Original Article

Can Montelukast Sodium be an Alternative Treatment in the Treatment of Interstitial Cystitis?

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Abstract

Background: The leukotriene D4 receptors have been detected in human bladder detrusor myocytes, and they can play the role of interstitial cystitis etiology. **Aim:** Our study aims to explain the role of mast cells histologically and immunohistochemically in the pathogenesis and the effectiveness of montelukast that leukotriene D4 receptor antagonist in the treatment of interstitial cystitis. **Subjects and Methods:** Twenty-four Wistar albino adult female rats were used. Group 1 (n = 8): control (sham) group, Group 2 (n = 8): interstitial cystitis group, and Group 3 (n = 8): treatment group. Groups 2 and 3 rats were administered 75 mg/kg cyclophosphamide four times every three days intraperitoneally. The rats in the treatment group were started on montelukast sodium as 10 mg/kg, 1 × 1/day per orally after the last administration of cyclophosphamide and were given for 14 days. Mast cells in the bladder tissues were examined histologically, and the presence of IL-6, 8, VEGF, and TNF alpha was examined immunohistochemically. **Results:** Thin transitional epithelium, loose connective tissue, weak smooth muscle bundles, and signs of chronic inflammation were observed in the interstitial cystitis group. Regenerated transitional epithelium, intact basement membrane, compact lamina propia, thick smooth muscle bundles, and rare inflammatory cells were observed after the treatment with the montelukast. Mast cells were decreased in bladder tissue after treatment. IL-6, IL-8, VEGF, and TNF alpha levels were significantly decreased after treatment. **Conclusions:** We found that inflammatory mediators were significantly reduced after treatment with montelukast in the interstitial cystitis group. Montelukast can be used as an effective drug in the treatment of interstitial cystitis.

Keywords: *Chronic pelvic pain, Interstitial cystitis, Leukotriene D4, Montelukast sodium*

Introduction

Interstitial cystitis (IC) is a chronic disease of the bladder that presents with the symptoms of severe urinary urgency, frequency, nocturia, and suprapubic or perineal pain.[1] This disease is commonly confused with urinary tract infections, as the symptoms are similar. It is five times more common in males than females. The prevalence rate of IC is 2.7–5.6% in females.[2] The most frequently used criteria in diagnosing IC are those determined by the European Society for the Study of IC/PBS (ESSIC) and the American Urological Association (AUA). These diagnostic criteria are chronic pelvic pain (>six months) and the feeling of pressure or discomfort about the urinary bladder (with persistent urgency to void or frequency). Normal cystoscopy findings and histopathological findings as Hunner lesions, glomerulations, intrafascicular fibrosis, inflammatory infiltrates, granulation tissue, and detrusor mastocytosis are the other diagnostic findings.[3]

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It is a common disease, but there are not enough studies about the etiology and treatment in the literature. Therefore, its etiology is still unclear. Researchers believe that inflammation plays a central role in the pathogenesis of IC. The presence of leukotriene (LT) receptors on detrusor muscle cells and increased urinary LTD4 levels in IC suggest pro-inflammatory mediators role in IC.[4] Leukotrienes play an important role in the activation of mast cells. There is an increased concentration of mast cells around the detrusor muscle.[5] These mast cells release LTD4, a pro-inflammatory mediator that causes spasmogenic effects. It is produced from phospholipids found in the perinuclear membrane.

Different pharmacologic treatments have been used to treat this condition with limited success. Oral tricyclic antidepressants, pentosan, and intravesical administration of dimethyl sulfoxide, heparin, and bacillus Calmette-Guerin have been tried in the treatment.[2] Montelukast is a drug that blocks LTD4 receptors and is frequently used to treat allergic rhinitis and asthma in clinical practice.[6] In previous studies, the presence of LTD4 receptors has been detected in human bladder detrusor myocytes.[7] It has been shown that it has an anti-inflammatory effect by inhibiting leukotriene receptors in the bladder.[8] Therefore, it can be predicted that montelukast can be used to treat IC.

