Continuous Infusion of Dexmedetomidine Improves Renal Ischemia-reperfusion Injury in Rat Kidney

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Abstract

**Background:** Dexmedetomidine has shown beneficial effects in several inflammatory models, including ischemia-reperfusion injury (IRI). This study investigated whether the continuous infusion of dexmedetomidine could improve renal IRI in rats.

**Methods:** Rats were subjected to either a sham operation and given pentobarbital (10 mg/kg/h; n=6) or were subjected to 45 minutes of renal ischemia and anesthetized with pentobarbital (10 mg/kg/h; n=6), dexmedetomidine (10 or 20 μg/kg/h; both n=6), or both pentobarbital (10 mg/kg/h) and dexmedetomidine (1.0 μg/kg/h; n=6) for 6 hours of reperfusion. Blood urea nitrogen and serum creatinine were measured 6 hours after reperfusion. Gene expression mediated by inflammatory systems in the kidney was measured with the real-time reverse-transcriptase polymerase chain reaction.

**Results:** Treatment with 10 or 20 μg/kg/h of dexmedetomidine reduced renal dysfunction. The increases in the messenger RNA expression of interleukin-6, intercellular adhesion molecule 1, and inducible nitric oxide synthase caused by renal IRI were suppressed. Under In rats under pentobarbital anesthesia, 1.0 μg/kg/h of dexmedetomidine also improved renal dysfunction after renal IRI.

**Conclusion:** The present study demonstrates that continuous infusion of dexmedetomidine improves renal IRI. Moreover, with pentobarbital anesthesia, a dose of dexmedetomidine lower than the sedative dose also improves renal IRI.

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**Key words:** dexmedetomidine, kidney, ischemia-reperfusion injury

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**Introduction**

Dexmedetomidine, a selective α2-adrenoceptor agonist, has been used as an adjunct to anesthesia, analgesia, and intensive care unit sedation. In addition to the sedative and analgesic effects of dexmedetomidine, organ-protective effects of dexmedetomidine have been shown in several inflammatory models, including ischemia-reperfusion injury (IRI)\(^3\).

Renal IRI is a major cause of acute kidney injury (AKI) and occurs in various clinical conditions, such as severe hypotension, transplantation, renal artery stenosis, embolic disease, and cardiovascular surgeries\(^2\). IRI continues to be associated with...
significant morbidity and mortality despite advances in preventive strategies and supportive measures. 1/4 R induces some injury in the cortical proximal tubules and a more severe, generally lethal injury in the outer medullary proximal tubules. Infiltration of neutrophils and macrophages in the kidney occurs after reperfusion. Oxidative stress and the generation of reactive oxygen species (ROS) during ischemia-reperfusion increase the production of proinflammatory cytokines that can further accelerate apoptotic signaling and cellular damage.

Although the involvement of the immune system in renal IRI is extremely complex, a major insight into the pathogenesis of AKI is the recognition that the initial ischemic insult elicits maladaptive responses that exacerbate the injury and might be modified if effective therapy can be developed.

Recently, in a histopathological study of the rat kidney, intraperitoneal injection of dexmedetomidine improved renal IRI. In this model, rats treated with dexmedetomidine show normal glomeruli and slight edema of the tubular cells 24 hours after renal IR. In mice, intraperitoneal injection of dexmedetomidine suppresses the toll-like receptor 4 (TLR-4)-mediated inflammatory circuitry. Dexmedetomidine provides long-term functional renoprotection and increases survival following nephrectomy.

However, the effect of intravenous infusion of dexmedetomidine on renal IRI remains unclear. We hypothesized that intravenous administration of dexmedetomidine could also attenuate renal IRI. This study investigated the role of dexmedetomidine in renal dysfunction and inflammation caused by renal IR.

Materials and Methods

The experimental procedures were approved by the Institutional Committee on Laboratory Animals of Nippon Medical School. The experimental protocol was performed on male Wistar rats weighing 200 to 250 g (Saitama Experimental Animals Supply, Saitama, Japan). Animals were housed in a temperature-controlled room with an alternating 12:12-hour light and dark cycle. Animals had free access to water and food until 2 hours before the surgical procedure.

