Renal Failure, 31:70–74, 2009

ISSN: 0886-022X print / 1525-6049 online
DOI: 10.1080/08860220802546487

informa
healthcare

LABORATORY STUDY

Effect of Dexmedetomidine on Ischemia-Reperfusion Injury in Rat Kidney: A Histopathologic Study

Hasan Kocoglu
Faculty of Medicine, Department of Anesthesiology, Abant Izzet Baysal University, Bolu, Turkey

Hulya Ozturk
Faculty of Medicine, Department of Pediatric Surgery, Duzce University, Düzce, Turkey

Hayrettin Ozturk
Faculty of Medicine, Department of Pediatric Surgery, Abant Izzet Baysal University, Bolu, Turkey

Fahri Yilmaz
Faculty of Medicine, Department of Pathology, Abant Izzet Baysal University, Bolu, Turkey

Nebahat Gulcu
Faculty of Medicine, Department of Anesthesiology, Abant Izzet Baysal University, Bolu, Turkey

Ischemia-reperfusion (I-R) injury remains the leading cause of acute renal failure. The purpose of this experimental study was to determine the role of dexmedetomidine on histologic alterations induced by renal I-R in rats. In the present study, thirty male Sprague-Dawley rats weighing 200–220 g were randomly assigned into three groups: the sham-control group (group 1, n = 10), the R/untreated group (group 2, n = 10), and the I-R/dexmedetomidine-treated group (group 3, n = 10). For group one, we performed a sham operation. The abdomen was dissected, the right kidney was harvested, and then the left renal pedicle exposed. Renal clamping was not applied. For group 2, rats underwent left renal ischemia for 60 minutes followed by reperfusion for 45 minutes. For group 3, the same surgical procedure as in group 2 was performed, and dexmedetomidine (100 μg/kg, intraperitoneal) was administrated at the starting time of reperfusion. The rats were sacrificed after reperfusion, and the kidney tissue was harvested. The histopathological score in the kidney of the I-R/dexmedetomidine-treated group rats was significantly lower than that of I-R/untreated group rats. This score in I-R/untreated group rats was higher than the other two groups, which was statistically significant. In the I-R/untreated group rats, kidneys of untreated ischemia rats showed tubular cell swelling, cellular vacuolization, pyknotic nuclei, medullary congestion, and moderate to severe necrosis. Treatment with dexmedetomidine shows normal glomeruli and slight edema of the tubular cells. These findings provide the first evidence that dexmedetomidine can reduce the renal injury caused by I-R of the kidney, and may be useful in enhancing the tolerance of the kidney against renal injury.

Keywords renal ischemia-reperfusion injury, dexmedetomidine, kidney

Received 8 August 2008; revised 10 September 2008; accepted 25 September 2008.

Address correspondence to Hasan Kocoglu, MD, Abant Izzet Baysal University, Faculty of Medicine, Department of Anesthesiology, Bolu, Turkey; Tel.: +90 374 253 46 56; Fax: +90 374 253 46 16; E-mail: hasankocoglu@yahoo.com

INTRODUCTION

Ischemia-reperfusion (I-R) injury remains the leading cause of acute renal failure.[1] Hence, kidney injury caused by ischemia and reperfusion is a major focus for both fundamental and clinical research.[1] It has been demonstrated histologically that acute tubular necrosis develops during/after renal ischemia-reperfusion (I-R) injury.[2,3] The organ dysfunction that accompanies this condition is generally associated with increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, and parenchymal cell dysfunction and necrosis.[4]

Dexmedetomidine, a selective and potent α2-adrenoceptor agonist, was approved by the U.S. Food and Drug Administration in 1999 for sedation of patients hospitalized in intensive care settings. Since then, a growing number of research articles have emerged reporting other possible indications, such as regional[5] and general[6] anesthesia. Dexmedetomidine was reported to be effective in protecting against focal ischemia in rabbits,[7] in cardiac I-R injury in rats,[8] and in incomplete forebrain ischemia in rats.[9] Despite its increased clinical use, many times in critically ill patients,[10] the effect of dexmedetomidine on renal I-R injury has not been yet investigated. The purpose of this experimental study was to determine the role of dexmedetomidine on histologic alterations induced by renal I-R in rats.

MATERIALS AND METHODS

In the present study, thirty male Sprague-Dawley rats weighing 200 to 220 g were used. All of the experimental protocols were performed according to the guidelines for the ethical treatment of experimentation animals.

