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Administration of minocycline ameliorates damage in a renal ischemia/reperfusion injury model

Abstract

Purpose: The purpose of this study was to investigate the effects of minocycline on the renal dysfunction and injury caused by bilateral ischemia/reperfusion (I/R) of murine kidneys *in vivo*.

Methods: Male C57BL/6 mice were administered minocycline (45 mg/kg i.v.) or saline (0.9%, v/v, NaCl) 36 hours prior to I/R. Mice were subjected to bilateral renal ischemia (35 min) followed by reperfusion (6 hours). Serum creatinine (sCr) and blood urea nitrogen (BUN) levels were measured. Additionally, renal superoxide dismutase (SOD) levels, malondialdehyde (MDA) levels and myeloperoxidase (MPO) activity were determined. The expression of intercellular adhesion molecule-1 (ICAM-1), caspase-3, caspase-8 and caspase-9 was determined using real time RT-PCR and Western blot analysis.

Results: Minocycline administration significantly reduced the increases in sCr and BUN caused by I/R, indicating attenuation of renal dysfunction and injury, and reduced histological evidence of renal damage caused by I/R. Minocycline administration also markedly reduced the evidence of oxidative stress (MPO activity, SOD and MDA levels), inflammation (ICAM-1 expression and MPO activity) and apoptosis (caspase-3, caspase-8 and caspase-9 expression) in mouse kidneys subjected to I/R.

Conclusion: These findings provide good evidence that minocycline can reduce the renal dysfunction and injury caused by I/R of the kidney. Its mechanism may involve suppression of apoptosis, inflammatory response and oxidative stress.

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Ischemia reperfusion (I/R) is the main mechanism of organ injury during stroke, myocardial infarction and organ transplantation [1-3]. Renal I/R injury, which occurs during renal transplantation, surgical revascularization of the renal artery, or severe burn with delay resuscitation, is a complex pathophysiological process leading to a major cause of acute renal failure [4]. To date, mortality from acute renal failure in hospitalized patients remains high. Many successful strategies for the treatment of renal I/R in animal models have failed to improve clinical outcomes when attempted in patients [5]. Although the pathophysiological mechanism of renal I/R is very complex and still incompletely understood, some key events leading to tissue injury and renal failure have been identified. Recent studies have demonstrated that both apoptosis and necrosis are implicated in the induction of inflammation in the course of I/R, an important cause of tissue injury induced by renal I/R [6-8]. Inhibition of certain elements of inflammatory responses and apoptotic pathway seemed to ameliorate renal I/R injury.

Using animal models, researchers have already demonstrated that tetracyclines have protective effects against I/R injury in heart [9], brain [10], skeletal muscle [11], liver [12] and kidney [13]. Although many possible mechanisms have been proposed, the exact protective mechanism of tetracyclines is still unclear [14]. Minocycline, a tetracycline derivative antibiotic, has been well characterized by its long half life and broad-spectrum antimicrobial activity, and safely used in clinical situations for acne vulgaris and rheumatoid arthritis [15]. Minocycline also has other outstanding advantages such as excellent bioavailability and superior blood-brain barrier permeability because of its small size and high lipophilicity. Additionally, it possesses cytoprotective properties by acting as an anti-apoptotic, anti-inflammatory agent and reactive oxygen species scavenger [16]. Recent studies have indicated that minocycline causes significant reduction in inducible nitric oxide synthase and reduced loss of hippocampal pyramidal neurons in a gerbil model of global ischemia [17]. Minocycline inhibits caspase-1 and caspase-3 mRNA expression in a transgenic mouse model of Huntington's disease and delayed disease progression in mice transgenic for the G93A mutant superoxide dismutase 1, familial amyotrophic lateral sclerosis allele [18]. Thus, anti-oxidant, anti-inflammatory and anti-apoptotic activities are considered to underlie I/R-mediated protective mechanisms.

