

Effects of ketamine and dexmedetomidine on ischemia-reperfusion injury in an experimental renal ischemia model

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Abstract. Kidneys are one of the organs most affected by ischemia-reperfusion (I/R) injury during surgical interventions. The present study aimed to investigate the effects of ketamine and dexmedetomidine administered in a rat model of renal I/R injury prior to ischemia. A total of 24 male Sprague-Dawley rats were included in the present study and divided into four groups. The Sham group underwent laparotomy and right nephrectomy, while the other groups underwent laparotomy, right nephrectomy and 45 min of ischemia to the left kidney, followed by reperfusion for 24 h. No medication was administered to the I/R group. The Ketamine group received 2 mg/kg intraperitoneal ketamine prior to ischemia. The Dexmedetomidine group received an initial dose of 1.0 mcg/kg dexmedetomidine intravenously over 10 min before ischemia, followed by a continuous infusion of 1.0 mcg/kg/h during the operation. Blood was drawn for serum creatinine level measurements before the operation and at sacrifice. Renal samples were taken for histopathological examination right before the rats were sacrificed. The final creatinine measurements of the I/R and Ketamine groups were higher compared with those of the Sham group ($P=0.012$ and $P=0.001$, respectively). The final creatinine measurements of the Dexmedetomidine group were lower compared with those of the Ketamine group ($P=0.040$). In addition, measurements of tubular dilation ($P=0.002$ and $P=0.001$, respectively), vacuolization in tubular epithelium ($P=0.002$ and $P=0.003$, respectively) and loss of brush border in tubular epithelium

($P=0.008$ and $P=0.001$, respectively) were significantly higher in the I/R and Ketamine groups compared with the Sham group, while there was no significant difference between the Dexmedetomidine group and the Sham group. Measurements in the Dexmedetomidine group were lower compared with the I/R group, but the difference was not statistically significant. Despite a reduction in serum creatinine levels and histopathological injury scores in subjects treated with dexmedetomidine compared with the I/R group, the differences were not found to be statistically significant. By contrast, the Ketamine group demonstrated significantly higher serum creatinine levels and histopathological injury scores compared with both the I/R and Dexmedetomidine groups. These findings suggested that dexmedetomidine may be associated with more favorable biochemical and histopathological outcomes compared with ketamine in the present experimental model of renal I/R injury; however, further studies are needed to validate these findings.

Introduction

Ischemia is defined as a temporary reduction or interruption of blood flow to a tissue or organ, whereas reperfusion refers to the restoration of blood flow following a period of ischemia. Although reperfusion is essential for tissue survival, it may paradoxically cause additional tissue injury beyond that induced by ischemia itself. Kidneys, due to their high energy requirements and complex microvascular network, are among the most susceptible organs to ischemia-reperfusion (I/R) injury (1). In numerous clinical conditions and surgical procedures, including kidney transplantation, partial nephrectomy, cardiopulmonary bypass, sepsis, urological interventions and hydronephrosis, renal I/R injury may occur and is often unavoidable (2). The clinical relevance of perioperative renal injury is substantial; a recent systematic review and meta-analysis that included 162 studies and 675,361 patients reported a pooled incidence of postoperative acute kidney injury of 16% (95% CI, 14-17%) following abdominal surgery (3). I/R injury can occur after re-supplying ischemic tissues with

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oxygenated blood, due to released free oxygen radicals and activated neutrophils, which can cause secondary organ dysfunction and ultimately lead to organ failure (1). Ischemic preconditioning, which involves brief episodes of ischemia followed by reperfusion to enhance tissue tolerance to a subsequent prolonged ischemic insult, has been identified as an important protective mechanism against I/R injury (4,5). Numerous experimental studies have investigated the potential protective effects of different pharmacological agents against I/R injury. Agents such as magnesium, N-acetylcysteine, activated protein C, α -melanocyte-stimulating hormone and statins have been shown to reduce I/R injury in these models (6-8). Dexmedetomidine, a highly selective and potent α_2 -adrenergic agonist, is an effective sedative, anxiolytic and analgesic agent with protective effects against I/R injury in a number of organs, including the heart, brain and the kidney, likely due to the antioxidant and anti-inflammatory properties of the compound (9). Ketamine, an N-methyl-D-aspartate (NMDA) antagonist, has been found to attenuate lipid peroxidation induced by I/R in numerous tissues such as the brain, myocardium and skeletal muscle (10). It has also been reported that ketamine reduces the positive effect of ischemic preconditioning on the heart (11). The present study was therefore designed to investigate the potential effects of preconditioning with ketamine and dexmedetomidine on renal I/R injury.

