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SCIENTIFIC ARTICLE

Comparison of the effects of dexmedetomidine administered at two different times on renal ischemia/reperfusion injury in rats

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Abstract

Background and objectives: We investigated the effect of dexmedetomidine on ischemic renal failure in rats.

Methods: In the present study, 26 male adult Wistar albino rats weighting 230–300 g were randomly separated into four groups: sham-operated ($n=5$), ischemia reperfusion (IR) (IR group, $n=7$), IR/reperfusion treatment with dexmedetomidine (Dex. R group, $n=7$) and IR/pre-ischemic treatment with dexmedetomidine (Dex. I group, $n=7$). In the first group, sham operation was achieved and renal clamps were not applied. For the IR group, renal ischemia was induced by occlusion of the bilateral renal arteries and veins for 60 min followed by reperfusion for 24 h. For the Dex. R and Dex. I groups, the same surgical procedure as in the IR group was performed, and dexmedetomidine (100 mcg/kg intraperitoneal) was administered at the 5th min after reperfusion and before ischemia. At the end of reperfusion, blood samples were drawn, the rats were sacrificed, and the left kidney was processed for histopathology.

Results: The blood urea nitrogen (BUN) levels in groups Dex. R and Dex. I were significantly lower than in the IR group ($p=0.015$, $p=0.043$), although urine flow was significantly higher in group Dex. R ($p=0.003$). The renal histopathological score in the IR group was significantly higher than in the other groups. There was no significant difference between the Dex. R and Dex. I groups.

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Conclusions: The results were shown that administration of dexmedetomidine reduced the renal IR injury histomorphologically. Administration of dexmedetomidine in the reperfusion period was considered as more effective due to increase in urinary output and decrease in BUN levels. © 2013 Sociedade Brasileira de Anestesiologia. Published by Elsevier Editora Ltda. All rights reserved.

Introduction

Acute renal failure is an acute ischemic response of the kidneys occurring due to hypoperfusion secondary to hypotension, hypovolemia and dehydration as well as ischemia/reperfusion (IR) injury presenting with high mortality and morbidity in clinical practice.¹⁻³

Increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, parenchymal cell dysfunction, and acute tubular necrosis (ATN) have been shown in the histopathological studies related to renal IR injury.⁴⁻⁶ These changes peaked at the 24th hours after reperfusion and they correlated with increased levels of blood urea nitrogen (BUN) and serum creatinine (Cr) which they are used as an indicator for renal function in clinical settings.^{7,8}

Dexmedetomidine is an active dextro-stereoisomer of medetomidine and selective α_2 -adrenoceptor agonist.⁹ Dexmedetomidine reduces the plasma levels of catecholamines,^{10,11} provides hemodynamic stability during surgery^{12,13} and increases the urinary flow rate.¹⁴ Villela et al.¹⁵, and Frumento et al.¹⁶ showed that dexmedetomidine caused aqueous diuresis by reducing central vasopressin secretion and significantly improved renal functions postoperatively in their both clinical and experimental studies. Kocoglu et al.¹⁷ have been studied the effects of dexmedetomidine on renal IR injury. They found improved histological scores at the end of the 45 min of reperfusion following 1 h of complete renal ischemia.

The aim of this study was to examine the histopathologic and biochemical effects of dexmedetomidine administered two different times (before ischemia and at the beginning of reperfusion) on renal IR injury at 24th hour of reperfusion.

Materials and methods

Twenty-six adult Wistar albino rats weighing 230–300 g were used in this study. The animals were housed in a light controlled room with a 12-h light/dark cycle and allowed access to food and water. Experimental protocols and animal care methods in the experiment were approved by the Experimental Animal Research Committee of our institution.