There is only one study in the literature on the effects of montelukast on bladder cells with IC, and the evaluation method in this study is limited. Our study aims to explain the role of mast cells histologically and immunohistochemically in the pathogenesis and the effectiveness of montelukast that leukotriene D4 receptor antagonist in the treatment of interstitial cystitis. Furthermore, as a result of our research, we would like to explain the usability of montelukast in IC.

Materials and Methods

Twenty-four Wistar albino adult female rats were used. All rats were followed up at an average room temperature of 23°C, in a 12-h dark-light cycle, and on an *ad libitum* diet. Rats were divided into three groups: Group 1 (n = 8): control (sham) group, Group 2 (n = 8): interstitial cystitis group, and Group 3 (n = 8): treatment group. Groups 2 and 3 rats were administered 75 mg/kg cyclophosphamide four times every three days intraperitoneally. The control group rats were injected with a similar volume of physiological saline (SF-0,9%NaCl) intraperitoneally. The rats in the treatment group were started on montelukast sodium as 10 mg/kg, 1 × 1/day per orally after the last administration of cyclophosphamide and were given for 14 days. The form to be used was prepared from the commercially available 4 mg sachets (Airfi × 4 mg sachet, Mentis). At the end of the treatment, the bladder was excised by laparotomy by applying intraperitoneal ketamine/xylazine anesthesia in all groups of rats. Bladder samples were fixed in 10% formalin. The procedures used and the care of animals were approved by the Institutional Animal Care and Use Committee (IACUC) in ***** University (approval number: *****).

Hematoxylin–Eosin staining and May Grunwald-Giemsa staining method

Sections taken from the experimental groups were kept in an oven at 60°C for 60 minutes, and then they were removed from xylol for 3 × 5 minutes and cleared of paraffin. Afterward, slides were passed through decreasing series of alcohol and washed in running water and then stained with Harris Hematoxylin and Eosin. May Grunwald-Giemsa stain was applied to evaluate mast cells. The slides were kept in Eosin for 2 minutes, passed through a series of increasing grades of alcohol, taken into xylene, and closed with Entellan.

Immunohistochemical staining method

Staining was done in Sequenza Immunostaining Center Shandon/ThermoScientific device. Sections of 3 μm thickness were taken from rat bladder tissue paraffin blocks on slides. Sections were applied with a 1/10 diluted CitratBuffer (PH: 6) (AP-9003-999 ThermoScientific) PT Module (A80400012 LabVision) to unmask the antigen. In the IHC Stainer, the slides were attached to the rack slots together with the cover plate. It was washed with PBS. Protein was blocked with solution (TA□125□PBQ ThermoFisherScientific) for 10 minutes and incubated for 2 h with IL-6 antibodies (orb539985 Biorbyt) 1/100), IL-8 antibodies (orb229133 Biorbyt) 1/50), TNF alpha antibodies (orb11495 Biorbyt) 1/100), and VEGF antibodies (orb191500 Biorbyt) 1/50). Washing was done with PBS. Staining was performed with DAB Chromogen (TA-125-HAThermoScientific) to identify positive cells.

Immunohistochemical and mast cell count evaluation

One hundred cells were counted to calculate percentages of IL-6, IL-8, VEGF, and TNF alpha positive cells in randomly selected high-power fields. Immunohistochemistry sections were evaluated for the presence or absence of cytoplasmic staining. In the evaluation of mast cells, mast cells in mm^2 were counted in the preparations prepared in May Grunwald-Giemsa stain.

