Clinical and Experimental Health Sciences

Originle Artical / *Özgün Araştırma*

Protective Effects of Montelukast Against Stress-Induced Degeneration of the Urinary Bladder

Stres ile Uyarılmış Mesane Hasarına Karşı Montelukastın Koruyucu Etkileri

Esra Çikler Dülger [1], Yasemin Ersoy Çanıllıoğlu [2], Şule Çetinel [3], Göksel Şener [4], Feriha Ercan[3]

[1] İstanbul Bilim University, School of Medicine, Department of Histology&Embryology, İstanbul
[2] Bahçeşehir University, School of Medicine, Department of Histology&Embryology, İstanbul
[3] Marmara University, School of Medicine, Department of Histology&Embryology, İstanbul
[4] Marmara University, School of Pharmacy, Department of Pharmacology, İstanbul

ORCID IDw of the authors: F.E. 0000-0003-2339-5669, Y.E.Ç. 0000-0002-3839-6931

Cite this article as: Dülger E.Ç, Ersoy Çanıllıoğlu Y., Çetinel Ş., Şener G., Ercan F. Protective Effects of Montelukast Against Stress-Induced Degeneration of the Urinary Bladder. Clin Exp Health Sci 2018; DOI: 10.5152/clinexphealthsci.2017.691

ABSTRACT

Objective: The aim of the study was to investigate the role of montelukast (ML), a cysteinyl leukotriene-1 receptor antagonist, on the water avoidance stress (WAS) – induced degeneration of the rat urinary bladder mucosa.

Methods: In WAS group, rats were exposed to WAS two hours daily for five days. In WAS+ML group, rats were administered ML (10 mg/kg; i.p.;) following every WAS exposure for 5 days. In control group, rats were injected vehicle solution only. The urinary bladder was evaluated for general morphology at light microscope. Mast cell activation and uroplakin distribution were assessed with immunohistochemistry. Glycosaminoglycan (GAG) distribution and urothelial permeability were observed using ruthenium red (RR) staining techniques in transmission electron microscope and luminal urothelial cells were observed with scanning electron microscope. Tissue malondialdehyde (MDA) and gluthatione (GSH) levels were also analysed.

Results: Irregular GAG layer and uroplakin distribution, penetration of RR in the intercellular spaces and dilated tight junctions in urothelial layer, increase in inflammatory cell infiltration, in number of both granulated and activated mast cells, and were observed in the WAS group. The MDA level was increased, and GSH level was decreased significantly in urinary bladder in the WAS group in comparison with the control group. Quite regular GAG layer, uroplakin distribution and tight junctions in most regions, decrease in inflammatory cell infiltration and both of activated and granulated mast cells in the mucosa, were observed in WAS+ML group. Moreover, significant decrease in MDA and increase in GSH levels were observed in this group.

Conclusion: Montelukast appears to exert a protective activity in WAS induced urinary bladder injury by inhibiting inflammatory and oxidative activity.

Keywords: Water avoidance stress, montelukast, urinary bladder, uroplakin, mast cell

ÖZ

Amaç: Bu çalışmanın amacı sisteinil lökotrien 1 reseptör antagonisti olan montelukastın (ML) sudan kaçınma stresi (SKS) ile uyarılmış sıçan mesane mukoza hasarı üzerine rolünü incelemek idi.

Yöntemler: SKS grubundaki sıçanlara beş gün boyunca günde iki saat SKS uygulandı. SKS+ML grubundaki sıçanlara, beş gün boyunca SKS uygulamasından sonra ML (10 mg/kg, i.p.,) uygulandı. Kontrol grubundaki sıçanlara sadece çözücü solüsyon enjekte edildi. Mesanenin genel morfolojik özellikleri ışık mikrsokobu ile değerlendirildi. Mast hücre aktivasyonu ve üroplakin dağılımı immunohistokimya ile değerlendirildi. Glikozaminoglikan (GAG) dağılımı ve ürotelyum geçirgenliği rutenyum kırmızısı (RK) boyama tekniği uygulanarak geçirimli elektron mikroskopi ile, luminal ürotelyal hücreler taramalı electron mikrsokopi ile değerlendirildi. Doku malondialdehit (MDA) ve glutatyon (GSH) düzeyleri de analiz edildi.