Few studies have investigated the effects of minocycline on the renal dysfunction and injury caused by I/R *in vivo*. The present study was designed to evaluate the effectiveness of minocycline in an established *in vivo* murine model of renal I/R injury. Subsequently, the ability of minocycline to reduce the apoptosis, inflammatory response and oxidative stress associated with renal I/R was evaluated using a combination of established biochemical, real time RT-PCR and Western blot analysis.

Materials and Methods

Animals

Animals were housed in facilities according to international guidelines, and studies were approved by and conducted in accordance with the Institutional Animal Care and Use committee. 40 male C57BL/6 mice (6 to 8 week-old, 25~30 g, Shanghai Animal Center of Chinese Academy of Science, Shanghai, P.R.China) were housed eight per cage with free access to food and water, and were kept in a constant environment ($22 \pm 2^\circ\text{C}$, $50 \pm 5\%$ humidity, 12 h light/dark cycle).

In Vivo Renal I/R Injury

Mice were anesthetized with ketamine hydrochloride (100 mg/kg body weight, i.v.). After medial laparotomy, all renal arteries and veins were clamped by microaneurysm clamps for 35 min. After the renal clamps were removed, the kidneys were observed for a further 5 minutes to ensure blood reflow then the incisions were sutured in two layers. All mice were kept at 37°C during the procedure via a homeothermic blanket and allowed to recover. Mice were then returned to their cages and were allowed free access to food and water. Mice with delayed recovery from anesthesia or with signs of hemorrhage were excluded from the study.

Grouping and drug administration

All mice were received intraperitoneal injection of 45 mg/kg minocycline (Sigma, St.Louis, MO, US) 36 hours before the surgery followed by intraperitoneal injection of 22.5 mg/kg minocycline every 12 hours for four times or intraperitoneal injection of 0.9% NaCl. Mice were randomly divided into four groups, with eight in each group: (1) Sham control group (Sham+vehicle), mice underwent identical surgical procedures to I/R mice, except that microaneurysm clamps were not applied, and received intraperitoneal injection of 0.9% NaCl; (2) Sham treatment group (Sham+minocycline), mice underwent identical surgical procedures to I/R mice, except that microaneurysm clamps were not applied, and received intraperitoneal injection of minocycline; (3) I/R control group (I/R+vehicle), mice received intraperitoneal injection of 0.9% NaCl and underwent renal I/R; (4) I/R treatment group (I/R+minocycline), mice underwent renal I/R and received intraperitoneal injection of minocycline as well.

Sample collection

24 hours after I/R or sham operation, peripheral blood samples were drawn from the mice in each group, and plasma was separated by centrifugation at $1000\times g$ for 10 min and stored at -20°C until measurements were performed. At the same time, the kidneys of the mice were removed under anesthesia. A piece of renal tissue including cortex and medulla was trimmed down and fixed by 10% buffered formalin for 24 h and embedded in paraffin for histological studies. An-

other portion of the kidney specimen was snap frozen in liquid nitrogen and conserved at -80°C for biochemical analyses.

Measurement of biochemical parameters

Serum creatinine (sCr) levels and blood urea nitrogen (BUN) were measured as an indicator of glomerular function by Automated Chemical Analyzer (7600, Hitachi, Japan).

Renal histopathology

Histology was based on paraformaldehyde-fixed, paraffin embedded tissue sections stained with hematoxylin and eosin (H&E) to evaluate the acute tubular necrosis in the kidney tissues. Samples were coded and examined in a blinded fashion. I/R injury was evaluated on a scale from 0 to 5 (0, none; 1, $\leq 10\%$ of section area with tubular necrosis; 2, 11~25% of section area with tubular necrosis; 3, 26~45% of section area with tubular necrosis; 4, 46~75% of section area with tubular necrosis; and 5, $\geq 76\%$ of section area with tubular necrosis). Two independent observers examined the slides by light microscopy in a blinded fashion.

