



# Effect of Montelukast on Spinal Cord Ischemia- Reperfusion Injury

## *Spinal Kord İskemi-Reperfüzyon Hasarına Montelukastın Etkisi*

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### ABSTRACT

**AIM:** Paraplegia due to ischemia-reperfusion (I/R) injury of the spinal cord is a devastating complication of thoracoabdominal aortic surgery. Cysteinyl leukotrienes are potent mediators of inflammation that are associated with I/R injury. The present study was designed to investigate the role of montelukast, a selective reversible CysLT1 receptor antagonist, on spinal cord I/R injury in an experimental model.

**MATERIAL and METHODS:** Twenty-one male Sprague-Dawley rats were randomly assigned to three groups (n=7 per group) as G1 (no aortic occlusion and montelukast administration), G2 (45 min. aortic occlusion; no montelukast administration) and G3 (45 min. aortic occlusion, 10 mg/kg montelukast administration). After neurologic evaluation using the Motor Deficit Index (MDI) score at the 48th hour of reperfusion, lumbar spinal cords were removed for histopathological evaluation and immunohistochemical staining for HSP70, interleukin-6 and myeloperoxidase (MPO).

**RESULTS:** All rats in the G1 group had a normal neurological status and their MDI score was 0 (p<0.05). The MDI score of G3 was significantly lower than G2 group (2.8 vs. 5.5; p<0.05). Vacuolar congestion was found to be significantly lower in G1 than the other groups (p=0.0001). The interleukin-6 receptor level was found to be significantly lower in G3 group than the control group (p=0.013). There was no statistically significant difference found among the groups in terms of the degree of HSP70 and MPO staining.

**CONCLUSION:** Increased generation of leukotrienes in postischemic organs play an important role in I/R injury. The findings of the current study demonstrated that montelukast improved motor recovery and decreased IL-6 levels in spinal cord I/R injury.

**KEYWORDS:** Spinal cord ischemia, Montelukast, Reperfusion injury, Rat, Experimental, CysLT1 receptor

### ÖZ

**AMAÇ:** Çalışma, deneysel bir modelde, seçici tersinir bir CysLT1 reseptör antagonisti olan montelukastın, spinal kord iskemi-reperfüzyon (I/R) hasarı üzerindeki rolünü incelemek için tasarlandı.

**YÖNTEM ve GEREÇLER:** Yirmi bir erkek Sprague-Dawley tipi sıçan, rasgele üç gruba (her grupta n = 7) ayrıldı; G1 (aort oklüzyonu ve montelukast uygulaması yok), G2 (45 dakika aort oklüzyonu; montelukast uygulaması yok) ve G3 (45 dk aort oklüzyonu, 10 mg / kg montelukast uygulaması). Reperfüzyonun 48. saatindeki Motor Defisit İndeksi (MID) skorlaması kullanılarak yapılan nörolojik değerlendirmeden sonra, lomber spinal kordlar, histopatolojik değerlendirme ve HSP70, interlökin-6 ve miyeloperoksidaz (MPO) için immünohistokimyasal boyama amacıyla çıkartıldı.

**BULGULAR:** G1 grubundaki tüm sıçanların nörolojik durumu normaldi ve MDI skorları 0'dı (p <0.05). G3'ün MDI skoru, G2 grubuna göre anlamlı derecede düşük bulundu (2.8 vs 5.5; p <0.05). G1'de vaküoler konjesyon, diğer gruplara göre anlamlı derecede düşük bulundu (p = 0.0001). İnterlökin-6 reseptörü düzeyi, G3 grubunda kontrol grubuna göre istatistiksel olarak anlamlı düzeyde daha düşük bulundu (p = 0.013). HSP70 ve MPO boyanma derecesi açısından gruplar arasında istatistiksel olarak anlamlı fark yoktu. Postiskemik organlarda lökotrienlerin artan üretimi, I/R hasarında önemli bir rol oynamaktadır.

**SONUÇ:** Çalışmanın bulguları, montelukastın motor iyileşmeyi geliştirdiğini ve spinal kordun I/R hasarında IL-6 düzeylerini azalttığını göstermiştir.

