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Protective effects of montelukast and *Hypericum perforatum* against intestinal ischemia-reperfusion injury in hamsters

Tarık OCAK[1,*], Arif DURAN[1], Gülzade ÖZYALVAÇLI[2], Zeynep OCAK[3], Elçin Hakan TERZİ[4], Mehmet TOSUN[5], Kemalettin ERDEM[6]

[1]Department of Emergency Medicine, Faculty of Medicine, Abant İzzet Baysal University, Bolu, Turkey
[2]Department of Medical Pathology, Faculty of Medicine, Abant İzzet Baysal University, Bolu, Turkey
[3]Department of Medical Genetics, Faculty of Medicine, Abant İzzet Baysal University, Bolu, Turkey
[4]Department of Histology and Embryology, Faculty of Medicine, Abant İzzet Baysal University, Bolu, Turkey
[5]Department of Medical Biochemistry, Faculty of Medicine, Abant İzzet Baysal University, Bolu, Turkey
[6]Department of Cardiovascular and Thoracic Surgery, Faculty of Medicine, Abant İzzet Baysal University, Bolu, Turkey

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Aim: To evaluate the effects of montelukast and *Hypericum perforatum* against ischemia/reperfusion (I/R)-induced intestinal damage.

Materials and methods: Twenty-eight hamsters were divided into 4 groups following midline abdominal laparotomy: control group (n = 7), I/R group (n = 7), montelukast and I/R (MIR) group (n = 7), and *Hypericum perforatum* and I/R (HPIR) group (n = 7). After 60 min of ischemia through obstruction of the superior mesenteric artery, 24 h of reperfusion was maintained. Ten minutes prior to the reperfusion period, the MIR group received 7 mg/kg of intraperitoneal montelukast and the HPIR group received 7 mg/kg of intraperitoneal *Hypericum perforatum*. Malondialdehyde, glutathione, myeloperoxidase, and cardiotrophin-1 levels were measured from blood samples. A semiquantitative histological evaluation was performed.

Results: Montelukast and *Hypericum perforatum* significantly reduced malondialdehyde levels and increased glutathione levels compared to the I/R group ($P < 0.008$). A statistically significant difference was also found between the I/R group and MIR and HPIR groups in terms of myeloperoxidase levels ($P < 0.008$). The MIR and HPIR groups showed increased cardiotrophin-1 levels compared to the control and I/R groups ($P < 0.008$ for all). The MIR and HPIR groups showed significantly lower histological scores compared to the I/R group ($P = 0.03$ and $P = 0.007$, respectively).

Conclusion: This study demonstrated the preventive effects of montelukast and *Hypericum perforatum* on I/R-induced intestinal injury.

Key words: Montelukast, *Hypericum perforatum*, intestinal ischemia-reperfusion injury

1. Introduction

Ischemia-reperfusion (I/R) injuries may occur in the early stages of shock, sepsis, and trauma. Recent developments in cell biology have started to solve the underlying mechanisms or processes involved in I/R injuries (1). The consequences of such an injury are both local and remote tissue destruction, and sometimes death. Initially, I/R injuries appear to be mediated by free oxygen radicals and, at a later stage, by the infiltration and activation of polymorphonuclear leukocytes (PMNLs). The initial ischemic damage is further worsened by a subsequent reperfusion injury. Small intestinal I/R injury may cause deterioration of the mucosal layers, activation of inflammatory processes, and microbial translocation (2). The barrier function of the intestines may be impaired significantly due to this injury, which results in increased permeability or bacterial overgrowth (1). Intestinal I/R might also stimulate injury to secondary organs including the kidneys, lungs, liver, and heart (3).

I/R injury induces apoptosis attributable to increases in PMNL infiltration and reactive oxygen species (ROS) (4). The ROS may cause lipid peroxidation, disrupt membrane integrity, and eventually lead to cell death (5). Once migrated into the ischemic zone, PMNLs release protease, elastase, myeloperoxidase (MPO), and various cytokines, which are involved in I/R-related tissue injury (6).

Montelukast (MK-0476), a reversible cysteinyl leukotriene receptor-1 antagonist, has recently been used in the treatment of asthma and is reported to diminish eosinophilic infiltration into the respiratory tract (7).

