



Protective Role of Dexmedetomidine on Ileum and Kidney Damage Caused by Mesenchymal Ischaemia in Rats

Deksmedetomidinin Ratlarda Mezenterik İskemiye Bağlı İleum ve Böbrek Hasarındaki Koruyucu Rolü

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Objective: The aim of this study was to demonstrate ischaemia reperfusion (IR) injury on the ileum and kidney tissue in rats and to evaluate the effect of dexmedetomidine administered at different doses and dosing schedules on recovery.

Methods: A total of 30 rats were randomly divided into five groups. Group I: sham; Group II: control; Group III: dexmedetomidine before ischaemia; Group IV: dexmedetomidine after ischaemia; and Group V: dexmedetomidine before and after ischaemia. The malondialdehyde (MDA) and signal peptide-CUB-EGF (epidermal growth factor) domain-containing protein 1 (SCUBE-1) levels of all subjects were studied from the serum, ileum, and kidney tissues. Moreover, the histopathology of ileum and kidney tissues was examined.

Results: The SCUBE-1 levels were found to be highly similar to the MDA levels in ischaemic groups. The serum SCUBE-1 levels obtained were significantly lower in Group V compared to Groups II, III and IV ($p < 0.001$, $p = 0.003$, $p = 0.013$, respectively). The apoptosis indexes were found to be lower in groups receiving dexmedetomidine compared to Group II. The groups receiving dexmedetomidine were detected to have normal morphological appearance when compared to Group II.

Conclusion: In this study, the use of dexmedetomidine in the preoperative and peroperative periods may be beneficial in reducing the negative effects of IR injury.

Keywords: Dexmedetomidine, SCUBE-1, malondialdehyde, ischaemia reperfusion injury

Amaç: Bu çalışmayla akut mezenterik iskemi oluşturulan ratlarda iskemi reperfüzyon (IR) hasarının ileum ve böbrek dokusu üzerinde gösterilmesi ve farklı doz ve zamanlarda uygulanan deksmedetomidinin iyileşme üzerine etkisini değerlendirilmesi amaçlandı.

Yöntemler: Bu randomize kontrollü çalışma için ağırlıkları 200-250 gr arasında değişen Sprague-Dawley cinsi dişi 30 adet sıçan seçildi. Grup I= sham grup, Grup II= kontrol grup, Grup III= deksmedetomidin iskemi öncesi (İÖ) grup, Grup IV= deksmedetomidin iskemi sonrası (İS) grup ve Grup V= deksmedetomidin iskemi öncesi ve sonrası (İÖ+S) grup olmak üzere rastgele 6'şarlı 5 gruba ayrıldı. Grup I haricindeki diğer gruplardaki deneklerin superior mezenter arterleri 30 dk boyunca klemplenip sonrasında reperfüze edildi. Tüm deneklerin serum, ileum ve böbrek dokularından MDA ve SCUBE-1 düzeyleri çalışıldı. Ayrıca ileum ve böbrek dokularının histopatolojisi incelendi.

Bulgular: İskemi oluşturulan gruplarda serum, ileum ve böbrek SCUBE-1 düzeyleri MDA düzeyleriyle benzer şekilde yüksek bulunmuştur. Serum SCUBE-1 düzeyleri Grup V'te Grup II, Grup III ve Grup IV'e oranla anlamlı olarak düşük saptanmıştır ($p < 0,001$, $p = 0,003$, $p = 0,013$, respectively). Deksmedetomidin verilen gruplarda Grup II'ye oranla ileum ve böbrek apoptozis indeksleri anlamlı düşük bulundu. Grupların histopatolojik incelenmesinde deksmedetomidin verilen grupların sadece IR modeli oluşturulan gruba oranla normal morfolojik görünüme sahip oldukları saptanmıştır.

