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Effects of Low-Molecular-Weight Heparins on Bacterial Translocation in an Experimental Mesenteric Ischemia Reperfusion Injury Model

Moleküler Ağırlıklı Heparinlerin Bakteriyel Translokasyon Üzerine Etkileri

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ABSTRACT

Background: Low molecular weight heparin (LMWH) has been reported to prevent intestinal bacterial translocation (BT), although this is not certain. The aim of this study was to determine the effect of LMWH used to prevent BT after mesentery ischaemia-reperfusion (I/R).

Materials and Methods: In this controlled experimental study, 21 female Wistar-Albino rats with an average weight of 250-350 grams were used. The rats were randomly divided equally (n=7) into 3 groups (control group, I/R group and I/R+LMWH group). Control group, no procedure other than explorative laparotomy (Ex-lab.) was performed. I/R group, superior mesenteric artery was clamped for 45 minutes and reperfusion was performed for 60 minutes after Ex-lab. I/R+LMWH group, 1 mg/kg enoxaparin sodium was given to the rats 4 hours before the I/R group. At the end of the experiment, samples taken from the ileum were evaluated histopathologically. The number of microorganisms per gram of tissue was analysed in blood, mesenteric lymph node (MLN), spleen and liver samples. Serum nitric oxide (NO) levels were also measured.

Results: When the groups were evaluated histopathologically in terms of ileal tissue damage, the difference between I/R and I/R+LMWH groups was found to be statistically insignificant (p=0.318). However, BT levels in tissue cultures of I/R+LMWH group were significantly decreased compared to I/R group (39% vs. 75%, p<0.001). There was no statistical difference between the bacterial counts per gram of total tissue of MLN, spleen and liver tissues of I/R and I/R+LMWH groups (4458.37 vs. 3157.14 colony-forming units/g, respectively, p=0.101). Although there was no statistical difference between them, NO levels in I/R+LMWH group tended to be higher than control and I/R groups [295 (149-437), 165 (89-298) and 192 (80-263) pg/mL; p=0.0626].

Conclusion: Since LMWH decreased BT and increased NO levels in patients with mesenteric ischaemia, it was determined that LMWH could be used as a therapeutic option to prevent sepsis.

Keywords: Low molecular weight heparin, ischemia-reperfusion, bacterial translocation, nitric oxide

ÖZ

Amaç: Düşük moleküler ağırlıklı heparinin (DMAH), kesin olmamakla birlikte intestinal bakteriyel translokasyonu (BT) önleyebileceği bildirilmektedir. Bu çalışmanın amacı, mezenter iskemi-reperfüzyon (İ/R) sonrası gerçekleşen BT engellemek için kullanılan DMAH'nin etkisini belirlemekti.

Gereç ve Yöntemler: Bu kontrollü deneysel araştırmada ortalama 250-350 gram ağırlığında 21 adet dişi Wistar-Albino sıçan kullanıldı. Sıçanlar eşit şekilde (n=7) randomize olarak 3 gruba ayrıldı (kontrol grubu, İ/R grubu ve İ/R+DMAH grubu). Kontrol grubuna eksploratif laparotomi (Ex-lab.) dışında, herhangi bir işlem yapılmadı. İ/R grubunda; Ex-lab. sonrasında, süperior mezenterik arter 45 dakika klemplendikten sonra 60 dakika reperfüzyon yapıldı. İ/R+DMAH grubunda ise İ/R grubuna uygulanan işlemler yanı sıra, 4 saat

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ÖZ

önce sıçanlara 1 mg/kg enoksaparin sodyum verildi. Deney sonunda ileumdan alınan örnekler histopatolojik olarak değerlendirildi. Kan, mezenterik lenf nodu (MLN), dalak ve karaciğer örneklerinde ise doku gramı başına düşen mikroorganizma sayısına bakıldı. Ayrıca serum nitrik oksit (NO) düzeyleri ölçüldü.

Bulgular: Gruplar histopatolojik olarak ileal doku hasarı açısından değerlendirildiğinde, İ/R ve İ/R+DMAH grupları arasındaki farkın istatistiksel olarak anlamsız olduğu saptandı (p=0,318). Ancak İ/R+DMAH grubunun doku kültürlerindeki BT düzeylerinin, İ/R grubuna kıyasla anlamlı düzeyde azaldığı görüldü (%39 vs. %75, p<0,001). İ/R ve İ/R+DMAH gruplarının MLN, dalak ve karaciğer dokularına ait total doku gramı başına düşen bakteri sayıları arasında istatistiksel olarak fark saptanmadı (sırasıyla, 4458,37'e karşı 3157,14 koloni oluşturan birimler/g, p=0,101). Aralarında istatistiksel fark saptanmamakla birlikte İ/R+DMAH grubunun serum NO düzeyleri, kontrol ve İ/R gruplarından daha yüksek çıkma eğilimindeydi [295 (149-437), 165 (89-298) and 192 (80-263) pg/mL; p=0,0626].