Materials and methods

Ethics approval and study setting. Ethics approval for the use of experimental animals was obtained from the Yeditepe University Animal Experiments Ethics Committee in October 2020 (approval no. 2020-871; decision no. 2020/10-1). As Şanlıurfa Mehmet Akif İnan Training and Research Hospital did not have an appropriate experimental animal research facility, the experiments were conducted at the Experimental Research Center of Yeditepe University Faculty of Medicine (Istanbul, Turkey), from which the experimental animals were also obtained. There was no objection from Şanlıurfa Mehmet Akif İnan Training and Research Hospital to conducting the experiments at this external facility. The minimum number of subjects required for the present study was calculated to be 24, based on an effect size of 0.6, a power of 0.80 and an α error level of 0.05 using G*Power (version 3.1.9.7; Heinrich Heine University Düsseldorf).

The present study included 24 male Sprague-Dawley rats, aged 10-12 weeks and weighing ~370 g, obtained from the Experimental Research Center of Yeditepe University Faculty of Medicine (Istanbul, Turkey). Throughout the experiment, the animals were housed in rooms with controlled temperature (20-24°C) and humidity (40-60%), under a 12/12-h light/dark cycle, with *ad libitum* access to standard rat pellet feed and purified tap water. A heating lamp was used on the operating table to protect the rats from hypothermia, maintaining their body temperature between 37.0 and 37.5°C as measured using a rectal probe. To prevent dehydration, physiological saline (30 ml/kg) was administered subcutaneously once during the experimental procedure. The rats were randomized into four groups: Group I: A group that would not undergo ischemic procedures (Sham group; n=6), Group II: A group that would undergo ischemia followed by reperfusion but no

preconditioning procedure (I/R group; n=6), Group III: A group that would receive ketamine along with ischemia-reperfusion (Ketamine group; n=6) and Group IV: A group that would receive dexmedetomidine along with I/R (Dexmedetomidine group; n=6).

All rats were anesthetized with isoflurane (Isoflurane USP 100 ml inhalation solution; Adeka Pharmaceutical and Chemical Products Industry and Trade Inc.), administered at 4% for induction and 1.5% for maintenance, together with intraperitoneal xylazine hydrochloride (5 mg/kg; Rompun® 2%; Bayer AG). The depth of anesthesia was assessed using the toe pinch test. Once adequate anesthesia depth was achieved, the surgical area was shaved and cleaned antiseptically with three layers of povidone-iodine. Before operations were conducted, 0.1 ml blood was drawn from the jugular vein for pre-operative creatinine measurement. Ophthalmic ointment was applied to both eyes to prevent corneal drying during anesthesia. The rats were then positioned supine on the operating table, and a midline abdominal incision was performed to expose both kidneys. The renal pedicles were subsequently dissected.

Renal I/R model. For the kidney I/R model, total ischemia of the left kidney was induced using an atraumatic microvascular clamp to occlude the renal pedicle. Adequate occlusion was determined by the absence of pulsation in the renal pedicle and a color change in the kidney. Following the completion of the ischemia period, the microvascular clamp was removed to allow reperfusion. The procedures applied to the present study groups were as follows: Group I (Sham): Abdominal dissection and right nephrectomy. No surgical procedures were performed on the left kidney, which was left undisturbed under anesthesia for 45 min before the abdomen was closed. Group II (I/R): Abdominal dissection and right nephrectomy, followed by clamping of the left renal artery to induce ischemia for 45 min. Afterwards, the clamps were removed and the abdomen was closed. Group III (Ketamine): In addition to isoflurane and xylazine anesthesia, this group received 2 mg/kg intraperitoneal ketamine (Ketasol 10%; İnterhas Tıbbi ve Kimyevi Ürünler San. ve Tic. A.Ş.) before undergoing abdominal dissection and right nephrectomy, followed by ischemia through clamping of the left renal artery for 45 min. After removing the clamps, the abdomen was closed. Group IV (Dexmedetomidine): In addition to isoflurane anesthesia, this group received a 10-min bolus of 1.0 mcg/kg intravenous dexmedetomidine (DEKSTOMID 200 mcg/2 ml intravenous concentrated infusion solution; POLİFARMA İLAÇ SAN. VE TİC. A.Ş.) followed by a continuous infusion at a rate of 1.0 μ g/kg/h during the surgical procedure. After abdominal dissection and right nephrectomy, the left renal artery was clamped to induce 45 min of ischemia. Subsequently, the clamps were removed and the abdomen was closed. Blood samples (0.1 ml) were collected from all rats after the operation for postoperative serum creatinine measurements. A total of 24 h after the I/R procedure, the rats were re-anesthetized with isoflurane (4% for induction and 1.5% for maintenance). Under deep anesthesia, blood samples were collected through cardiac puncture and kidneys were harvested for biochemical and histopathological analyses. Subsequently, the animals were euthanized through cervical dislocation. The predetermined humane endpoint criteria included severe or persistent

