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Histopathologic evaluation of anti-ulcerogenic effect of montelukast in indomethacin-induced experimental ulcer model

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Background/aims: *The effects of anti-ulcerogenic drugs are dependent on the increase in prostaglandin production and reduction in leukotriene production in the gastric mucosa. Montelukast is an anti-asthmatic drug, a selective reversible cysteinyl leukotriene D4 receptor antagonist. In this study, we aimed to evaluate the anti-ulcerogenic effect of montelukast and to investigate the relationship between its anti-ulcerogenic effect and polymorphonuclear leukocyte infiltration in the gastric tissues.* ***Materials and Methods:*** *Male Sprague-Dawley rats were separated into five groups. Distilled water (control group), famotidine (40 mg/kg), and montelukast (5, 10 and 20 mg/kg) were given orally (gavage). Thirty minutes later, indomethacin (25 mg/kg) was administered to all the groups. Six hours later, the animals were sacrificed by decapitation. The ulcer indexes for each stomach and the ulcer inhibition rates for each group were calculated, and the stomachs were later evaluated histopathologically (polymorphonuclear leukocyte infiltration).* ***Results:*** *Ulcer inhibition rates were as follows: famotidine 96.14% and montelukast 59.96%, 72.65% and 76.97% (5, 10 and 20 mg/kg, respectively). Montelukast (10 and 20 mg/kg) showed effects similar to those of famotidine histopathologically.* ***Conclusions:*** *In this study, it was observed that there was a relationship between the anti-ulcerogenic effect of montelukast and polymorphonuclear leukocyte infiltration in the gastric mucosa, and montelukast behaved as an anti-ulcerogenic drug both macroscopically and microscopically.*

Key words: Montelukast, indomethacin, gastric ulcer, polymorphonuclear leukocytes

Montelukastin anti-ülserojenik etkisinin indometazinle oluşturulan deneysel ülser modelinde histopatolojik olarak değerlendirilmesi

Amaç: *Antiülserojenik ilaçların etkileri mide mukozasında prostoglandin üretiminin artışına ve lökotrien üretiminin azalmasına bağlıdır. Montelukast selektif reversibil sisteinil lökotrien D4 reseptör antagonisti, antiastmatik bir ilaçtır. Bu çalışmada, montelukastın antiülserojenik etkisi ve bu etkisinin polimorfnükleer lökosit infiltrasyonu ile ilişkisi araştırılmıştır.* ***Gereç ve Yöntem:*** *Erkek Sprague-Dawley sıçanlar beş gruba ayrıldı. Gruplara distile su (kontrol grubu), famotidin (40 mg/kg) ve montelukast (5- 10 ve 20 mg/kg) ağız yoluyla verildi. 30 dakika sonra bütün gruplara yine ağız yoluyla indometazin (25 mg/kg) uygulandı. 6 saat sonra, denekler sakrifiye edildi. Her mide için ülser indeksleri ve her grup için ülser inhibisyon oranları ölçüldü, daha sonra mide dokuları histopatolojik olarak (polimorfonükleer lökosit infiltrasyonu) değerlendirildi.* ***Bulgular:*** *Ülser inhibisyon oranları famotidin için 96.14%, 5-10 ve 20 mg/kg montelukast için sırasıyla 59.96%, 72.65% ve 76.97% bulundu. Montelukast (10-20 mg/kg) histopatolojik olarak famotidine benzer etkiler gösterdi.* ***Sonuç:*** *Bu çalışmada, montelukastın antiülserojenik etkisi ile mide mukozasındaki polimorfonükleer lökosit infiltrasyonu arasında bir ilişki olduğu gözlenmiştir ve montelukast hem makroskopik olarak hem de mikroskopik olarak antiülserojenik bir ilaç gibi davranmıştır.*

Anahtar kelimeler: Montelukast, indomethazin, mide ülseri, polimorfonükleer lökosit