Sonuç: DMAH'nin mezenter iskemi geçiren hastalarda oluşan BT'yi azalttığından ve NO düzeylerini arttırdığından, terapötik bir seçenek olarak sepsisi engellemek amacıyla kullanılabileceği saptandı.

Anahtar Kelimeler: Düşük moleküler ağırlıklı heparin, iskemi-reperfüzyon, bakteriyel translokasyon, nitrik oksit

Introduction

Acute Mesenteric Ischemia (AMI), a vascular condition with an increasing incidence worldwide, has a mortality rate of 60-80% and requires urgent intervention. Reperfusion-induced damage may also occur after therapeutic procedures to restore mesenteric blood circulation without the development of intestinal necrosis. Therefore, an early recognition of pathology is important for effective treatment.

I/R injury in the small intestine after mesenteric ischemia is characterized by microvascular and mucosal changes. Due to decreased resistance of damaged tissues and mucosa to endogenous microorganisms, bacteria translocate to extraintestinal areas, such as the bloodstream, mesenteric lymph node (MLN), liver, and spleen. The physiopathological basis of microvascular changes due to ischaemia-reperfusion (I/R) is attributed to nitric oxide (NO) and oxidative events, which are vascular tone regulators, anti-aggregants, and regulators of leukocyte activation/migration. This can progress to Systemic Inflammatory Response Syndrome, in which many organs are affected. Therefore, correcting the pathology causing microcirculatory dysfunction in the intestine is one of the main goals of treatment. Research is increasingly focused on this issue (1-3). Recently, it has been suggested that heparin-based anticoagulant therapy may be effective in preventing I/R injury, although this is not certain. Low molecular weight heparin (LMWH), which is obtained by depolymerization of unfractionated heparin with an average of 5000 Da, can be effective even at low doses because it binds to plasma proteins. In addition, when administered subcutaneously, its bioavailability is 100%, reaching a peak level in an average of 4 hours and inactivating factor Xa just like unfractionated heparin (4). Many studies have shown that LMWH is also anti-inflammatory (5,6). Moreover, LMWH has been shown to have a protective effect against endothelial damage by preventing leukocyte adhesion in rats with endotoxemia (7). The possible reason for this effect has been attributed to the effect of LMWH to prevent microcirculatory dysfunction. In other words, this effect may be considered as the repair of pathophysiological mechanisms that occur with reperfusion after mesenteric ischemia. Bleeker et al.(8) reported that heparin prevented endothelial cell dysfunction and decreased leukocyte adhesion after ischemia. Another study supporting this finding is the report by Zapata-Sirvent et al. (9) that heparin decreases the incidence of computed tomography (CT) in burn injury.

In light of the above information, we investigated the effects of LMWH, which has been proven to have properties other than its anticoagulant effect, on reperfusion injury, CT, and NO in mesenteric ischemia, which is difficult to treat and manage, and has a high mortality rate.

Materials and Methods

Experimental Animals and Group Creation

In the study, 21 female Wistar-Albino rats, 6-8 weeks old, with an average weight of 250-350 g, and given standard rat chow and water ad libitum, were used. The rats were randomly divided into 3 groups, each group consisting of 7 rats.

Group 1. Sham group (n=7): Rats underwent laparotomy only, and mesenteric ischemia was not induced.

Group 2. Mesenteric I/R group (n=7): 45 minutes mesenteric ischemia followed by 60 minutes reperfusion.

Group 3. Mesenteric I/R+LMWH group (n=7): A single subcutaneous dose of 1 mg/kg LMWH was administered 4 hours before mesenteric ischemia was induced and 45 minutes of mesenteric ischemia was followed by 60 minute of reperfusion.

Enoxaparin sodium (Clexan, Aventis Pharma, France) was used as the LMWH.

After these procedures, blood, MLN, intestinal (ileum), liver, and spleen tissue samples were obtained from all 3 groups.