The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg; Nembutal, Abbott Laboratories, Abbott Park, IL, USA) and provided satisfactory anesthesia as determined by loss of reflex responses to painful stimuli (endotracheal intubation and skin incision). After endotracheal intubation with a 16-G catheter (BD InsyteTM IV Catheter, Becton, Dickinson and Company, Franklin Lakes, NJ, USA), the animals were ventilated with a ventilator (Harvard Rodent Ventilator 683, Harvard Apparatus, Holliston, MA, USA). The fraction of inspiratory oxygen was maintained at 33%, and the ventilator rate was set at 60 to 70 strokes per minute with a tidal volume of 7 mL/kg to maintain the PaCO₂ at 35 to 45 mmHg. The left femoral vein was cannulated with a 24-G catheter (BD Insyte IV Catheter, Becton, Dickinson and Company) for drug and fluid administration, and the left femoral artery was cannulated for blood gas analysis and direct monitoring of mean arterial blood pressure (MAP) and heart rate (HR). Body temperature was monitored with a rectal thermometer and maintained at 36°C to 37°C with a heating pad.

A midline incision was made, and both sides of the kidneys were visualized. Bilateral pedicle clamping was performed with microvascular clamps. Forty-five minutes later, the vascular clamps were removed and allowed blood flow to return to the kidneys. Visual inspection, showing a change in the color of the kidneys from dark blue to bright red, confirmed successful reperfusion after the clamps were released. The surgical incision was sutured in 2 layers. Pentobarbital and dexmedetomidine (Precedex®; Hospira, Inc., Lake Forest, IL, USA) were dissolved in normal saline, and the infusion rate was determined on the basis of a pilot study and previous studies. The anesthesia due to pentobarbital or dexmedetomidine or both during the reperfusion period was indicated by the loss of reflex responses to painful stimuli (skin suture and intermittent needle skin prick).

The animals were divided into 5 groups (each group, n=6). Rats in the sham operation group (P group) underwent laparotomy without induction of
ischemia; the kidneys were visualized, and 45 minutes later pentobarbital was administrated intravenously (i.v.) at 10 mg/kg/h (1 mg/mL) for 6 hours. Rats in the ischemia group (IR+P group) were subjected to bilateral renal IR and received pentobarbital at 10 mg/kg/h i.v. (1 mg/mL) for 6 hours after reperfusion. Dexmedetomidine-treated groups were subjected to bilateral renal IR and received dexmedetomidine at 10 (1 μg/mL) (IR+D[10] group) or 20 μg/kg/h i.v. (2 μg/mL) (IR+D[20] group) for 6 hours after reperfusion. The dexmedetomidine and pentobarbital-treated group was subjected to bilateral renal I/R and received pentobarbital at 10 mg/kg/h i.v. (1 mg/mL) and dexmedetomidine at 1.0 μg/kg/h (0.1 μg/mL) (IR+P+D[1] group) for 6 hours after reperfusion.

Six hours after reperfusion, the sufficiency of anesthesia was checked, all animals were killed, and blood was obtained via cardiac puncture. Blood was allowed to clot at room temperature for 1 hour and then subjected to centrifugation at 3,000 × g for 10 minutes and frozen until blood urea nitrogen (BUN) and serum creatinine were measured. The BUN and serum creatinine were measured with an autoanalyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). The kidneys were removed and immediately stored at −80°C until use for the real-time reverse-transcriptase polymerase chain reaction (RT-PCR).

**Real-time RT-PCR Analysis**

Total mRNA was isolated from each kidney by means of the acid guanidinium phenol chloroform method with Isogen (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Total RNA (8 μg) was used as a template for the reverse transcription reaction with 8 μL of 5 × RT buffer (Invitrogen, Carlsbad, CA, USA), 6 μL of 1.25 mM deoxyribonucleoside triphosphate mixture (Takara Bio Inc., Otsu, Japan), 2 μL of random primer (hexadecyldimethylammonium chloride mixture; Takara Bio Inc.), 2 μL of 0.1 M dithiothreitol (Invitrogen), 1 μL of Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen), and 0.2 μL of ribonuclease inhibitor (Takara). The reverse transcription reaction was carried out with PCR Express® (Thermo Fisher Scientific, Waltham, MA, USA) at 42°C for 60 minutes, at 99°C for 10 minutes, and at 4°C for 5 minutes.