Bulgular: SKS grubunda, ürotelyum tabakasında düzensiz GAG tabakası ve üroplakin dağılımı, hücrelerarası boşluklara RK geçişi ve genişlemiş sıkı bağlantıların varlığı, enflamatuvar hücre infiltrasyonunda ve hem granüllü hem de degranüle olmuş mast hücre sayısında artış gözlendi. Kontrol grubu ile kıyaslandığında SKS grubunda mesanede anlamlı olarak MDA seviyesinde artış, GSH seviyesinde düşüş gözlendi. SKS+ML grubunda bir çok alanda oldukça düzenli GAG tabakası, üroplakin dağılımı ve sıkı bağlantıların varlığı, enflamatuvar hücre infiltrasyonunda ve hem granüllü hem de degranüle olmuş mast hücre sayısında düşüş gözlendi. İlaveten, bu grupta anlamlı bir şekilde MDA seviyesinde düşüş, GSH seviyesinde artış gözlendi.

Sonuç: Montelukastın SKS ile uyarılmış mesane hasarı üzerine enflamtuvar ve oksidatif aktiviteyi engelleyerek koruyucu etki göstermeye çalıştığı görülmektedir.

Anahtar Kelimeler: Sudan kaçınma stresi, montelukast, mesane, üroplakin, mast hücresi

INTRODUCTION

Interstitial cystitis (IC) is one of the inflammatory bladder diseases that occurs mostly in women (90%) with urinary frequency, urgency, burning and suprapubic pain (1). Although the pathophysiological basis of IC is not been fully understood, experimental and epidemiological

Correspondence Author/Sorumlu Yazar: Feriha Ercan **E-mail/E-posta:** fercan@marmara.edu.tr
 DOI: 10.5152/clinexphealthsci.2017.691

data show that urothelial damage, increase and degranulation of mast cells and activation of afferent fibers have a role in this disease. It was indicated that stress conditions aggravates IC symptoms (2). Continuous exposure to stressful stimuli has been directly related to the onset, progression or outcome of many pathological processes in several organs such as stomach and urinary bladder. should be corrrected as “In a previous study it was observed that cold-restraint stress induced urothelial damage, activated and increased mast cells and activation of afferent fibers in rats (3). When activated, mast cells release many inflammatory mediators as histamine, proteases, prostaglandins, leukotrienes and cytokines (3). It was reported that the mast cells have a critical role in inflammatory and allergic reactions including stress-induced inflammatory diseases (4). Water avoidance stress (WAS), one of the used stress models, mimics psychological stimuli so it may be a model of life stress as its duration is 5 days (5). In recent studies it is shown that WAS cause both histopathological (6) and functional abnormalities as micturition frequency, interval and volume of rat urinary bladder (7).

Leukotrienes are lipid mediators involved in allergic and inflammatory diseases (8). There are two classes of leukotrienes, leukotriene B4 (LTB4) and the cysteinyl leukotrienes C4 (LTC4), D4 (LTD4) and E4 (LTE4) (9). The cysteinyl leukotrienes are potent pro-inflammatory mediators, and are produced by inflammatory cells, such as mast cells, eosinophils, basophils and macrophages (10). Leukotrienes are potent spasmogens that promote mucous secretion and have primarily been studied with regard to their action on reactive preparations from guinea-pig and human airways, and guinea-pig ileum (11). Leukotriene receptor antagonists and synthesis inhibitors have been shown to be effective on several inflammatory models in rats, such as experimentally induced mucosal damage of stomach (12), colitis (13), burn induced multiorgan damage (14) and renal ischemia/ reperfusion injury (15). Montelukast is cysteinyl leukotriene receptor 1 antagonist, selectively inhibited the LTD4 mediated calcium response and used to treat asthma and other diseases (16, 17). Oral treatment daily has inhibited bronchoconstriction and decreased blood eosinophils. (18). Recent studies were shown that montelukast decreased urinary frequency and pain in eosinophilic cystitis (19) and IC (17).

It was aimed to evaluate the protective effects of montelukast on WAS induced urinary baldder damage in rats. Morphologic alterations, mast cell activity, uroplakin distribution, GAG layer and tight junctions were evaluated by histological techniques, oxidative stress markers; malondialdehyde (MDA) and glutathione (GSH) were analysed by biochemical methods.

METHODS

Animals

Wistar Albino adult female rats (180-200 gr) were used in this study. They were housed individually in standard laboratory conditions and fed with standard pellet laboratory chow and w*ater ad libitum. All* experimental protocols were approved by the Marmara University, Animal Care and Use Committee (33.2017.mar9).

Water avoidance stress procedure

Rats were handled daily by the same investigator for 2 weeks before the study and then subjected to WAS according to the Santos et all. (5). The procedure works by placing the rat on a glass platform (8 X 6 cm^2) in the middle of a plastic container with 90 cm diameter and 50 cm height, filled with warm water (25 º C) to 1 cm below the height of the platform. Rats avoided the aversive stimulus (water) by remaining on the platform for 2 h (5).