Surgery Procedure

Group 1 (Sham group): A mixture of Xylazine (5 mg/kg) and Ketamine (40 mg/kg) was administered as a single dose via subcutaneous injection. The rats were placed on the experimental table in the supine position and were secured by their front and hind legs. The abdominal skin of all experimental animals was shaved on an operating table and cleaned with 10% povidone iodine. The abdomens of the rats were then opened (Figure 1A), and a 3-cm laparotomy was performed. The cecum and large intestines were removed from the incision site and preserved by superior mesenteric artery (SMA) observation (Figure 1B). Without any procedure, 2 cm of the terminal ileum was resected and placed in formalin solution for pathological examination. Then, approximately 1x1 cm samples were taken from the MLN, liver, and spleen and placed in microbiologic tubes. Finally, blood was collected from the v. port and placed in microbiological tubes, after which the subjects were sacrificed.

Group 2 (I/R group): The same procedures were performed in the I/R group until SMA was detected. The ligament of Treitz was found and cut, the SMA was occluded with an atraumatic microvascular clamp from the aorta, and the intestines were left to ischemia for 45 minutes. After 45 minutes of ischemia following the observation of pallor in the intestines and disappearance of pulse, reperfusion was performed for 60 minutes after the intestines turned pink and pulses returned when the clamps were opened. The following reperfusion, tissue and blood samples were collected as in the first group.

Group 3 (I/R+LMWH group): Enoxaparin sodium was administered subcutaneously at a single dose of 1 mg/kg 4 hours before the procedure (peak plasma level reached 3-4 hours). Subsequently, the same procedures were applied to the second group.

Histopathological Investigations

For histopathological examination, ileal tissue sections were fixed with 10% formaldehyde. After dehydration with alcohol and embedding in paraffin blocks, thin sections were obtained. These sections were stained with Hematoxylin and Eosin stain. Histologic changes were quantitatively evaluated by light microscopic examination. Tissue damage in the terminal ileum was graded using the Chiu classification (10).

Microbiological Analysis

Sterile tissue (MLN, spleen and liver) samples were weighed on a precision balance and placed in sterile tubes containing 1 mL of thioglycolate broth with known weights. The tissues were crushed under sterile conditions and homogenized by cortexin. Aerop cultures were inoculated on 5% sheep blood agar, chocolate agar, and eosin methylene blue (EMB) agar (RTA, Türkiye) and incubated at 37 °C for 24-48 hours. Colonies in cultured Petri dishes were stained with Gram-stained slides. Bacterial colony counts (Phoenix 100, BD Diagnostics, USA) were determined.

In anaerobic media prepared using gas kits (Anaero-Gen, Oxoid, UK), samples were inoculated with blood, EMB, and Schaedler medium (RTA, Türkiye) and incubated at 37 °C for 72 hours. The morphology of the colonies in growth media was compared with that of the colonies in aerobic media, and gram-stained slides were prepared and analyzed. For aerotolerance control, blood was passaged and incubated at 37 °C for 24 hours in aerobic medium.

The following formula was used to calculate the number of microorganisms per gram of tissue as the CT index in tissues with growth:

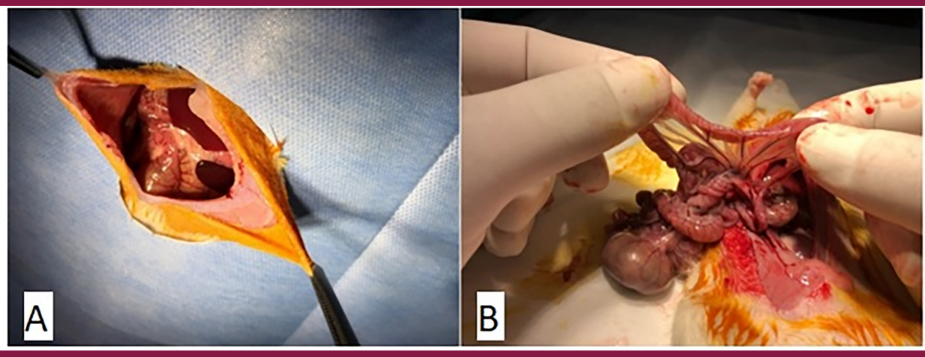

Figure 1. Explorative laparotomy procedure. A) Open the abdomen of rats after skin cleansing with povidone-iodine, B) Visualizing and protecting the Superior Mesenteric Artery

Number of colonies per tissue (CFU/g)=[Number of colonies (CFU)xReconstitution valuex10]/[Tissue weight]

Blood samples were added to automated blood culture bottles (BACTEC 9120, BD Diagnostics, USA) and monitored for 7 days. Samples that did not show any growth signals within this period were Gram-stained slides, passaged on 5% sheep blood agar media, and confirmed as negative. Signaling samples were passaged onto 5% sheep blood agar and EMB medium and incubated at 37 °C for 24 hours. At the end of this period, the colonies in the medium that grew in aerobic cultures were first placed on Gram-stained slides and then identified by microbiological methods.