The amplification of tumor necrosis factor (TNF)-α, interleukin (IL)-6, intercellular adhesion molecule 1 (ICAM-1), neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS), and endothelial nitric oxide synthase (eNOS) was performed in a Fast® 96-well reaction plate (Applied Biosystems Japan, Tokyo, Japan). Reactions were carried out in a 20-μL volume containing 10 μL of TaqMan® Universal PCR master mix (Applied Biosystems), 1 μL of TaqMan Gene Expression Assays (Applied Biosystems), 8 μL of RNase-free water (Wako Pure Chemical Industries, Ltd.), and 20 ng of complementary (c) DNA. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to assess DNA integrity. Assay IDs are shown in Table 1.

PCR consisted of initial denaturation at 95°C for 20 seconds, followed by 40 cycles at 95°C for 3 seconds and at 60°C for 30 seconds; the fluorescence signal was measured after each cycle. The TaqMan probe labeled with 6-carboxyfluorescein was cleaved during amplification, generating a fluorescent signal. The assay used an instrument capable of measuring fluorescence in real time (ABI PRISM 7500 Fast Sequence Detector; Applied Biosystems). The results
of real-time RT-PCR are represented as the threshold cycle (Ct) values, where Ct is a unitless value defined as the fractional cycle number at which the sample fluorescence signal passed a fixed threshold above baseline. The relative amounts of all messenger (m) RNAs were calculated with the comparative Ct method (Applied Biosystems) using the equation $2^{-\Delta \Delta Ct}$.

The $\Delta Ct$ was the difference between the Ct values derived from the unknown sample and the GAPDH control, whereas the $\Delta \Delta Ct$ was calculated with the formula: $\Delta \Delta Ct = \Delta Ct - \Delta Ct$ of the calibrator sample.

**Statistical Analysis**

All values are expressed as mean ± SD. The data were analyzed with Student’s $t$-test when comparing means between groups or with one-way analysis of variance. A $P$-value of less than 0.05 was considered indicate statistical significance.

**Results**

**Hemodynamic Changes**

Changes in MAP and HR between the P group and the IR+P group were similar during the procedures (Fig. 1A and B). The MAP change in the IR+D[10] group was not significantly different from that in the IR+P group, and the MAP in the IR+D[20] group was significantly higher than that in the IR+P group after reperfusion. Slight bradycardia was observed in the IR+D[10] group, and bradycardia was also observed in the IR+D[20] group.

Changes in MAP in the IR+P+D[1] group was not significantly different from that in the IR+P group. Slight bradycardia was observed in the IR+P+D[1] group.

**Effect of Dexmedetomidine on BUN and Serum Creatinine**

The BUN and serum creatinine in the IR+P group was significantly greater than that in the P group 6 hours after renal IR. Rats in the IR+P+D[1], IR+D[10], and IR+D[20] groups had significantly lower BUN and serum creatinine levels than did rats in the IR+P group (Fig. 2A and B).

**Result of Real-time RT-PCR Analysis**

The expression of TNF-α, IL-6, and ICAM-1 mRNA was up-regulated in the IR+P group (Fig. 3 A–C). TNF-α mRNA expression of the IR+P+D[1], IR+P[10], and IR+P[20] groups was not significantly different from that in the IR+P group. The expression of IL-6 and ICAM-1 mRNA in the IR+P+D[1], IR+P[10], and IR+P[20] groups was significantly lower than that in the IR+P group. The expression of nNOS mRNA in the IR+P group was downregulated 6 hours after reperfusion (Fig. 3D). nNOS mRNA expression of the dexmedetomidine-
Dexmedetomidine improves renal IRI

Fig. 2  Effect of dexmedetomidine on renal injury. Renal function indicators—blood urea nitrogen (BUN) (A) and serum creatinine (B)—were measured 6 hours after renal ischemia-reperfusion (IR) injury (each group, n=6). The groups were as follows: the sham operation + pentobarbital (10 mg/kg/h) group (F); IR + pentobarbital (10 mg/kg/h) group (IR + P); IR + dexmedetomidine (1.0 μg/kg/h) group (IR + D[1]); IR + dexmedetomidine (10 μg/kg/h) group (IR + D[10]); IR + dexmedetomidine (20 μg/kg/h) group (IR + D[20]). Data in bar graphs are presented as means ± SD. *P<0.05 vs. P group, **P<0.01 vs. P group.

The present study has shown that continuous infusion of dexmedetomidine (10 and 20 μg/kg/h) reduces the renal dysfunction caused by renal I/R compared with pentobarbital (10 mg/kg/h), and inhibits increases in the expression of IL-6, ICAM-1, and iNOS mRNA caused by renal I/R.

