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Original Article

Effects of Dexmedetomidine on Renal Ischaemia Reperfusion Injury in Streptozotocin-Induced Diabetic Rats

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Abstract

Objective: The aim of this study was to investigate the effects of dexmedetomidine before and after ischaemia in diabetic rat kidney ischaemia reperfusion (IR) injury in the experimental diabetic rat model.

Methods: Data belonging to 35 rats weighing between 250 and 300 g were analysed. Diabetes mellitus (DM) was induced using streptozotocin. Groups had bilateral renal vasculature clamped for 45 min ischaemia before clamps were removed, and 4 hours reperfusion was applied. Rats were divided into five groups: Group I or nondiabetic sham group (n=7), Group II or diabetic sham group (n=7), Group III or diabetic IR group (n=7), Group IV or diabetic IR+prophylactic Dex P (before ischaemia) (n=7) and Group V or diabetic IR+therapeutic Dex T (following reperfusion) (n=7). Dexmedetomidine was administered at a dose of 100 μg kg^{-1} intraperitoneally. Histomorphological and biochemical methods were used to assess the blood and tissue samples.

Results: The proximal tubule injury score in the control sham group was significantly lower than in other groups. The proximal tubule and total cell damage scores of the diabetic IR group were significantly higher than the diabetic IR+Dex T group, and no significant difference was detected in the diabetic IR+Dex P group. The biochemical parameters of the IR group were significantly increased compared to Groups I and II; however, there was no significant reduction in these parameters in the groups administered dexmedetomidine.

Conclusion: Although administration of dexmedetomidine after ischaemia in the diabetic rat renal IR model was found to be more effective on the histopathological injury scores compared to preischaemic administration, this study has not shown that dexmedetomidine provides effective and complete protection in DM.

Keywords: Acute renal injury, experimental diabetes mellitus, reperfusion injury, dexmedetomidine

Introduction

Currently, diabetes mellitus (DM) is accepted as an epidemic disease in many developed and developing countries. Among the causes of the increase in DM prevalence is a combination of genetic, environmental, behavioural, socioeconomic and cultural factors (1). Diabetic nephropathy is a significant cause of mortality in DM patients. In developed countries, one-third of patients receiving end-stage renal failure treatment in dialysis units are diabetics. In Europe and America, 30%-50% of type 1 diabetic patients and 5%-15% of type 2 diabetic patients develop diabetic nephropathy (2).

In experimental studies, diabetic rats are reported to develop renal dysfunction faster compared to nondiabetic rats (3). DM is defined as a risk factor for acute renal injury development after radio-contrast nephropathy or cardiopulmonary bypass, with many publications documenting an increasing tendency for acute renal injury, whether clinical or in experimental models (4). Additionally, the mechanisms increasing the tendency toward renal ischaemia in DM are still not fully known (3).

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The kidneys are particularly sensitive to ischaemia reperfusion (IR) injury caused by the cessation of blood flow to the tissue and renewal of the blood flow to the ischaemic tissue. Several different methods including pharmacologic and nonpharmacologic have been used to prevent renal IR injury. Various studies on reducing renal IR injury have been conducted with different drugs. It has been reported that agents, including magnesium sulfate, N-acetylcysteine, activated protein C, captopril, insulin and dexmedetomidine, reduce renal IR injury (5-8). The effect of dexmedetomidine on IR injury has been studied by many teams. Gonullu et al. (9) administered dexmedetomidine before ischaemia and at the start of reperfusion and showed it reduced histopathologic renal IR injury. They found that dexmedetomidine administered in the reperfusion period was more effective compared with the IR injury group. Bagcik et al. (10) administered dexmedetomidine both alone and with remote ischaemic preconditioning (RIPC) and found it reduced histomorphological renal IR injury at significant levels. They identified that the efficacy of the combination of both methods on active caspase 3 prevented apoptosis.

The aim of this study was to evaluate the effects of dexmedetomidine administration before ischaemia (prophylactic) or after reperfusion (therapeutic) by using biochemical (BUN, Cr) and histomorphological methods in a diabetic rat renal IR injury model.