* Correspondence: tarikocak78@gmail.com

Additionally, cysteinyl leukotriene receptor antagonists or biosynthesis inhibitors have been reported to ameliorate alcohol-induced gastric damage (8) and experimental colitis (9). Recently, it has been documented that montelukast improved multiorgan damage induced by burn or sepsis through a PMNL-dependent mechanism (10).

Hypericum perforatum, also called St. John's wort, has been used in various ways for wound-healing in the alternative medicine fields of several countries (11). Extracts of *H. perforatum* have been used as a medicinal herb for centuries. It has been proposed to have activity against bacteria, viruses, inflammation, and pain. *H. perforatum* extract, which contains flavonoids and phenolic acids, demonstrated antioxidant and antiinflammatory effects in animal models of acute inflammation (12,13). Oral administration of *Hypericum* tincture was shown to improve wound-healing in an experimental study on rats (14) and, in a clinical study, Samadi et al. reported that *H. perforatum* facilitated cesarean wound healing (15).

In this study, we aimed to evaluate the protective effects of montelukast and *H. perforatum* against I/R-induced intestinal tissue damage in hamsters.

2. Materials and methods

2.1. Subjects

A total of 28 Golden Syrian hamsters were used. The experimental procedures were conducted according to the guidelines for the ethical treatment of experimental animals and approved by the Abant İzzet Baysal University Animal Care and Use Local Ethics Committee.

All animals were kept under 12-h light/dark cycles at a constant room temperature (22 ± 2 °C). All subjects were fed standard hamster chow (210 kcal 100 g^{-1} day^{-1}) and were provided tap water. The animals were fasted for 12 h prior to commencement of the experiments but had free access to water. Ketamine hydrochloride (50 mg/kg, Ketalar, Eczacıbaşı, İstanbul, Turkey) and xylazine hydrochloride (10 mg/kg, Rompun, Bayer, Germany) were administered intramuscularly to induce general anesthesia. The animals were positioned on the operating table in a supine position, immobilized at 4 points, and subjected to abdominal trichotomy using antiseptic techniques with povidone detergent. A midline abdominal laparotomy, with exposure of the abdominal cavity, was performed. The superior mesenteric artery (SMA) was then exposed.

2.2. Experimental groups

All animals (n = 28) were randomly divided into 4 groups as follows:

1. Sham (control) group (n = 7): Hamsters were subjected to a laparotomy and the superior mesenteric artery was exposed. The operations were finished at this stage in this group.

2. Ischemia-reperfusion group (I/R, n = 7): In each hamster, the SMA was isolated using microvascular clips. The ischemic phase was maintained with a complete occlusion of the SMA using microvascular clips for 60 min, thereby interrupting the mesenteric blood flow. We used the procedure developed by Megison et al. (16) to obstruct collateral blood supply from the right colic and jejunal arteries. The atraumatic vascular clamp was carefully removed and the reperfusion period followed for the duration of the next 24 h.

3. Montelukast and ischemia-reperfusion group (MIR, n = 7): The I/R injury was created by the same technique described above. The animals in this group received 7 mg/kg of intraperitoneal montelukast 10 min before the reperfusion period.

4. *H. perforatum* and I/R group (HPIR, n = 7): The I/R injury was created by the same technique discussed previously. In this group, hamsters received 7 mg/kg of intraperitoneal *H. perforatum* 10 min prior to the reperfusion period.

Table. Mean ± SEM values for malondialdehyde (MDA), glutathione (GSH), myeloperoxidase (MPO), and cardiotrophin-1 (CT-1) and significance levels between the groups.