NO measurements

NO levels in sera obtained from rats were measured in a spectrophotometer (Pharmacia Biotech, Novaspec II, Cambridge, England) using 550 nm wavelength with a colorimetric method kit (Elabscience, USA). Test principle; NO is oxidized by the formation of NO_2 in solution *in vivo*. With a color-developing agent, it gives a red (azo compound). The color intensity is proportional to the concentration.

Ethical Approval

This study was reviewed by the Bolu Abant İzzet Baysal University Faculty of Medicine Animal Experiments Ethics Committee and approved on 10/05/2017 with the decision number 2017/26. The surgeries were performed in Bolu Abant İzzet Baysal University Faculty of Medicine Experimental Animals and Research Laboratory.

Statistical Analysis

The chi-square test was used to compare the presence of categorical data in the control, I/R, and I/R+LMWH groups. In addition, the I/R and I/R+LMWH groups were compared according to the number of bacteria per gram of tissue and ileal damage scoring. Since the data for these comparisons did not show a normal distribution and the number of bacteria in the control group had a value of 0, the non-parametric Mann-Whitney U test was used. Kruskal-Wallis test was used to compare the parametric data of the three independent groups. SPSS 22.0 software was used for data analysis and α=0.05 was used for statistical significance.

Results

Histopathological Examination Results

As a result of histopathological examination of ileal tissue samples obtained from the subjects (Table 1), no difference was found between the I/R and I/R+LMWH groups in terms of damage scoring [3 (3-5) vs. 5 (2-5), p=0.318]. In addition, the histopathological differences between the two groups is as shown in Figure 2. According to these findings, although the I/R+LMWH group had higher damage scores than the I/R group, the difference was not statistically significant. This effect was not interpreted as tissue damage by LMWH.

Microbiological Analysis Results

Bacterial growth in MLN, spleen, liver, and blood tissue samples obtained from subjects was calculated in a sterile environment. When the percentages of bacterial growth in the tissue samples of the subjects in the control, I/R, and I/R+LMWH groups were statistically compared, no bacterial growth was observed in any of the tissue samples of the control group (Table 2). In the chi-square statistic in which the three groups were evaluated together, while there was a statistical difference in the number of MLN,

Table 1. The results of the damage score determined by Chiu Scoring of histopathological changes in the tissue samples of the subjects separately according to the groups

Group Rat number	Control	I/R	I/R+LMWH
1	0	3	5
2	0	3	5
3	0	3	3
4	0	4	5
5	0	5	5
6	0	3	4
7	0	4	2
Mean ± SD Median (Min.-Max.)	0	3.6±0.8 3 (3-5)	4.1±1.2 5 (2-5)
Mann-Whitney U test. P-value.		0.318	

The statistical significance level was taken as α=0.05. Control: The group that underwent only laparotomy without mesenteric ischemia. I/R: Ischemia/reperfusion, LMWH: Low molecular weight heparin, SD: Standard deviation, Min.: Minimum, Max.: Maximum

spleen, and liver tissue samples in which microbiological translocation (growth) was detected (p=0.001; p=0.005; p=0.001, respectively), when only the I/R and I/R+LMWH groups were compared, translocation was detected in 7 MLN tissues of the I/R group (n=7), while translocation was detected in 5 tissues in the I/R+LMWH group. The difference between the two groups was not statistically significant (p=0.127). There were translocations in 6 splenic tissues in the I/R group (n=7) and 3 splenic tissues in the I/R+LMWH group. The difference between the two groups was statistically significant (p=0.031). There were translocations in 7 liver tissue in the I/R group (n=7) and 3 liver tissue in the I/R+LMWH group. The difference between the two groups was statistically significant (p=0.018). However, there was no significant difference in terms of microbiological growth in blood samples obtained from the I/R and I/R+ LMWH groups (p=0.299). In total, bacterial growth was detected in 75% of the tissue samples of the I/R group and 39% of the tissue samples of the I/R+LMWH group. The difference was statistically significant (p<0.001) (Figure 3 and Table 3). According to these results, no growth was observed in any tissue sample from the control group. In addition, we found that bacterial translocation was significantly decreased in the spleen and liver tissue samples of LMWH-treated subjects.