In the present study, rats were subjected to bilateral renal ischemia for 45 minutes and subsequent reperfusion for 6 hours, as described previously. Histological examination of the kidney 6 hours of reperfusion demonstrates glomerular degeneration, tubular dilatation, tubular swelling and necrosis, luminal congestion, and the presence of eosinophilia. Forty-five minutes of ischemia followed by 24 hours of reperfusion is a commonly used animal model that is more suitable for simulating the changes in renal function induced by hemodynamic changes in humans. However, no significant increases in plasma urea or creatinine levels were observed between 6 and 24 hours of reperfusion in a similar model of renal I/R in rats. Moreover, early infiltrating macrophages play an important pathogenetic role in the ischemic AKI produced by proinflammatory cytokines and chemokines, and the injury after ischemic AKI comprises the initial insult and subsequent maladaptive responses, including the inflammatory responses. Thus, we assessed the effects of dexmedetomidine on early inflammatory responses after renal IRI.

IRI induces renal tubular cell production of TNF-α, and the cellular localization of renal TNF-α production is injury-specific. TNF-α production is induced by ROS through phosphorylation of p38 mitogen-activated protein kinase, which activates nuclear factor-B to drive stress-induced inflammation. TNF-α is a proinflammatory cytokine capable of inducing renal dysfunction. Furthermore, TNF-α induces renal cellular apoptosis and reduces glomerular blood flow and the glomerular filtration rate by stimulating the production of a variety of vasoactive mediators. In the present study, although dexmedetomidine did not affect the expression of TNF-α mRNA, it suppressed the upregulation of IL-6 protein and mRNA. Dexmedetomidine itself might not have antioxidative effects and might not regulate phosphorylation of p38 mitogen-activated protein kinase in the kidney. However, TNF-α mRNA expression reportedly

treated groups did not differ significantly from that in the IR+P group. The expression of iNOS mRNA was up-regulated in the IR+P group (Fig. 3E). The iNOS mRNA expression of the IR+P+D(I), IR+P(10), and IR+P(20) groups was significantly lower than that in the IR+P group. The eNOS mRNA expression was not significantly changed in any of the groups (Fig. 3F).

Discussion

The present study has shown that continuous infusion of dexmedetomidine (10 and 20 μg/kg/h)
peaks 24 hours after reperfusion$^{22}$. Further study might be needed to investigate the long-term regulation of TNF-α expression by dexmedetomidine.

IL-6 is a pleiotropic cytokine primarily involved in the regulation of immune and inflammatory responses$^{23}$. In mice, maximal IL-6 production occurs at an early phase of inflammation in renal IRI. Macrophages infiltrate areas adjacent to the vascular bundles in the outer medulla within hours of reperfusion and produce IL-6 mRNA: IL-6 exacerbates ischemic murine acute renal failure$^{25}$. Moreover, early IL-6 production by macrophages is mediated by TLR-4 activation$^{26}$. Dexmedetomidine attenuates the high-mobility group box-1/TLR-4 pathway, preserves tubular architecture, and reduces cell death 24 hour after renal IRI$^{23}$. Together with these findings, our results suggest that dexmedetomidine improves renal IRI by attenuating early IL-6 production and that this regulation might be, in a part, mediated by TLR-4.

IL-6 promotes the expression of adhesion molecules, such as ICAM-1 and P- and E-selectin, which results in an excessive accumulation of polymorphonuclear leukocytes within the kidney and subsequent nitrosative stress$^{27,28}$. Nitric oxide (NO) production is dependent on the activities of NOS enzymes. Previous authors have demonstrated different effects of the 3 NOS isoforms, 2 of which are constitutive (nNOS and eNOS) and 1 of which is inducible (iNOS), in apoptosis regulation under ischemic or hypoxic conditions in a variety of cells and tissues$^{29,30}$. nNOS is expressed largely in the macula densa cells.
Diminished nNOS expression in the macula densa might contribute to the reduction in glomerular filtration in postischemic AKI3. iNOS, which is expressed in several segments of the renal tubule as well as in the glomerulus of the normal rat kidney, can be induced during immune and inflammatory responses32. Reductions in iNOS expression and NO production can provide protection against renal IRI30. Previous studies have revealed that an increase in eNOS expression in the endothelium might ameliorate renal IRI by mediating vasodilation and by inhibiting leukocyte adhesion and platelet aggregation. Although a change in eNOS expression due to dexmedetomidine has been observed in several earlier studies, no change in eNOS mRNA expression was observed in the present study. Assessing the long-term activity of each NO isoform at the gene and protein levels and measuring NO production are the necessary next steps in determining the role of dexmedetomidine in NO regulation31,34.