Statistical analyses

All results were expressed as means ± standard deviation (SD). Significant values in the groups were

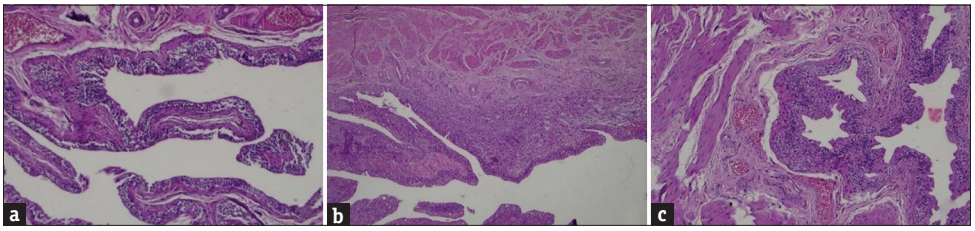

Figure 1: Hematoxylin and Eosin staining of the bladder mucosa: A—control group, B—ISC group, and C—ISC-ML group. Smooth stratified transitional epithelium, intact basement membrane, compact lamina propia, and thick smooth muscle bundles in bladder tissue of control group (a). Thin transitional epithelium, loose connective tissue, weak smooth muscle bundles, and signs of chronic inflammation were observed in bladder tissue of ISC group (b). Regenerated transitional epithelium, intact basement membrane, compact lamina propia, thick smooth muscle bundles, and rare inflammatory cells in bladder tissue of ISC + ML treatment group (c).*(ISC: interstitial cystitis, ISC-ML: interstitial cystitis and treatment with montelukast sodium)*

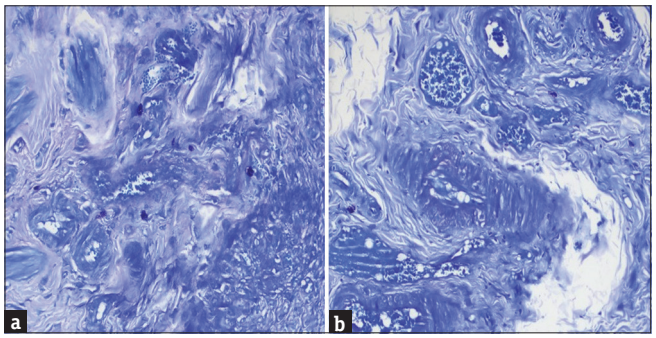

Figure 2: May Grunwald-Giemsa staining of the bladder mucosa: A—ISC group and B—ISC-ML group.Mast cells were counted as 6–8 per mm2 in ISC group (a) Mast cells were counted as 3–4 per mm2 in ISC + ML treatment (b) *(ISC: interstitial cystitis, ISC-ML: interstitial cystitis and treatment with montelukast sodium)*

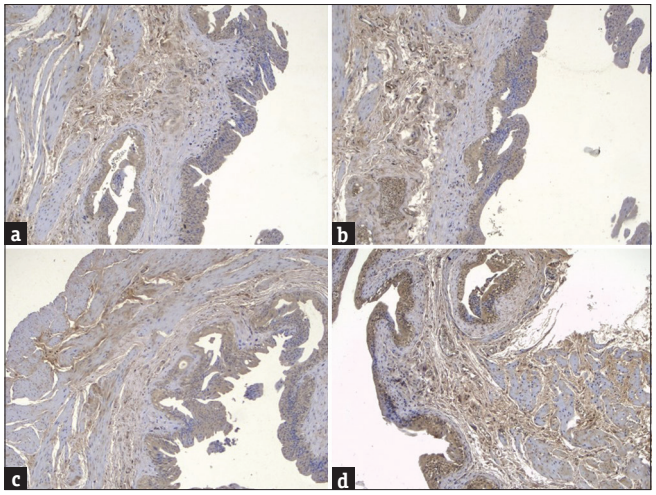

Figure 3: Immunohistochemical staining results of control group rats: A—staining with IL-6, B—staining with IL-8, C—staining with TNF alpha, and D—staining with VEGF. Staining rates with IL-6(a) and IL-8 varied between 0 and 25% (b) and TNF alpha and Vascular Endothelial Growth Factor (VEGF) stained moderately at a 25–50% (c and d) *(IL: interleukin, TNF: tumor necrosis factor, VEGF: Vascular Endothelial Growth Factor)*

assessed with one-way ANOVA. IHC staining results in groups were evaluated with the Kruskall–Wallis and Tamhane's *post hoc* test using SPSS software version 22 (SPSS Inc., Chicago, IL, USA). P <0,05 was considered significant.