Rats were divided into four groups: sham operated group ($n=5$), IR/untreated group (IR group, $n=7$), reperfusion treatment with dexmedetomidine ($100 \mu\text{g}/\text{kg}$ at 5 min after the reperfusion, ip) (Dex. R group, $n=7$), preischemic treatment with dexmedetomidine ($100 \mu\text{g}/\text{kg}$ at 5 min before the ischemia, ip) (Dex. I group, $n=7$). The rats were anesthetized with ketamine ($50 \text{mg}/\text{kg}$ ip) and xylazine hydrochloride ($10 \text{mg}/\text{kg}$ ip) and bilateral renal pedicles were exposed after laparotomy. After anesthesia, the rats

were heated with a heating lamp to maintain a rectal body temperature of 37°C . Isotonic saline solution accounted of 25% of rat body weight was given intraperitoneally before closure of abdomen. For ischemia and reperfusion injury induced, bilateral renal pedicle occlusion was performed with hemostasis clip for 60 min. At the end ischemic period, the clips were removed for blood reperfusion. In sham operated group, bilateral renal pedicles were exposed without any intervention after laparotomy. The animals exposed to 60 min ischemia were housed in metabolic cages 24 h after reperfusion; 24 h urine samples were collected. At the beginning of study, 1 ml of blood sample was drawn from the lateral tail vein for the measurement of basal renal function parameters before abdominal incision. At the end of reperfusion, the animals were anesthetized, the blood samples were drawn from the right atrium for the measurement of renal function parameters and left kidneys were excised. The kidneys were fixed in 10% buffered formalin and embedded in paraffin wax, cut at $4\text{--}5 \mu\text{m}$ and stained with hematoxylin and eosin for histological studies using light microscope.

Histopathologic changes were analyzed for mononuclear cell infiltration, erythrocyte extravasation, capillary dilatation, renal corpuscle morphology, vacuolization of proximal tubules, apoptosis, loss of tubular brush border, tubular dilatation and cast formation. Tubulointerstitial injury was scored as follows: 0 = none, 1 = 0–10%, 2 = 11–25%, 3 = 26–45%, 4 = 46–75%, and 5 = 76–100%.¹⁸ The scoring of the histological data was performed by blind investigator.

Blood urea nitrogen and plasma Cr levels were measured. Fractional sodium excretion (FNa_{Na}) and Cr clearance (CCr) were calculated from the following formula: $\text{FNa}_{\text{Na}} = \text{UNaV}/(\text{PNa} \times \text{creatinine clearance}) \times 100$ (UNaV: urinary sodium, PNa: plasma sodium);¹⁹ $\text{CCr} = (\text{Urine Cr} \times \text{urine volume})/(\text{Plasma Cr} \times \text{time})$.²⁰

For statistical analysis, SPSS 15.0 (Statistical Package for the Social Sciences ver. 15, Chicago, IL, USA) was used. All data were expressed as mean \pm standard deviation (mean \pm SD). Univariate analysis was conducted via Mann–Whitney *U* test to compare two independent groups. The level of statistical significance was accepted as $p < 0.05$.

Results

A total of 26 rats were included in the study. One rat in the IR group died during the ischemia period and was excluded from the study; thus, 25 subjects completed the study. The histopathological scores of the rats in all groups are presented in Table 1.

The histomorphologic injury scores of the sham operated group were statistically significant lower than IR, Dex. I and

Table 1 Histopathologic scores in groups.

Groups	Proximal tubulus	Mononuclear cell infiltration	Erythrocyte extravasation
Sham operated (n=5)	0.00 ± 0.00	0.20 ± 0.44	0.00 ± 0.00
IR (n=6)	2.50 ± 0.55	1.33 ± 0.52	2.17 ± 0.75
Dex. R (n=7)	1.57 ± 0.53	0.86 ± 0.38	1.29 ± 0.49
Dex. I (n=7)	1.71 ± 0.49	1.00 ± 0.58	1.57 ± 0.53
<i>p values</i>			
P ₁₂	0.003	0.010	0.004
P ₁₃	0.002	0.029	0.002
P ₁₄	0.002	0.030	0.002
P ₂₃	0.018	0.081	0.036
P ₂₄	0.026	0.291	0.133
P ₃₄	0.591	0.593	0.298

IR, ischemia reperfusion group; Dex R, ischemia reperfusion/reperfusion treatment with dexmedetomidine; Dex I, ischemia reperfusion/preischemic treatment with dexmedetomidine. Mann Whitney-*U* test was conducted to compare two independent groups.

p₁₂: comparison of sham operated and IR.

p₁₃: comparison of sham operated and Dex. R.

p₁₄: comparison of sham operated and Dex. I.

p₂₃: comparison of IR and Dex. R.

p₂₄: comparison of IR and Dex. I.

p₃₄: comparison of Dex. R and Dex. I.