**ANAHTAR SÖZCÜKLER:** Omurilik iskemisi, Montelukast, Reperfüzyon hasarı, Sıçan, Deneysel, CysLT1 reseptörü

### INTRODUCTION

Paraplegia due to ischemia-reperfusion (I/R) injury of the spinal cord is a devastating and undesired complication of thoracoabdominal aortic surgery, ranging in incidence from 2.9% to 38% (9, 32). Temporary or permanent ischemia of the spinal cord is inevitable during the operation and caused by interruption of the blood supply during aortic cross-clamping

(33). Several strategies have been implemented to increase spinal cord perfusion and modulate the immune system (20). These strategies seem to be partly helpful in reducing the risk of spinal cord deficits. However, despite these protective approaches, paraplegia still remains a devastating complication of thoracoabdominal aortic surgeries.

Oxidative stress due to the reperfusion of recruit leukocytes is the main mechanism of inflammation. This further exacerbates the inflammatory reaction by immune cell infiltration (10). The release of pro-inflammatory cytokines, chemokines, proteolytic enzymes, adhesion molecules and free oxygen radicals result in neuronal edema, membranous lipid peroxidation, calcium overload, breakdown of blood-nerve barrier, nerve fiber degeneration and cellular apoptosis (10, 23, 36).

Interleukin-6 (IL-6) is a pleiotropic cytokine engaged in the differentiation of B lymphocytes and an important mediator of the inflammatory response in ischemia. It is generally considered as a non-specific marker of inflammation that is released in response to infection, burns, trauma, neoplasia (30).

Myeloperoxidase (MPO) is one of the distinct indicators for the tissue infiltration of neutrophilic granulocytes. MPO activity increases in response to I/R injury (14).

Heat shock proteins (HSPs) are cellular stress proteins which have been shown to have an important role for the survival of cells under stress conditions (2). Zhang et al. pointed out that HSP70 could respond to a wide variety of stress conditions such as ischemia, and inflammation (43). It can prevent the irreversible denaturation of proteins (17). It has been shown that overexpression of HSP70 attenuates the release of inflammatory factors and interfere with apoptotic cell death (5, 11, 22).

Cysteinyl leukotrienes (CysLTs), namely leukotriene (LT) C<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>, which are the 5-lipoxygenase (5-LO) metabolites of arachidonic acid, are potent mediators of inflammation that are associated with I/R injury (21, 31). Montelukast is a selective reversible CysLT<sub>1</sub> receptor antagonist. It is clinically used for the treatment of asthma by reducing the eosinophilic inflammation in the airways (7, 41). Anti-inflammatory and antioxidant protective effects of montelukast against I/R injury of kidneys, ovaries, liver, brain and skin flaps have recently been shown in several experimental studies (6, 19, 24, 25, 31, 42). Therefore, the present study was designed to investigate the role of montelukast on spinal cord I/R injury in an experimental model.

## MATERIAL and METHODS

### Animal Care

This study was approved by the Ethical Committee of Düzce University Graduate School of Medicine. All rats received humane care in compliance with the European Convention on Animal Care. Twenty-one male Sprague-Dawley rats weighing 250 to 350 g were housed in cages and maintained on a 12-hour light/dark cycle with free access to food and water. The animals were kept within the same unit at a room temperature between 18°C and 21°C. The animals were followed for 15 days before the procedure. None of the animals had any neurological abnormality before anesthesia and surgery.

### Anesthesia and Monitoring

Rats did not receive food or water within 8 hours before anesthesia. 50 mg/kg ketamine and 5 mg/kg xylazine was administered for induction of anesthesia. Maintenance of anesthesia was accomplished by intermittent delivery of ketamine (25 mg/kg). Animals were allowed to breathe spontaneously without mechanical ventilation and core temperature was maintained between 36.5 and 37.5°C by external measures. The animals received oxygen at 200 mL/minute via a pediatric face mask throughout the procedure. Each operation was performed in the same operating room at ambient temperature. Ensuring adequate depth of anesthesia, a 24G catheter was surgically inserted into the left jugular vein to provide intravenous fluid replacement (0.9% isotonic saline solution). An arterial 24G catheter was inserted into the left carotid artery for monitoring the arterial blood pressure. The core temperature above 36°C was followed with a rectal probe. The animals received prophylactic antibiotics (procaine penicillin, 200,000 units administered intramuscularly twice a day) for 2 days in the immediate postoperative period. 400 IU/kg of heparin was administered to all animals immediately before the procedure. Postoperative analgesia was maintained by subcutaneous injection of tramadol.