Animals and Experimental Protocol

The rats were housed individually in cages and were allowed free access to standard rat chow and water before and after the experiments. The animal rooms were windowless with temperature (22 ± 2°C) and lighting controls. The animals were fasted overnight before the experiments but were given free access to water. They were anesthetized by 50 mg/kg ketamine and 20 mg/kg xylazine body weight, i.p. The animals were randomly separated into three groups, each containing 10 rats:

- *Sham-control group (group 1, n = 10).* We performed a sham operation. The abdomen was dissected, the right kidney was harvested, and the left renal pedicle exposed. However, renal clamping was not applied.
- *I-R/untreated group (group 2, n = 10).* Rats were subjected to the surgical procedures described below, and underwent left renal ischemia for 60 minutes followed by reperfusion for 45 minutes.
- *I-R/dexmedetomidine-treated group (group 3, n = 10).* The same surgical procedure as in group 2 was performed. Dexmedetomidine [Dexmedetomidine hydrochloride 100 µg/kg, i.p. (Precedex 100 mcg/2 ml, Abbott®, Abbott Laboratory, North Chicago, Illinois, USA)] was administrated at the starting time of reperfusion.[11,12]

Ischemia Reperfusion Model

The abdomen was dissected under anesthesia, the right kidney was harvested, and then the left renal artery and vein were clamped with a hemostasis clip for 60 minutes. The abdomen was closed during I-R. The clip was subsequently removed to permit reperfusion. Sham-operated control rats underwent the same surgical procedure, including dissection of the renal pedicle; however, renal clamps were not applied. The rats were sacrificed after reperfusion, and the kidney tissue was harvested.

Histopathological Evaluation

The extracted kidneys were fixed in 10% buffered formalin, and embedded in paraffin. Sections were slided to 5 µm-thick pieces and coded. The coded kidney specimens were stained with hematoxylin and eosin and examined in blinded fashion. Histological changes were evaluated by quantitative measurement of tubulointerstitial injury, which was assessed by counting the number of necrotic and apoptotic cells, loss of tubular brush border, tubular dilatation, cast formation, and neutrophil infiltration. The scoring was 0 = none; 1 = 0–10%; 2 = 11–25%; 3 = 26–45%; 4 = 46–75%; and 5 = 76–100%.[13]

Statistical Analysis

All data were expressed as median ± standard error of mean (SEM). Significance of differences was evaluated using the Mann-Whitney U test. The level of statistical significance was accepted as p less than 0.5.

RESULTS

The histopathological score of the rats in all groups are presented in Figure 1. The histopathological score in the kidney of I-R/dexmedetomidine-treated group rats was significantly lower than that of I-R/untreated group rats ($p < 0.05$). This score in I-R/untreated group rats was higher than the other two groups, which was statistically significant ($p < 0.05$ for all differences of the scores).

The sham-operated group did not show any morphological changes (see Figure 2A). By contrast, kidneys of untreated ischemia rats showed tubular cell swelling, cellular vacuolization, pyknotic nuclei, medullary congestion, and moderate to severe necrosis (see Figure 2B). Kidneys in the rats of the dexmedetomidine-injected group showed normal glomeruli and slight edema of the tubular cells (see Figure 2C).

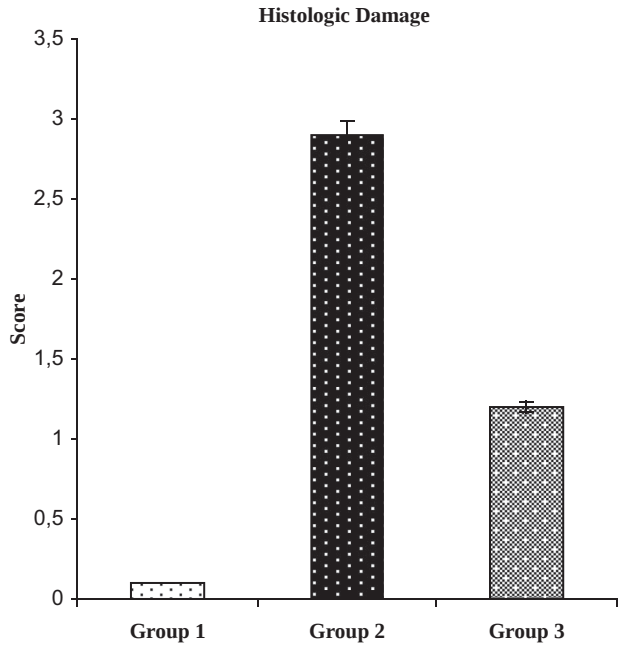

Figure 1. Histologic scores in groups. *$p < 0.05$ compared with group 1. †$p < 0.05$ compared with group 2. Values are mean ± SEM.