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INTRODUCTION

Montelukast is a selective leukotriene (LT) D4 receptor antagonist, anti-asthmatic and anti-inflammatory drug that interferes directly with LT production (5-lipoxygenase inhibitors) (1,2). LTs are lipoxygenase pathway products of arachidonic acid metabolism. They can be classified according to their chemical structures and biological activities into two classes: the dihydroxy leukotriene (LTB4) and the cysteinyl LTs (CysLTs). LTB4 has been known as a potent chemo-attractant mediator of inflammation; it stimulates neutrophil chemotaxis, chemokinesis and adherence to endothelial cells, and activates neutrophils, leading to release of enzymes and mediators and degranulation (3). The CysLTs are also intimately involved in changes within the vasculature, and LTC4 and LTD4 cause vasoconstriction (4-6).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain, fever and inflammation, and cause gastric ulcers. The ulceration induced by indomethacin and NSAIDs is attributed mainly to the biosynthesis of cytoprotective prostaglandin resulting in overproduction of LTs and other products of the 5-lipoxygenase pathway (7). These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H+ and Na+ ions and a drop in the transmucosal potential difference, and induce the formation of erosions and ulcers (8,9). Other major factors include a decrease in the secretion of mucus, an inhibition of bicarbonate secretion, a reduction in the mucosal blood flow (10), an alteration in microvascular structures and microvascular injury (11), an increase in acid and pepsinogen secretion, and neutrophil infiltration (12). Neutrophil infiltration into the gastric mucosal tissues is a critical process in the pathogenesis of a variety of gastric ulcers. A relationship between the anti-oxidant and anti-ulcerogenic effects of montelukast was shown in our previous study (13). The purpose of the present study was to investigate the relationship between the gastroprotective effect of montelukast and polymorphonuclear leukocytes (PMNL) infiltration in the gastric tissues.

MATERIALS AND METHODS

Drugs and chemicals

Montelukast (Merck Sharp & Dohme, Turkey), famotidine (Nobel A.Ş., Turkey) and indomethacin (Merck Sharp & Dohme) were used, and all of them were given orally (gavage) with a metal orogastric tube. Indomethacin, montelukast and famotidine were dissolved in distilled water.

Animals

The male Sprague-Dawley rats (150-200 g) used in the study were obtained from the Animal Laboratory of the Pharmacology Department (Atatürk University, Medical Faculty, Erzurum). The animals were housed under standard laboratory conditions. Food was withdrawn 24 hours (h) before the experiment, but the animals were allowed free access to water. Experiments were in accordance with the recommendations from the Declaration of Helsinki and the internationally accepted principles in the care and use of experimental animals.

Procedure

The effects of montelukast on PMNL infiltration in an ulcer model produced by indomethacin were investigated (14). Animals were separated into five groups as follows: control (distilled water - 5 ml/kg), famotidine (40 mg/kg), and montelukast (5-10-20 mg/kg). Distilled water, famotidine and montelukast were given orally, and 30 minutes (min) later, indomethacin (25 mg/kg–per oral) was administered to all the groups. Six hours later, the animals were sacrificed by decapitation. The stomachs were removed, then opened along the great curvature and washed with tap water to remove the gastric contents, and examined under a dissecting microscope with square-grid eyepiece to assess ulcer formation. For each stomach, ulcerated and total areas were measured in mm2. The ulcer indexes (UIs) for each stomach were calculated using the following formula:

UI = [Ulcerated area / Total stomach area] X 100, and

the anti-ulcerogenic activity (AUA) for each group was calculated by the following formula:

$$AUA\ (\%) = [\ (UI_{CONTROL} - UI_{TREATED} / UI_{CONTROL})\ ] \times 100.$$

Pathological analysis

The stomachs were fixed in 10% formalin solution and routinely processed for paraffin embedding. From each sample, 4 Ìm-thick sections were obtained and stained with hematoxylin-eosin for evaluation. Intramucosal PMNLs infiltration was scored in 10 separate microscopic fields (X 40 high power field [hpf]): score 0=PMNLs <10%; score 1= PMNLs 10–50%; score 2= PMNLs 50–75%; and score 3= PMNLs >75%.

Analysis of results

All data were expressed as mean ± standard error of the mean (SEM). A value of $p<0.05$ was considered to be statistically significant. UIs of the groups were analyzed by one-way analysis of variance (ANOVA) and post-hoc LSD test. The histological scores were statistically analyzed using the Kruskal-Wallis test, and differences between the groups were evaluated using the Mann-Whitney U test.

RESULTS

Montelukast and famotidine showed protective effects on indomethacin-induced ulcer (Table 1). The anti-ulcerogenic effect of famotidine was 96.14% (p=0.000) in comparison to the control group. The AUAs presented by montelukast at 5, 10 and 20 mg/kg doses were 59.96% (p=0.000), 72.65% (p=0.000) and 76.97% (p=0.000), respectively. Although famotidine showed more AUA than montelukast, the difference was not statistically significant.

According to PMNL infiltration in gastric tissues, famotidine and montelukast caused a decrease in the PMNL count (Table 1, Figures 1A, B, C, D and E). It seemed as if montelukast (10 and 20 mg/kg) was more effective than famotidine, but these decreases were not statistically significant. Among all groups of the study, PMNL infiltration was the least with 10 mg/kg dose of montelukast. In comparison to the famotidine group, the statistical difference was 0.058.