Results

Histopathological evaluation

Group 1: Smooth stratified transitional epithelium, intact basement membrane, compact lamina propia, and thick smooth muscle bundles [Figure 1a]. Mast cells were counted as 1–2 per mm^2.

Group 2: Thin transitional epithelium, loose connective tissue, weak smooth muscle bundles, and signs of chronic inflammation were observed [Figure 1b]. Mast cells were counted as 6–8 per mm^2 [Figure 2a]

Group 3: Regenerated transitional epithelium, intact basement membrane, compact lamina propia, thick smooth muscle bundles, and rare inflammatory cells [Figure 1c]. Mast cells were counted as 3–4 per mm^2 [Figure 2b].

Immunohistochemical evaluation

Group 1: Staining rates with IL-6 and IL-8 varied between 0 and 25%. TNF alpha and Vascular Endothelial Growth Factor (VEGF) stained moderately at a 25–50% [Figure 3].

Group 2: 75–100% intense staining pattern was observed with IL-6, IL-8, TNF alpha, and VEGF stain [Figure 4].

Group 3: IL-6 and IL-8, and TNF alpha were slightly stained at 0–25%. Vascular Endothelial Growth Factor (VEGF) stained moderately at a 25–50% [Figure 5].

We found an increase in IL-6, IL-8, TNF alpha, and VEGF levels in the patient group by the immunohistochemical analysis compared to the control and treatment groups. After treatment with ML, a statistically significant decrease was found in IL-6, IL-8, TNF alpha, and VEGF levels (P = 0,000, P = 0,000, P = 0,000, P = 0,003) [Figure 6]. While six–eight mast cells were detected in the interstitial cystitis group, a decrease was observed in the number of these cells with treatment, and they were counted as 1–4. However, no statistical correlation was found between the groups in this decrease (P = 0,054).

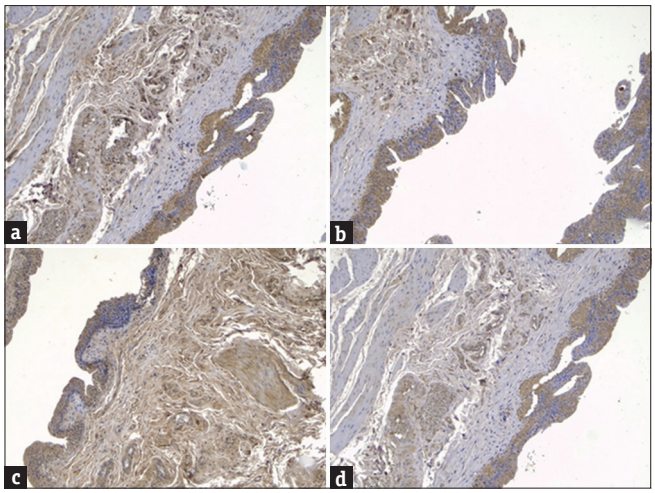

Figure 4: Immunohistochemical staining results of ISC group rats: A—staining with IL-6, B—staining with IL-8, C—staining with TNF alpha, and D—staining with VEGF 75–100% intense staining pattern was observed with IL-6, IL-8, TNF alpha, and VEGF stain (a-d) *(ISC: interstitial cystitis, IL: interleukin, TNF: tumor necrosis factor, VEGF: Vascular Endothelial Growth Factor)*