Experimental Groups

In the study four groups were present as control, montelukast (ML), WAS (WAS) and WAS exposed ML-treated (WAS+ML) groups. Eight rats were used in each group. In control and ML groups rats were placed on the platform in a waterless container for 2h daily for 5 days afterwards, each day received an i.p injection of physiological saline or 10 mg/kg ML. In WAS and WAS+ ML groups rats were exposed to WAS for 2h daily for 5 days. Right after WAS exposure, rats received either physiological saline or 10 mg/kg ML injected i.p. for 5 days. The animals were fed with a standard pellet lab chow and water *ad libitum* during the experimental procedures. Rats were euthanized by decapitation 2 h after the last stress exposure and/or treatments, the urinary bladders were removed from the animals and processed for microscopic and biochemical investigations.

Light Microscopic Preparation

The urinary bladder samples were fixed in 10% buffered formalin for 48 hours and processed routine paraffin embedding. Approximately 5mm thick paraffin sections were stained with hematoxylin and eosin (H&E) for morphological observation.

Triptase and uroplakin Immunohistochemistry

For triptase immunohistochemistry, paraffine sections were rehydrated, endogenous peroxidase activity was quenched with 3% H_2O_2 in methanol 10 min and the sections were rinsed in phosphate buffered saline (PBS). Non-specific labeling was blocked by incubation in a blocking solution (Super Block, Scy Tek UltraTek HRP, Anti-Polivalent, UHP 125, USA) for 25 min and then the sections were incubated with a mouse anti-mast cell tryptase (diluted 1:100, Novo Castra, NCL-MCTRYP, UK), overnight at 4 ºC. In the negative controls, the sections were incubated with PBS without mouse anti-mast cell tryptase overnight at 4 ºC. An anti-polyvalent biotinylated antibody (Scy Tek Ultra Tek HRP, Anti – Polivalent, UHP 125, and USA) was applied as a secondary antibody (supplied ready-to-use) for 30 min. The sections were then incubated with horseradish peroxidase (HRP) – labeled avidin (supplied ready-to-use, Scy Tek UltraTek HRP, Anti-Polivalent, UHP 125, USA) for 25 min, and then labeling revealed by incubation with a freshly prepared solution of 3-amino-9-ethylcarbazole (AEC, 20 ml AEC chromogen with each 1

ml of AEC substrate, Scy Tek ACJ 125 AEC Chromogen/Substrate Bulk Pack, USA) for 5–10 min. Sections were coverslipped with glycerol gelatine (20).

For the uroplakin immunohistochemistry, tissue sections were deparaffinized and rehydrated and endogenous peroxidase activity was quenched with 1% H_2O_2 in methanol for 5 minutes and the sections were rinsed in PBS two times. The sections were treated with trypsin solution for epitope retrieval for 10 minutes at 37°C, incubated with normal goat serum (1:50) in PBS, and then incubated with mouse monoclonal antibodies UPIII (the AU1 antibody, undiluted, catalog# RDI-PRO651108, Fitzgerald, USA) overnight at 4ºC. After then the other procedures were similar as triptase immunohistochemistry mentioned above (21).

Light microscopic sections were evaluated and photographed with a digital camera (Olympus C-5060; Olympus, Tokyo, Japan) attached to a photomicroscope (BX51; Olympus, Tokyo, Japan).

Electron Microscopic Preparation

Transmission electron microscopy (TEM) was used to demonstrate the glycosaminoglycan (GAG) layer and leakage of the thight junction. The urinary bladder samples were fixed in 4% phosphate buffered glutaraldehyde (0.13 M and pH 7.4), stained en bloc with ruthenium red (RR) and postfixed with OsO4 (ratio used 1 part of stock RR solution: 4 parts of 1% OsO4) for 1 hour. Samples were stained en bloc within a range of 1/5 stock RR solution and 2% uranyl acetate (22), then dehydrated in a ascending alcohol series and embedded in epon 812. Sixty nanometer thick sections were stained with uranyl acetate and lead citrate. These sections were investigated and photographed with digital camera (Olympus Morada Soft Imaging System) attached to a transmission electron microscope (Jeol 1200 EXII,Tokyo, Japan).

Scanning electron microscopy (SEM) was used to demonstrate the alterations of luminal surface of urinary bladder. The samples were fixed in 4% phosphate buffered glutaraldehyde (0.13M, pH: 7.4) for 4 h, postfixed with 1% OsO_4 for 1h, and processed for routine scanning electron micrsocopy. Then, the specimens were examined under Jeol JSM SEM (Tokyo, Japan).

Mast Cell Counts

The paraffin sections stained with triptase immunohistochemistry were used for mast cell counting. Similar ten areas in each sample were selected and tryptase immunoreactive (ir) mast cells were counted at X400 magnification in the mucosa.