DISCUSSION

The present study evaluated both macroscopically and microscopically the gastroprotective effects of different doses of montelukast on indomethacin-induced gastric damage in rats. The results were compared these those obtained with famotidine as a classic anti-ulcerogenic drug, and montelukast presented gastroprotective effects.

We previously showed the anti-ulcerogenic and anti-oxidant activities of montelukast in the same ulcer model (13). In this study, we aimed to determine the relationship between the anti-ulcerogenic effects and PMNL infiltration into the gastric mucosa.

Indomethacin- and other NSAIDs-induced ulcer formations are known to be related with the inhibition of cyclooxygenase, which prevents prostaglandin biosynthesis, which in turn inhibits the release of mucus, a defensive factor against gastrointestinal damage (15), and also decreases the blood flow in gastric mucus (10). Gastric ulcer results from an imbalance of the interactive process of aggressive and defensive factors in the stomach. Recruitment and activation of neutrophils in the gastric mucosa are crucial in indomethacin-induced gastric injury, and gastric mucosal damage reaches the highest point at six hours (16). In our study, animals were sacrificed after six hours, gastric samples were evaluated histopathologically, and both montelukast and famotidine decreased the PMNL infiltration in comparison to the control group; these decreases were statistically significant in the montelukast (10 and 20 mg/kg) and famotidine groups. In this study, an interesting finding was that it seemed as if famotidine was the most gastroprotective drug according to the UI, but montelukast at 10 and 20 mg/kg doses was histopathologically more effective than famotidine according to PMNL infiltration.

Our previous study (13) and the study of Şener et al. (17) had shown that pretreatment with montelukast prevented the increase in myeloperoxidase (MPO) activity. MPO is an essential enzyme for normal neutrophil function, and is also an index of neutrophil infiltration. Neutrophils are a good source of LTs, and it is possible that LTs released by

Table 1. Effects of montelukast on indomethacin-induced ulcer model in rats

Groups	Ulcerated animal	Ulcer index (%) (Mean±SEM)	Anti-ulcerogenic activity (%)	PMNL infiltration (score)
Control (distilled water) 5 ml/kg	6/6	8.08±0.48	-	3.00±0.00
Famotidine 40 mg/kg	4/6	0.31±0.14*	96.14	2.00±0.37 *
Montelukast 5 mg/kg	6/6	3.23±0.81*	59.96	2.50±0.34
Montelukast 10 mg/kg	4/6	2.21±0.80*	72.65	1.00±0.26 **
Montelukast 20 mg/kg	6/6	1.86±0.37*	76.97	1.33±0.33 **

For ulcer index, * p=0.000; in comparison with the control group (post-hoc LSD test).
For PMNL infiltration, * p=0.022, ** p=0.002; in comparison with the control group (Mann-Whitney U test).
PMNL: Polymorphonuclear leukocyte.