Methods

After obtaining permission from Dokuz Eylul University School of Medicine (DEUSM) Local Animal Experiments Ethics Committee (Date: 03/09/2014, protocol number: 27/2014), the research was carried out at the Dokuz Eylul University. Forty adult Wistar albino male rats weighing between 230 and 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.

Induction of diabetes

Streptozocin was used to induce diabetes as described previously (11). To induce the diabetes model, 45 mg kg^{-1} streptozocin (STZ) (STZ, Sigma Chemical Co., St. Louis, MO, USA) was administered intraperitoneally in a single dose. STZ was prepared in a 0.1 M phosphate-citrate buffer (pH: 4.5), and an equal volume of buffer was injected intraperitoneal into the control sham group without induced diabetes. STZ was prepared freshly and used immediately. Three days after this application, a blood sample was taken from the tail. Rats with blood sugar >250 mg dL^{-1} on glycometry of the sample were accepted as diabetic (11). The rats were monitored for 1 month in the experimental animal laboratory, and then the study began. Within this time, weight changes and blood glucose measurements were recorded.

Study design

Rats were divided into five groups: Group I or nondiabetic sham group (n=7), Group II or diabetic sham group (n=7), Group III or diabetic IR group (n=7), Group IV or diabetic IR+prophylactic Dex P (before ischaemia) (n=7), Diabetic IR+prophylactic preischaemic administration (100 μg kg^{-1}, intraperitoneally [i.p.], 5 min before ischaemia) of dexmedetomidine (Dexmedetomidine, *Precedex* 100 μg/2 mL flk., Abbott Laboratory, Illinois, USA); and Group V or diabetic IR+therapeutic Dex T (following reperfusion) (n=7), diabetic IR + therapeutic postischaemic administration (100 μg kg^{-1}, i.p., 5 min after reperfusion) of dexmedetomidine.

The rats were anaesthetised with ketamine (50 mg kg^{-1}, i.p.) and xylazine hydrochloride (10 mg kg^{-1}, i.p.), and to maintain anaesthetic depth, supplemental ketamine (25 mg kg^{-1}, i.p.) was administered considering reflex responses.

Following anaesthesia, all rats were secured to the operation table in the supine position and warmed with a heating lamp to maintain a rectal body temperature between 37 and 37.5°C throughout the procedure. Laparotomy was performed with a midline abdominal incision, and bilateral renal pedicles were carefully exposed. To prevent hypovolemia, isotonic saline solution (3 mL kg^{-1}, i.p.) was administered hourly, and the abdomen was closed with a moist sterile pad during the reperfusion period. In the sham groups (Group I+Group II), bilateral renal pedicles were exposed without any intervention after laparotomy, and rats were kept under anaesthesia for an additional 285 min (ischaemia+reperfusion duration) to standardise the anaesthesia duration for all groups. In Groups III+IV+V, for the IR injury model, bilateral renal pedicle occlusion was performed with atraumatic microvascular clamps for 45 minutes. Adequate occlusion was confirmed by the lack of pulsation in renal pedicles and presence of pallor in the kidneys. This sustained ischaemia model using the same clamps was confirmed in our previous studies by using a laser flow meter (Laser Flo BPM2, Vasamedic, St Paul, MN, USA) (9, 12). At the end of the ischaemic period, the clamps were removed to start the 4-hour reperfusion phase. Renal reperfusion was confirmed by the reflow of renal perfusion for 5 minutes after removing the clamps from renal vasculature. In Group IV (IR+Dex P), dexmedetomidine (100 μg kg^{-1}, i.p.) was administered 5 min before renal ischaemia (prophylactic), and then renal IR (45 min ischaemia+4 h reperfusion) was induced in both kidneys. Different from Group IV, dexmedetomidine (100 μg kg^{-1}, i.p.) was administered 5 min after reperfusion (therapeutic) in Group V (IR+Dex T). During the waiting time, the abdomen was closed with a moist sterile pad and surgical forceps. At the end of reperfusion, the animals were anaesthetised, blood samples were drawn from the right atrium for the measurement of renal function parameters, and kidneys were excised. The kidneys were fixed in 10% buffered formalin and embedded in paraffin for histomorphological examination.

Exclusion criteria

Rats in need of resuscitation were excluded from the study.