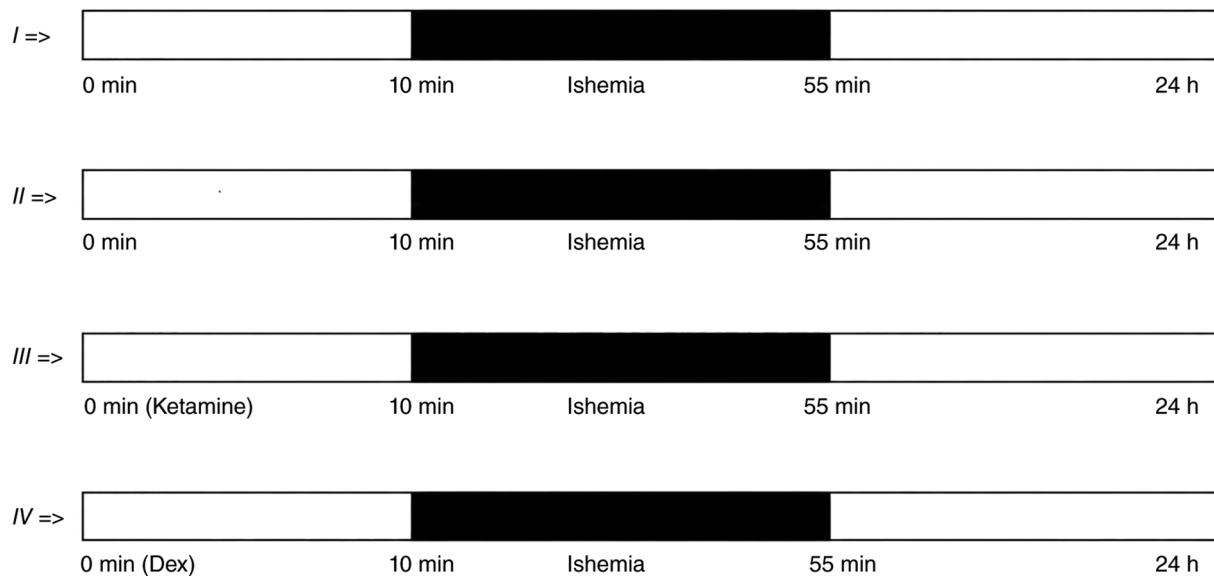


Figure 1. Schematic view of the experimental protocol including Group I (Sham group), Group II (Ischemia-reperfusion group), Group III (Ketamine group) and Group IV (Dex group). The black bar represents the 45-min ischemic period. Blood and kidney tissue samples were collected 24 h after the experimental procedure. Dex, dexmedetomidine.

signs of distress, markedly reduced activity, inability to access food or water, severe respiratory distress and signs of uncontrolled pain. Animals meeting any of these criteria were to be euthanized before the scheduled end of the experiment. None of the rats met the humane endpoint criteria or required early euthanasia before the scheduled end of the study. Mortality was determined by the absence of heartbeat, spontaneous respiration and corneal reflex. The experimental protocol schematic is shown in Fig. 1.

Histopathological examination. Kidney tissue samples were fixed in 10% neutral-buffered formalin (equivalent to ~4% formaldehyde) for 36 h at room temperature (20–25°C). Following routine tissue processing, the samples were embedded in paraffin and sectioned at a thickness of 4 μ m.