	Control group (n = 7)	I/R group (n = 7)	MIR group (n = 7)	HPIR group (n = 7)	P
MDA (pmol/mL)	19.45 (18–21)[a,b,c]	36.75 (36–37.4)[d,e]	22.50 (21–27)	24.35 (21–29.6)	<0.001
GSH (μM)	25.95 (24.9–27.4)[a,b,c]	17.25 (15.2–19.1)[d,e]	41.35 (38.9–45.1)	40.50 (38.1–47)	<0.001
MPO (ng/mL)	1.71 (1.4–2.1)[a,b]	3.34(3.2–4)[d,e]	2.38 (2.1–2.6)[f]	1.54 (1.5–2)	<0.001
CT-1 (pg/mL)	150.8 (146–159.2)[a,b,c]	41.75 (25.9–67.4)[d,e]	220.90 (206.1–264.8)	237.40 (209.7–252.9)	<0.001

*: Kruskal–Wallis test, statistical significance was accepted as P< 0.05. [a]: Statistically significant difference between the control and IR groups, [b]: statistically significant difference between the control and MIR groups, [c]: statistically significant difference between the control and HPIR groups, [d]: statistically significant difference between IR group and MIR group, [e]: statistically significant difference between IR group and HPIR group, [f]: statistically significant difference between MIR group and HPIR group (P < 0.008 accepted as statistically significant for these comparisons).

In all groups, blood and intestinal tissue samples were obtained 60 min following the reperfusion period. All hamsters were then euthanized with an intracardiac puncture.

2.3. Biochemical analysis

Blood samples were collected in serum separator tubes and allowed to clot for 2 h. Serum was separated by centrifugation for 15 min at 1000 × *g* and stored at –80 °C until analysis. Malondialdehyde (MDA) and cardiotrophin-1 (CT-1) levels and MPO activities were measured with specific enzyme-linked immunosorbent assays using Cusabio Biotech reagents (Hubei, P.R. China). Glutathione (GSH) was measured using a colorimetric assay (Cayman Chemical Company, Ann Arbor, MI, USA).

2.4. Histopathologic Evaluation

Tissue samples were fixed in 10% formaldehyde for 48 h, then embedded in paraffin and cut into 5-μm sections. Slides were stained with hematoxylin-eosin and examined under a light microscope. A pathologist evaluated the slides in a blinded manner. A semiquantitative histological evaluation scoring system was used to determine histopathological changes (17).

2.5. Statistical analysis

SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Biochemical data were analyzed by a Kruskal–Wallis test and expressed as mean ± standard error of the mean (SEM). Mann–Whitney U tests with Bonferroni corrections were used for dual comparisons between the groups. Chi-square tests were used for possible histological score differences between the groups. Statistical significance was accepted at $P < 0.05$ for the Kruskal–Wallis tests and $P < 0.008$ for Mann–Whitney U tests with Bonferroni corrections.

3. Results

Montelukast and *H. perforatum* treatments significantly reduced the MDA levels and increased the GSH levels compared to the I/R group ($P < 0.008$ for both). There was a statistically significant difference between the I/R group and MIR and HPIR groups in terms of MPO levels ($P < 0.008$ for both). The MIR and HPIR groups showed increased CT-1 levels compared to the control and I/R groups ($P < 0.008$ for all). Mean MDA, GSH, MPO and CT-1 levels of the groups and significance status are shown in the Table.

Median histologic values were 0 (0–1) in the control group, 2 (2–3) in the I/R group, 1 (0–2) in the MIR group, and 0 (0–1) in the HPIR group. There was a statistically significant difference between the groups in terms of histologic values. The MIR and HPIR groups showed significantly lower histologic scores compared to the I/R group ($P = 0.03$ and $P = 0.007$, respectively). However, histologic score differences of the control group and the MIR and HPIR groups were not significant ($P = 0.2$ and $P = 0.7$, respectively). There was no significant difference between the MIR and HPIR groups in terms of histologic score ($P = 0.2$). The control group had better histologic scores compared to the I/R group ($P = 0.007$). The architecture of the ileal section in control hamsters demonstrated normal histological structural features (Figure 1a). However, significant mucosal injury with a loss of villi, hemorrhage, and ulceration was observed in hamsters subjected to I/R (Figure 1b). Ileal sections of the MIR and HPIR groups showed minimal alterations characterized with moderate lifting of the epithelial layer from the lamina propria (Figures 1c and 1d).

4. Discussion

In the present study, we found that montelukast and *H. perforatum* are both effective chemotherapeutics against I/R-induced intestinal ischemia model in hamsters. Montelukast and *H. perforatum* administration in addition to I/R injury significantly decreased MDA and MPO levels and increased GSH and CT-1 levels compared to the untreated I/R group. Consistent with these biochemical results, montelukast and *H. perforatum* also showed their effectiveness at the histological level.