Sonuç: Bu çalışmayla IR hasarın organlar üzerindeki olumsuz etkisinin azaltılması için preoperatif ve peroperatif dönemde deksmedetomidin kullanımının faydalı olabileceği gösterilmiştir. Dexmedetomidinin IR hasarındaki etki mekanizmalarının aydınlatılması ve ortak bir tedavi protokolünün belirlenebilmesi için geniş serilerde yapılacak ileri araştırmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Deksmedetomidin, SCUBE-1, malonilaldehit, iskemi reperfüzyon hasarı

Introduction

Ischaemia causes both reversible and irreversible cell and tissue damage due to the inadequacy of blood flow perfusing the organ or tissue. The release of cellular energy deposits and the accumulation of toxic metabolites lead to cell death and tissue damage due to ischaemia (1). Post-ischaemic reperfusion paradoxically increases the damage caused by mechanisms such as oxidative

stress and lipid peroxidation. These harmful effects that occur during ischaemia and reperfusion (IR) periods are classified as IR injury (2). Lipid peroxidation, caused by free radicals after ischaemia, forms highly toxic products such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). Due to the effects of these toxic products, membrane structures are degraded, which results in permeability changes, affecting their functions such as ion transport and enzymatic activities (3). With the demonstration of the decrease in antioxidants or the increase in metabolites, it can be evaluated whether IR injury occurs and the level of severity.

Intestinal ischaemia occurs after partial or complete occlusion of the intestinal blood flow, which is usually arterial. The cause in the majority of mesenteric ischaemia cases is the superior mesenteric artery (SMA) embolism due to aortic atheromatous plaques (4). The damage to distant organs following intestinal IR injury, primarily the lungs, can lead to multiple-organ failure and death (5). The intestinal IR also causes a decrease in renal blood flow; thus, the adenosine triphosphate (ATP) level in kidney tissue decreases, resulting in inulin clearance and Na^+ decrease due to renal tubular dysfunction (6). Because of inadequacies in the specific diagnosis and effective treatment of mesenteric ischaemia, whose morbidity and mortality are still quite high, studies related to this issue remain important.

Signal peptide-CUB-EGF 'epidermal growth factor' domain-containing protein 1 (SCUBE-1) is a newly defined cell surface protein, which emerges in early embryogenesis and is secreted. SCUBE-1 is stored in alpha granules of inactive platelets. Activated by thrombin, it is translocated to the platelet surface, secreted as small soluble fragments, and then incorporated into the thrombus. Previous studies have shown that the platelet activation and aggregation are responsible for ischaemic complications (7, 8).

Dexmedetomidine is a selective α -adrenoreceptor agonist commonly used in intensive care units for sedation and analgesia, which has sympatholytic, anxiolytic and haemodynamic stabilising effects. It has been shown in a limited number of studies conducted in ischaemic and toxic inflammatory response models that dexmedetomidine has anti-inflammatory activity and is protective against ischaemia (9). Further studies are necessary to develop treatment models that can prevent progressive cell death triggered by inflammatory mechanisms due to IR injury.

The aim of this study is to investigate the effect of dexmedetomidine administered at different times in preventing tissue damage, measuring the serum, kidney and ileum MDA, and SCUBE-1 levels and conducting the histopathologic examination in rats with an intestinal IR model.

Methods

Experimental animals

Animal testing was performed in accordance with the National Research Council of the National Academies Guide for

the Care and Use of Laboratory Animals and was approved by the local Animal Ethics Committee of Karadeniz Technical University (Ethics Committee Number 2014/3). All rats were provided by Ataturk University's Medical Experimental Application and Research Center. A total of 30 Sprague Dawley female rats, whose food and water were provided under standard laboratory conditions and whose weight ranged from 200 g to 250 g, were used in this study. The rats were randomly divided into five groups of six rats each—Group I: sham group, Group II: control group, Group III: dexmedetomidine before ischaemia (BI), Group IV: dexmedetomidine after ischaemia (AI), and Group V: dexmedetomidine before and after ischaemia (B+AI).

Surgical technique and treatment administration

After administering 50 mg kg^{-1} ketamine (Ketalar®, Pfizer Pharmaceutical Co. Ltd., Istanbul) and 10 mg kg^{-1} xylazine (Rompun®, Bayer Turkish Chemical Industries Inc., Istanbul) intraperitoneally, a vascular access was aesthetically established in all rats under sterile conditions for laparotomy. The rats were administered 10 mL $\text{kg}^{-1} \text{h}^{-1}$ of 0.9% physiological saline solution (PSS) for maintenance. The SMA clamping was not established in Group I: sham group, and rats, whose tissues were taken at the end of 280 minutes, were sacrificed.