DISCUSSION

Acute renal failure produced by ischemia and reflow is a clinical and experimental syndrome characterized by major reductions in glomerular filtration rate, extensive tubular damage, tubular cell necrosis, glomerular injury, and signs of tubular obstruction with cell debris.[14–16] Acute renal failure due to ischemia is a complex syndrome involving renal vasoconstriction, extensive tubular damage, tubular cell necrosis, glomerular filtration failure, and glomerular injury.[17,18] Studies in animals have revealed a number of factors that could contribute to the injury associated with ischemic acute renal failure (ARF).[19] The mechanisms proposed to explain the ischemia–reperfusion injury include anoxia, release of reactive oxygen species (ROS) during reperfusion, neutrophil accumulation, and the subsequent release of additional ROS and lytic enzymes.[18,20,21] In this study, we have reported (for the first time, to our knowledge) that ischemia-reperfusion of the rat kidney results in a significant renal injury, and the administration of dexmedetomidine, a highly potent and selective α_2-adrenoreceptor agonist, administered at the starting time of reperfusion of the kidney, can provide varying degrees of protection against renal injury.

Dexmedetomidine has been widely investigated in a variety of cerebral ischemia models. It has been reported to protect against incomplete ischemia in rats[9] and against focal ischemia in rabbits.[22] Decreased sympathetic tone and inhibition of N-methyl-d-aspartate receptor-mediated ion currents were believed to mediate the reduction of necrotic cell death.[23] In a recent study, Engelhard et al.[23] reported that dexmedetomidine increased the concentration of the anti-apoptotic proteins Bcl-2 and Mdm-2. It was also shown in their study that neuroprotection mediated by the α_2-agonist dexmedetomidine might also involve apoptosis-regulating proteins. In another study, Duminda et al.[24] suggested that α_2 adrenergic agonists reduce mortality and myocardial infarction following vascular surgery. Frumento et al.[25] showed a significant association between dexmedetomidine infusion and improved postoperative renal function in post hoc analysis of postoperative thoracic surgical patients without renal disease, including indices of glomerular filtration as well as urine flow rate. In the present study, we found better histopathological structure in the kidney of dexmedetomidine-treated group rats compared to the untreated group. In the untreated group, the kidneys showed tubular cell swelling, cellular vacuolization, pyknotic nuclei, medullary congestion, and moderate to severe necrosis more apparently. Treatment with dexmedetomidine prevented the injury, and showed normal glomeruli and a slight edema of the tubular cells.

The role of dexmedetomidine in the protection of renal injury is not very clear. However, it was previously reported that dexmedetomidine decreases the sympathetically mediated presynaptic release of norepinephrine in the kidney, attenuates stress-induced increases in circulating norepinephrine, and may maintain renal blood flow and glomerular filtration.[26] Indeed, it is well established that the administration of α_2-adrenergic agonists can inhibit the surgical stress response[27–29] and thereby protect the kidney against the detrimental effects of adrenergic-mediated vasoconstriction.[30] Dexmedetomidine could promote renal arterial vasodilatation as well. There may be direct vascular effects in the kidney also. Although we have not studied the mechanisms by which dexmedetomidine protects the kidney, we have found that it clearly protects the kidney against I-R injury. This protection may be related to the effects of dexmedetomidine explained above.

As a result, it was shown for the first time that dexmedetomidine reduces the renal injury caused by ischemia-reperfusion. We conclude that, in conditions of renal ischemia and consequent reperfusion such as arterial occlusion, shock, and organ transplantation, dexmedetomidine may be useful in enhancing the tolerance of the kidney against renal injury.