Measurement of Malondialdehyde (MDA) and Glutathione (GSH) Levels

Tissue samples were homogenized in ice cold 150 mM KCl (w/v, 10 %) for determination of MDA and GSH levels. The MDA levels were used as index of the products of lipid peroxidation and used as a biomarker of oxidative damage in urinary bladder. Results are expressed as nmol MDA/g tissue (23). GSH was determined by the spectrophotometric method using the Ellman's reagent (24). Results are expressed as mmol GSH/g tissue.

Statistics

Statistical analysis was performed using GraphPad Prism 4.0 (GraphPad Software, San Diego; CA; USA). All data were expressed as means ± SEM. Groups of MDA and GSH data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Mast cells evaluation was done by the Mann-Whitney non-parametric U-test. Values of $p<0.05$ were regarded as significant.

RESULTS

Morphological Results

The control group had a regular layout of mucosa with urothelium (Fig.1A1) and a few numbers of granulated mast cells (Fig. 1A2). Uroplakin immunoractivity (ir) was observed throughout the urothelium as a brown color (Figure 1A3). Regular luminal mucosal topography at SEM level (Fig. 1A4), GAG layer and tight junctions (Fig. 1A5) were obsereved at TEM level.

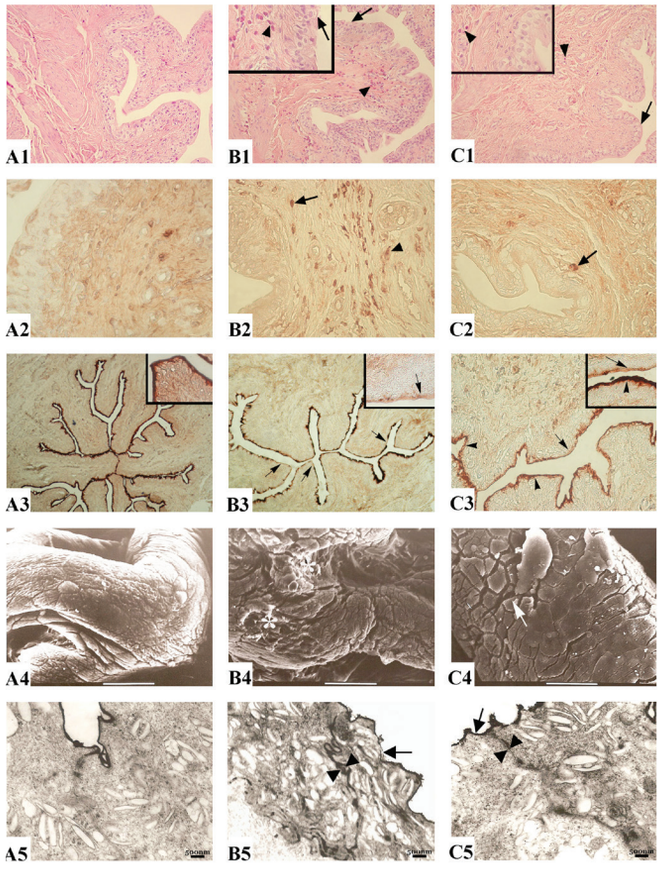

Figure 1: Representative micrographs of the experimental groups. Urothelial mucosa with regular mucus layer and urothelial cells **(A1)**, a few triptase immunostained mast cells **(A2),** regular uroplakin-ir in apical surface of the urothelium (**A3**), regular luminal surface of the bladder with mucosal foldings and polygonal shaped apical cells **(A4)** and regular apical surface of GAG layer with impermeable tight junctions **(A5)** are seen in control group. Degeneration of apical urothelial cells (arrows) with severe accumulation of neutrophils (arrowheads) **(B1)**, increased granulated (arrow) and degranulated

(arrowhead) mast cells (**B2**), localised uroplakin-ir in apical surface of the urothelium (arrows, **B3**), ulcerations and epithelial cell loss in the urothelium (white*, **B4**), irregular GAG layer (arrow) and prominent dilatation of the intercellular spaces (arrowheads, **B5**) are seen in WAS group. Quite regular apical urothelial cells (arrow) in most areas, a few number of neutrophils (arrowhead**, C1**), decreased granulated (arrow) degranulated mast cells (**C2**), weak uroplakin-ir (arrowheads) in renewed regions and strong uroplakin-ir in regular regions (arrows) of urothelium (**C3)**, quite regular apical urothelial cells in most region and localised dilatation of apical urothelial cells (white arrow) of the urothelial mucosa (**C4**) and regular GAG layer (arrow) and impermeable intercellular junctional complex (arrowheads) are seen in WAS+ML group **(C5)**. Fig 1 A1-C1: H&E staining, Fig 1 A2 – C2: tryptase immunostaining, Fig. 1 A3-C3: uroplakin immunostainig, Fig 1 A4-C4: SEM micrograph, Fig. 1 A5-C5: TEM micrograph, original magnifications: A1-C1 and A3-C3: x200, insets and A2-C2: x400, A4-C4: x750, A5-C5: x15000.