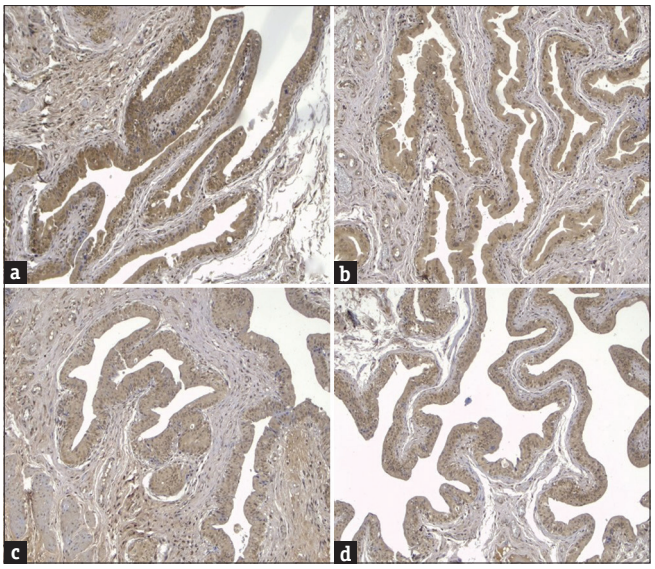

Figure 5: Immunohistochemical staining results of treatment group rats: A—staining with IL-6, B—staining with IL-8, C—staining with TNF alpha, and D—staining with VEGF. IL-6 and IL-8, and TNF alpha were slightly stained at 0–25% (a-c), and Vascular Endothelial Growth Factor (VEGF) stained moderately at a 25–50% (d) *(ISC: interstitial cystitis, IL: interleukin, TNF: tumor necrosis factor, VEGF: Vascular Endothelial Growth Factor)*

Discussion

Interstitial cystitis is a chronic disease characterized by bladder and pelvic pain, urinary urgency, and frequency. It is always easily confused with urinary tract infections. It is a disorder about the sensory dysregulation of the bladder. As a result, perceived pain in the organ and a feeling of increased urgency and/or frequency develop.[1] There are multiple theories relating to the etiology of the IC. It is thought that various causes such as infection, overactivation of mast cells, overproduction of leukotrienes, changes in urothelial permeability, and autoimmunity play a role in the etiology of this disease.[9] In the literature, it has been reported that mast cell numbers have increased in many studies.[5] In interstitial cystitis, partial or completely degranulated mast cells can be diagnosed in the lamina propia, submucosa, and bladder muscle. Fall *et al*.[10] reported that 20 mast cells/mm^2 in the bladder muscle have 88% specificity and 95% diagnostic specificity for IC. Mast cells are more consistently increased in classic IC with Hunner's ulcers.[11] In non-ulcer IC, bladder mast cells show large standard deviations due to heterogeneous study groups and methodological differences. Although the reason for the increase in mast cells in IC is unknown, cytokines such as growth factor (NGF) and stem cell factor (SCF) produced by damaged urothelial cells are known mast cell stimulators.[5] Pro-inflammatory, vasoactive, and nociceptive molecules released from mast cells are thought to be responsible for neuropathic pain and sensory neuronal hyperreactivity seen in IC.[12] While six–eight mast cells were detected in the interstitial cystitis group, a significant decrease was observed in the number of these cells with treatment, and they were counted as 1–4. However, no statistical correlation was found between the groups in this decrease.

Mast cells perform their functions through vasoactive and pro-inflammatory mediators previously synthesized and stored in granules or synthesized de novo.[5,13] Histamine, serotonin, TNF, kinins, and proteases are preformed molecules stored in secretory granules. Vascular endothelial growth factor (VEGF), leukotrienes (LT), platelet-activated factor (PAF), prostaglandins, different types of interleukin (IL), nitric oxide (NO), and tumor necrosis factor (TNF) are synthesized de novo. Therefore, it is an indicator of these amines and cytokines released into the environment without degranulation in mast cells. The presence of these mediators is another indication that mast cells are active.[14] IL-6–8, tumor necrosis factor alpha (TNFα), and VEGF are potent inflammatory cytokines. Inflammatory mediators activate corresponding receptors on cells in the bladder to stimulate different molecular signaling pathways. IL-6 is secreted by inflammatory cells such as macrophages and mast cells. It has been shown that serum and urinary IL-6 levels are high in IC patients, and their levels are associated with symptom severity.[15] In previous studies in the literature, it has been reported that IL-6 positive mast cells are found in the mucosa and detrusor muscle in the IC.[16] In another study, it has been shown that IL-8 does correlate positively with mast cell infiltration