Histomorphological evaluation of renal tissue

All histomorphological analyses described below were performed by two histologists blinded to experimental groups. Each kidney tissue was fixed with 10% formaldehyde. Kidney tissues were processed with routine histological methods and embedded in paraffin blocks. Paraffin blocks were placed in a rotary microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany) with disposable metal microtomeblades (Type S35, Feather Company, Osaka, Japan). Three chosen transverse sections of 4-5 μm thickness from each sample (left and right kidneys) were evaluated. From these sections, 15 cortical images were scored. The chosen transverse sections from each sample were stained with haematoxylin eosin. The sections were examined under light microscopy (Olympus BX-51, Olympus, Tokyo, Japan) for structural changes in proximal tubules (tubular atrophy, loss of tubular brush border, vacuolisation, tubular dilatation, cast forma-

Table 1. Histomorphological scores in groups

Groups	Group I (n=7)	Group II (n=7)	Group III (n=7)	Group IV (n=7)	Group V (n=7)
Proximal tubular structural variations (mean±SD)	0.00±0.00[a,b,c,d]	1.14±0.38[a]	1.43±0.54[f]	1.14±0.38	0.86±0.38
Mononuclear cell infiltration (mean±SD)	0.14±0.38[a,b,c,d]	0.57±0.54[e]	1.29±0.49	0.71±0.49	0.71±0.49
Capillary vasodilatation (mean±SD)	0.29±0.49[a]	0.43±0.54	0.86±0.69	0.43±0.49	0.29±0.49
Total cell injury scores (mean±SD)	0.00±0.00[a,b,c,d]	1.00±0.00	1.43±0.53[f]	1.14±0.38	0.86±0.38

Group I: nondiabetic sham; Group II: diabetic sham; Group III (diabetic IR): renal ischaemia reperfusion in diabetic rats; Group IV (diabetic IR+Dex P): renal ischaemia reperfusion diabetics rats with prophylactic dexmedetomidine; Group V (Diabetic IR+Dex T): renal ischaemia reperfusion in diabetic rats with therapeutic dexmedetomidine. Data are presented as mean±standard deviation (SD). For two-way comparison of groups, the Mann-Whitney U test was used.
[a]p<0.05: Comparison of the nondiabetic sham with diabetic sham group, [b]p<0.05: Comparison of the nondiabetic sham with diabetic ischaemia reperfusion group, [c]p<0.05: Comparison of the nondiabetic sham with diabetic ischaemia reperfusion dexmedetomidine before reperfusion group, [d]p<0.05: Comparison of the nondiabetic sham with diabetic ischaemia reperfusion dexmedetomidine after reperfusion group, [e]p<0.05: Comparison of the diabetic sham with diabetic ischaemia reperfusion group, [f]p<0.05: Comparison of the diabetic ischaemia reperfusion with diabetic ischaemia reperfusion dexmedetomidine after reperfusion group

Table 2. Biochemical parameters of groups after 4-hour reperfusion: blood urea nitrogen, creatinine and serum-neutrophil-gelatinase-associated lipocalin levels

Groups	Group I (n=7)	Group II (n=7)	Group III (n=7)	Group IV (n=7)	Group V (n=7)
BUN (mean±SD)	22.81±4.44[a,b,c,d,e]	37.43±10.83[f]	56.00±14.62[h]	61.00±9.08	71.33±9.05
Creatinine (mean±SD)	0.23±0.05[a,b,c,d,e]	0.36±0.0f[g]	0.61±0.20	0.48±0.15[i]	0.81±0.27
NGAL (mean±SD)	365.57±83.19	309.71±87.87	364.57±70.46	394.14±71.93	466.00±367.63