I/R injuries are important clinical situations that can be observed following abdominal aortic aneurysm surgery, bowel transplantation, cardiopulmonary bypass, and strangulated hernias. Mesenteric ischemia can also be encountered secondary to hypovolemic and septic shock due to the collapse of systemic circulation. Excited bacterial translocation by I/R injury is thought to contribute to the development of septic multiple organ failure syndrome through portal and/or systemic achievement of bacteria or endotoxins within the gut (18,19). The occurrence of I/R injury has been reported at the end of 30 min of intestinal ischemia followed by 15 min of reperfusion in rats (20). Sun et al. also concluded that a period of intestinal ischemia as short as 45 min can result in serious irreversible changes and blood flow cannot be restored after 120 min of intestinal ischemia (21). In the present study, we used an experimental model of 60 min of ischemia followed by 24 h of reperfusion in hamsters, which resulted in significant I/R injury on the basis of biochemical markers and histological values.

ROS degrade unsaturated fatty acids, forming MDA, which is a sensitive marker of reperfusion injury and lipid peroxidation. Hazinedaroglu et al. found a significant increase in tissue MDA levels in an intestinal I/R model (22). The authors did not find evidence of any preventive effects of N-acetylcysteine on I/R injury by means of histopathologic findings. Other studies showed increased MDA levels after 60 min of reperfusion in rats following 60