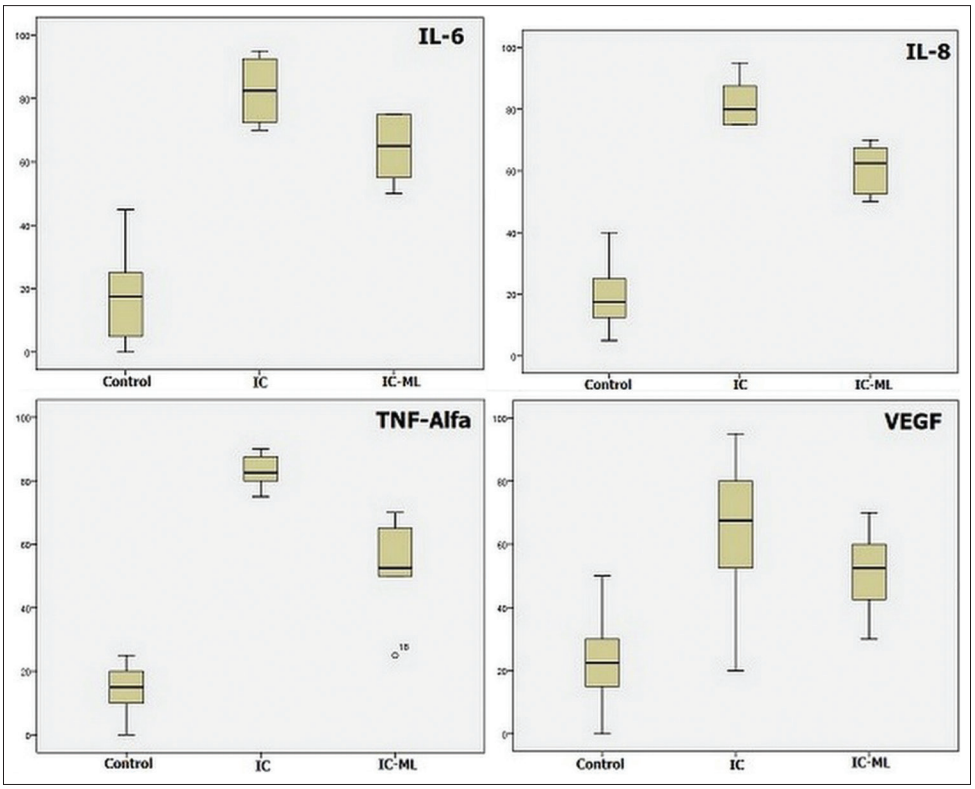

Figure 6: Statistical analysis graph of immunohistochemical staining results. ***Immunohistochemical analysis results:*** It was observed that IL-6, IL-8, TNF alpha, and VEGF levels were significantly decreased in the treatment group

into the bladder wall.[17] IL-8, which has chemotactic properties, increases the inflammatory response through neutrophil, eosinophil, and T lymphocyte migration. Although it has been shown that IL-8 levels can be a highly sensitive biomarker for the diagnosis of IC, a significant correlation was found with the clinical features of the disease.[18] In the previous studies, urinary IL-6 and IL-8 levels in IC have been found at different levels, the relationship of this increase with the presence and severity of the disease in IC has not been fully demonstrated.[17] In our study, we found a significant increase in IL-6/8 in the patient group in the immunohistochemical analysis compared to the control and treatment groups.