BUN: blood urea nitrogen; NGAL: neutrophil gelatinase-associated lipocalin. Group I: nondiabetic sham; Group II: diabetic sham; Group III (diabetic IR): renal ischaemia reperfusion in diabetic rats; Group IV (diabetic IR+Dex P): renal ischaemia reperfusion diabetics rats with prophylactic dexmedetomidine; Group V (Diabetic IR+Dex T): renal ischaemia reperfusion in diabetic rats with therapeutic dexmedetomidine. Data are presented as mean±standard deviation (SD). For two-way comparison of groups, the Mann-Whitney U test was used.
[a]P12: Comparison of the nondiabetic sham with diabetic sham group, [b]P13: Comparison of the nondiabetic sham with diabetic ischaemia reperfusion group, [c]P14: Comparison of the nondiabetic sham with diabetic ischaemia reperfusion dexmedetomidine before reperfusion group, [d]P15: Comparison of the nondiabetic sham with diabetic ischaemia reperfusion dexmedetomidine after reperfusion group, [e]P23: Comparison of the diabetic sham with diabetic ischaemia reperfusion group, [f]P24: Comparison of the diabetic sham with diabetic ischaemia reperfusion dexmedetomidine before reperfusion group, [g]P25: Comparison of the diabetic sham with diabetic ischaemia reperfusion dexmedetomidine after reperfusion group, [h]P35: Comparison of the diabetic ischaemia reperfusion with diabetic ischaemia reperfusion dexmedetomidine after reperfusion group, [i]P45: Comparison of the diabetic ischaemia reperfusion dexmedetomidine before reperfusion with diabetic ischaemia reperfusion dexmedetomidine after reperfusion group

Table 3. Blood glucose levels

	Group I (n=7)	Group II (n=7)	Group III (n=7)	Group IV (n=7)	Group V (n=7)
Basal blood glucose (mean±SD)	116.0±11.4	116.0±7.9	116.7±11.0	114.4±7.5	116.0±7.0
Post-streptozosin (3rd day) (mean±SD)	114.0±7.6	348.7±58.6	362.9±67.2	359.3±41.8	403.3±110.6
p	0.611*	0.018*	0.018*	0.018*	0.018*

*p<0.05. SD: standard deviation

tion), mononuclear cell infiltration, capillary dilatation, interstitial structural changes, renal corpuscle morphology, and necrotic/apoptotic cells. Histomorphological injury scoring was carried out using a semiquantitative method based on a scale from 0 to 4 as follows: 0=None, 1=1%-25%, 2=26%-50%, 3=51%-75%, and 4=76%-100%.

Biochemical evaluation

The blood urea nitrogen, blood creatinine level and serum neutrophil gelatinase-associated lipocalin (NGAL) levels were measured 4 hours after reperfusion in Dokuz Eylul University Medical Faculty Hospital Biochemistry Laboratory. Blood urea nitrogen and blood creatinine levels were analysed photometrically with a *Beckman AU 5800* autoanalyser. Serum NGAL levels were analysed with the enzyme-linked immunosorbent assay, using a Boster trade kit (Boster Biological Technology Co., CA; cat number: EK0855, USA). According to the manufacturer's prospectus, the NGAL detection limit is 10 pg mL^{-1} with measurement interval from 78 to 5000.

Statistical analysis

The Statistical Package for the Social Sciences version 15.0 (SPSS Inc.; Chicago, IL, USA) was used. Continuous variables are presented as the mean standard deviation and median (minimum-maximum). For univariate analysis, the Mann-Whitney U test was used for comparison of two groups. To determine the weight and blood glucose level fluctuations over time, the Friedman repeated measurement was conducted. The level of statistical significance was accepted as $p<0.05$.