After the administration of anaesthesia, Groups II and IV were given 10 mL kg^{-1} PSS, and Groups III and V were given 10 mcg kg^{-1} dexmedetomidine through the tail vein for 10 minutes, followed by a 30-minute rest period. Except for the sham group, subjects were laid in the supine position, surgical regions were cleaned using 10% povidone iodine (Dsolol, Central Lab, Turkey), and traditional asepsis and antisepsis rules were followed. Laparotomy was performed through a midline incision. The SMA was found and clamped by atraumatic microvascular clamp from the aorta. Before clamping, heparin (Nevparin® flakon, Mustafa Nevzat Pharmaceutical Industries Inc. Istanbul, Turkey) at a 200 U kg^{-1} dose was administered intravenously through the tail vein catheter. Intestinal ischaemia was confirmed by the loss of arterial pulsation and the paleness in the intestinal colour. To minimise heat and fluid loss, the intestines were placed in the abdomen, and laparotomy incision was closed with 4/0 atraumatic silk suture. At the end of the 60-minute ischaemia period, the sutures were removed, the clamp of the SMA was removed, and the reperfusion process was initiated.

Reperfusion was confirmed by the loss of paleness in the intestinal colour, an increased pink colouration of the intestines, and the recurrence of arterial pulsation. The intestines were placed back into the abdomen, and the incision was again closed. The intestines were left untouched for 180 minutes during the reperfusion period.

Group IV and V were administered dexmedetomidine through the tail vein during reperfusion as an infusion of 10 mcg $\text{kg}^{-1} \text{h}^{-1}$.

Table 1. The comparisons of serum, ileum and renal MDA and SCUBE-1 values in groups

| | | Group I | Group II | Group III | Group IV | Group V |
|--------------------------------|-------|--------------------------------|--------------------------------|--------------|--------------|--------------------------------|
| MDA (nmol mL ⁻¹) | Serum | 0.35±0.12 | 0.63±0.14 ¹ | 0.43±0.29 | 0.37±0.10 | 0.30±0.06 |
| | Ileum | 89.82±14.70 ^{2,3,4,5} | 280.38±30.10 ⁶ | 193.87±37.04 | 214.83±73.33 | 201.78±58.24 |
| | Renal | 306.94±29.61 ⁷ | 487.08±83.30 ^{8,9} | 353.81±50.30 | 372.97±52.42 | 333.62±99.00 |
| SCUBE-1 (ng mL ⁻¹) | Serum | 17.15±6.10 ^{10,11,12} | 39.60±3.05 | 36.35±13.81 | 33.73±6.95 | 17.80±2.46 ^{13,14,15} |
| | Ileum | 5.04±1.24 | 6.28±0.66 | 5.00±1.39 | 4.60±1.08 | 4.72±1.02 |
| | Renal | 23.29±1.54 ¹⁶ | 35.58±3.04 ^{17,18,19} | 21.91±3.73 | 22.14±5.64 | 21.61±2.22 |

Values are mean±standard deviation.
 SCUBE-1: signal peptide-CUB-EGF (epidermal growth factor) domain-containing protein 1; MDA: malondialdehyde.
¹Group II vs. Group V p=0.017, ²Group I vs. Group II p<0.001, ³Group I vs. Group III p=0.009, ⁴Group I vs. Group IV p=0.001, ⁵Group I vs. Group V p=0.004, ⁶Group II vs. Group III p=0.043, ⁷Group I vs. Group II p=0.001, ⁸Group II vs. Group III p=0.022, ⁹Group II vs. Group V p=0.006, ¹⁰Group I vs. Group II p<0.001, ¹¹Group I vs. Group III p=0.002, ¹²Group I vs. Group IV p=0.009, ¹³Group II vs. Group V p<0.001, ¹⁴Group III vs. Group V p=0.003, ¹⁵Group IV vs. Group V p=0.013, ¹⁶Group I vs. Group II p<0.001, ¹⁷Group II vs. Group III p<0.001, ¹⁸Group II vs. Group IV p<0.001, ¹⁹Group II vs. Group V p<0.001