Number of Bacteria per Gram of Tissue

No bacterial growth was observed in any tissue samples from the control group. Bacterial growth was observed in blood samples from one rat in the I/R group. The mean numbers of bacteria per gram of tissue in MLN, spleen, and liver samples of the groups are shown in Figure 4. Although the number of bacteria in the spleen and liver tissues of

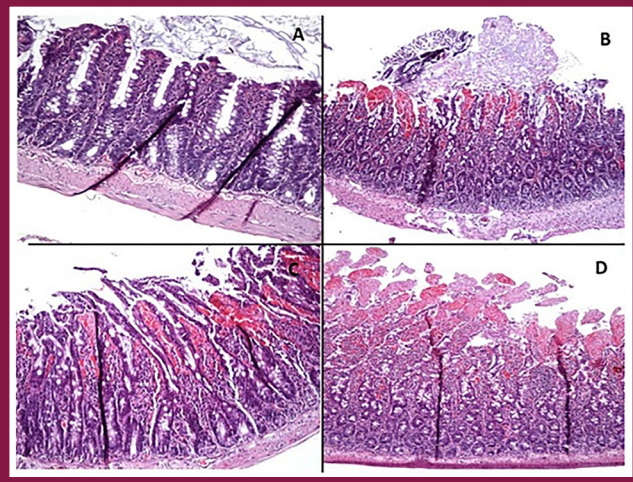

Figure 2. Chiu Scores for Hematoxylin and Eosin stain. A) 0; B) 3; C) 4; D) 5

Table 2. Bacterial growth in mesenteric lymph node, spleen, liver, and blood tissue samples according to groups

Tissue	Groups	NTS, n	NRTD, n (%)	p-value
MLN	A: Control	7	0 (0%)	0.001[a]
	B: I/R	7	7 (100%)	
	C: I/R+LMWH	7	5 (71%)	
Comparison p	0.127			
Spleen	A: Control	7	0 (0%)	0.005[a]
	B: I/R	7	6 (86%)	
	C: I/R + LMWH,	7	3 (43%)	
Comparison p	0.031			
Liver	A: Control	7	0 (0%)	0.001[a]
	B: I/R	7	7 (100%)	
	C: I/R + LMWH	7	3 (43%)	
Comparison p	0.018			
Blood	A: Control	7	0 (0%)	0.350[a]
	B: I/R	7	1 (14%)	
	C: I/R + LMWH	7	0 (0%)	
Comparison p	0.299			

[a]Chi-square test. The comparison of p with the chi square was made only between B and C. The statistical significance level was taken as α=0.05. MLN: Mesenteric lymph node, Control: Group that underwent only laparotomy without mesenteric ischemi, I/R: Ischemia/reperfusion, LMWH: Low molecular weight heparin, NTS: Number of tissue samples, NRTD: Number of reproductive tissues detected

the I/R+LMWH group was lower than that of the I/R group (spleen: 1400.84±2288.82 vs. 5534.18±6811.85 CFU/g p=0.165; liver: 961.42±1551.84 vs. 4099.74±5105.15 CFU/g p=0.165), this difference was not statistically significant. In MLN tissue, the number of bacteria observed in the I/R+LMWH group was higher than that in the I/R group, but the difference was not statistically significant (7109.17±8196.16 vs. 3741.20±3853.67 CFU/g p=0.535, respectively).

NO Levels

Although there was no statistical difference between the groups (Figure 5), the NO levels of the I/R+LMWH group tended to be higher than those of the control and I/R groups [295 (149-437), 165 (89-298) and 192 (80-263) pg/mL, respectively].

Discussion

Mesenteric ischemia remains an important health problem with a high mortality rate (11). Intestinal obstruction, incarcerated hernia, small-bowel volvulus, pneumoperitoneum, and necrotizing colitis are encountered in I/R injury due to AMI in various clinical situations. Mesenteric ischemia causes various morbidities, such as malabsorption, severe diarrhea, short-bowel syndrome, and high mortality rates. The most important reason for the high mortality rate in AMI is the lack of disease-specific investigations and physical examination methods that can be used for early disease diagnosis. Therefore, treatment is delayed. Early diagnosis can prevent the release of several vasoactive mediators (cytokines, endothelins and free oxygen radicals) that block mesenteric blood circulation and reduce leukocyte activation, endothelial dysfunction,