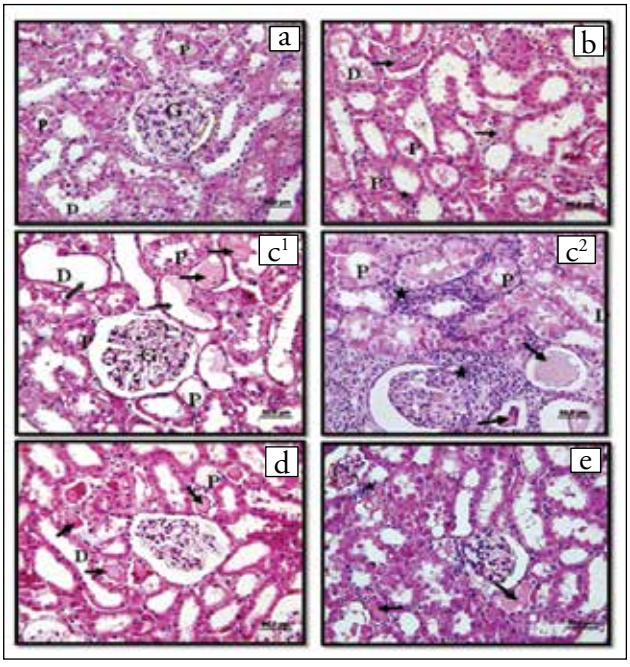

Figure 1. a-e. Representative histochemical staining (H&E) micrographs of experimental groups. (a) Group I (nondiabetic sham), (b) Group II (diabetic sham), (c1,2): Group III (diabetic IR), (d) Group IV (diabetic IR+Dex P) diabetic IR+prophylactic preischaemic administration (100 μg kg^{-1}, i.p., 5 min before ischaemia) of dexmedetomidine, and (e) Group V (diabetic IR+Dex T), diabetic IR+therapeutic postischaemic administration (100 μg kg^{-1}, i.p., 5 min after reperfusion) of dexmedetomidine, bar: 50μm. In this figure, G: renal corpuscule, P: proximal tubule, D: distal tubule, (★): proteinous material accumulation in tubule lumen, (⟶) mononuclear cell infiltration and (⟹): tubular dilatation.

Results

This study included a total of 40 rats. The study was completed with 35 rats, as 3 rats could not have type 1 diabetes model induced, and 2 rats were exitus during the experimental stage.

The histopathological score and biochemical assessments of rats in all groups are presented in Tables 1 and 2.

Table 3 shows the results of blood glucose levels in groups.

Renal histomorphological injury score

Structural changes to proximal tubules

When the nondiabetic sham (0.00±0.00), diabetic sham (1.14±0.38), diabetic IR (1.43±0.54), diabetic IR+Dex P (1.14±0.38) and diabetic IR+Dex T (0.86±0.38) groups are investigated, the proximal tubular injury score was significantly lower in the control sham group compared to the other groups ($p<0.001$, $p=0.001$, $p<0.001$, $p=0.002$, respectively). The diabetic IR group injury score was significantly higher than the score in the diabetic IR+Dex T group ($p=0.044$), and there was no significant difference identified with the diabetic IR+Dex P group ($p=0.254$), although the diabetic IR group injury scores were moderately higher. There was no clear difference identified between the other groups (Figure 1).

Mononuclear cell infiltration

When the nondiabetic sham (0.14±0.38), diabetic sham (0.57±0.54), diabetic IR (1.29±0.49), diabetic IR+Dex P (0.71±0.49) and diabetic IR+Dex T (0.71±0.49) groups are investigated, the mononuclear cell infiltration score in the control sham group was significantly lower than the scores in the diabetic IR, diabetic IR+Dex P and diabetic IR+Dex T groups ($p=0.001$, $p=0.001$, $p=0.002$, respectively). There was no significant difference identified between the nondiabetic sham and diabetic sham groups; however, the mononuclear cell infiltration scores in the diabetic sham group were relatively higher. The scores in the diabetic sham group were significantly lower than in the diabetic IR group ($p=0.030$). There was no significant difference identified between the diabetic IR group and the diabetic IR+Dex P and diabetic IR+Dex T groups ($p=0.054$, $p=0.054$), although the mononuclear cell infiltration scores in the diabetic IR group were moderately higher (Figure 1).

Capillary vasodilatation

When the nondiabetic sham (0.29±0.49), diabetic sham (0.43±0.54), diabetic IR (0.86±0.70), diabetic IR+Dex P (0.43±0.49) and diabetic IR+Dex T (0.29±0.49) groups are

examined, there was no significant difference ($p > 0.05$) between the capillary vasodilatation scores in the groups. The injury score in the diabetic IR group was moderately higher (Figure 1).