Table 2. Histopathological evaluation of the terminal ileum and kidney tissue

| | | Group I | Group II | Group III | Group IV | Group V |
|----------------|--|-------------------------------|----------------------------|----------------------------|-----------|-----------|
| Terminal Ileum | Inflammatory cell infiltration | 0.17±0.41 ^{1,2,3} | 1.67±0.52 | 1.50±0.55 | 1.33±0.52 | 1.0±0.63 |
| | Villus fusion | 0.33±0.52 ⁴ | 1.50±0.55 | 1.33±0.52 | 1.00±0.63 | 0.67±0.52 |
| | Villus apical facial epithelium degeneration | 0.17±0.41 ^{5,6} | 2.50±0.55 ⁷ | 1.83±0.41 | 0.83±0.41 | 1.00±0.00 |
| | Haemorrhage | 0.17±0.41 ^{8,9} | 2.50±0.55 ^{10,11} | 1.83±0.41 ^{12,13} | 0.83±0.75 | 0.83±0.41 |
| Kidney | Degeneration in tubular cells | 0.33±0.52 ^{14,15,16} | 2.50±0.55 ^{17,18} | 1.83±0.41 | 1.33±0.52 | 1.17±0.41 |
| | Intertubular congestion | 0.17±0.41 ¹⁹ | 0.33±0.52 | 0.67±0.52 | 0.83±0.41 | 1.00±0.00 |

Values are mean±standard deviation
¹Group I vs. Group II p<0.001, ²Group I vs. Group III p=0.002, ³Group I vs. Group IV p=0.008, ⁴Group I vs. Group II p=0.025, ⁵Group I vs. Group II p<0.001, ⁶Group I vs. Group III p=0.005, ⁷Group II vs. Group IV p=0.02, ⁸Group I vs. Group II p<0.001, ⁹Group I vs. Group III p<0.001, ¹⁰Group II vs. Group IV p<0.001, ¹¹Group II vs. Group V p<0.001, ¹²Group III vs. Group IV p=0.028, ¹³Group III vs. Group V p=0.028, ¹⁴Group I vs. Group II p<0.001, ¹⁵Group I vs. Group III p<0.001, ¹⁶Group I vs. Group IV p=0.014, ¹⁷Group II vs. Group IV p=0.003, ¹⁸Group II vs. Group V p=0.001, ¹⁹Group I vs. Group V p=0.019

Table 3. Terminal ileum and kidney apoptosis

| | | Group I | Group II | Group III | Group IV | Group V |
|------------------|------------------------------|-------------------------------|------------------------|-----------|-----------|---------|
| Ileum apoptosis | 0.83±0.41 ^{1,2} | 2.83±0.41 ^{3,4} | 2.50±0.55 ⁵ | 1.67±0.52 | 1.50±0.55 | |
| Kidney apoptosis | 0.24±0.05 ^{6,7,8,9} | 0.70±0.08 ^{10,11,12} | 0.57±0.05 | 0.48±0.08 | 0.46±0.06 | |

Values are mean±standard deviation.
¹Group I vs. Group II p<0.001, ²Group I vs. Group III p<0.001, ³Group II vs. Group IV p=0.004, ⁴Group II vs. Group V p=0.001, ⁵Group III vs. Group V p=0.016, ⁶Group I vs. Group II p<0.001, ⁷Group I vs. Group III p<0.001, ⁸Group I vs. Group IV p<0.001, ⁹Group I vs. Group V p<0.001, ¹⁰Group II vs. Group III p=0.023, ¹¹Group II vs. Group IV p<0.001, ¹²Group II vs. Group V p<0.001

After the experiment was concluded, the abdomens of rats with an adequate anaesthesia level were opened, 2 mL of blood was taken from the abdominal aorta for the MDA and SCUBE-1 analyses, the right kidney and 4 cm of the ileal tissue from the proximal terminal ileum were removed, then the extracted ileal tissue was washed using cold saline solution. Half of the extracted kidney tissue and a 2 cm piece of the ileal tissue were stored in 10% buffered formaldehyde for histopathological examination. The remaining kidney and ileal tissue were dried using a surgical buffer, and it was then placed in microcentrifuge tubes (Eppendorf) for biochemical

processing and maintained at -80°C until the day of measurement.

Biochemical examination

The MDA level in the serum samples was determined using the thiobarbituric acid method, whereas the MDA levels in the kidney and ileal tissues were determined by the method developed by Miahara and Uchiyama (10). The serum and tissue SCUBE-1 levels were measured using an enzyme-linked immunosorbent assay kit (Cusabio Biotech Co., Catalog No CSBE15005h, P.R. China).