Determination of malondialdehyde (MDA) and superoxide dismutase (SOD) levels

Levels of MDA and SOD in kidneys were determined as an indicator of lipid peroxidation. Briefly, kidney tissue was weighed and homogenised in a 1.15% (w/v) potassium chloride solution. A 100 ml aliquot of homogenate was then removed and the levels of MDA and SOD were measured using MDA and SOD Detection Kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions.

Determination of myeloperoxidase (MPO) activity

MPO activity in kidneys was used as an indicator of polymorphonuclear leukocyte (PMN) infiltration. Briefly, kidney tissue was weighed and homogenised in a solution containing 0.5% (w/v) hexadecyltrimethylammonium bromide dissolved in 10 mM potassium phosphate buffer (pH=7.4) and centrifuged for 30 min at 20,000×g at 4°C. An aliquot of supernatant was then removed and the MPO levels in tissue homogenates were determined by MPO Detection Kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions.

Fluorimeter-based quantitative RT-PCR assay

The mRNA expression level of intercellular adhesion molecule-1 (ICAM-1), caspase-3, caspase-8 and caspase-9 in kidney tissues of mice in each group were detected by fluorescein-based quantitative RT-PCR.

Total RNA was extracted from the tissues with a phenol/guanidine isothiocyanate based reagent (Trizol™, Gibco BRL, Scotland). Briefly, 1 ml of Trizol and 200 µl of chloroform were added

to each tube. The RNA was then precipitated with 500 µl of isopropanol (v/v), washed with 75% ethanol and air dried. The purified RNA was then dissolved in 10 µl of diethyl pyrocarbonate (DEPC) treated water, the optical density measured at 260/280 wavelength and stored at -80°C prior to being used for the synthesis of cDNA.

RNA was reverse transcribed using the Super script™ Preamplification System (Life Technology, Guangzhou, Guangdong, China). Total RNA were mixed with oligo (dT) primers and incubated at 70°C for 10 min. The mixture was then chilled on ice and incubated with a 1×reverse transcriptase buffer (50 mM Tris-HCl, pH 8.4, 75 mM KCl), 3 mM MgCl₂, 500 µM of each deoxynucleotide, 10 mM dithiothreitol and 200 U of Superscript II RT reverse transcriptase at 42°C for 50 min. The 20 µl samples were further incubated at 70°C for 15 min and 2 U RNase H was added to each tube. Following a final incubation at 37°C for 20 min, the cDNAs were stored at -80°C until use.

PCR was performed in MJ Opticon Monitor 2.0 (MJ Research, Alameda, CA, USA) using SYBR Green I (Biogene, Valencia, CA, USA) as fluorescein. The sequences of the primers used were as following: 5'-AGCACCTCCCCACCTACTTT-3' and 5'-AGCTTGACGACCCTTCTAA-3' for ICAM-1; 5'-TGGACTGTGGCATTGAGACAG-3' and 5'-CGACCCGTCCTTTGAATTTC-3' for caspase-3; 5'-CCTAGA-CTGCAACCGAGAGG-3' and 5'-GCAGGCTCAAGTCATCTTCC-3' for caspase-8; 5'-GGATGCTGTGTCAAGTTTGCC-3' and 5'-CTTTTCGCAGAACAGCATTGG-3' for caspase-9; 5'-ATGCCATCACTGCCAC TCAG-3' and 5'-CAGGGATGATGTTCTGGGT-3' for GAPDH. All primers were synthesized by Sangon Co., Shanghai, P.R.China. Reverse transcription and amplification was performed using a thermocycler (Biometa, Göttingen, Germany) that was programmed with the following parameters: (1) reverse transcription at 50°C for 30 min; (2) PCR activation at 95°C for 15 min; (3) 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min; and (4) final extension at 72°C for 10 min.

Sample quantification was carried out by the software of Sequence Detection System (SDS2.0) with constructing a standard calibration curve using serial dilutions of GAPDH DNA of known concentration, from which the concentration of an unknown sample could be determined. To analyze inter-assay variation, a least square curve fitting test was used to construct a fit, and the standard error of the data points to the curve was estimated by plotting the log of the sample concentration against the number of cycles the PCR machine undertook to yield a set of fluorescent data. The standard error was estimated to be 15% on the log and was within the values described for GAPDH quantification using competitive PCR.