Dex. R groups (respectively, $p=0.003$, $p=0.002$, $p=0.002$). The scores were significantly higher in the IR group than Dex. I and Dex. R groups (respectively, $p=0.018$, $p=0.026$). The difference between the scores of the Dex. R and Dex. I groups was not statistically significant ($p=0.59$).

Mononuclear cell infiltration

The histomorphologic injury scores of the sham-control group were significantly lower than of the IR, Dex. I and Dex. R groups (respectively, $p=0.01$, $p=0.029$, $p=0.03$). The differences between the scores of the IR, Dex. I and Dex. R groups were not statistically significant (respectively, $p=0.08$, $p=0.29$). The difference between the scores of the Dex. R and Dex. I groups was not statistically significant ($p=0.59$).

Erythrocyte extravasation

The histomorphologic injury scores of the sham-control group were statistically significant lower than IR, Dex. I and Dex. R groups (respectively, $p=0.004$, $p=0.002$, $p=0.002$). The scores of the IR group were significantly higher than Dex. R group ($p=0.03$), but there was no statistically significant difference between the scores of the IR and Dex. I groups ($p=0.29$).

The sham operated group showed normal morphological features. No cell infiltration or loss of tubular brush border was observed (Fig. 1).

For the IR group, infiltration of mononuclear cells in the peritubular area, especially the cortical area, brush border loss in the proximal tubule cells, tubular atrophy, tubular dilatation, and vacuolization were observed. In some tubules, proteinaceous material deposition with cast formation and cell debris in the lumen of the tubule were

observed. In some areas in the cortex, vasodilatation and erythrocyte extravasation were prominent (Fig. 2A–C).

In the Dex. R group, mononuclear cell infiltration in the peritubular area and erythrocyte extravasation was observed less than in the IR group.

Tubular atrophy, tubular dilation and vacuolization, proteinaceous material deposition in the tubules, and loss of cell debris and brush border to tubule lumen, which were observed in the IR group, were observed less in the Dex. R group (Fig. 3A, Fig. 3B).

In the Dex. I group, reductions in mononuclear cell infiltration in the cortical region, especially in the peritubular area and in degeneration tubule cells and erythrocyte extravasation were observed compared to the IR group. (Fig. 4A, Fig. 4B).

In the histomorphologic comparison of the Dex. R and Dex. I groups, the brush border loss observed in proximal

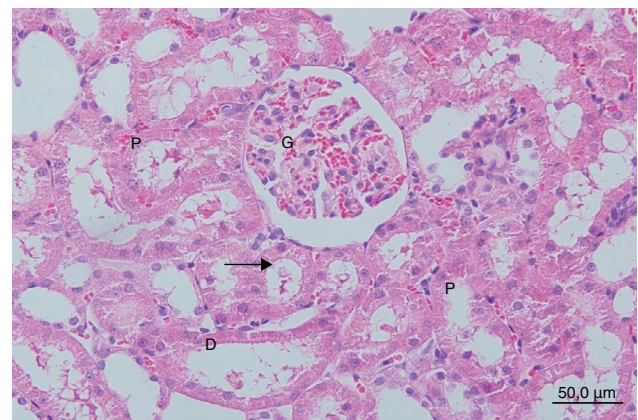


Figure 1 The sections of sham operated group. G: glomerulus, P: proximal tubule, D: distal tubule, (→) tubular brush border.