Total cellular injury scores

When the nondiabetic sham (0.00±0.00), diabetic sham (1.00±0.00), diabetic IR (1.43±0.53), diabetic IR+Dex P (1.14±0.38) and diabetic IR+Dex T (0.86±0.38) groups are investigated, the histomorphological total cellular injury scores in the control sham group were significantly lower than the scores in the diabetic sham, diabetic IR, diabetic IR+Dex P and diabetic IR+Dex T group scores ($p<0.001$, $p=0.001$, $p<0.001$, $p=0.002$, respectively). There was no significant difference identified between scores in the diabetic sham group and the diabetic IR ($p=0.060$), diabetic IR+Dex P ($p=0.317$) and diabetic IR+Dex P ($p=0.317$) group scores. However, when the diabetic IR group is compared with the diabetic IR+Dex T group, the scores in the diabetic IR+Dex T group were significantly lower ($p=0.044$). There was no significant difference identified between the diabetic IR+Dex P and diabetic IR+Dex T groups. The injury score in the diabetic IR group was moderately higher than the score in the diabetic IR+Dex T group (Figure 1).

Discussion

According to the results of this study, the renal effects of diabetes were evident, especially in proximal tubular injury (proteinous material accumulation) with a significant reduction occurring in histopathologic total cellular injury scores. The addition of IR injury in diabetic rats increased the level of renal injury causing renal mononuclear cell infiltration, as well as proximal tubular injury. The administration of dexmedetomidine after ischaemia in a renal IR model in diabetic rats prevented this injury was more effective on histopathological injury scores compared to administration before ischaemia, but this study did not show that dexmedetomidine provided effective and full protection in DM.

The positive effects on IR injury of one of the methods used to prevent or treat renal IR injury of dexmedetomidine have been shown by many studies (9, 13, 14). The preventative mechanism of dexmedetomidine on renal injury is not fully known, but reduced release of renal noradrenalin, fall in increasing noradrenalin levels in circulation due to stress, and regulation of glomerular filtration and renal blood flow have been shown to be effective. When dexmedetomidine is administered during surgery, it lowers catecholamine plasma levels, ensures haemodynamic stability and increases urinary output. Thus, renal changes linked to the endocrine-metabolic response are reduced (15, 16).

A study by Billings et al. (17) showed that clonidine and dexmedetomidine regulated the reduction in renal perfusion developing after IR, also after another cause of renal failure of radio-contrast injection and reduced the development of nephropathy. Sugita et al. (18) showed that 10-20 $\mu g\ kg^{-1}\ hr^{-1}$ dexmedetomidine infusion reduced renal function disorder, and suppressed the increase in nitric oxide synthesis, messenger RNA and intracellular adhesion molecule-1 induced by renal IR injury. Si et al. (19) found dexmedetomidine protection against renal IR injury occurred by inhibition of the Janus kinase pathway and stated that as a result, it may be used in the prevention and treatment of perioperative IR injury. A study by Gonullu et al. (9) administered 100 $\mu g\ kg^{-1}$ dose of dexmedetomidine intraperitoneally 5 minutes before ischaemia or 5 minutes after reperfusion and showed that dexmedetomidine administered before ischaemia and after reperfusion significantly reduced the histopathological injury scores at 24 hours, reporting a positive effect on IR injury. Bagcik et al. (10) administered dexmedetomidine alone and with RIPC in a rat renal IR model. This study found that dexmedetomidine administered alone or with RIPC provided nearly full renal protection. In both groups, IR injury was significantly reduced in terms of renal mononuclear cell infiltration, glomerulotubular variations and total injury scores. Additionally, these researchers assessed the immunoreactivity of active caspase-3, a marker of apoptosis, and they found that the administration of dexmedetomidine with RIPC significantly reduced active caspase 3 immunoreactivity and stated that the combination of these two administrations may prevent apoptosis, a significant pathway in IR injury.

Diabetes mellitus a common and increasing chronic metabolic disease. Experimental studies of diabetic rats reported more rapid development of renal dysfunction compared to nondiabetic rats (3, 20). DM is defined as a risk factor for acute renal injury development after radio-contrast nephropathy or cardiopulmonary bypass; it is described as increasing the tendency toward acute renal injury whether in clinical or experimental models (21, 22). To induce the type 1 diabetes model in this study, 45 $mg\ kg^{-1}$ STZ was administered i.p. in a single dose based on the study by Guneli et al. (11). Three days after administration, blood sugar was examined with a glycometer in a sample from the tail, and rats with blood sugar >250 $mg\ dL^{-1}$ were accepted as diabetic. For development of chronic effects of diabetes, rats were left for 4 weeks. In this study, the proximal cell injury scores in the diabetic sham group were significantly high compared to nondiabetic rats (control sham). With normal renal corpuscle morphologies, the diabetic sham group was observed to have noteworthy proteinous material accumulation in the tubules, especially the proximal tubular lumen. The significant increase in BUN and creatinine levels in these rats show the effect of diabetes on renal functions.

