendectomy specimens, reported between 1990 and 2009, using the key words ‘mucin’, ‘mucinous’, ‘adenoma’ and ‘adenocarcinoma’. Slide review was performed on cases meeting the search criteria. Results: After excluding secondary mucinous tumors, only 4 LAMN cases were confirmed (total = 8 including the 4 recent cases). These occurred in 4 males and 4 females with a mean age of 44.8 years (range 31–67). The appendices were invariably grossly dilated, up to 3 cm in diameter, with a wall thickness of 0.8–1.2 cm. Out of 7 cases with available slides, 5 (71%) showed mucin pools confined to the appendix histologically, and 2 (29%) showed mucin extending into periappendiceal tissues (no
clonal information available on the presence/absence of PMP). Acellular mucin was seen in 5/7 (71%) cases and luminal epithelial dysplasia in 7/7 (100%). Coexisting appendicitis was noted in 5 cases. **Conclusions:** LAMN was not diagnosed frequently between 1990 and 2009. It may have been overlooked for several reasons: (a) appendices affected by LAMN usually look like a benign mucocele grossly, (b) LAMN is not obviously invasive histologically and (c) lack of awareness amongst surgeons and pathologists. Any dilated mucin-filled appendix should be viewed with caution. This finding should prompt a search for extravesical mucin intraoperatively and careful pathological assessment. LAMN patients must be referred to a multidisciplinary oncology team.

3. Graduate PhD (Basic Science)

1

**Associations of Leukocyte Telomere Length with Cardiometabolic Risk Factors and Circulating Biomarkers of Inflammation and Oxidative Stress**

R. Al Khaldi, O.A. Mojiminiyi, F. Al Mulla, N.A. Abdella

Departments of Pathology and Medicine, Faculty of Medicine, Kuwait University, Kuwait

**Introduction:** Telomeres are tandem sequences at the end of chromosomes necessary for chromosomal integrity, and activity of telomerase enzyme prevents telomere exhaustion. Telomeres and telomerase were linked to aging-associated diseases, namely obesity and type 2 diabetes mellitus (T2DM). We hypothesize that shortened telomere length would be associated with cardiometabolic risk factors, and that this relationship might be mediated by obesity-induced metabolic changes. **Methods:** Body mass index, waist circumference (WC), serum human telomerase reverse transcriptase (hTERT), total adiponectin, insulin, myeloperoxidase (MPO), total oxidative skin stress (TOS) and leukocyte telomere length (LTL) were measured in 225 T2DM patients and 245 age- and sex-matched controls. Insulin resistance (IR) was estimated using the homeostasis model assessment (HOMA) calculator. **Results:** T2DM patients had a significantly (p < 0.0001) lower LTL compared to controls (mean ± SD: 2.1 ± 0.2 vs. 4.1 ± 0.1, respectively). Levels of hTERT were higher in controls compared to T2DM patients (mean ± SD: 32.9 ± 8.9 vs. 21.4 ± 4.7 ng/ml). LTL was negatively associated with WC (β = –5.7, p = 0.004), HOMA-IR (β = –1.1, p = 0.003), MPO (β = –0.6, p < 0.0001) and TOS (β = –2.2, p < 0.0001). hTERT showed similar trends. LTL and hTERT were associated significantly and positively associated with adiponectin (β = 3.1, p = 0.02; β = 1.5, p = 0.003). Shorter LTLs were associated significantly with a higher risk of T2DM (odds ratio = 7.5, p = 0.003). **Conclusions:** We show a link between telomere biology, cardiometabolic risk factors and T2DM in the Kuwaiti population which has not been studied before. Metabolic changes such as the dysregulation of adiponectin, hyperinsulinemia, IR and obesity-associated inflammatory processes could play a role in mediating telomere shortening. Obesity and T2DM are increasing at an epidemic pace in Kuwait; telomere attrition and telomerase levels could be potential cardiometabolic risk markers of obesity and T2DM. **Funding Agency:** College of Graduate Studies, Research Sector, Kuwait University, Kuwait, grant No. YM06/11.

**Basic Sciences**

1

**Identification of the First Gene CCNO Causing a Novel Congenital Ciliary Respiratory Disorder**


aDepartment of Pathology, Faculty of Medicine, Health Sciences Center, Kuwait University, and bZain Hospital for Ear, Nose and Throat, Kuwait, Departments of Pediatrics and Pathology, University Hospital Münster, Münster, Germany; 

cDepartment of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

**Introduction:** We recently discovered and reported the first gene causing a novel congenital respiratory disorder, CCNO (cyclin O). We reported this phenotype as a pulmonary clearance disorder that was misdiagnosed before as primary ciliary dyskinesia. **Methods:** CCNO was firstly discovered in a multiplex consanguineous Kuwaiti family with 5 affected individuals using whole exome sequencing and autozygos mapping technologies. The segregation of the identified mutations was performed using the Sanger sequencing method. The ultrastructural defect was detected using transmission electron microscopy (TEM). **Results:** Linkage analysis showed an identical by descent (IBD) region at chromosome 5 that was shared by all affected individuals with an interval of [50, 317, 612–65, 419, 300]. Exome sequencing showed a mutation in the CCNO gene that was located within the IBD region. Sanger sequencing and segregation analysis showed a founder homozygous loss-of-function mutation (c.252_253insTGCCC; p.Gly85Cysfs*10) in the CCNO gene within all affected individuals who shared the autozygous interval across the CCNO gene locus. TEM photographs of respiratory epithelial cells of the patients with CCNO mutations after in vitro ciliogenesis showed severe reduction in the numbers of motile cilia and basal bodies in the apical cell region and mislocalization of basal bodies and rootlets within the cytoplasm. **Conclusions:** This indicates that this congenital pulmonary disease is caused by a marked reduction of the number of multiple motile cilia (MMCs) covering the cell surface of respiratory epithelial cells. CCNO is the first gene reported to cause a defect in centriole amplification and migration due to reduced MMCs and consequently develops an inherited defective mucociliary clearance disorder which leads to a severely defective respiratory system (the collaborative paper was published in *Nature Genetics*). **Funding Agency:** This work was funded by Dubai Harvard Foundation, grant No. 08-MED497-20.