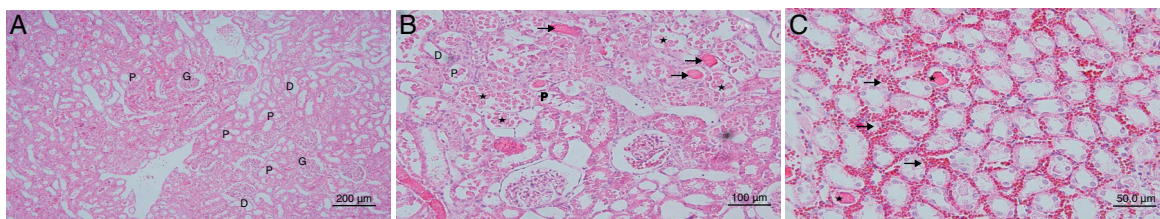


Figure 2 (A) The sections of ischemia reperfusion group. G: glomerulus, P: proximal tubule, D: distal tubule, (→) the accumulation of proteinaceous material at tubules, (★): proximal tubule epithelia cells poured into the lumen. (B) The sections of ischemia reperfusion group. G: glomerulus, P: proximal tubule, D: distal tubule, (→) mononuclear cell infiltration, (★): proximal tubule epithelia cells poured into the lumen. (C) The sections of ischemia reperfusion group. (→) erythrocyte extravasation, (★): tubules and the accumulation of proteinaceous material at tubules.

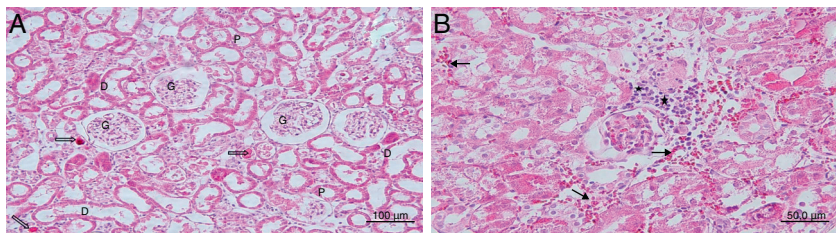


Figure 3 (A–B) The sections of reperfusion treatment with dexmedetomidine group. G:glomerulus, P:proximal tubule, D: distal tubule, (→) the apperence that the accumulation of proteinaceous material at tubules reduced compared to other groups.

tubule cells, tubular atrophy, tubular dilatation, vacuolization, proteinaceous material deposition in some tubules, cast formation, and cell debris in the lumen of tubules seen in the Dex. I group were observed less in Dex. R group. In the statistical analysis of the semiquantitative light microscopy scoring for these two groups, no statistically significant differences were determined in the scores of erythrocyte extravasation, proximal tubule and mononuclear cell infiltration.

Biochemical parameters

For the IR group, urine volume and CCr were significantly lower (respectively, $p=0.006$, $p=0.025$) and BUN, blood Cr and FA_{Na} excretion levels were significantly higher than in the sham-control group (respectively, $p=0.006$, $p=0.006$, $p=0.025$).

Urine volume was significantly higher in the Dex. R group than in the IR and Dex. I groups (respectively, $p=0.003$, $p=0.030$) and BUN value was significantly lower in the Dex. R and Dex. I groups than in the IR group (respectively,

$p=0.015$, $p=0.043$). Biochemical data of the groups were presented in [Table 2](#).

Discussion

In this experimental study, a 60-min rat renal IR model was used to create a life-threatening renal injury, which was induced by bilateral clamping of renal vascular structures. Renal injury was examined according to the changes in histomorphology and renal function at the 24th hour of reperfusion. At current study compared to the sham operated group, as signs of this impairment in renal function, a significant decrease in UV and CCr and significant increase in the levels of FA_{Na} , BUN and Cr were found in IR group.