### Study Groups

Twenty-one male Sprague-Dawley rats weighing between 250 and 350 g were used in the study. Intraperitoneal heparin was administered immediately before the procedure to animals in the study groups. The animals were divided into 3 experimental groups:

- Group G1 (Vehicle-treated Sham-operation group) (n=7): The operation was performed in the same fashion, but without aortic occlusion and montelukast administration.
- Group G2 (Vehicle-treated ischemia group)(n=7): Aorta was cross-clamped for 45 minutes. No montelukast administration.
- Group G3 (Montelukast treated group)(n=7): Aorta was cross-clamped for 45 minutes and 10 mg/kg of montelukast solution was administered for 30 minutes before and during the ischemic period.

### Montelukast Sodium Parenteral Preparation Method

We did not have a commercial parenteral form of montelukast. For this reason, the parenteral form was prepared from oral tablets. Ten tablets of montelukast (Onceair®; Merck & Co. Inc., Whitehouse Station, NJ, USA, 10 mg) were dissolved in 10 milliliters of ethanol. The solution was centrifuged for 5 minutes at 5000 rpm to precipitate ethanol-insoluble excipients and the resultant supernatant was filtered using a filter with pores of 0.2µm. The filtered solution was concentrated using an evaporation method and reduced to a volume of 3 ml. In this solution, there was approximately 30 mg/mL concentration of montelukast sodium. 100 µL of this solution was injected into the semi-preparative high-performance liquid chromatography (HPLC) system and fractionated according to

time of extracting from chromatogram. We use a method in the HPLC device which was previously validated (28).

The peak of montelukast sodium was at the 12.115th minute in HPLC chromatogram. Fraction was collected from the initiation till the termination of the peak. The same process was repeated until the collected amount of montelukast sodium reached a desired concentration in the fraction (approximately 20 times). All fractions were collected together.

The concentration of drug obtained from the fraction was measured by analytical HPLC. 1 tablet dissolved in 10 ml of ethanol and stock solution was obtained. Diluting this stock solution, three different calibration standards were constituted. The proportion of the peak areas of montelukast sodium was used to obtain a calibration curve. 5 µL from this fraction was injected into the analytical system. A mixture of water-acetonitrile (ratio 5:95) was used as mobile phase at a rate of 1 mL/min. The RP Semi-preparative (ACE) column, which was filled with 4.6x250 mm C18 (5 mm particle diameter), was used. Measurements were taken with a 225 nm wavelength UV detector.

As a result of this analysis, the purity and concentration of montelukast sodium, was obtained by fractioning method.

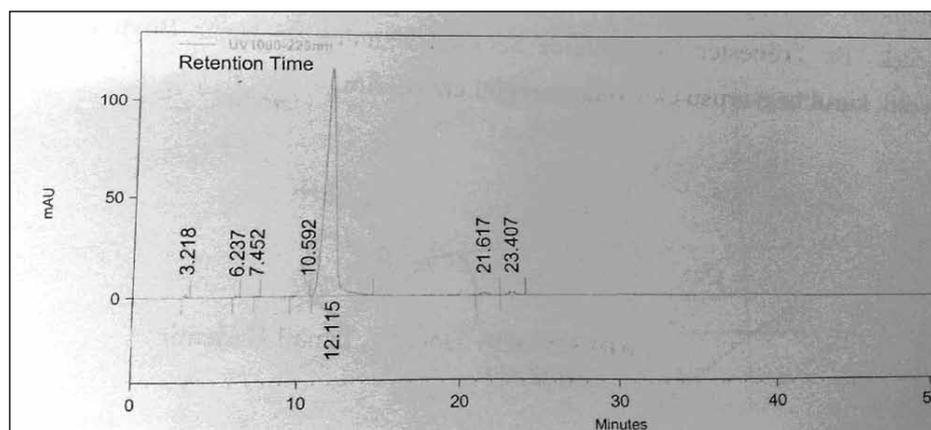
The amount of montelukast sodium was determined by placing the peak area of obtained fraction in HPLC into calibration curve (Figure 1).

The liquid phase of the fraction evaporated. The remaining solid part was substantially diluted with distilled water to obtain a 10 mg/mL of solution. The purity ratio was calculated to be 97.874% respectively (Figure 2).