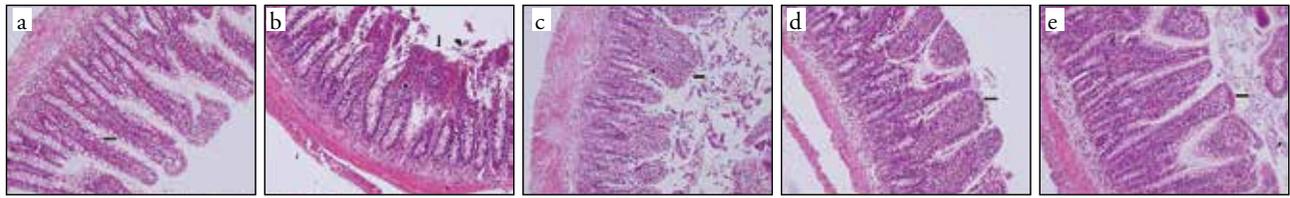


Figure 1. a-e. Light microscopic evaluation of the ileum preparations. (a), Group I; (b), Group II; (c), Group III; (d), Group IV; (e), Group V (see the text for explanation)

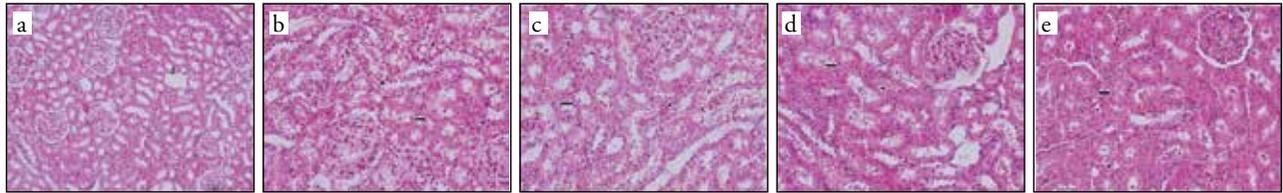


Figure 2. a-e. Light microscopic evaluation of the kidney preparations. (a), Group I; (b), Group II; (c), Group III; (d), Group IV; (e), Group V (see the text for explanation)

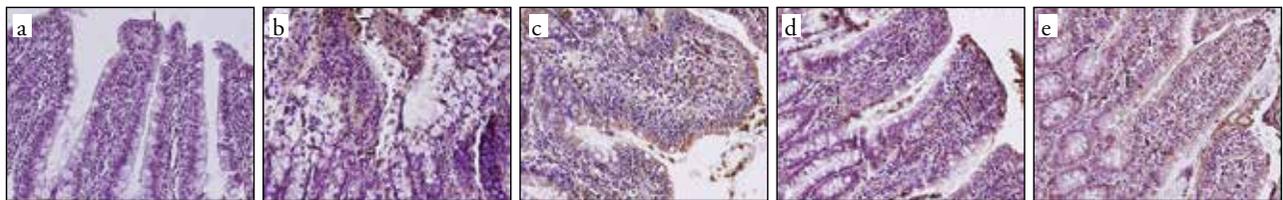


Figure 3. a-e. The degree of ileum apoptosis. (a), Group I; (b), Group II; (c), Group III; (d), Group IV; (e), Group V (see the text for explanation)

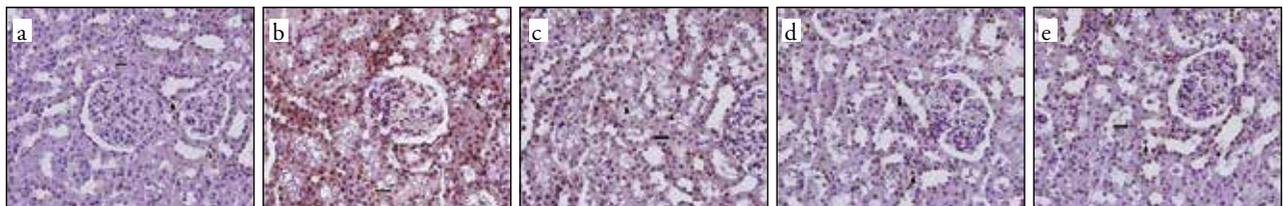


Figure 4. a-e. The degree of kidney apoptosis. (a), Group I; (b), Group II; (c), Group III; (d), Group IV; (e), Group V (see the text for explanation)

Histopathological examination

The tissue samples taken for histopathologic examination were fixed in 10% formaldehyde and placed in paraffin blocks. Then, sections with a thickness of 5 microns were taken and stained with haematoxylin-eosin. In the evaluation of ileal tissues, each field was scored semi-quantitatively between 0 and 3 in terms of inflammatory cell infiltration, haemorrhage, villus fusion, and villus apical facial epithelium degeneration. The following scoring was applied: 0, none; 1, mild; 2, moderate; and 3, severe (11).