Western blot analysis

Kidney tissues for each of the four groups were homogenized in lysis buffer [PBS, 1% nonidet P-40 (NP-40), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 100 µg/ml aprotinin, 100 µg/ml phenylmethylsulfonyl fluoride (PMSF), sodium orthovanadate]. Samples were kept at 4°C throughout all procedures. Samples were

FIGURE 1. Effect of minocycline on renal dysfunction. Levels of sCr (A) and BUN (B) were measured subsequent to sham-operation (Sham) or renal I/R (Renal I/R) in the absence or presence of minocycline or vehicle, administered 36 hours prior to renal I/R. Values are means $\pm$ SD. *, $p < 0.01$, comparison with sham+vehicle group, #, $p < 0.01$, comparison with sham+minocycline group, \$, $p < 0.05$, comparison with sham+minocycline group, &, $p < 0.05$, comparison with I/R+vehicle group (24 hours), @, $p < 0.01$, comparison with I/R+vehicle group (24h).

sonicated for 70 s, 300 μ g PMSF per gram of tissue added, incubated on ice for 30 min, then centrifuged at 15,000 rpm for 20 min. The protein content was determined according to Bradford's method, with bovine serum albumin used as a standard. Protein samples (30 μ g) were boiled with 2 $\times$ sample buffer containing 5% β -mercaptoethanol for 5 min, separated by size on 15% polyacrylamide gel under SDS denaturing conditions, and transferred to a nitrocellulose membrane at 90 V for 2 hours. The nitrocellulose membranes were stained with ponceau S to assess the efficiency of transfer. Non-specific binding was blocked by incubation in block buffer (5% non-fat dry milk, 0.05% Tween-20, 1 $\times$ tris-Cl-buffered saline) overnight at 4 $^{\circ}$ C. The membranes were hybridized with a 1:100 dilution of monoclonal rabbit anti-mouse ICAM-1 (200 μ g/ml, #sc-107, Santa Cruz Biotechnology, CA, USA), 1:150 dilution of monoclonal rabbit anti-mouse caspase-3 (200 μ g/ml, #sc-7272, Santa Cruz Biotechnology, CA, USA), 1:100 dilution of monoclonal rabbit anti-mouse caspase-8 (200 μ g/ml, #sc-81661, Santa Cruz Biotechnology, CA, USA), and 1:500 dilution of monoclonal rabbit anti-mouse caspase-9 (200 μ g/ml, #sc-56076, Santa Cruz Biotechnology, CA, USA), respectively, then incubated with a horseradish peroxidase-labeled goat anti-rabbit IgG (1:500). The bound secondary antibody was detected by enhanced chemiluminescence (Amersham Life Science, Little Chalfont, UK). Housekeeping protein β -actin was used as a loading control. Positive immunoreactive bands were quantified densitometrically (Leica Q500IW image analysis system) and expressed as ratio of ICAM-1, caspase-3, caspase-8 and caspase-9 to β -actin in optical density units, respectively.

Statistical Analysis

The software of SPSS version 13.0 for Windows (SPSS Inc, IL, USA) was used for statistical analysis. Data obtained were expressed as mean $\pm$ S.D. and analyzed by one-way ANOVA with the post-hoc Tukey's test applied for paired comparisons. A difference between means was considered significant if the P value was less than 0.05.

Results

Effect of minocycline on renal dysfunction caused by I/R

To investigate the effect of minocycline on I/R induced renal dysfunction, sCr and BUN levels were determined in three separate groups: at 24, 48 and 72 hours after a 35 min ischemic period, respectively. Compared to sham-operated animals, mice that underwent renal I/R exhibited a significant increase in sCr (0.08 ± 0.01 mg/dl vs. 1.03 ± 0.25 mg/dl) and BUN concentration (13.4 ± 2.66 mg/dl vs. 103.5 ± 20.40 mg/dl), suggesting a significant degree of glomerular dysfunction, respectively. Administration of vehicle for minocycline to mice prior to I/R did not result in any significant alterations of sCr and BUN concentration (Figure 1A and B). Pre-treatment of mice with minocycline prior to I/R produced significant reduction in serum levels of sCr and BUN (Figure 1A and B).