Degeneration of apical urothelial cells, inflammatory cell infiltration (Fig. 1B1) and increased both granulated and degranulated mast cells (Fig. 1B2), were observed at light microscopic level in the WAS group. Uroplakin immunohistochemistry showed focal uroplakin-ir in apical surface of the urothelium (Fig. 1B3). Desquamation of urothelial cells and ulcerated areas were observed in SEM level (Fig. 1B4). Irregular layout of GAG layer and penetrations of RR in the intercellular spaces were observed in TEM level (Figs. 1B5).

In the WAS + ML group, quite regular urothelium, mild inflammatory cell infiltration (Fig. 1C1) and reduced number of both granulated and degranulated mast cells (Fig. 1C2) were observed at light microscope level. Besides regular uroplakin-ir in most regions, weak uroplakin-ir in the renewed urothelial regions was observed (Fig.1C3). SEM observations showed quite regular luminal urothelial morphology in most regions and focal dilatation of apical urothelial cells of the mucosa (Fig. 1C4). TEM observations demonstrated quite regular GAG layer with impermeable intercellular junctional complex (Fig. 1C5).

The average number of both granulated and degranulated mast cells per unit area in the mucosa of the bladder was significantly higher in the WAS group when compared with the control and WAS+ML groups (Fig. 2).

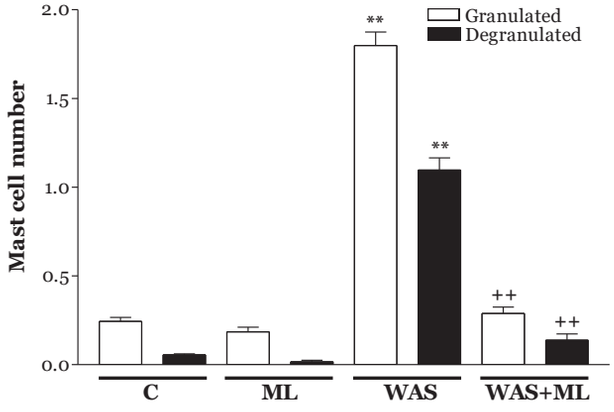

Figure 2: Mean number of mast cells per 0.0789 mm^2 in control (C), montelukast-treated control (ML), WAS and WAS + ML groups. Each group consists of 8 rats. *p<0.05 and **p<0.01 compared to control group; ++p<0.01 compared to WAS group.

Biochemical results

WAS induction caused an increase in MDA levels (P<0.001), compared to control group showed a significant decrease in MDA levels compared to WAS group (P<0.001). The GSH level (P<0.001) diminished in the WAS group and increased in WAS +ML group (P<0.01, Fig. 3).