#### Surgical Procedure

Spinal cord I/R was performed according to the method described by Lafci et al. (16) and Korkmaz et al. (15). After adequate anesthesia was maintained, supine positioning and sterile preparation of the animals was made. A midline laparotomy incision was used to expose infrarenal abdominal aorta. Careful dissection was made to isolate the aorta from the left renal artery to aortic bifurcation. Heparin was administered before clamping the aorta and not reversed by protamin in the study groups after removal of the aortic clamps. The aorta was cross-clamped after the left renal artery and before the aortic bifurcation (3).

Loss of aortic pulse was confirmed by palpation. The duration of the ischemic insult was 45 minutes. Following removal of the cross-clamp, distal perfusion was observed visually. Upon completion of the procedure, the abdominal wall was closed



**Figure 1:** Pure Montelukast Sodium Obtained with preparative HPLC chromatogram device.

Retention Time (Minute)	Area (AU)	Area Percent (%)	Integretion Codes
3.218	17195	0.327	MM
6.237	6228	0.119	MM
7.452	5368	0.102	MM
10.592	5119	0.097	MM
12.115	5143884	97.874	MM
21.617	33156	0.631	BD
23.407	44671	0.850	VB
<b>TOTAL:</b>	<b>5255621</b>	<b>100.000</b>	

**Figure 2:** Purity ratio of Montelukast Sodium in HPLC chromatogram.

in a double-layer fashion with 4-0 prolene sutures. After anesthesia was discontinued, the rats were separated from each other to allow optimal recovery from anesthesia.

#### **Postoperative Care and Neurological Evaluation**

At 1 hour of reperfusion, arterial and venous lines were removed, and all medications were stopped. The animals were returned to their cages following their recovery from anesthesia. The Crede maneuver was used to empty the bladders of the paraplegic animals at least twice daily.

An independent observer, who was blinded to the protocol and group assignments, assessed the motor deficit index (MDI) (34) of the animals after 48 hours of reperfusion. MDI was scored using the assessment of ambulation using the hindlimbs and by the placing/stepping reflex. Assessment of the ambulation of lower extremities was quantified as: 0: normal (symmetric and coordinated ambulation)

1: toes flat beneath the body while walking but ataxia is present

2: knuckle walking

3: unable to knuckle walk but there is some movement in lower extremities

4: no movement of the lower extremities

The placing/stepping reflex was evaluated by dragging the dorsum of the hindpaw along the edge of the surface. This movement causes a response of coordinating lifting and placing. It was graded as:

0: normal

1: weak

2: no stepping response

The MDI score of a rat was the sum of ambulation and placing/stepping reflex score. The maximum deficit was indicated by a score of 6. Animals with MDI<3 were considered as non-paraplegic, and animals with MDI≥3 were considered as paraplegic.

#### **Sacrifice and Tissue Preparation**

Immediately after functional assessment at the postoperative 48th hour, the animals were killed by an intracardiac injection of sodium pentobarbital (100 mg/kg). The spinal cord segments of the animals between 1st lumbar and 1st sacral vertebrae were harvested immediately via an anterior approach. Each spinal cord was longitudinally divided into 2 equal parts with a fine scalpel. One of the halves was fixed in 10% neutral buffered formalin solution and embedded in paraffin. The other half was snap-frozen for histopathological examination. The experimental model was carried out according to the experimental studies in the literature that investigate the spinal cord and visceral organ damage after cross-clamping the aorta (12, 29, 39).

#### **Histopathological Evaluation**

The paraffin-embedded spinal cord samples were sectioned

into 4-µm-thick transverse sections, which were then stained with hematoxylin-eosin (HE). The histopathological investigation was carried out by two histopathologists blinded to the group assignments. The slides were examined using a light microscope (Olympus BX51; Olympus Corp., Tokyo, Japan) at x400 magnification to assess the degree of spinal cord injury. The gray matter (motor neurons) and white matter (axonal structure and glial cells) were assessed for ischemic injury. A semi-quantitative scale was devised to assess ischemic features. Neurons with an eosinophilic cytoplasm and without a nucleus were classified as injured or dead. Neurons were classified as viable if they had a prominent nucleus with chromatin and nissl bodies in their cytoplasm (3). Four spinal cord injury parameters were evaluated: neuronal degeneration, axonal vacuole formation, edema and inflammation. At least 10 fields from each spinal cord section were examined for the severity of these changes.