In addition, the presence of renal tubular injury was supported by significantly increased histopathologic injury scores compared with the sham operated group. These histological and biochemical findings in accordance with prior studies have shown that IR injury caused both glomerular and tubular dysfunction in the kidney.^{17,19,21}

In the previous studies in which renal IR injury models were created, different ischemia and reperfusion durations

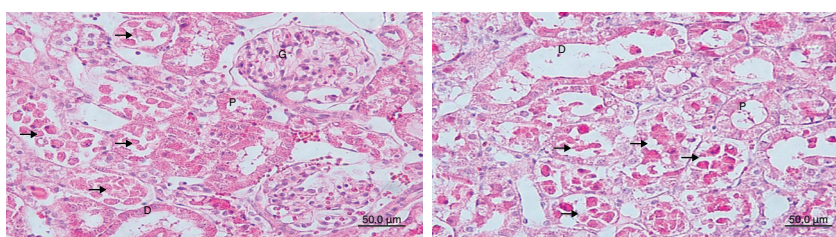


Figure 4 (A–B) The sections of preischemic treatment with dexmedetomidine group. G:glomerulus, P:proximal tubule, D: distal tubule, (→) proximal tubule epithelial cells poured into the lumen.

Table 2 Biochemical data of the groups at 24th hour after reperfusion; blood urea nitrogen, blood creatinine level, creatinine clearance, urine volume, and fractional excretion of Na⁺.

Groups	BUN	Cr	CCr	UV	FANa ⁺
Sham operated (n=5)	21.80 ± 3.42	0.55 ± 0.04	0.64 ± 0.11	7.72 ± 0.66	125.23 ± 28.56
IR (n=6)	129.33 ± 11.32	2.98 ± 0.84	0.01 ± 0.02	0.95 ± 1.25	19542.82 ± 27244.54
Dex.R (n=7)	113.71 ± 16.46	2.80 ± 1.00	0.05 ± 0.04	5.92 ± 1.69	4735.43 ± 7441.64
Dex.I (n=7)	92.00 ± 43.23	2.35 ± 1.23	0.17 ± 0.19	2.9 ± 2.44	943.28 ± 1378.02
<i>p values</i>					
P ₁₂	0.004	0.004	0.036	0.004	0.036
P ₁₃	0.003	0.003	0.003	0.073	0.030
P ₁₄	0.003	0.003	0.008	0.003	0.032
P ₂₃	0.014	0.628	0.267	0.001	0.267
P ₂₄	0.051	0.445	0.143	0.138	0.071
P ₃₄	0.805	0.620	0.432	0.026	0.268

IR, ischemia reperfusion group; Dex R, ischemia reperfusion/reperfusion treatment with dexmedetomidine; Dex I, ischemia reperfusion/preischemic treatment with dexmedetomidine; BUN, blood urea nitrogen; Cr, blood creatinine level, CCr, creatinine clearance; UV, urine volume; FANa⁺, fractional excretion of Na⁺.

Mann Whitney-U test was conducted to compare two independent groups.

p₁₂: comparison of sham operated and IR

p₁₃: comparison of sham operated and Dex. R

p₁₄: comparison of sham operated and Dex. I

p₂₃: comparison of IR and Dex. R

p₂₄: comparison of IR and Dex. I

p₃₄: comparison of Dex. R and Dex. I

were applied.^{7,17,19,20,22} Williams et al.⁷ created ischemia for 45 min with application of clips at the bilateral renal arteries and veins and investigated the effects of IR injury on blood BUN and Cr levels and renal histology, at reperfusion for 0, 0.5, 1, 2, 4, 6, 9, and 24 h and after 1 week. These investigators reported that the earliest renal injury started at the 4th h following ischemia for 45 min and peaked at the 24th hour. Similarly, Yamamoto et al.²³ and Arendshorst et al.²⁴ showed that ATN and medullary perfusion defect became more manifest after 22–48 h of reperfusion. Gu et al.²² created a moderate renal damage with application of clips on bilateral renal pedicles for 25 min. For a life-threatening renal injury in rats, they performed that clip application on the right renal pedicle for 40 min, harvesting the left kidney. These researchers showed that plasma mean Cr and urea levels increased more than 7 times at the 24th hour of reperfusion following ischemia for 40 min. Kocoglu et al.¹⁷ documented histological data of reperfusion injury for 45 min following 60-min ischemia on the left kidney of rats subjected to right kidney nephrectomy. In our study, a 60-min ischemia model was created with application of clips on bilateral renal pedicles, and the effects of IR and dexmedetomidine treatments were studied 24 hours after reperfusion. Acute renal failure due to ischemia is a complex syndrome involving renal vasoconstriction, tubular damage, tubular cell necrosis, glomerular filtration failure, and glomerular damage.^{25,26} The medications that may be effective on the various factors that contribute to this damage have been used in prophylaxis and treatment. Sympathetic activation due to presynaptic release of noradrenaline and increase in noradrenaline levels in the circulation induced by stress in the kidney, and accordingly, reduction in renal blood flow and glomerular filtration, has been suggested as one of the possible mechanisms of acute renal failure produced by IR injury.