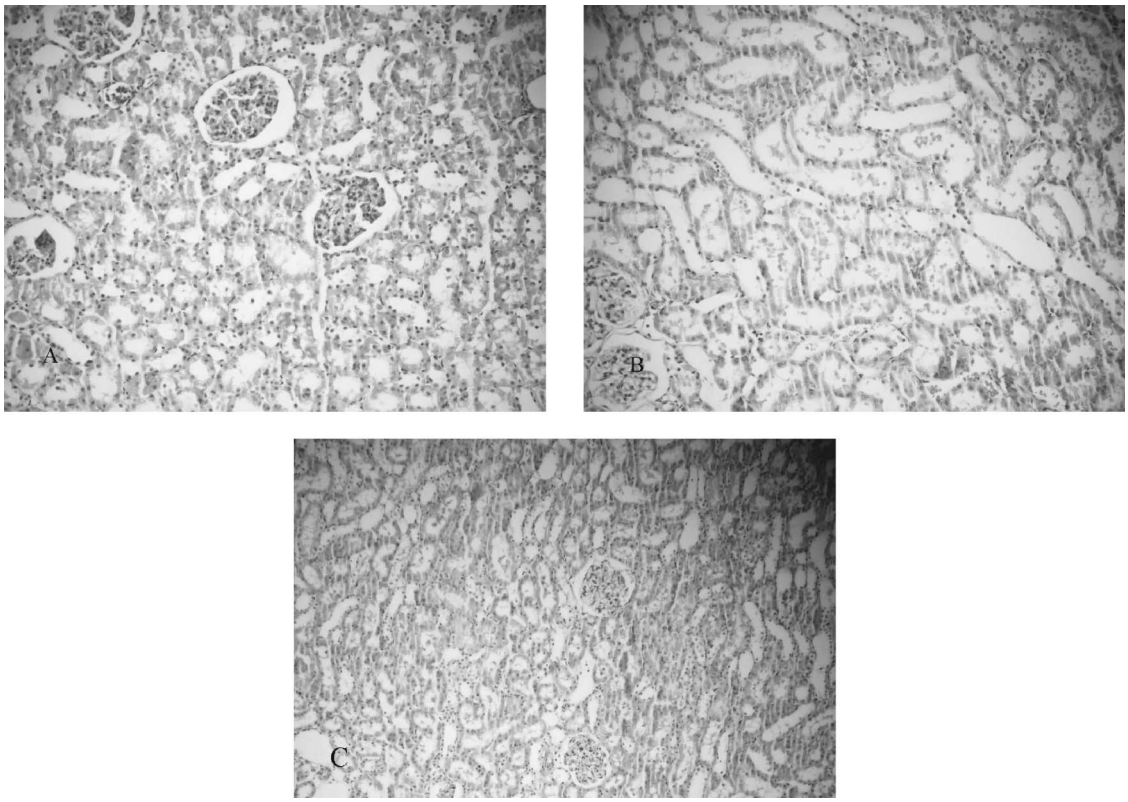

Figure 2. (A) The sham-operated group did not show any morphological changes. (B) The kidneys of untreated ischemia rats showed tubular cell swelling, cellular vacuolization, and moderate to severe necrosis. (C) Treatment with dexmedetomidine shows slight edema of the tubular cells.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Caron A, Desrosiers RR, Béliveau R. Kidney ischemia-reperfusion regulates expression and distribution of tubulin subunits, beta-actin, and rho GTPases in proximal tubules. *Arch Biochem Biophys.* 2004;431:31–46.
2. Brezis M, Rosen S. Hypoxia of the renal medulla—its implications for disease. *N Engl J Med.* 1995;332:647–655.
3. Chiao H, Kohda Y, McLeroy P, Craig L, Housini I, Star RA. Alpha-melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. *J Clin Invest.* 1997;99:1165–1172.
4. Granger DN, Korthuis RJ. Physiologic mechanisms of postischemic tissue injury. *Annu Rev Physiol.* 1995;57:311–332.
5. McCutcheon CA, Orme RM, Scott DA, Davies MJ, McGlade DP. A comparison of dexmedetomidine versus conventional therapy for sedation and hemodynamic control during carotid endarterectomy performed under regional anesthesia. *Anesth Analg.* 2006;102:668–675.
6. Ramsay MA, Luterman DL. Dexmedetomidine as a total intravenous anesthetic agent. *Anesthesiology.* 2004;101:787–790.
7. Maier CM, Sun GH, Kunis DM, Giffard RG, Steinberg GK. Neuroprotection by the N-methyl-D-aspartate receptor antagonist CGP 40116: In vivo and in vitro studies. *J Neurochem.* 1995;65:652–659.
8. Kocoglu H, Karaaslan K, Gonca E, Bozdogan O, Gulcu N. Preconditioning effects of dexmedetomidine on myocardial ischemia/reperfusion injury in rats. *Curr Ther Res Clin Exp.* 2008;69:150–158.
9. Hoffman WE, Kochs E, Werner C, Thomas C, Albrecht RF. Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat. Reversal by the alpha 2-adrenergic antagonist atipamezole. *Anesthesiology.* 1991;75:328–332.
10. Snapir A, Posti J, Kentala E, et al. Effects of low and high plasma concentrations of dexmedetomidine on myocardial perfusion and cardiac function in healthy male subjects. *Anesthesiology*. 2006;105:902–910.
11. Hoffman WE, Baughman VL, Albrecht RF. Interaction of catecholamines and nitrous oxide ventilation during incomplete brain ischemia in rats. *Anesth Analg.* 1993;77:908–912.