Spinal cord injury was scaled relatively as 0, 1 and 2 for absent, moderate, and severe injury, respectively. Edema and vacuolar congestion was scaled as 0 and 1 for absent and present. The intensity of the inflammatory response was evaluated by number of leukocytes which infiltrated the tissue sample, as "0" if there were no leukocytes, "1" if the number of leukocytes was less than 20, "2" if the number of leukocytes was between 20 and 50 and "3" if the number of leukocytes was more than 50 (3).

#### **Immunohistochemistry Examination**

Blood samples from each rat were obtained at the end of 48h immediately before the sacrifice. The chest wall was cleansed with chlorohexidine in spirit, and a sterile 10ml syringe was then used to obtain a blood sample by direct cardiac puncture. Blood samples for cytokine assay were collected into heparinized (20 unit/ml blood) sterile tubes and immediately transferred on ice to be centrifuged at 2000 rpm (at 4°C) for 10 minutes. They were stored at -70°C until the time of assay for IL-6, MPO and HSP-70.

**Analysis of HSP70:** Paraffin sections (4 µm thick) were prepared. Tissue sections were deparaffinized and hydrated in xylenes and graded alcohol. The sections were incubated with primary anti-HSP70 (clone BRM.22, dilution 1/80, Biogenex, San Ramon, California) diluted in buffer. PBS was used as negative control.

**Analysis of IL-6:** The polyclonal anti-human IL-6 receptor antibody C-20 (Santa Cruz Biotechnologies, Santa Cruz, CA, USA) was used for the detection of IL-6 receptor. This antibody was diluted 1:20. IL-6 receptor immunostaining was also performed according to a streptavidin-biotin-peroxidase protocol. The secondary anti-rabbit antibody was diluted 1:500. Negative controls were performed by omitting the first antibody.

**Analysis of MPO:** Evaluation of the MPO activity of the spinal cord was made by using an anti-MPO kit (Cytostore Inc., Calgary, Alberta, Canada). Tissue samples were put on polylysine-coated slides. After deparaffinization and

**Table I:** Comparison of the Degree of Neuronal Degeneration, Edema, Vacuolar Congestion and Inflammatory Response in the Spinal Cord Specimens

H&E	Grade	G1		G2		G3		P value
		Number	%	Number	%	Number	%	
Edema	0	4	57.1	0	0	3	42.9	0.06
	1	3	42.9	6	85.7	4	57.1	
	2	0	0	1	14.3	0	0	
Neuronal Degeneration	0	4	57.1	2	28.6	1	14.3	0.389
	1	3	42.9	4	57.1	5	71.4	
	2	0	0	1	14.3	1	14.3	
Inflammation	0	4	57.1	3	42.9	3	42.9	0.689
	1	1	14.3	1	14.3	1	14.3	
	2	2	28.6	1	14.3	1	14.3	
	3	0	0	2	28.6	2	28.6	
Vacuolar Congestion	0	7	100	1	14.3	1	14.3	0.0001
	1	0	0	6	85.7	6	85.7	

rehydration, they were incubated in a 3% H<sub>2</sub>O<sub>2</sub> solution for inhibiting endogenous peroxidase activity. Sections were incubated with a blocking solution to prevent non-specific background staining. The incubation process was continued by incubation with primary anti-MPO antibody, biotinylated goat-mouse antibody and chromogenic substrate. Following this process, the sections were counterstained with hematoxylin-eosin (8). The ration of the stained MPO in the cytoplasm of the neutrophils was evaluated by means of intensity and frequency of staining (26).

The intensity of staining (I) was graded as:

0: negative

1: weak

2: moderate

3: strong.

The frequency of staining (F) was expressed with percentage ratio as:

0: less than 3%

1: between 3-25%

2: between 25-50%

3: between 50-75%

4: more than 75%

Grading the intensity and frequency of staining, the "index score" was calculated by multiplying the intensity and frequency grades:

0: IxF=0

1: IxF=1 or 2.

2: IxF=3 or 4.

3: IxF= 6-12

### Statistical Analysis

Statistical analysis and calculations were performed by using SPSS 15 for Windows (Chicago, IL). Results were expressed as the mean (standard error mean). Kruskal-Wallis analysis of variance and Chi-square test was used to detect differences between groups. Statistical comparisons were made using the Mann-Whitney U test. P values less than .05 were considered as statistically significant.