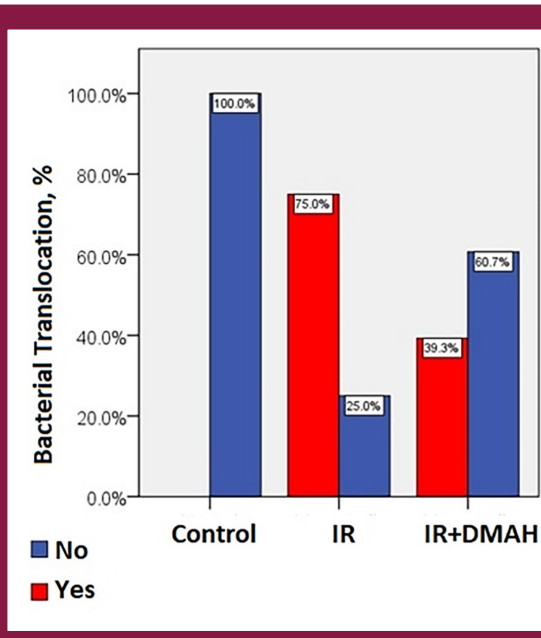

Figure 3. Bacterial growth rates by groups. It appears that bacterial translocation occurs at a higher rate in the I/R group
I/R: Ischaemia-reperfusion, DMAH: Low molecular weight heparin (LMWH)

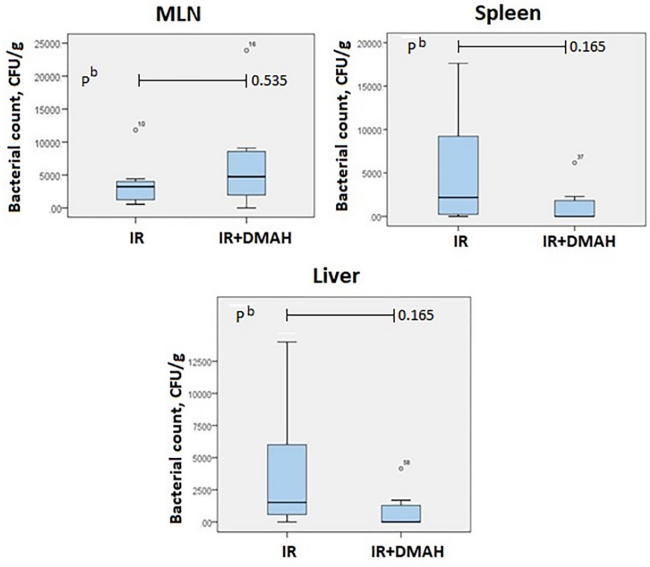

Figure 4. Average bacterial counts of the groups. It is observed that the average bacterial counts are higher in the spleen and liver tissue samples of the I/R group
[b]Mann-Whitney U test, I/R: Ischaemia-reperfusion, DMAH: Low molecular weight heparin (LMWH), MLN: Mesenteric lymph node

Table 3. Comparison of total bacterial reproduction rates among the groups

Groups	NTS	NRTD	p-value
Control	28	0 (0%)	<0.001[a]
I/R	28	21 (75%)	
I/R+LMWH	28	11 (39%)	
Total	84	32 (38%)	

[a]Chi-square test. The statistical significance level was taken as α=0.05. Control: Group that underwent only laparotomy without mesenteric ischemia, I/R: Ischemia/reperfusion, LMWH: Low molecular weight heparin, NTS: Number of tissue samples, NRTD: Number of reproductive tissues detected