The infusion rate of dexmedetomidine is an important factor because dexmedetomidine may cause significant hemodynamic changes that affect renal blood flow. In humans, the doses of dexmedetomidine were established on the basis of the recommended dose of 0.2 to 0.7 μg/kg/h, which achieves a target plasma concentration of 0.6 to 2.1 ng/mL35. On the other hand, a higher infusion rate of dexmedetomidine is needed to achieve the target plasma concentration in rats36–38. On the basis of these findings, we chose sedative doses of dexmedetomidine (10 and 20 μg/kg/h) as infusion rates. At a low dexmedetomidine concentration, binding to α2 receptors in the vasomotor centers of the brainstem is thought to cause a reduction of sympathetic tone, resulting in decreases in HR and blood pressure. However, significant hypotension is usually observed in patients with pre-existing hypovolemia and vasoconstriction. At higher concentration of dexmedetomidine, binding to α2 adrenergic receptors in the peripheral vascular bed results in vasoconstriction and an increase in blood pressure39.

In the present study we found that despite the hemodynamic changes being dose-dependent, both 10 and 20 μg/kg/h of dexmedetomidine reduced renal dysfunction and altered gene expression to the same degree. This finding suggests that the ability of dexmedetomidine to attenuate renal IRI might not be dose-dependent. However, the anti-inflammatory effects of lower doses of dexmedetomidine have been controversial32,33. Thus, we also examined whether a lower dose of dexmedetomidine (1.0 μg/kg/h) under anesthesia can improve renal IRI. Evidence suggests that several anesthetics and opioids have protective effects against renal IRI in vivo or in vitro or both30–41. Thus, we chose pentobarbital as an anesthetic because there is little evidence it has any protective effects against renal IRI. In the present study we found that 1.0 μg/kg/h of dexmedetomidine under pentobarbital anesthesia improved renal function in the same manner. However, we did not examine whether higher doses of dexmedetomidine improves renal IRI under pentobarbital anesthesia because the considerable interaction between these 2 drugs could not be assessed. Thus, further studies are needed to investigate the interactions between dexmedetomidine and other anesthetics.

This study had several limitations. First, we investigated dexmedetomidine-induced hemodynamic changes as indicated by MAP and HR, but not renal blood flow. In previous studies, the effects of α2-adrenoreceptor agonists on cardiovascular responses have been reported, but the effect on renal blood flow has been complicated42–44. Dexmedetomidine increased glomerular filtration rate and decreased renal vascular resistance but did not change renal blood flow after acute hemorrhage in rats. Moxonidine, an α2-adrenoreceptor and imidazoline receptor agonist, injected into the lateral cerebral ventricle increases renal blood flow, while injected into the 4th cerebral ventricle moxonidine reduces renal blood flow in rat. Moreover, the mechanism of cardiovascular responses of moxonidine was regulated by central NO synthesis in rat. Thus, renal blood flow and tissue perfusion should be evaluated, and tissue oxygen delivery should be examined. Second, the mechanism of the organ-protective effects of dexmedetomidine has not been fully clarified.
Although some studies have found that dexmedetomidine reduces TNF-α production, in the present study, dexmedetomidine did not affect the renal expression of TNF-α mRNA. Further studies should focus on the expression of proteins, including the mediators of inflammatory responses and the latter inflammatory responses. Third, we could not explain fully why the lower dose of dexmedetomidine with pentobarbital altered gene expression in the same manner as the higher doses of dexmedetomidine. Moreover, the role of α2-adrenoceptors and the specific subtypes (α2A, α2B, and α2C) mediating the effects of dexmedetomidine on regulating the expression of inflammatory molecules remains to be elucidated. If our study were repeated with an α2-adrenergic receptor antagonist such as atipamezole, it would provide better support for α2-adrenergic effects protecting the kidney.

In conclusion, the present study has demonstrated that a sedative dose of dexmedetomidine improves the renal dysfunction caused by renal IRI. Moreover, under pentobarbital anesthesia, a dose of dexmedetomidine lower than the sedative dose also improves renal IRI. Understanding how to take advantage of the protective effects of dexmedetomidine on renal IRI may be of great benefit for patients in the intensive care unit and in the perioperative setting, and the further investigation is needed.

Conflicts of Interest: None

References

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