In the evaluation of kidney tissues, cortex and external medullar areas were examined. Kidney sections were scored semi-quantitatively between 0 and 3 in terms of degeneration in tubular cells and intertubular congestion. The following scoring was applied: 0, none; 1, mild; 2, moderate; and 3, severe. In the evaluation of tubular degeneration, spillage in tubular epithelial cells and vacuolisation in tubular cells were examined.

In the evaluation of apoptosis, homogeneously stained TUNEL (+) cells without necrotic areas were identified as apoptotic cells (12). In the evaluation of renal tissue preparations, a total of 100 tubulus epithelial cells were counted in five different areas at 400X magnification on each tissue. Apoptotic and normal cells were recorded, and apoptotic cell percentage was calculated as an apoptotic index. Semi-quantitative scoring between 0 and 4 was used to evaluate the apoptosis of the ileal tissue. According to this scoring, Score 0 meant a few apoptotic cells were present in villus epithelial cells; Score 1, the aggregation of apoptotic cells at the villus epithelial tips; Score 2, apoptotic cells in all villus epithelium except crypts; Score 3, apoptotic cells in villus and crypts; and Score 4, apoptotic cells in all layers (13).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL,

USA) version 13.0.1 (licence number: 9069727). The normality of variables was examined using the Kolmogorov-Smirnov/Shapiro-Wilk tests. Descriptive analyses were shown using the mean and standard deviation for normally distributed variables. The homogeneity of variances was assessed by the Levene test. Pairwise post-hoc comparisons were conducted when there was a significant difference between the groups.

In a comparison of group data, the analysis of variance and Kruskal-Wallis tests were used according to the suitability of data. The Bonferroni correction was performed in pairwise comparisons when there was a significant difference between groups. P-values of less than 0.05 were considered as statistically significant results.

Results

Evaluation of biochemical results

The comparisons of serum, ileum, and renal MDA and SCUBE-1 values in groups are shown in Table 1. In serum MDA measurements, the serum MDA level was found to be lower in Group V compared to Group II ($p=0.017$). In ileum MDA measurements, the ileum MDA level was found to be lower in Group I compared to Groups II, III, IV and V ($p<0.001$, $p=0.009$, $p=0.001$, $p=0.004$, respectively). The ileum MDA level was significantly lower in Group III compared to Group II ($p=0.043$). In kidney MDA measurements, the kidney MDA level was significantly higher in Group II compared to Groups I, III and V ($p=0.001$, $p=0.022$, $p=0.006$, respectively).

In serum SCUBE-1 measurements, the serum SCUBE-1 level was found to be significantly lower in Group I compared to Groups II, III and IV ($p<0.001$, $p=0.002$, $p=0.009$, respectively). The serum SCUBE-1 level was significantly lower in Group V compared to Groups II, III and IV ($p<0.001$, $p=0.003$, $p=0.013$, respectively). There was no significant difference among groups with ileum SCUBE-1 levels.

In kidney SCUBE-1 measurements, the kidney SCUBE-1 level was higher in Group II compared to Groups I, III, IV and V ($p<0.001$ for all comparisons).

There was no significant difference among other groups.

Evaluation of histopathological results

In light microscopic evaluation of the ileum preparations, a normal ileal tissue villus histological structure was observed in Group I. A widespread haemorrhage was detected in the ileal tissue in Group II. There were severe degenerations and inflammatory cell infiltrations in the villus structure and epithelium. In Group III, villus fusions were observed in the ileal tissue. In this group, moderate degeneration, inflammatory cell infiltration, and haemorrhage were seen in villus epithelial cells. The villus structure was detected to have near-normal morphology in Groups IV and V. In both of these groups, the villus fusions decreased, and goblet cells were found in the

villus epithelium. Mild haemorrhage and inflammatory cell infiltration were observed (Figure 1).