Hematoxylin and eosin staining was performed at room temperature (20–25°C) using a Leica ST5020 automated slide stainer (Leica Biosystems Nussloch GmbH). Following deparaffinization and rehydration, the sections were stained with Harris hematoxylin (cat. no. 05-12011E; Bio-Optica Milano S.p.A.) for 4 min and counterstained with alcoholic Eosin Y solution (cat. no. BS-009; BESLAB/Histomed) for 30 sec. The sections were subsequently dehydrated through graded ethanol, cleared in xylene and mounted.

Masson's trichrome staining was performed manually at room temperature (20–25°C) using a Masson trichrome kit with aniline blue (cat. no. 04-010802; Bio-Optica Milano S.p.A.), according to the manufacturer's instructions. After the sections were rinsed with distilled water, six drops each of reagents A and B (Weigert's iron hematoxylin solutions A and B) were applied for 10 min. Without washing, the slides were drained and 10 drops of reagent C (alcoholic picric acid solution) were applied for 4 min. The sections were rapidly washed in distilled water for 3–4 sec and treated with 10 drops of reagent D (Ponceau acid fuchsin) for 4 min. After washing in distilled water, 10 drops of reagent E

(phosphomolybdic acid solution) were applied for 10 min. Without washing, the slides were drained and 10 drops of reagent F (Masson aniline blue) were applied for 5 min. Finally, the sections were washed in distilled water, rapidly dehydrated through ascending concentrations of ethanol, kept in the final absolute ethanol bath for 1 min, cleared in xylene and mounted.

Histopathological evaluation was performed using an Olympus CX43 light microscope (Olympus Corporation). The histopathological changes evaluated included Bowman's capsule dilation, tubular dilation, vacuolization in the tubular epithelium, loss of brush border in the tubular epithelium, hyaline casts in the tubules, tubular atrophy, mononuclear cell infiltration in the interstitium, erythrocyte extravasation in the interstitium and interstitial fibrosis. The examinations were performed in a blinded manner by two experienced pathologists from the Department of Pathology at Istanbul Medeniyet University. Tissue damage was scored according to the percentage of affected tubules as follows: 0, normal kidney (no damage); 1, minimal damage (<25%); 2, mild damage (25–50%); 3, moderate damage (50–75%); and 4, severe damage (>75%). After each histopathological change had been scored, the individual scores were combined to obtain the total histopathological injury score.

Biochemical evaluation. For the biochemical assessment of kidney damage, creatinine levels were measured. Blood samples used for serum creatinine measurements were coded prior to laboratory analysis and laboratory personnel were unaware of group allocation. The collected blood samples were centrifuged at ~2,400 $\times$ g for 9 min at 4°C using a Hettich Universal 320R centrifuge (Andreas Hettich GmbH), and were stored at 4°C. The blood samples were analyzed at the Yeditepe University Animal Experiments Laboratory (Istanbul, Turkey) using an IDEXX Vet Test 8008 (IDEXX Laboratories, Inc.) device.

Table I. Comparison of creatinine levels between groups.

Creatinine measurement	Sham(n=6)	I/R(n=6)	Ketamine (n=6)	Dexmedetomidine (n=6)	P-value
Pre-procedure	48.33±6.38	47.00±4.73	47.17±9.54	42.67±4.84	0.388
Post-procedure	61.33±1.75	418.00±79.00 ^a	634.00±150.25 ^a	241.50±52.66 ^b	0.001 ^c
Within-group comparison (pre vs. post)	0.027 ^d	0.028 ^d	0.028 ^d	0.028 ^d	

^aP<0.05 vs. Sham group and ^bP<0.05 vs. Ketamine group. ^cKruskal-Wallis test and ^dWilcoxon test. I/R, ischemia-reperfusion.

Table II. Comparison of histomorphological changes between groups.

Histomorphological changes	Sham (n=6)	I/R (n=6)	Ketamine (n=6)	Dexmedetomidine (n=6)	P-value
Bowman capsule dilatation	0	0.50±0.55	1.00±0.89	0.33±0.52	0.083
Tubular dilatation	0	3.50±0.55 ^a	3.67±0.52 ^a	1.33±0.82	0.001
Vacuolization in tubular epithelium	0.17±0.41	3.50±0.55 ^a	3.33±0.82 ^a	1.50±0.84	0.001
Loss of brush border in tubular epithelium	0	3.00±0.63 ^a	3.33±1.21 ^a	1.00±0.89	0.001
Hyaline casts in tubules	0.17±0.41	1.83±1.17	2.50±1.38 ^a	0.83±0.98	0.011
Tubular atrophy	0	0	1.33±2.07	0	0.099
Mononuclear cell infiltration in interstitium	0	0	0	0	
Red blood cell extravasation in interstitium	0	0	0	0.17±0.41	0.392
Interstitial fibrosis	0	0	0	0	

^aP<0.05 vs. Sham group.