Effect of minocycline on renal injury caused by I/R

The administration of minocycline to sham-operated animals (Figure 2B) did not result in a change in histological features relative to those seen in the sham+vehicle mice (Figure 2A). In contrast, renal I/R produced a distinct abundance of cast formation, degeneration of

FIGURE 2. Effect of minocycline on renal injury. Histological features of mouse kidney in sham+vehicle (A), sham+minocycline (B), I/R+vehicle (C) and I/R+minocycline (D) groups and histological score (E) were measured subsequent to sham-operation (Shams) or renal I/R (Renal I/R) in the absence or presence of minocycline, administered 36 hours prior to renal I/R, respectively. Values are means $\pm$ SD. *, $p<0.01$, comparison with sham+vehicle group, '#', $p<0.01$, comparison with sham+minocycline group, '&', $p<0.05$, comparison with I/R+vehicle group.

tubular structure, tubular dilatation, loss of tubular cells, swelling and necrosis (Figure 2C), suggesting that tubular injury was significantly reduced by the administration of minocycline prior to I/R (Figure 2D). When compared to the histological score measured from kidneys obtained from sham-operated animals, I/R produced a significant increase in histological score suggesting marked renal injury caused by I/R (Figure 2E). Histological evidence of renal injury was significantly reduced by administration of minocycline prior to I/R (Figure 2E). Administration of minocycline to sham-operated mice did not have a significant effect on histological score when compared to the histological scores measured in sham+vehicle mice (Figure 2E).

FIGURE 3. Effect of minocycline on kidney MPO activity (A), MDA (B) and SOD (C) levels. MPO activity, MDA and SOD levels were measured subsequent to sham-operation (Shams) or renal I/R (Renal I/R) in the absence or presence of minocycline, administered 36 hours prior to renal I/R. Values are means $\pm$ SD. *, $p<0.01$, comparison with sham+vehicle group, '#', $p<0.01$, comparison with sham+minocycline group, '&', $p<0.05$, comparison with I/R+vehicle group.

Effects of minocycline on kidney MPO activity

Mice subjected to renal I/R exhibited a substantial increase in kidney MPO activity (1.38 ± 0.130 U/g vs. 3.84 ± 0.833 U/g) suggesting increased PMN infiltration into renal tissues (Figure 3A). Pretreatment of mice with minocycline prior to I/R produced a significant reduction of MPO activity on comparison with the activity obtained from I/R+vehicle mice kidneys (3.84 ± 0.833 U/g vs. 2.80 ± 0.772 U/g) (Figure 3A).

Effects of minocycline on kidney MDA and SOD levels

Mice subjected to renal I/R exhibited a substantial increase in kidney MDA (0.41 ± 0.07 nmol/mg vs. 0.88 ± 0.06 nmol/mg) and decrease in kidney SOD (303.00 ± 54.20 U/mg vs. 195.00 ± 49.90 U/mg) activity both suggesting increased lipid peroxidation and reduced oxidative stress (Figure 3B and C). The pre-treatment of mice with minocycline produced a significant reduction of the MDA (0.88 ± 0.06 nmol/mg vs. 0.62 ± 0.15 nmol/mg) and elevation of the SOD (195.00 ± 49.90 U/mg vs. 288.00 ± 58.30 U/mg) levels associated with I/R (Figure 3B and C). Regulation of both parameters by minocycline produced levels, which were not significantly different to those measured from the renal tissues of sham+vehicle mice (Figure 3B and C).