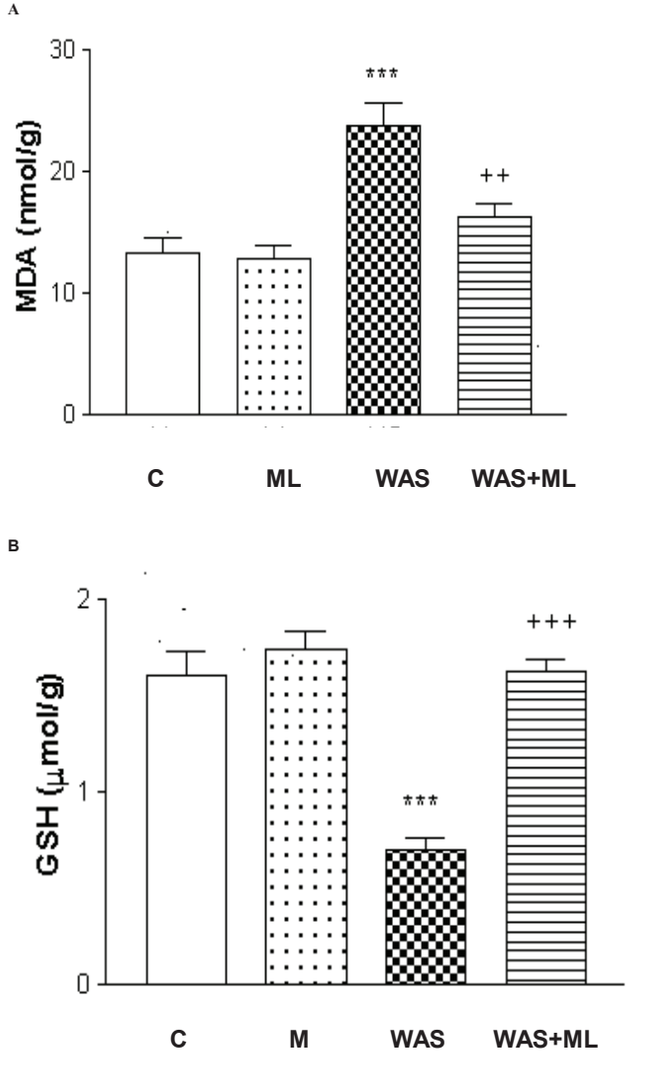

Figure 3: Malondialdehyde (MDA, A) and gluthatione (GSH, B) levels of urinary bladder in control (C), montelukast-treated control (ML), WAS and WAS+ML groups. Each group consists of 8 rats. *** p<0.001: compared to control groups, ++ p<0.01 and +++p<0,001: compared to WAS group.

Discussion

In our study we showed that induction of WAS caused urothelial infiltration and urothelial damage, increased number of both granulated and activated mast cells increased malondialdehyde and decreased endogenous glutathione levels. The leukotriene antagonist montelukast ameliorated both oxidative damage and morphology in urinary bladder.

Stress conditions worsen the symptoms of IC in patients (25) and experimental studies aim to explain the mechanism of the stress conditions on urinary bladder. One of the experimental studies that could explain the life stress with duration of 5 days is WAS including psychological stimuli (5). Stress conditions induce the increase and activity of mast cells, moreover mast cell activity increases in many diseases as irritable bowel disease, psoriasis and IC (4). The mast cell activation has been indicated in many different stress models as well in WAS. Acute immobilization stress induces mast cell activation in urinary bladder (26) and dura mater (27). Moreover, cold-restraint stress causes an increase and activation of mast cells in urinary bladder mucosa (3). In this study, WAS exposure caused an increase in the number and activation of mast cells in urinary bladder. These results suggest that WAS-induced activation of mast cells occurs by activating an inflammatory reaction and stimulating mast cells, to release inflammatory mediators such as histamine and leukotriens to the extracellular matrix, and this can be prevented by montelukast treatment via its anti-inflammatory and antioxidant effects.

Surface glycosaminoglycan (GAG) layer maintains the integrity of the urothelium, tight junctions and urothelial permeability (28 – 30). Generally, degeneration in the mucous layer of the urothelium has an initiating role in IC (29). In IC patients dilatations in the cells of the superficial and intermediate layers were demonstrated (31). A decreased mucous layer, degeneration of the cell junctions and increased number of microvilli (32) were observed in the SEM observations of IC patients. Our light and electron microscopic observations of WAS group showed a reduction of the GAG layer, decrease of uroplakin-ir density, leaky tight juntions, desquamation and ulceration at the apical surface. Because of the reduction of GAG layer, dilatation of tight junctions, reduction of density of microridges may be related to inflammation.

MDA is a good indicator of the rate of lipid peroxidation and it is related to inflammation in tissue (33). GSH is one of the endogenous antioxidant against a number of noxious stimuli including free oxygen radicals and several reports indicate that reduction of cellular GSH is accompanied by lipid peroxidation. It was shown that montelukast suppresses the release of oxidative stress markers and enhances enzymatic antioxidant activities in urinary bladder in spinal cord injury induced secondary degeneration (34) and kidney in cisplatin induced renal disfunction (35). In this study, the MDA level was significantly increased and GSH level was significantly decreased by WAS exposure. However, montelukast treatment causes significant decrease of MDA level indicating a reduction in lipid peroxidation and cellular damage, and prevention of GSH reduction, might be related by its free radical scavenging activity.

It was shown that cysteinyl leukotrienes induce the activation of mast cells. Cysteinyl leukotrienes can provoke interleukine 5 and tumor necrotic factor-α production in mast cells, and this effect is blocked by cysteinyl leukotrienes inhibition (36). One of cysteinyl leukotriene antagonist montelukast down regulates the human monocyte chemotaxis induced by monocyte chemotactic protein-1 which is chemotactic for eosinophils and mast cells realize the release of histamine and leukotriene (37). Cysteinyl leukotrienes mediate histamine hypersensitivity by increase of histamine H1 receptors (38). Cysteinyl leukotrienes initiate histamine responses by recruiting additional histamine receptors in immunologically related cells (38). Moreover, montelukast was effective for micturation symptoms and urinary bladder pain in IC patients (39). In the present study it was observed that, montelukast treatment reduces the number and activity of mast cells in WAS induced bladder damage. In accordance to the inhibition of mast cell activation regular urothelium was observed in both light and electron microscopy levels. These results might be related by the anti-inflammatory effects of montelukast.