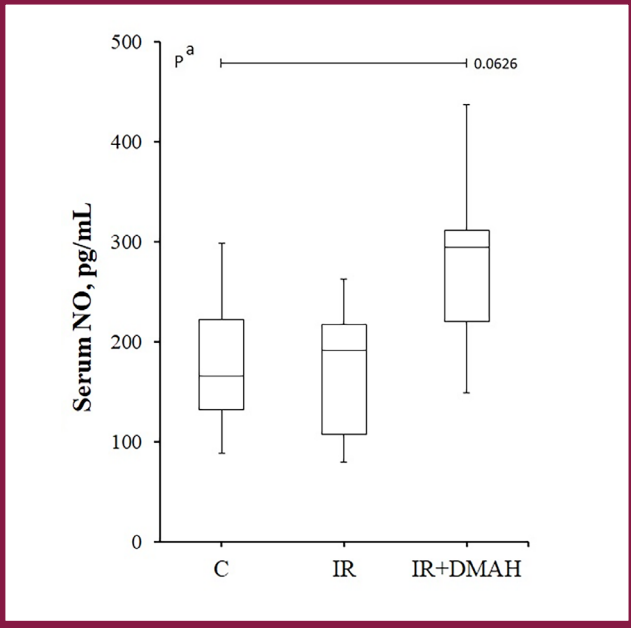

Figure 5. Box plot graph showing NO levels of groups. It was observed that the NO levels of the I/R group are higher than those of the control and I/R+LMWH groups, although there was no statistical difference
[a]Kruskal-Wallis test (non-parametric analysis of variance), I/R: Ischaemia-reperfusion, DMAH: Low molecular weight heparin (LMWH), NO: Nitric oxide

and tissue edema. Therefore, mucosal damage that may occur in the reperfused tissue will be mitigated.

In the studies conducted to date, single or combined pharmacological agents have been tried, but only partial benefits have been found in intestinal I/R injury (12). The main objective of most studies was to prevent or eliminate the formation of inflammatory and toxic mediators that trigger mechanisms that cause multiple damage during the I/R process. In this study, we aimed to investigate whether ischemia-induced tissue damage can be minimized by LMWH in premedication.

One of the consequences of I/R injury in the small intestine after mesenteric ischemia is the translocation of live bacteria and/or their products, termed bacterial translocation (BT), across the intestinal barrier to other tissues. This translocation mostly occurs at sterile body sites such as MLN, spleen, liver, and blood circulation. In a study by Ozban et al. (13) In which intestinal I/R injury was induced by SMA occlusion in rats, BT was demonstrated. Berg (14) described the three primary mechanisms leading to BT. These mechanisms were explained by intestinal bacterial overgrowth, host immune defense inadequacy, and increased or damaged intestinal mucosal permeability. It has also been reported that BT occurring in mesenteric I/R injury may be triggered by oral ricinoleic acid, endotoxemia, zymosan injection, thermal damage, and hemorrhagic shock (14,15). Morehouse et al. (16) showed that intragastric inoculation of ricinoleic acid damages the intestinal mucosa and causes translocation of many endogenous bacteria. In our study, tissue damage caused by mesenteric ischemia in the ileum was demonstrated by pathological examinations and was considered the cause of the increase in CT. Although LMWH was administered, no expected decrease in ileal tissue damage scores in group 3 was not observed. This result was attributed to the small number of subjects or biological differences. Therefore, this finding should be investigated in a larger population.

Prevention of I/R-induced vasoconstriction, oxidative stress, neutrophil migration, platelet aggregation, microcirculatory dysfunction, and BT are considered therapeutic targets. In this context, LMWH, which has a protective effect against endothelial damage and thus is thought to prevent microcirculatory dysfunction, was thought to be used in the treatment of AMI, and its effects on gastrointestinal anatomical changes and CT findings after mesenteric ischemia were investigated. In this context, the fact that NO levels tended to be higher in the I/R+LMWH group than in the control and I/R groups in the blood obtained from rats was interpreted as LMWH prevented tissue damage by inhibiting platelet aggregation and neutrophil migration via NO and regulating microvascular function. In a study by Waisman et al. (17) it was reported that NO was important in the protection of microvascular function and decreased vascular resistance and neutrophil-endothelial cell interaction, which explains the physiopathological

mechanism of our study finding. However, a larger sample size is required to confirm this conclusion.

Alexander et al. (18) have shown that enteric bacteria first settle in the MLN, which is the most vulnerable region, through damaged epithelial cells or cell components along the mucosa. Some bacteria may survive in the MLN or spread to the liver, kidney, and spleen via blood. In our study, although the number of bacteria spreading to the MLN was not statistically significant, a higher bacterial load was found in the MLN compared with the liver and spleen when the number of bacteria per gram of tissue was compared, which is consistent with the literature. This showed that the BT first spread from the MLN, and the bacteria primarily settled in this region.