Statistical analysis. For statistical analyses, NCSS 2020 Statistical Software (version 20.0.3; NCSS, LLC) was used. Descriptive statistical methods (mean, SD and median) were employed in evaluating the present study data. Kruskal-Wallis tests were used for comparisons among three or more groups that did not display normal distribution, while for pairwise comparisons, the Bonferroni-Dunn test was employed. For within-group comparisons of parameters that did not follow a normal distribution, the Wilcoxon Signed Ranks test was used. P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed by an investigator who was not blinded to group allocation.

Results

Initial and final creatinine measurements for the groups are shown in Table I. No significant difference was found in the initial creatinine measurements between the groups (P>0.05). However, a significant difference was found in the final creatinine measurements between the groups (P=0.001). Pairwise comparisons revealed that the final creatinine measurements in the I/R and Ketamine groups were significantly higher compared with those of the Sham group (P=0.012 and P=0.001, respectively). In addition, the final creatinine measurement in the Ketamine group was significantly higher compared with that of the Dexmedetomidine group (P=0.040). No significant

differences were found between the final creatinine measurements of the other groups (P>0.05). Compared with the initial measurements, the final creatinine measurements were significantly increased in all four groups (Sham, P=0.027; I/R, P=0.028; Ketamine, P=0.028; Dexmedetomidine, P=0.028).

The comparison of histomorphological evaluations among the groups is shown in Table II. Representative histopathological findings of renal I/R injury are shown in Fig. 2. No significant differences were found among the groups with regard to Bowman's capsule dilation (P=0.083), tubular atrophy (P=0.099) or erythrocyte extravasation in the interstitium (P=0.392). However, significant differences were observed among the groups regarding tubular dilation (P=0.001), vacuolization in the tubular epithelium (P=0.001), loss of brush border in the tubular epithelium (P=0.001) and hyaline casts in the tubules (P=0.011). Pairwise comparisons showed that tubular dilation (P=0.002 and P=0.001, respectively), vacuolization in the tubular epithelium (P=0.002 and P=0.003, respectively) and loss of brush border in the tubular epithelium (P=0.008 and P=0.001, respectively) were significantly higher in the I/R and Ketamine groups compared with the Sham group. In addition, hyaline cast measurements in the Ketamine group were significantly higher compared with those in the Sham group (P=0.014). Mononuclear cell infiltration in the interstitium and interstitial fibrosis were recorded as zero in