Dexmedetomidine is a selective and potent α_2 -adrenoceptor agonist. Dexmedetomidine has been reported to have effective protective efficacy on focal ischemia in rabbits, and cardiac ischemia, reperfusion injury, and incomplete forebrain ischemia in rats.^{27–29} The precise mechanism of the protective effect of α_2 -adrenergic agonists in the brain is unclear. Catecholaminergic neurotransmission is considered to possibly be related to this effect. Dexmedetomidine reduces excessive secretion of noradrenaline due to ischemia by activating presynaptic α_2 -adrenoceptor. This prevents excessive noradrenaline metabolism, which causes the formation of free radicals. It is suggested that protection in brain injury provided with H₂O₂ production decreases in reperfusion due to prevention of oxidative deamination of catecholamines.^{30,31} In addition, dexmedetomidine is considered to cause reduction in necrotic cell death by decreased sympathetic tone, as well as inhibition of ion flow mediated by N-methyl-D-aspartate receptor.³² In addition to these possible mechanisms, Engelhard et al.³² reported that dexmedetomidine caused an increase in the concentration of antiapoptotic proteins. In another study, Wijesundera et al.³³ suggested that α_2 -adrenergic agonists reduced mortality and myocardial infarction in vascular surgery. In the earlier studies, the anti-ischemic effect of dexmedetomidine was demonstrated to occur in high-dose administration, as 100 μ g/kg; therefore, in our study, 100 μ g/kg dose of dexmedetomidine was used for renal IR injury.^{17,27,34}

Kocoglu et al.¹⁷ reported that dexmedetomidine dose of 100 μ g/kg i.p. administered at the start of reperfusion prevents the injury produced by 60-min ischemia and followed by reperfusion of 45 min, which caused acute renal failure, and they showed normal glomeruli and a slight edema of tubular cells. In our study, different from that of Kocoglu et al.¹⁷, dexmedetomidine was used at two different times:

at the beginning of reperfusion and before ischemia. Additionally, whether the renoprotective effect continued until the 24th hour when IR injury peaked, was evaluated by using histopathological examination and renal function tests. Our study has shown that mononuclear cell infiltration, brush border loss of proximal tubule cells, tubular atrophy, tubular dilatation, vacuolization, protein and cell debris accumulation in some tubules, and erythrocyte extravasation were seen in the IR group. We also showed that proximal tubule changes, which were seen in the IR group, decreased significantly in both groups treated with dexmedetomidine, and further, erythrocyte extravasation changes decreased significantly in the group administered dexmedetomidine in the reperfusion period. These histologic findings have shown that, dexmedetomidine had a partial renoprotective effect in both groups in the 24th hour of reperfusion.