In light microscopic evaluation of the kidney preparations, normal kidney glomerulus and tubule histological structures were observed in Group I. Group II had a widespread vacuolisation and degeneration in proximal and distal tubule epithelial cells. In Group III, epithelial cells showing extensive degeneration were seen besides normal cells in proximal and distal tubules. In Groups IV and V, epithelial cells with normal morphology were extensive, despite rare degeneration in epithelial cells in proximal and distal tubules of the kidney. In these groups, extensive vascular congestion presented in the intertubular area (Figure 2).

Results of the histopathological evaluation of the terminal ileum and kidney tissue among the groups are shown in Table 2.

In evaluating ileum apoptosis, the degree of apoptosis was lower in Group I compared to Groups II and III ($p<0.001$ for both). The degree of apoptosis was found to be higher in Group II compared to Groups IV and V ($p=0.004$ and $p=0.001$, respectively). The degree of apoptosis was lower in Group V compared to Group III ($p=0.016$) (Table 3, Figure 3).

In evaluating kidney apoptosis, the degree of apoptosis was found to be lower in Group I compared to Groups II, III, IV and V ($p<0.001$, for all comparisons). The degree of apoptosis was higher in Group II compared to Groups III, IV and V ($p=0.023$, $p<0.001$ and $p<0.001$, respectively) (Table 3, Figure 4).

Discussion

Ischaemic injury is seen in intestines due to various causes, such as occlusions in arteries feeding the intestines due to emboli, thrombosis or atherosclerosis. Mechanical vascular factors causes various issues such as volvulus, intestinal strangulation, invagination and obstruction in venous return of the intestine (14). Ischaemia lasting less than 20 minutes in the small intestines does not significantly change the mucosa; on the other hand, ischaemia lasting more than 2 hours may cause permanent injury, leading up to transmural necrosis (15). The passage of toxic products into systemic circulation after mucosal injury can cause a multiple-organ failure, affecting organs such as the kidney, liver, heart and most commonly, the lungs (16).

In the literature, the serum MDA, ischaemia-modified albumin, lactate, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), superoxide dismutase and glutathione peroxidase levels have been shown to be reliable parameters in determining ischaemic damage in experimental studies investigating IR injury (7, 11, 17). However, SCUBE-1 is a new marker for ischaemic damage (8). In a study conducted by Turkmen et al. (7), the SCUBE-1, MDA, TAS, TOS and OSI levels were measured in rats with acute

mesenteric ischaemia, and it was discovered that SCUBE-1 showed an increase similar to MDA. Similar to this study, we found a significant increase in the ileum and kidney MDA and the serum and kidney SCUBE-1 values in rats with IR injury. These findings indicate that SCUBE-1 is as sensitive as MDA in the diagnosis of acute mesenteric ischaemia. Moreover, the decrease in SCUBE-1 after treatment suggests that it can be used as an effective marker during follow-up care.

The efficacy of many drugs and methods in preventing IR injury have been investigated in the literature (17, 18). The efficacy of dexmedetomidine in preventing distant organ damage has been investigated in IR models; anti-inflammatory and anti-apoptotic properties of dexmedetomidine have also been demonstrated in previous studies (18, 19). Dexmedetomidine has been suggested to be effective in treating IR injury through K-ATP channels. It has been detected with the opening of K-ATP channels that mitochondrial Ca^{++} accumulation during an ischaemic process diminishes, which affects ion channels, leading to anti-apoptotic activity (20). It has also been reported that dexmedetomidine could reduce the pro-apoptotic protein Bax expression and increase the anti-apoptotic protein Bcl-2 expression, thereby attenuating apoptosis by inhibiting the activation of the intrinsic apoptotic cascade (21). Engelhard et al. (22) also showed in their study that dexmedetomidine has a neuroprotective effect by increasing anti-apoptotic proteins in cerebral ischaemia. Gencer et al. (23) compared the IR group and treatment group in retinal IR injury and found that the apoptosis score of the group receiving dexmedetomidine was lower. Similarly in our study, there was a significant decrease in the ileum and kidney apoptosis index in the groups administered dexmedetomidine compared to the group with IR injury. The apoptosis index in the ileal tissue was found to be significantly lower in the group administered dexmedetomidine as an infusion before and after ischaemia compared to the group administered dexmedetomidine only before ischaemia. However, there was no statistically significant decrease in the apoptosis index values in Group IV compared to Group III. This is likely due to the difference in time and duration of dexmedetomidine administration. Studies in the literature about the effect of dexmedetomidine on IR injury regarding higher dexmedetomidine doses and a prolonged administration time have shown a reduction in the apoptosis index. The results of our study were found to be similar to these results.