Conclusion

It is demonstrated that montelukast, a cysteinyl leukotriene receptor 1 antagonist, alleviates WAS-induced bladder damage by inhibiting mast cell activation and preserve the urothelial mucous layer through its antioxidant effects. Thus, montelukast is a highly promising agent for the protection of urinary bladder from pathologic conditions in which oxidant-antioxidant balance is altered. Moreover, montelukast might be also used as therapeutic agent for IC symptoms which are worsening by stressful conditions.

Acknowledgement: This study was supported by L'Oreal Turkey, the Supporting Grant of the Young Women Scientists (2005).

REFERENCES

[1] van de Merwe JP, Nordling J, Bouchelouche P, Bouchelouche K, Cervigni M, Daha LK, et al. Diagnostic criteria, classification, and nomenclature for painful bladder syndrome/interstitial cystitis: an ESSIC proposal. Eur Urol. 2008; 53(1):60-7. Epub 2007 Sep 20.

[2] Lutgendorf SK, Kreder KJ, Rothrock NE, Ratliff TL, Zimmerman B. Stress and symptomatology in patients with interstitial cystitis: a laboratory stress model. J Urol 2000; 164: 1265-69.

[3] Ercan F, San T, Cavdar S. The effects of cold-restraint stress on urinary bladder wall compared with interstitial cystitis morphology. Urol Res 1999; 27: 454-61.

[4] Sismanopoulos N, Delivanis DA, Alysandratos KD, Angelidou A, Therianou A, Kalogeromitros D, et al. Mast cells in allergic and inflammatory diseases. Curr Pharm Des 2012; 18: 2261-77.

[5] Santos J, Benjamin M, Yang PC, Prior T, Perdue MH. Chronic stress impairs rat growth and jejunal epithelial barrier function: role of mast cells. Am J Physiol Gastrointest Liver Physiol 2000; 278: G847– 54.

[6] Sağlam B, Cikler E, Zeybek A, Cetinel S, Sener G, Ercan F. An aqueous garlic extract alleviates water avoidance stress-induced degeneration of the urinary bladder. BJU Int 2006; 98: 1250-54.

[7] Smith AL, Leung J, Kun S, Zhang R, Karagiannides I, Raz S, et al. The effects of acute and chronic psychological stress on bladder function in a rodent model. Urology 2011; 78: 967.e1-967.e7.

[8] Hedi H and Norbert G. 5-Lipoxygenase pathway, dendritic cells, and adaptive immunity. J Biomed Biotechnol 2004; 2: 99-105.

[9] Dahlen SE, Hedqvist P, Hammarstrom S and Samuelsson B. Leukotrienes are potent constrictors of human bronchi. Nature 1980; 288: 484-86.

[10] Back M. Functional characteristics of cysteinyl-leukotriene receptor subtypes. Life Sci 2002; 71: 611-22.

[11] Samuelsson B, Borgeat P, Hammarstrom S, Murphy RC. Leukotrienes: a new group of biologically active compounds. Adv Prostaglandin Thromboxane Leukot Res 1980; 6: 1-18.

[12] Wallace JL, Beck PL, Morris GP. Is there a role for leukotrienes as mediators of ethanol-induced gastric mucosal damage? Am J Physiol 1988; 254: G117-23.

[13] Holma R, Salmenperä P, Riutta A, Virtanen I, Korpela R, Vapaatalo H. Acute effects of the cys-leukotriene-1 receptor antagonist, montelukast, on experimental colitis in rats. Eur J Pharmacol 2001; 429: 309-18.

[14] Şener G, Kabasakal L, Cetinel S, Contuk G, Gedik N, Yeğen BC. Leukotriene receptor blocker montelukast protects against burn-induced oxidative injury of the skin and remote organs. Burns 2005; 31: 587-96.

[15] Sener G, Sehirli O, Velioğlu-Oğünç A, Cetinel S, Gedik N, Caner M, et al. Montelukast protects against renal ischemia/reperfusion injury in rats. Pharmacol Res 2006; 54: 65-71.

[16] Dahle´n SE, Haeggstro¨m JZ, Samuelsson B, Rabe KF, Leff AR. Leukotrienes as targets for treatment of asthma and other diseases. Am J Respir Crit Care Med 2000; 161: S1.

[17] Bouchelouche K, Nording J, Hald T, Bouchelouche P. The cysteinyl leukotriene D4 receptors antagonist montelukast for the treatment of interstitial cystitis. J Urol 2001; 166: 1734-37.