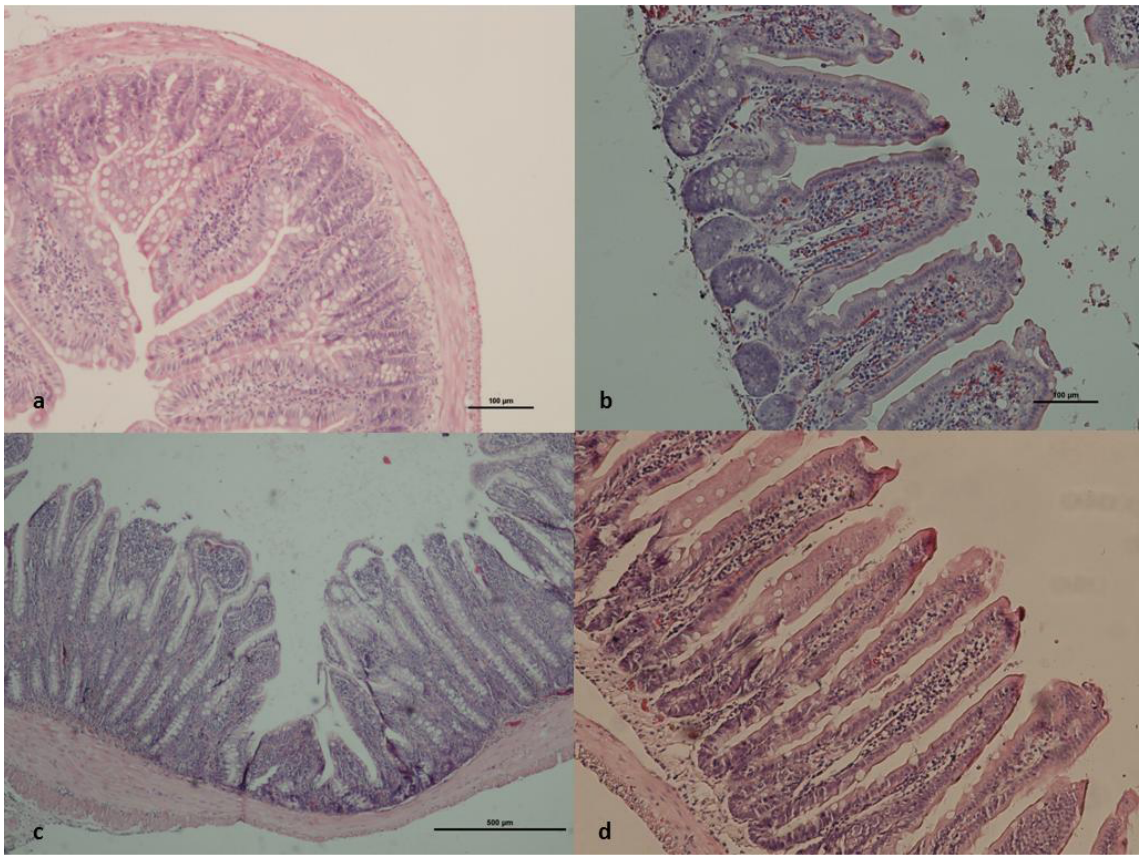

Figure 1. Photomicrographs of hematoxylin and eosin stained sections. **a)** Normal appearance of mucosa in the control group (bar = 100 µm). **b)** The mucosa is almost completely destroyed in the specimens from hamsters in the I/R group. Massive subepithelial lifting and a denuded tip with lamina propria were observed (bar = 100 µm). **c)** Moderate epithelial lifting confined to the tips of the villi was demonstrated in hamsters treated with montelukast (bar = 500 µm). **d)** Moderate epithelial lifting confined to the tips of the villi was demonstrated in hamsters treated with *Hypericum perforatum* (bar = 100 µm).

or 45 min of SMA occlusion (23,24). Önder et al. reported a significant increase in serum MDA levels in a mesenteric I/R injury model of 30 min of ischemia followed by a 1-h reperfusion period (25). The authors concluded that curcumin ameliorated histopathological damage in the intestine and distant organs and prevented the increase in serum MDA levels with mesenteric I/R injury. In the present study, we have detected increased serum MDA levels after 60 min of ischemia by SMA occlusion followed by a 24-h reperfusion period in hamsters. Consistent with previous studies, our findings support the occurrence of oxidative stress-induced tissue destruction after I/R injury. Montelukast and *H. perforatum* administration prevented the increase in MDA levels compared to the I/R group. However, both treatment groups showed increased serum MDA levels compared to the control group.

GSH is an antioxidant provided by dietary intake and can prevent damage to important cellular components caused by ROS, such as free radicals and peroxides. Reduced GSH is the main intracellular endogenous antioxidant produced and can nonenzymatically act directly with ROS (e.g., superoxide radicals and hydroxyl radicals) for their removal. Previous in vivo and in vitro studies showed that midgut I/R injury can activate oxidative stress responses with subsequent ROS generation and GSH depletion (26–28). In our study, decreased serum GSH levels in the I/R group were detected. We also found increased GSH levels in the MIR and HPIR groups compared to the control group. These results indicate that montelukast and *H. perforatum* may protect against intestinal I/R injury through the regulation of downstream antioxidant factors such as GSH.

MPO is the most abundant cytoplasmic enzyme in neutrophils and macrophages. Watson et al. found significant time-dependent increases in MPO activity in mice undergoing intestinal I/R injury. Increased MPO activity in mesenteric I/R injury was also reported by other authors (29–31). Lojek et al. reported that elevated MPO activity was already evident at the end of the ischemic period and maximum MPO activity was observed 3 h

after the onset of reperfusion (31). In the present study, increased serum MPO levels in the I/R group supported I/R-related oxidative stress. Montelukast and *H. perforatum* treatments decreased the MPO levels significantly when compared to the I/R group. The lowest level of MPO was observed in the HPIR and control groups in our study. CT-1 is a member of a family of cytokines that has been demonstrated to have cytoprotective properties against I/R injury (32). The results of our experiments indicate that montelukast and *H. perforatum* treatment reduced some of the ischemia-induced damage and increased CT-1 levels in hamster intestines.

There are some limitations of the present study. First of all, we did not determine the additive effects of montelukast and *H. perforatum* on I/R injury. Secondly, preventive effects of these agents on early I/R injury models were not tested in our study. We used dosages of montelukast and *H. perforatum* according to the current literature. Separately, the dose titration of these agents may provide additional information about the effective dosages of the agents. Two or more additional groups for determining the additive efficacy of these agents and dose titration curve may be more helpful in this context.

In conclusion, the results of the present study revealed that montelukast and *H. perforatum* play an important role in the protection of the intestine against I/R-induced intestinal damage. These agents markedly reduced the intestinal tissue damage due to the impact of both oxidative stress and neutrophils. Further studies are necessary to clarify the dose-response effects of these agents and their applicability in human subjects.

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