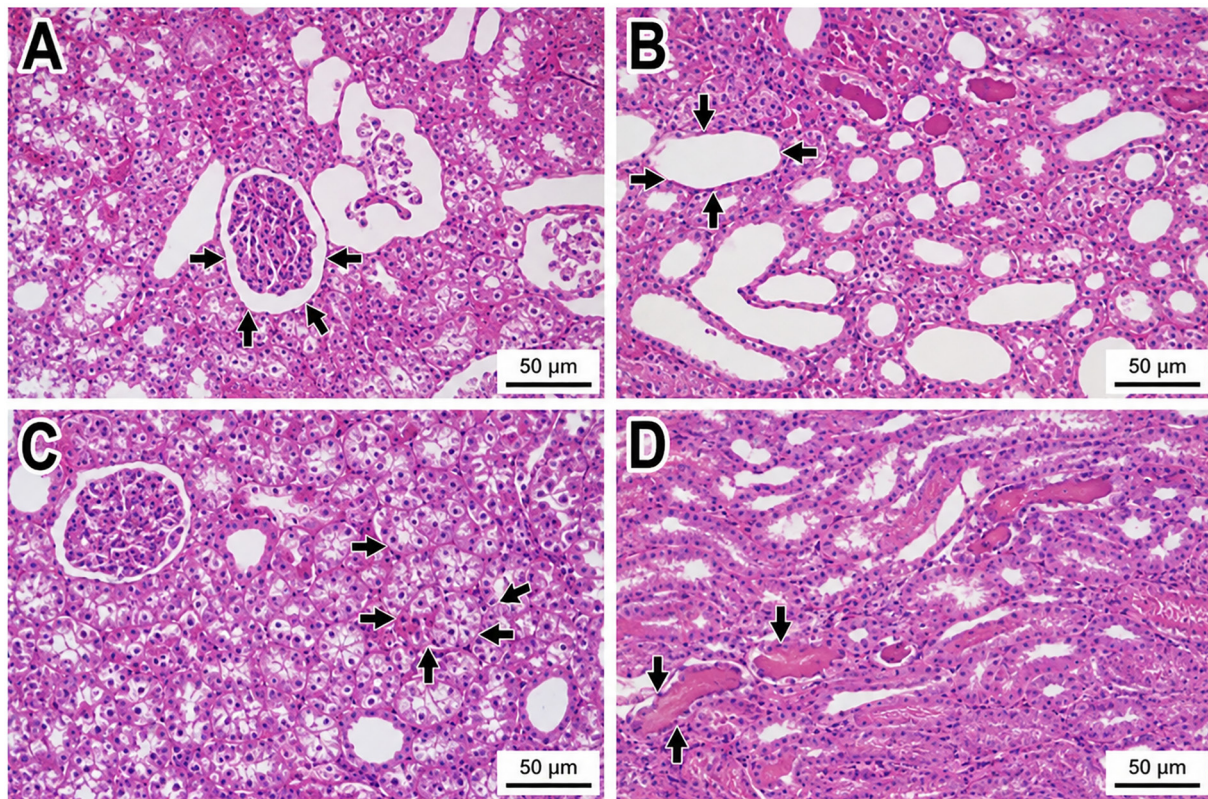


Figure 2. Representative histopathological findings in renal tissue samples obtained from rats in the I/R group. (A) Bowman's capsule dilatation (black arrows), (B) tubular dilatation (black arrows), (C) vacuolization in tubular epithelium (black arrows) and (D) hyaline casts in tubules (black arrows). Hematoxylin and eosin staining. Scale bars, 50 μ m.

all groups; therefore, statistical comparisons could not be performed.

Discussion

Within the present study, the effects of dexmedetomidine and ketamine on I/R injury were investigated. The main findings of the present study were as follows: i) In subjects treated with dexmedetomidine, serum creatinine levels and histopathological involvement were similar to the Sham group; ii) dexmedetomidine-treated subjects exhibited significantly lower serum creatinine levels compared with the Ketamine group and; iii) subjects treated with ketamine exhibited significantly higher final serum creatinine levels and histopathological involvement compared with the Sham group. Results suggested that dexmedetomidine might be more preferable than ketamine for protecting against I/R where renal ischemia is likely, such as transplantation surgery and vascular surgery.

Ischemia is defined as a reduction or cessation of blood flow to a tissue or organ, while reperfusion is the restoration of blood flow to the tissue or organ after ischemia (12). The condition characterized by endothelial damage caused by free oxygen radicals and superoxide radicals generated during reperfusion, increased microvascular permeability and tissue edema is known as I/R injury (13,14). Experimental renal I/R injury has been modeled using two different methods in previous literature. As used in the present study, one of these methods involves unilateral nephrectomy and clamping of

the opposite renal artery or renal pedicle. Orvieto *et al* (15) compared nephrectomies where both the renal artery and vein were clamped, with nephrectomies where only the renal artery was clamped, in 36 pigs undergoing laparoscopic nephrectomy. Significant differences were found in serum creatinine levels on postoperative days 1, 3, 8 and 15 after leaving the right kidney ischemic for 120 min. Subjects where only the renal artery was clamped exhibited significantly lower creatinine levels compared with those where both the renal artery and vein were clamped. Similarly, additional studies have shown that clamping both the renal artery and veins is a more accurate method for inducing ischemic damage (16,17). In the present study, the renal artery and vein were clamped together to induce renal ischemia in accordance with the literature.