TNF alpha is an inflammatory molecule and decreased wound healing in chronic doses.[19] TNFα is released from activated macrophages. It causes an increase in free oxygen radicals and the expression of adhesion factors in the vascular endothelium. At the same time, it provides an inflammatory response by acting as a chemotactic agent for monocyte and polymorph-nucleated leukocytes.[20] TNF alpha is a major component of the soluble factors released by mast cells that mediate the urothelial response. In cell culture studies, it has been shown that inflammation in uroepithelial cells induced by mast cells is suppressed by TNF alpha blockers.[21] Batler *et al*.[21] suggested that TNFα acts in synergy with other inflammatory mediators to elicit urothelial responses to mast cells. Another cell culture study showed that mast cells and TNFα contributed to apoptosis in interstitial cystitis.[22] They found that administration of a monoclonal antibody of TNFα significantly decreased the mast cell in the lamina propria, stabilized the bladder permeability barrier, reported that anti-TNF can be used to treat the bladder permeability barrier, and reported that anti-TNF could be used to treat IC. In our study, we found a significant increase in TNF alpha levels in the patient group. We can suggest that TNF alpha plays a role in ulcerative lesions and inflammation in IC.

Angiogenesis is an important factor in chronic inflammatory diseases. VEGF, an angiogenic cytokine, has been reported to increase the severity of the disease in colitis, asthma, and some cancers.[23] VEGF is expressed in healthy bladder tissue, urothelial, lamina propria, and intramural ganglion cells of the bladder wall. It is also a mediator secreted by stimulated mast cells. In the immunohistochemical analysis, a significant increase in VEGF was observed in our patient group compared to the other groups. VEGF expression is increased in these areas during inflammation.[24] Kiuchi *et al*.[24] showed an increase in VEGF expression and an association with the degree of pelvic pain in bladder

biopsies from patients with IC. Angiogenic factors, such as VEGF, are associated with and contribute to various neurological conditions. It is thought that VEGF may modulate visceral hypersensitivity through neuronal cells and epithelial cells in the inflammatory bladder.[24]

Leukotrienes are a group of bioactive lipids that emerge from arachidonic acid metabolism.[4] Cysteinyl leukotrienes LTC4, LTD4, and LTE4 are known as the slow-acting agents of anaphylaxis and play an important role in inflammation. LTA4 is a common step in all leukotriene synthesis and is metabolized to produce LTC4. It is converted to LTD4 in the extracellular space in LTC4.[25] It has been shown that the frequency of symptoms and complaints of pain are reduced with montelukast treatment in IC, which is known to have a role in the pathophysiology of mast cells. Therefore, it has been found to be a potential treatment alternative.[5] Despite clinical and basic research on interstitial cystitis, a health problem with an increasing incidence, an effective standard treatment has not yet been determined. In the literature, the presence of LTD4 receptors in human detrusor myocytes has been demonstrated in a study.[7] LTD4 is a pro-inflammatory mediator produced from mast cells in the detrusor muscles and causes a spasmogenic effect on the bladder. And it is responsible for the symptoms and pain in IC.[7] Montelukast, which is widely used in the treatment of allergies, blocks LTD4 receptors, and it has been shown in previous studies to have an anti-inflammatory effect through this pathway.[6,8,26] We thought that ML could play an active role in treating IC by reducing inflammatory mediators. After ML treatment, it was observed that the thinned transitional epithelium regenerated, the basement membrane became intact, and the weakened smooth muscles became thickened. Furthermore, it was determined that the inflammatory cells in the tissue decreased. In addition, we found a statistically significant decrease in IL-6, IL-8, TNF alpha, VEGF, and mast cell levels in the ML treatment group. With these findings, we think that ML treatment may play an important role in treating interstitial cystitis.

Conclusions

In conclusion, we have determined the active role of mast cells in inflammation in IC. With these findings, we suggest that IL-6, IL-8, VEGF, and TNF alpha have an important role in the etiopathogenesis of IC. And we found that these inflammatory mediators were significantly reduced after treatment with montelukast in the IC group. According to these results, montelukast can be used as an effective drug in the treatment of IC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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