Abu-Saleh et al. (23) in a diabetic rat model induced with STZ identified histological changes in both diabetic and nondiabetic rats after 30 min renal ischaemia and reported that for all pathological parameters, including congestion and inflammation in the interior of diabetic ischaemic renal me-

dulla, the morphologic score was 2.5 times higher. Our study identified a significant increase in proximal tubular injury in both diabetic sham and diabetic IR groups compared to the nondiabetic group. Mononuclear cell infiltration and erythrocyte extravasation were observed with tubular dilatation in addition to proteinous material accumulation in tubular lumens with IR injury. Also, although all injury scores in the diabetic IR group were higher than the diabetic sham group, this only reached statistical significance for mononuclear cell infiltration.

As typically known, DM nephropathy causes lesions in the renal corpuscule, but in our study, morphological changes were mostly prominent in renal tubules. The proximal tubule plays a vital role in the pathophysiology of the diabetic kidney. We are beginning to better understand the molecular basis of the complex interactions between the proximal tubule and tubulointerstitium. Tubular glucose uptake is important for detrimental renal effects of diabetes (24, 25). A study by Fouad et al. (8) observed dilatation especially in the proximal tubules and vacuolar degeneration with widespread necrosis in the diabetic IR group, similar to our results.

To date, the effects of dexmedetomidine on diabetic rat IR injury were investigated for various organs, such as myocardium and cerebral tissue (7, 26). However, in the literature, there are insufficient studies related to the effect of dexmedetomidine on renal IR injury in diabetic rats (12, 27). As a result, in our study, we aimed to investigate the effect of 100 μg kg^{-1} i.p. administration of dexmedetomidine, with known protective effect on renal IR injury in nondiabetic rats, by administering it before ischaemia and after reperfusion and examining early period effects on histopathology and renal functions. According to the results of our study, dexmedetomidine administered at the start of reperfusion reduced mononuclear cell infiltration in the peritubular area and erythrocyte extravasation in the cortex compared to the IR group with less tubular degeneration, tubular dilatation and proteinous material accumulation in the tubules compared with the group administered dexmedetomidine before ischaemia. The results relating to effects on temporary hyperglycaemia, an acute oxidising factor, of renal IR injury preventative methods are still not clear. To date, many studies in the literature about pharmacologic and non-pharmacologic methods to prevent the effects of renal IR injury have reported that positive effects resolve temporary acute hyperglycaemia (28, 29), although a few studies have reported hyperglycaemia does not prevent the renal protective effect (30). Previous studies have shown that the renal protective effects of isoflurane, propofol, melatonin and RIPC have resolved temporary hyperglycaemia (30, 31). Similarly, Wang et al. (32) studied the effects of dexmedetomidine on acute hyperglycaemia in renal IR injury in rats. These researchers used a very low dose (50 μg kg^{-1} i.p.) and administered dexmedetomidine only before ischaemia and reported positive effects on IR resolved acute hyperglycaemia. In a study with ischaemia duration held below 45 minutes and erythropoietin only administered at low (600 U kg^{-1}) and high (5000 U kg^{-1}) doses, Caetano et al. (30) showed that erythropoietin did not prevent tubular necrosis in rats with temporary hyperglycaemia; however, it reduced apoptosis and improved glomerular functions. There are two studies showing positive effects of dexmedetomidine on IR injury in diabetic rats (12, 27). The first of these studies researched the role of dexmedetomidine on the kidneys with lower extremity IR injury to a distant organ. The other study showed positive effects of dexmedetomidine on direct renal IR in diabetic rats using histopathologic tissue samples, and it indicated this positive effect formed due to P38-MAPK/TXNIP signalling activation inhibition. Different from this study, we applied a longer ischaemia period (45 minutes compared to 25) and administered dexmedetomidine a shorter period before ischaemia (5 minutes compared to 30 minutes) and 5 minutes after reperfusion at higher doses (100 microgram compared to 50 microgram) to compare the effects. In this study, we showed that administration in the reperfusion period was better for IR injury and only histologically; however, we did not identify improvements in renal function tests. We believe the reason for this is that these tests were performed in the early period; if measured after longer periods like 24 hours, this positive effect may be observed.