Furthermore, previous studies have demonstrated that the duration of renal ischemia is an important determinant of the severity of I/R injury, with longer ischemic periods generally resulting in more pronounced functional and histopathological renal damage (18). In experimental renal I/R models, ischemia periods of 30-60 min are commonly used, with 45 min being frequently employed in rat models (19). While prolonged renal ischemia >60 min leads to acute tubular necrosis and renal failure, ischemia <30 min has been shown to result in rapid proliferation of tubular epithelial cells and repair of damaged renal tubules, improving renal function (6,20). A review by Simmons *et al* (17) compared ischemia durations in different studies and concluded that the appropriate ischemia duration for rats was 40-45 min. The present study applied a

45-min ischemia duration based on this literature. In addition, Williams *et al* (20) investigated the effects of renal I/R injury after 45 min of ischemia followed by reperfusion at a number of time points up to 1 week and found that the effects of renal I/R injury began as early as 4 h, before peaking at 24 h. Cochrane *et al* (21) also noted that renal damage following reperfusion was observed as early as 4 h post-event. Therefore, a 24-h reperfusion period was targeted in the present experimental model. The significant differences in histomorphological and biochemical data between the I/R group and the Sham group compared in the present study suggest that the selected I/R durations were adequate and the I/R model was correctly applied.

Serum creatinine is one of the most commonly used laboratory markers for the assessment of kidney function as it is widely available, inexpensive and easy to measure (22-24). However, creatinine alone often falls short, especially in detecting mild to moderate kidney damage. Notable increases in creatinine levels can occur even without any meaningful renal disorder following heavy exercise or trauma (25). Therefore, in certain cases, more sensitive markers such as β -Trace protein, serum cystatin-C levels and β -2-microglobulin are more effective in assessing renal function (26,27). Despite no renal ischemia being induced in the present Sham group, a significant difference was observed between the initial and final creatinine values, whereas no difference was observed in the histopathological evaluation; this may be attributed to the low sensitivity of creatinine measurement. The present study considered that this significant difference, despite the absence of renal ischemia, was associated with the surgical stress endured.

I/R is a common clinical scenario in transplantation surgery and vascular surgery (28). A number of mechanisms activate and release toxic products in ischemic tissue after revascularization, leading to systemic inflammatory response syndrome, which can cause multiorgan failure (1,12,29). The histomorphological findings in the kidneys following I/R injury include increased microvascular permeability, interstitial edema, inflammatory cell infiltration and parenchymal cell dysfunction (30). A number of agents considered to be effective on the mechanisms causing I/R injury have been used in prophylaxis (pharmacological preconditioning) and treatment (pharmacological postconditioning).

Propofol is one of the pharmacological agents researched for its effect in pharmacological preconditioning to protect against renal ischemia. Yang *et al* (31) investigated the protective effects of propofol on renal I/R injury in 32 rats and reported that propofol exhibits protective effects against I/R injury (32). An additional study comparing the effects of propofol and sevoflurane on I/R injury also showed that propofol exhibited more positive effects compared with sevoflurane (33). Although both ketamine and dexmedetomidine have been investigated in experimental I/R models, relatively few studies have directly compared their effects on renal I/R injury. Curtis *et al* (8) directly compared S(+)-ketamine and dexmedetomidine in a rat renal I/R model involving 45 min of renal ischemia and reported lower post-reperfusion serum creatinine levels in the dexmedetomidine group than in the S(+)-ketamine group, although dexmedetomidine did not completely prevent renal injury.

To the best of our knowledge, the effects of dexmedetomidine on renal I/R injury in rats were first demonstrated in a study by Koçoğlu *et al* (6). This study reported that intraperitoneal administration of 100 mcg/kg dexmedetomidine at the beginning of reperfusion markedly reduced histopathological damage scores at 45 min and exhibited a renoprotective effect in their study, where they clamped the left renal artery of rats for 60 min following a right nephrectomy. In addition, this study found that the renal protective effect was not only limited to the early post-reperfusion period but also continued at 24 h, which is considered the peak effect time for I/R injury. Similarly, Gu *et al* (34) studied the effects of dexmedetomidine on renal I/R injury in rats both *in vivo* and *in vitro* and reported that treatment with dexmedetomidine notably reduced changes in tubular structures at 24 h post I/R, including improvements in renal function. The protective effect of dexmedetomidine on renal I/R injury is not fully understood; however, it is considered to increase renal blood flow and glomerular filtration by reducing norepinephrine release (34). Similar to previous research, the present study also observed significant reductions in both creatinine values and histomorphological variables, such as Bowman's capsule dilation, tubular dilation, vacuolization in tubular epithelium, loss of brush border in tubular epithelium and hyaline casts in the group treated with dexmedetomidine compared with the I/R group. Similarly, Lempiäinen *et al* (35) demonstrated that dexmedetomidine preconditioning attenuated renal I/R injury in rats; a 10 μ g/kg dexmedetomidine bolus reduced serum creatinine levels by 30% and ameliorated I/R-induced acute tubular necrosis, characterized by loss of brush border, tubular dilation, cast formation and cell lysis (35).