Gu et al.²² investigated the effects of dexmedetomidine in both human cell cultures as in vitro and with rats as in vivo studies. Their in vitro studies demonstrated that dexmedetomidine provided protection of tubular structure and prevention of cell death with activation of the Akt pathway and weakening of the HMGB1/TLR4 pathway mediated by α_2 -adrenergic receptor. They demonstrated in their in vivo studies with rats that the administration of dexmedetomidine at a dose of 25 $\mu\text{g}/\text{kg}$ i.p. before ischemia caused a significant reduction in the levels of urea, and Cr increased at the 24th hour, due to the effect of ischemia produced by a 25-min application of clips to bilateral renal pedicles. The investigators also showed a reduction in histological damage scores, at 53% and 38%, respectively, with pre- and post-ischemic dexmedetomidine. Again, in that same study, it was reported that renal function deterioration produced by ischemia for 40 min, which was more serious (seven fold at 24th hour after IR injury), significantly decreased with both pre- and post-ischemic treatment and this effect was dependent on α_2 adrenoreceptor. In our study, similarly with the results of Gu et al.²², we found that renal histology and function (for only BUN levels) improved significantly with dexmedetomidine used pre- and post-ischemia in more severe IR injury. Furthermore, Curtis et al.³⁵ studied the effects of dexmedetomidine on renal histological changes and blood Cr level after IR injury in rats anesthetized with ketamin. These investigators, different from us, administered an α_2 -adrenoceptor agonist dose of 1 $\mu\text{g}/\text{kg}$ intravenous (i.v.) for 10 min and continued with a dose of 1 $\mu\text{g}/\text{kg}/\text{h}$. In that study, ischemia time was 45 min, and nephrectomy and blood sampling were performed 48 h after reperfusion.

In accordance with our and Gu et al.²² studies, they also demonstrated that dexmedetomidine protected the kidneys partially against IR injury at late period of reperfusion.

In their experimental study, Villela et al.¹⁵ reported that, followed by administration of low dose dexmedetomidine (1 and 2 $\mu\text{g}/\text{kg}$ bolus dose application followed by 1 and 2 $\mu\text{g}/\text{kg}$, 1-h i.v. infusion) to the dogs under anesthesia caused free water diuresis with decrease in urine osmolality and plasma vasopressin levels. Frumento et al.¹⁶ demonstrated improvement in postoperative renal function with infusion of dexmedetomidine in patients without renal disease who underwent thoracic surgery, by using glomerular filtration indicators involving urine flow, serum Cr and fractional changes of serum Cr level. In that study, decrease in

serum Cr levels peaked in the first postoperative week. The decrease of Cr levels identified in the period of without drug administration was accepted as proof of the beneficial effect of the drug on glomerular filtration. According to Frumento et al.¹⁶, the improvement in renal function was related with decreased renal vasoconstriction by dexmedetomidine. In our study, different from these investigators, we determined limited recovery in biochemical renal parameters in IR injury. In this study, in both groups administered dexmedetomidine, BUN values were determined to be significantly lower than in the IR group. In addition, administration of dexmedetomidine at the 5th min of reperfusion provided a significant increase in uriner output. However, this improvement determined in diuresis and BUN levels could not be shown in blood Cr levels and CCr, which are more specific, tests for the kidney. The results of our study suggest that, a partial histomorphological and a functional protection were obtained with dexmedetomidine in renal IR injury.

In the present study, Ketamine-Xylazine (KX) was used as the anesthetic regimen which was appropriate for ischemia-reperfusion studies. As ketamine is compatible with the other medications and has a wide confidence interval, it is one of the anesthetic drugs with the widest usage range in experimental studies. Previous studies in the medical literature had conflicting results on the effect of ketamine in renal IR injury.³⁵⁻³⁸ These studies in the literature that compare different anesthetics lack a true control group. Similarly, one limitation to our study is lack of a true control (no anesthetic drug) group, as all animals received KX as the anesthetic regimen in accordance with our institutional ethics committee. Therefore we could not determine the extent of protection induced by the KX anesthesia compared with a 'no-drug' group. In order to standardize the effect of anesthetic regimen in our study, the anesthesia was provided by KX in all the groups. In addition; there were sham and control (IR) groups in our study and when we evaluated the results of these groups, in which no active material was used apart from the KX anesthesia, renal I/R injury was not observed in the sham group but occurred in the control group. Therefore, we are convinced that the results we obtained were independent from the effect of KX anesthesia.

In conclusion, dexmedetomidine used both before ischemia and after reperfusion reduced the effects of renal IR injury at the 24th hour histomorphologically. Although no histomorphologic significant difference was determined between the two methods, administration of dexmedetomidine in the reperfusion period was considered more effective due to the decrease in BUN levels and increase in urinary output.

Conflicts of interest

The authors declare no conflicts of interest.

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