To date, many pharmacologic and nonpharmacologic methods have been applied with the aim of preventing or treating renal IR injury in diabetic rats (33-35). The effects of these methods on renal IR injury in rats are very variable, with some methods increasing negative results such as temporary hyperglycaemia. According to the results of histomorphologic assessment of local ischaemic preconditioning (LIPC), a nonpharmacological method, in diabetic rats by Ozbilgin et al. (35), there was no reducing or protective effect on IR injury. According to the results of this study, in addition to LIPC not providing protective effect, the histological scores for mononuclear cell infiltration in proximal tubules, capillary vasodilatation and structural variations in this group were higher compared to other groups. This led to the opinion that in renal IR injury, LIPC causes a more negative effect. Additionally, in accordance with histopathological findings, the biochemical parameters of BUN, creatinine and NGAL showed no protective effect of LIPC on renal IR injury.

There are studies assessing the effects of pharmacological agents on a diabetic rat renal IR model (33). To the best of our knowledge, dexmedetomidine was not used previously for renal IR injury in diabetic rats. Kip et al. (26) administered dexmedetomidine after reperfusion at the same doses as our study and reported it prevented the development of pulmonary injury after myocardial ischaemia in diabetic rats. Zeng et al. (36) administered 5 μg kg^{-1} hr^{-1} i.v. dose of dexmedetomidine over 90 minutes and showed it significantly reduced global cerebral IR injury in diabetic rats. According to these researchers, the positive effect of dexmedetomidine on cerebral IR injury is provided by antiapoptotic protein expression and Bcl-2 up-regulation and inhibition of the proapoptotic protein Bax expression.

In our study, dexmedetomidine administered in the reperfusion period reduced mononuclear cell infiltration in the peritubular area and erythrocyte extravasation in the cortex compared with both the IR group and the group administered dexmedetomidine before ischaemia, but only proximal tubular structural changes were statistically significantly different. These results show that in renal IR injury in diabetic rats, dexmedetomidine at this dose only provides a partial histopathologic renal amelioration when administered for treatment. In situations with high oxidant stress, like DM, higher doses or infusion of dexmedetomidine or longer administration is required.

In addition to BUN and creatinine showing acute renal injury, in recent years, the highly sensitive, specific and determinant marker in the early period of NGAL has begun to be examined (35). Serum NGAL levels in blood samples taken 2 hours after 30 minutes of bilateral renal artery ischaemia were identified to be higher and change earlier compared to other markers. Neutrophil gelatinase-associated lipocalin may be identified in both urine and serum 2-6 hours after acute renal injury. As a result, NGAL is proposed as the parameter with best sensitivity and specificity for determination of acute renal injury (37-39). A study by Si et al. (19) identified an increase in BUN and creatinine values in blood samples examined 0, 12, 24 and 48 hours after renal ischaemia, while NGAL was increased in blood samples from 12 hours. Additionally, another study reported NGAL was a weak marker, and there may be extrarenal production independent of renal injury, especially in the presence of systemic stress (40). In our study, there was no significant difference between the groups in terms of NGAL, BUN and creatinine levels. Just as these results may be related to the time the blood samples were taken, we believe it may be related to extrarenal production of NGAL related to stress, unique to NGAL.

This study has some limitations. An example may be the lack of a longer reperfusion period like 24-48 hours to identify variations in biochemical and histopathological parameters. Additionally, as it was not an aim of the study, the effect of dexmedetomidine on apoptosis pathways was not assessed, which is another limiting factor. Another limiting aspect is the lack of haemodynamic monitoring in this experimental model.

Conclusion

According to histopathologic injury scores in this study, administration of dexmedetomidine after ischaemia in a renal IR model in diabetic rats was only more effective in terms of tubular injury compared to administration before ischaemia. There is a need for new studies using different doses and durations to explain the underlying mechanisms of the efficacy of dexmedetomidine on diabetic renal IR injury.

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