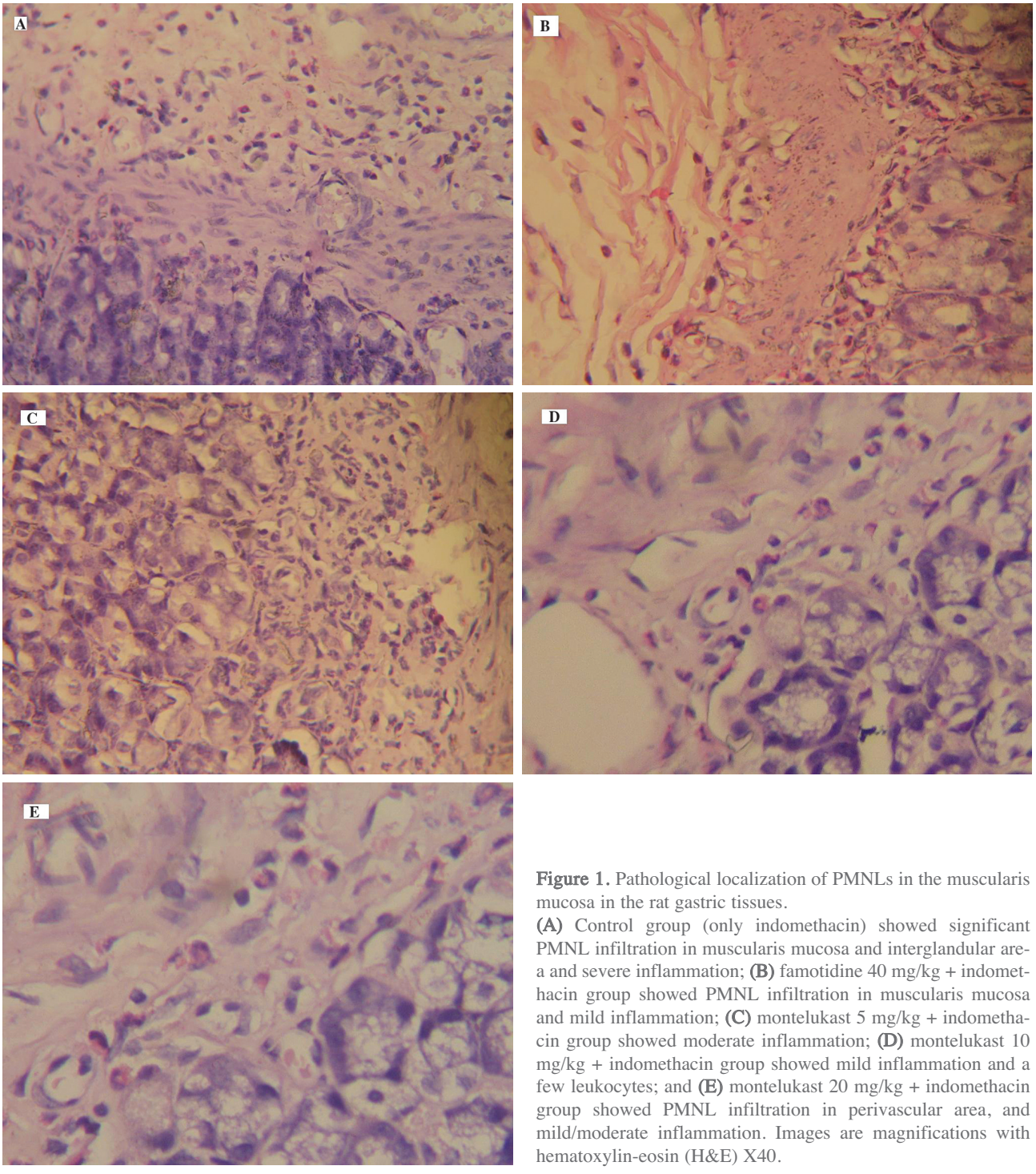

Figure 1. Pathological localization of PMNLs in the muscularis mucosa in the rat gastric tissues.
(A) Control group (only indomethacin) showed significant PMNL infiltration in muscularis mucosa and interglandular area and severe inflammation; **(B)** famotidine 40 mg/kg + indomethacin group showed PMNL infiltration in muscularis mucosa and mild inflammation; **(C)** montelukast 5 mg/kg + indomethacin group showed moderate inflammation; **(D)** montelukast 10 mg/kg + indomethacin group showed mild inflammation and a few leukocytes; and **(E)** montelukast 20 mg/kg + indomethacin group showed PMNL infiltration in perivascular area, and mild/moderate inflammation. Images are magnifications with hematoxylin-eosin (H&E) X40.

the neutrophils contribute to the damage by their vasoconstrictive effect (18,19). Since we previously determined MPO activity in gastric tissue with montelukast, we did not determine MPO activity in this study. In our previous study, the MPO activity in the stomach tissues of the indomethacin group had increased in comparison with that occurring in the tissues of healthy rats. The activity levels of these enzymes had been alleviated with each dose of montelukast, acting counter to the indomethacin. In the previous study, we did not determine the MPO activity of famotidine in gastric tissues, but we determined that ranitidine caused an increase in this enzyme activity. It has been re-

ported that the release of MPO from gastric cells is another indication of the degree of ulceration, with NSAIDs such as indomethacin also exerting their effects via inhibition of MPO pathways (20). Tissue MPO from the antioxidant enzymes has been in widespread use as an index of neutrophil infiltration in various gastric injuries (11,21). Neutrophils are considered to be a major effector cell in the tissue damage occurring in several inflammatory diseases (18,19). Activated neutrophils are known to cause tissue injury through the production and release of reactive oxygen metabolites and cytotoxic proteins (e.g. proteases, MPO, lactoferrin) into the extracellular fluid (19,22).

In conclusion, the gastroprotective action of montelukast, also observed previously (13), was supported with a decrease in PMNL infiltration in gastric tissues. We think that this effect of montelukast is related with its decreasing effect on MPO activity and/or with possible local increases in the synthesis of cytoprotective prostaglandin, inhibition of LTs and gastric mucosal permeability to H+ and Na+ ions. Furthermore, the anti-ulcerogenic action (both macroscopically and microscopically) and antioxidant effects of montelukast may be promising for patients with asthma who suffer from gastric discomfort related with steroid therapy.

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