In recent years, researchers have suggested that antithrombotic molecules such as enoxaparin may also have antioxidant effects. In traumatic brain injury, this molecule has been shown to reduce COX-2 overexpression, increase thiobarbituric acid reactive substances, and increase oxidized protein levels (19,20). In support of these findings, Okutan et al. (21) showed that LMWH (deltaparin, enoxaparin, nadroparin) reduced acute inflammation by reducing early neutrophil infiltration in the vein wall in rats with venous thrombosis. In more detail, Wang et al. (22) reported that the potent anti-inflammatory effects of heparins were mediated by the blockade of P- and L-selectins. Harada et al. (23) showed that a heparinoid derivative (danaparoid sodium) increased the release of the calcitonin gene-related peptide, which ameliorates neuronal damage in rats exposed to I/R injury. In the present study, we demonstrated that enoxaparin can reduce mesenteric ischemia-induced intestinal translocation. However, further studies are required to explain which molecular pathways are involved in this effect. The available data suggest that the antioxidant property of enoxaparin, a LMWH, contributes to this result.

The ability of enoxaparin to provide adequate perfusion in reducing CT has been attributed to its ability to reach sufficient density in small vessels and its anti-inflammatory properties (5,6,24). In a study conducted Iba and Miyasho (5) in rats, it was reported that enoxaparin decreased circulating pro-inflammatory cytokine levels, which may be related to mechanisms that prevent organ dysfunction. In a study in which mesenteric artery ischemia was induced in rats and CT was examined in MLN, it was observed that the decrease in inflammatory molecules was associated with a decrease in the amount of bacteria (25). Another study by Zhang et al. (26) LMWH reduced cerebral I/R injury by regulating energy metabolism and inhibiting apoptosis, in addition to its anti-inflammatory properties.

The anticoagulant properties of heparins are essential to prevent venous thrombosis and improve microcirculation during reperfusion therapy after ischemia. On the other hand, heparins are also known to cause bleeding complications after severe mesenteric I/R (27). In addition, many studies on intestinal I/R injury have reported that heparin sodium administered at therapeutic doses aggravates intestinal injury instead of benefiting (28-30). To investigate this problem, Walensi et al. (31) examined the effect of subtherapeutic doses of enoxaparin (heparin sodium) without anticoagulant effects on intestinal I/R injury in a model of SMA ischemia. This study showed that enoxaparin administered as a premedication provided intestinal protection independent of changes in the intestinal microcirculation and may reduce the risk of ischemia-reperfusion-induced gastrointestinal clinical complications. Again, in a study by Yeh et al. (3) it was reported that enoxaparin prevented intestinal microcirculatory dysfunction by preventing microvascular thrombosis and maintaining arterial pressure in rats with endotoxemia. In our study, when ileal damage classification according to the Chiu score was compared, we found that there was no significant increase or decrease in ileal damage in LMWH-treated rats, and the damage was predominantly observed in grades 4 and 5. This result supported that 1 mg/kg LMWH did not have a positive or negative effect on ulceration and hemorrhage in rats.

The groups included in our study were compared in terms of MLN, liver, spleen, and blood; CT, bacterial load; and ileal damage. Although no significant difference was found between the groups in terms of the number of bacteria per gram of tissue and ileal damage, BT was induced by mesenteric ischemia in all extraintestinal tissues, and this translocation was alleviated by LMWH treatment.

Study Limitations

Since this was an experimental animal study, we were able to work on rats with fewer subjects. Therefore, to clearly assess the effects of LMWH in mesenteric ischemia, studies in humans and more data are needed. The effects on tissues should have also been observed by administering LMWH alone without inducing mesenteric ischemia. This study could be confirmed by a study including other markers of mesenteric ischemia, such as D-dimer and intestinal free fatty acid-binding protein.

Conclusion

It was found that LMWH used in rats with mesenteric I/R was not effective in preventing tissue damage in the terminal ileum, but its effect on NO and microbiological CT was positive. This finding indicated that LMWH could be a therapeutic option for premedication.

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This publication is based on a thesis (Bolu-2018). NO measurements could not be written in the thesis due to the late arrival of the kit.

Ethics

Ethics Committee Approval: This study was reviewed by the Bolu Abant İzzet Baysal University Faculty of Medicine Animal Experiments Ethics Committee and approved on 10/05/2017 with the decision number 2017/26.

Informed Consent: Not required.

Authorship Contributions

Surgical and Medical Practices: S.K., N.A.K., E.B.K., Concept: S.K., N.A.K., E.B.K., Design: S.K., N.A.K., E.B.K., Data Collection or Processing: S.K., N.A.K., E.B.K., Analysis or Interpretation: S.K., N.A.K., E.B.K., M.Ş., O.H.Ö., Z.M.Y.K., Literature Search: S.K., N.A.K., E.B.K., Writing: S.K., N.A.K., E.B.K., M.Ş., O.H.Ö., Z.M.Y.K.

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