12. Engelhard K, Werner C, Eberspächer E, Bachl M, Blobner M, Hildt E, Hutzler P, Kochs E. The effect of the alpha 2-agonist dexmedetomidine and the N-methyl-D-aspartate antagonist S(+)-ketamine on the expression of apoptosis-regulating proteins after incomplete cerebral ischemia and reperfusion in rats. *Anesth Analg.* 2003;96:524–531.
13. Feng L, Xiong Y, Cheng F, Zhang L, Li S, Li Y. Effect of ligustrazine on ischemia-reperfusion injury in murine kidney. *Transplant Proc.* 2004;36:1949–1951.
14. Finn WF. Nephron heterogeneity in polyuric acute renal failure. *J Lab Clin Med.* 1981;98:21–29.
15. Barnes JL, Osgood RW, Reineck HJ, Stein JH. Glomerular alterations in an ischemic model of acute renal failure. *Lab Invest.* 1981;45:378–386.
16. Chatterjee PK, Cuzzocrea S, Thiemermann C. Inhibitors of poly (ADPribose) synthetase protect rat proximal tubular cells against oxidant stress. *Kidney Int.* 1999;56:973–984.
17. Bird JE, Milhoan K, Wilson CB, et al. Ischemic acute renal failure and antioxidant therapy in the rat: The relation between glomerular and tubular dysfunction. *J Clin Invest.* 1988;81:1630–1638.
18. Weinberg JM. The cell biology of ischemic renal injury. *Kidney Int.* 1991;39:476–500.
19. Okusa MD. The inflammatory cascade in acute ischemic renal failure. *Nephron*. 2002;90:133–138.
20. Bonventre JV. Mechanisms of ischemic acute renal failure. *Kidney Int.* 1993;43:1160–1178.
21. Baker GL, Corry RJ, Autor AP. Oxygen free radical induced damage in kidneys subjected to warm ischemia and reperfusion: Protective effect of superoxide dismutase. *Ann Surg.* 1985;202:628–641.
22. Maier C, Steinberg GK, Sun GH, Zhi GT, Maze M. Neuroprotection by the alpha 2-adrenoreceptor agonist dexmedetomidine in a focal model of cerebral ischemia. *Anesthesiology*. 1993;79:306–312.
23. Engelhard K, Werner C, Eberspächer E, et al. The effect of the alpha 2-agonist dexmedetomidine and the N-methyl-D-aspartate antagonist S(+)-ketamine on the expression of apoptosis-regulating proteins after incomplete cerebral ischemia and reperfusion in rats. *Anesth Analg.* 2003;96:524–531.
24. Wijeysundera DN, Naik JS, Beattie WS. Alpha-2 adrenergic agonists to prevent perioperative cardiovascular complications: A meta-analysis. *Am J Med.* 2003 Jun 15;114(9):742–752.
25. Frumento RJ, Logginidou HG, Wahlander S, Wagener G, Playford HR, Sladen RN. Dexmedetomidine infusion is associated with enhanced renal function after thoracic surgery. *J Clin Anesth.* 2006;18:422–426.
26. Taoda M, Adachi YU, Uchihashi Y, Watanabe K, Satoh T, Vizi ES. Effect of dexmedetomidine on the release of [3H]-noradrenaline from rat kidney cortex slices: Characterization of alpha2-adrenoceptor. *Neurochem Int.* 2001;38:317–322.
27. Helbo-Hansen S, Fletcher R, Lundberg D, et al. Clonidine and the sympatico-adrenal response to coronary artery by-pass surgery. *Acta Anaesthesiol Scand.* 1986;30:235–242.
28. Kulka PJ, Tryba M, Zenz M. Dose-response effects of intravenous clonidine on stress response during induction of anesthesia in coronary artery bypass graft patients. *Anesth Analg.* 1995;80:263–268.
29. Flacke JW, Bloor BC, Flacke WE, Wong D, Dazza S, Stead SW, Laks H. Reduced narcotic requirement by clonidine with improved hemodynamic and adrenergic stability in patients undergoing coronary bypass surgery. *Anesthesiology.* 1987;67:11–19.
30. Kulka PJ, Tryba M, Zenz M. Preoperative alpha2-adrenergic receptor agonists prevent the deterioration of renal function after cardiac surgery: Results of a randomized, controlled trial. *Crit Care Med.* 1996;24:947–952.