In previous organ-preserving studies, dexmedetomidine has been administered in various ways, before and/or after ischaemia, as a single dose at various amounts, and before and/or after ischaemia as an infusion (21, 24, 25). Gu et al. (26) found in a study conducted with rats that there was a decrease in IR injury in a group administered prophylaxis and $25 \mu\text{g kg}^{-1}$ dexmedetomidine 30 minutes preoperatively for therapy and intraperitoneal postoperatively, compared to the kidney IR group. Si et al. (27) conducted 45 minutes of ischaemia and 24 hours of reperfusion in a study of five groups on bi-

lateral renal artery clamping. Tubular epithelial cell apoptosis was observed less in a group administered 50 mcg kg^{-1} dexmedetomidine intraperitoneally for 30 minutes before ischaemia, compared to the IR group. Kocoglu et al. (28) investigated the effect of dexmedetomidine on the renal IR injury. They administered dexmedetomidine intraperitoneally at a dose of $100 \mu\text{g kg}^{-1}$ at the 5th minute of reperfusion, which was performed 1 hour following the completion of renal ischaemia, and it was demonstrated by histology that renal injury was prevented after 45 minutes of reperfusion. In accordance with these studies, it has also been suggested in our study that, based on histological evaluation, dexmedetomidine can reduce IR injury as MDA and SCUBE-1 levels were low in the dexmedetomidine groups and that there was a recovering effect found of ileum and kidney damage.

Zhang et al. (21) conducted a study with 10 groups using 2.5, 5 and $10 \text{ mcg kg}^{-1} \text{ h}^{-1}$ infusions of dexmedetomidine before and after acute mesenteric ischaemia. It was shown that a decrease in intestinal mucosal epithelial cell apoptosis can be achieved using an infusion of dexmedetomidine at a dose of $5 \text{ mcg kg}^{-1} \text{ h}^{-1}$ before ischaemia in intestinal IR injury. There was no beneficial effect of dexmedetomidine when administered after ischaemia. This was likely due to administration of dexmedetomidine an hour after reperfusion began. The corrective action mechanisms of dexmedetomidine on IR injury have not been fully elucidated yet, and dexmedetomidine has not been administered at different times and doses by clinicians. It has been observed in our study that ischaemia markers and ileum and kidney histopathological scores were lower in groups administered dexmedetomidine before and after ischaemia (Group V) compared to groups administered before or after ischaemia (Group III or Group IV). These results suggest that dexmedetomidine should be administered before ischaemia and during the reperfusion period to prevent IR injury.

It has been shown in previous studies that IR injury has adverse effects on kidney and that dexmedetomidine can reduce this injury (6, 27). In a study investigating the effect of intestinal IR injury on the lungs and the protective role of dexmedetomidine in rats, it was demonstrated that the administration of dexmedetomidine before treatment may be an effective method in the reduction of distant organ damage caused by IR (29). Another study showed that the negative effects of IR injury in the kidney extremities can be reduced by using curcumin and dexmedetomidine (30). In our study, distant organ damage was assessed through kidneys from the intestinal ischaemia groups, and the kidney MDA and SCUBE-1 levels and kidney apoptosis index were observed to increase. In accordance with the results of the mentioned studies, these values decreased in groups administered dexmedetomidine, and dexmedetomidine was likely useful against possible kidney IR injury.

Conclusion

This study demonstrated that dexmedetomidine infusion of 10 mcg kg⁻¹ h⁻¹ before and/or after ischaemia reduces small intestinal injury due to IR and kidney damage and that it leads to a significant reduction in the severity of histopathologic changes in the ileum and kidney tissue. Furthermore, this study showed that the preoperative and postoperative use of dexmedetomidine may be beneficial in reducing the negative effect of IR injury on organs. Further extensive studies are necessary to elucidate the mechanism of action of dexmedetomidine on IR injury and to determine a common treatment protocol.

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