### RESULTS

There was no significant difference in terms of body temperature, mean arterial pressure, heart rate and body temperature among the groups.

### Neurological Outcome

Neurological assessment based on the MDI is shown in Figure 3. All rats in the G1 group had a normal neurological status. The ranges of MDI scores were significantly higher in the other groups (spinal cord ischemia-induced rat groups) than the G1 group ( $p < 0.05$ ). Mean MDI score of G3 was 2.8 (range 0-4) and this value was significantly lower than 5.5 (range 5-6) in the G2 group ( $p < 0.05$ ).

### Histopathological Evaluation

As depicted in Table II, histopathological evaluation of spinal cord tissue samples showed no significant intergroup differences in terms of neuronal cell degeneration, edema, or inflammation; but vacuolar congestion was found to be significantly lower in G1 group ( $p = 0.0001$ ). Comparison of the degree of neuronal degeneration, edema, vacuolar congestion and inflammatory response in the spinal cord specimens is shown in Table I. An example of edema that was found in a specimen taken from an animal in G2 is shown in Figure 4.

**Immunohistochemical Evaluation**

There was no statistically significant difference found among the groups in terms of HSP70 staining ( $p=0.304$ ). The IL-6 receptor level was found to be significantly lower in G3 group than the control group ( $p=0.013$ ) (Table II). An example to grade-2 HSP70 staining in a sample taken from an animal in G3 is shown in Figure 5.

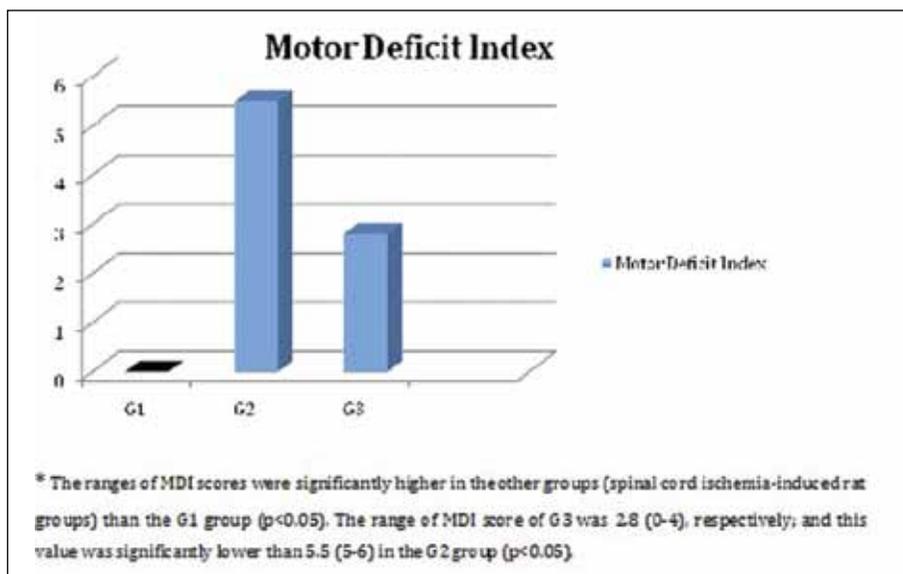
No significant difference was observed between the groups in terms of the degree of MPO staining.

**DISCUSSION**

It is well known that neural tissues are quite vulnerable to ischemia. Cross-clamping the aorta during the surgical treatment of descending thoracic and thoracoabdominal

**Table II:** Comparison of the Groups in Terms of HSP-70 and IL-6 Staining

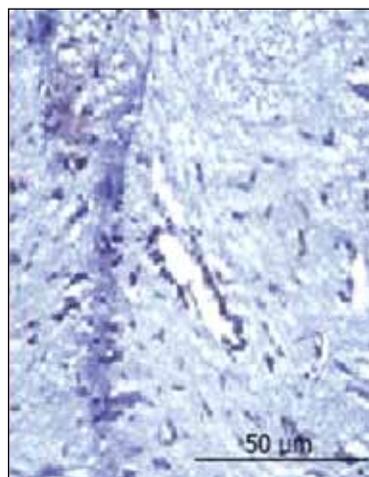
	Grade	G1		G2		G3		P value
		Number	%	Number	%	Number	%	
HSP	0	3	42.9	2	28.6	3	42.9	0.304
	1	4	57.1	3	42.9	4	57.1	
	2	0	0	2	28.6	0	0	
IL-6	0	2	28.6	0	0	4	57.1	0.013
	1	5	71.4	4	57.1	3	42.9	
	2	0	0	3	42.9	0	0	