[18] Reiss TF, Chervinsky P, Dockhorn RJ, Shingo S, Seidenberg B, Edwards TB. Montelukast, a once daily leukotriene receptor antagonist in the treatment of chronic asthma: a multicenter, randomised, double-blind trial. Montelukast Clinical Research Study Group. Arch Intern Med 1998; 158: 1213-20.

[19] Sterrett S, Morton J, Perry D, Donovan J. Eosinophilic cystitis: successful long-term treatment with montelukast sodium. Urology 2006; 67: 423.e19-e21.

[20] Chen Z, Irani AA, Bradford TR, Craig SS, Newlands G, Miuer H, et al. Localization of Rat Tryptase to a Subset of the Connective Tissue Type of Mast Cell. J Histochem Cytochem 1993; 41: 961-69.

[21] Liang FX, Riedel I, Deng FM, Zhou G, Xu C, Wu XR, et al. Organization of uroplakin subunits: transmembrane topology, pair formation and plaque composition. Biochem J 2001; 355: 13-18.

[22] Dixon JS, Holm-Bentzen M, Gilpin CJ, Gosling JA, Bostafte E, Hold T, et al. Electron microscopic investigation of the bladder urothelium and glycocalyx in patients with interstitial cystitis. J Urol 1986; 135: 621-25.

[23] Beuge JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52: 302-11.

[24] Beutler E. Glutathione in red blood cell metabolism. A manuel of biochemical methods. 2nd ed. New York: Grune and Stratton; 1975.

[25] Rothrock NE, Lutgendorf SK, Kreder KJ, Ratliff T, Zimmerman B. Stress and symptoms in patients with interstitial cystitis: a life stress model. Urology 2001; 57: 422-27.

[26] Theoharides TC, Sant GR, el-Mansoury M, Letourneau R, Ucci AA Jr, Meares EM Jr. Activation of bladder mast cells in interstitial cystitis: a light and electron microscopic study. J Urol 1995; 153: 629-36.

[27] Rozniecki JJ, Dimitriadou V, L ambracht-Hall M, Pang Theoharides TC. Morphological and functional demonstration of rat dura mater mast cell-neuron interactions in vitro and in vivo. Brain Res 1999; 849: 1-15.

[28] Lori A Birder, William C de Groat. Mechanisms of Disease: involvement of the urothelium in bladder dysfunction. Nat Clin Pract Urol 2007; 4: 46-54.

[29] Lilly JD, Parsons CL. Bladder surface glycosaminoglycans is a human epithelial permeability barrier. Surg Gynecol Obstet 1990; 171: 493-96.

[30] Eldrup J, Thourp J, Nielsen SL, Hald T, Hainau L. Permeability and ultrastructure of human bladder epithelium. Br J Urol 1983; 55:488-92.

[31] Johansson SL, Fall M. Pathology of interstitial cystitis. Urol Clin North Am 1994; 21: 55-62.

[32] Anderstro¨m CRK, Fall M, Johansson SL. Scanning electron microscopic findings in interstitial cystitis. Br J Urol 1990; 63: 270-75.

[33] Reiter RJ, Melchiorri D, Sewerynek E, Poeggeler B, Barlow-Walden L, Chuang J, et al. A review of the evidence supporting melatonin's role as an antioxidant. J Pineal Res 1995; 18: 1-11.

[34] Erşahin M, Çevik Ö, Akakın D, Şener A, Özbay L, Yegen BC, et al. Montelukast inhibits caspase-3 activity and ameliorates oxidative damage in the spinal cord and urinary bladder of rats with spinal cord injury. Prostaglandins Other Lipid Mediat 2012; 99: 131-39.

[35] Suddek GM. Montelukast ameliorates kidney function and urinary bladder sensitivity in experimentally induced renal dysfunction in rats. Fundam Clin Pharmacol 2013; 27: 186-91.

[36] Friesen CA, Neilan NA, Schurman JV, Taylor DL, Kearns GL, Abdel-Rahman SM. Montelukast in the treatment of duodenal eosinophilia in children with dyspepsia: effect on eosinophil density and activation in relation to pharmacokinetics. BMC Gastroenterol 2009; 9: 32.

[37] Hung CH, Li CY, Hua YM, Chen CJ, Yang KD, Jong YJ. Effects of leukotriene antagonists on monocyte chemotaxis, p38 and cytoplasmic calcium. Pediatr Allergy Immunol 2006, 17: 250-58.

[38] Pynaert G, Grooten J, van Deventer SJ, Peppelenbosch MP. Cysteinyl leukotrienes mediate histamine hypersensitivity ex vivo by increasing histamine receptor numbers. Mol Med 1999; 5: 685-92.

[39] Traut JL, Macdonald ES, Spangler ML, Saxena S. Montelukast for symptom control of interstitial cystitis. Ann Pharmacother 2011; 45: e49.