Studies regarding ketamine and I/R injury have yielded mixed results. While a number of studies have shown that ketamine reduces I/R injury (36), others have demonstrated that it increases renal ischemic damage (8). The present results demonstrated that the Ketamine group experienced more severe renal injury compared with the I/R group. This finding, while unexpected in the context of general anesthesia, aligns with recent evidence suggesting a detrimental role for NMDA receptor (NMDA-R) blockade in certain renal contexts.

NMDA-Rs are constitutively expressed in the renal cortex, medulla and podocytes, where they serve a key role in maintaining normal renal function. A possible mechanism for the worsened outcomes is the dichotomous effect of NMDA-R activity; while high-level activation is toxic, low-level basal activity drives nitric oxide synthase to produce nitric oxide, which is key in renal vasodilation. Therefore, the high-trapping blockade of these receptors by ketamine may induce sustained renal vasoconstriction, further reducing renal blood flow during the critical reperfusion phase. In addition, ketamine has been shown to induce epithelial phenotypic changes in renal tubular cells by suppressing E-cadherin expression and disrupting the apical junctional complex. This loss of cell-to-cell adhesion, potentially mediated by non-classical pro-fibrotic mechanisms, such as p38 MAPK activation and cytoskeletal reorganization, may impair tubular epithelial integrity (37). Finally, the disruption of glutamatergic signaling in podocytes by

ketamine may compromise the integrity of the glomerular filtration barrier, leading to exacerbated proteinuric injury. These combined hemodynamic and cellular mechanisms may explain why ketamine exacerbated the damage in the present experimental model (38). In the present study, both creatinine values and histomorphological variables such as Bowman's capsule dilation, tubular dilation, vacuolization in tubular epithelium, loss of brush border in tubular epithelium and hyaline casts were significantly increased in the Ketamine group compared with the Sham group. Despite the present findings supporting the data suggesting that ketamine increases renal ischemic damage, the present study suggests more comprehensive research is needed on this topic.

The present study exhibits a number of limitations. Only male rats were used, so the effects of fluctuating female hormones such as estrogen and progesterone on I/R injury are unknown. More rats would have been statistically more effective in demonstrating differences when significance is considered in the statistical analyses of histopathological examinations. Lastly, the assessment of additional renal biomarkers, such as β -trace protein, serum cystatin C and β 2-microglobulin, alongside serum creatinine may have provided complementary information regarding renal dysfunction and allowed a more comprehensive assessment of differences in renal injury between the experimental groups (26).

Furthermore, although the sample size was determined using an *a priori* power analysis, the relatively small number of animals per group ($n=6$) may have limited the ability to detect modest intergroup differences; therefore, a Type II error cannot be excluded. An additional limitation of this study is that dexmedetomidine and ketamine were administered through different routes (intravenously and intraperitoneally, respectively). Differences in absorption and pharmacokinetic profiles associated with these administration routes may have influenced the observed outcomes independently of the pharmacological effects of the drugs.

Overall, the present study aimed to investigate the effects of ketamine and dexmedetomidine, which are commonly used in anesthesia, on renal I/R injury. Despite a reduction in serum creatinine levels and histopathological evaluations having been observed in subjects treated with dexmedetomidine compared with the I/R group, the difference was not found to be statistically significant. In subjects treated with ketamine, both serum creatinine levels and histopathological evaluations were found to be significantly higher compared with those in both the I/R and Dexmedetomidine groups. In light of these findings, dexmedetomidine may represent a more favorable option compared with ketamine with regard to biochemical and histopathological outcomes in the present experimental model of renal I/R injury. However, further studies are needed to validate these findings.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MHE, HK, TBT and MGC designed the present study and experimental protocol. MHE, HK and TZ performed the experiments and collected and processed the data. MHE, HK, TBT and TZ analyzed and interpreted the results. MHE and HK prepared the manuscript draft and created the visual materials. TBT, MGC and TZ critically revised and edited the manuscript. MGC supervised the study. MHE, HK and MGC confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Yeditepe University Local Ethics Committee for Animal Experiments (approval no. 2020-871; decision no. 2020/10-1; 30.10.2020). All animal procedures were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. Animals were anesthetized and sacrificed using accepted humane methods to minimize suffering.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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