**Figure 3:** Motor deficit index (MDI) scores of the animals 48 hours after reperfusion (\*).



**Figure 4:** An example of edema that was found in a specimen taken from an animal in G2 (H&E, x200).



**Figure 5:** An example to grade-2 HSP70 staining in a sample taken from an animal in G3. (H&E, x200).

aortic disease inevitably results in temporary or permanent ischemia of the spinal cord. Several strategies have been implemented to maintain spinal cord blood flow (distal aortic perfusion, intrathecal vasodilators, reattachment of intercostal and lumbar vessels, decreasing cerebrospinal fluid pressure), to increase spinal cord tolerance to ischemia (hypothermia, anesthetic agents, calcium-channel blockers, excitatory amino acid antagonists), and to decrease reperfusion injury (free radical scavengers, immune system modulation, adenosine) (20). However, paraplegia remains to be an uncommon but devastating complication of thoracoabdominal aortic surgery.

In the present study, we focused on measures to eliminate or reduce reperfusion injury. Actually, ischemia and reperfusion is a chain reaction resulting in free O<sub>2</sub> radical generation, respiratory burst of activated neutrophils as a response to tissue injury and the autooxidation of catecholamines (20). Free and unstable radicals damage DNA, initiate protein degradation and lipid peroxidation. Ueno et al. showed that lipid peroxidation can spread to circumferential neuronal tissue (38). Temiz et al. showed that lipid peroxidation breaks down the membrane integrity and inactivates the critical membrane bound enzyme systems (37). The overexpression and release of inflammatory mediators, cytokines, activation of phospholipase A2 and complement system, effects of adhesion molecules, activation of arachidonic acid system make I/R injury very complicated and difficult to understand (4, 29, 38).

LTs, which are metabolites of arachidonate, are products of the 5-LO pathway. They are potent chemotactic factors involved in recruitment of cells at the inflammation site, and increase vascular permeability (40). CysLTs are secreted mainly by eosinophils, mast cells, monocytes and macrophages. Previous studies reported that, increased generation of 5-LO products, LTs, in postischemic organs play an important role in I/R injury (18, 31, 35).

Montelukast is a selective antagonist of CysLT1 receptor which is used in the treatment of bronchial asthma (27). It causes a significant dose-related inhibition of superoxide by interfering with the activation of NADPH oxidase. Anderson et al pointed out that besides its primary mechanism of therapeutic activity, antagonism of CysLT1 receptors, it causes an increase in basal cAMP and cGMP suggesting a non-specific PDE –targeted anti-inflammatory activity (1). Yu et al showed the neuroprotective effects of montelukast against I/R injury (42). In our study we aimed to investigate the protective effects of montelukast on spinal cord I/R injury.

Genovese et al. induced a spinal cord injury in a rat model and achieved a significant improvement of the limb function over 10 days (13). Their results are consistent with the present study. Treatment with montelukast significantly improved the MDI of the animals compared with the control group. The range of MDI score of G3 was 2.8 (0-4), respectively; and this value was significantly lower than 5.5 (5-6) in the G2 group ( $p < 0.05$ ).

It is well known that I/R induces potent tissue injury related to change in microvascular environment (23). We are unable to find a statistically significant difference among the groups in terms of edema, neuronal degeneration or inflammation. Vacuolar degeneration was significantly lower in the sham group as expected.

As mentioned earlier, inflammatory reaction plays a key role in spinal cord I/R injury. In order to evaluate the free radical scavenging effects of montelukast, we investigated the levels of IL-6, MPO and HSP-70. The degree of IL-6 staining in montelukast-treated group was significantly lower ( $p = 0.013$ ), suggesting the beneficial anti-inflammatory effect of the drug.

In conclusion, protection of spinal cord from I/R injury requires a multimodal management. Recent developments in operative techniques, anesthetic management and monitoring, pharmacological medications and postoperative care have decreased the risk of neurologic complications but obviously further investigation is necessary. The findings of the current study demonstrated that montelukast improved motor recovery and decreased IL-6 levels in spinal cord I/R injury. The protective effects of montelukast can be attributed to its potential of anti-oxidative, anti-inflammatory and neuroprotective actions.

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