FIGURE 4. Effect of minocycline on mRNA expression levels of caspase-3 (A), caspase-8 (B) caspase-9 (C) and ICAM-1 (D) in kidney tissues of different groups. Values are means $\pm$ SD. *, $p < 0.01$, comparison with sham+vehicle group, #, $p < 0.01$, comparison with sham+minocycline group, &, $p < 0.05$, comparison with I/R+vehicle group.

FIGURE 6. Effect of minocycline on protein expression levels of caspase-3 (A), caspase-8 (B) caspase-9 (C) and ICAM-1 (D) in kidney tissues of different groups. Values are means $\pm$ SD. *, $p < 0.01$, comparison with sham+vehicle group, #, $p < 0.01$, comparison with sham+minocycline group, &, $p < 0.05$, comparison with I/R+vehicle group.

FIGURE 5. Western blots for caspase-3, caspase-8, caspase-9 and ICAM-1 expression in kidney tissues of different groups with anti-caspase-3, caspase-8, caspase-9 and ICAM-1 monoclonal antibodies. Lanes 1: the expression of four proteins in kidney tissues of sham+vehicle group; Lanes 2: the expression of four proteins in kidney tissues of sham+minocycline group; Lanes 3: the expression of four proteins in kidney tissues of I/R+vehicle group; Lanes 4: the expression of four proteins in kidney tissues of I/R + minocycline group.

Effect of minocycline on caspase-3, caspase-8 and caspase-9 expression during renal I/R

In comparison with the mRNA concentration of sham-operated mice, the relative expression levels of caspase-3 (Figure 4A), caspase-8 (Figure 4B) and caspase-9 (Figure 4C) mRNA in kidney tissues were significantly up-regulated at 24 h after reperfusion, respectively, while down-regulated by administration of minocycline prior to I/R. Similar to the results of fluorimeter-based quantitative RT-PCR assay, the Western blot analysis suggested that the expression levels of caspase-3 (Figures 5 and 6A), caspase-8 (Figures 5 and 6B) and caspase-9 (Figures 5 and 6C) were increased in kidney tissues of mice subjected to renal I/R but decreased in animals that were administered minocycline prior to ischemia.

Effect of minocycline on ICAM-1 expression during renal I/R

In comparison with the mRNA concentration of sham-operated mice, the relative expression levels of ICAM-1 (Figure 4D) mRNA in kidney tissues were significantly up-regulated at 24 hours after reperfusion, but down-regulated by administration of minocycline prior to I/R. Similar to the results of fluorimeter-based quantitative RT-PCR assay, the Western blott analysis noted that ICAM-1 (Figures 5 and 6D) protein expression was increased in kidney tissues of mice subjected to renal I/R but treated with minocycline prior to ischemia.

Discussion

Recent *in vivo* and *in vitro* studies have demonstrated that minocycline has antioxidant, anti-inflammatory and anti-apoptotic activities [19-20]. This study evaluates the effects of minocycline administration on renal dysfunction and inflammatory reaction, necrosis and apoptosis after experimental I/R injury in mice. The administration of minocycline prior to renal I/R was found to significantly reduce the renal dysfunction and injury caused by I/R in mouse kidney. This conclusion is supported by the following key findings: in a murine model of renal I/R, minocycline administration significantly reduced the I/R-mediated increases in: (i) serum levels of creatinine, (ii) blood urea nitrogen, and (iii) histological evidence of I/R-mediated renal injury. Additionally, minocycline reduced the expression of caspase-3, caspase-8, caspase-9, ICAM-1 in mRNA and protein levels, and MPO activity, indicating attenuation of apoptosis, inflammatory and PMN infiltration into kidney tissues and reduced MDA levels, indicating decreased lipid peroxidation. Moreover, minocycline reduced oxidative stress of the kidney by increasing SOD levels. Together, with these findings suggest that the antioxidant, anti-inflammatory and anti-apoptotic function of minocycline plays a key role in improving recovery of renal function.

Renal I/R is a main cause of acute kidney injury in both native and transplanted kidneys [4]. It results in a strong inflammatory response, believed to be initiated mainly by neutrophil infiltration. Inflammatory responses mediated by both innate and adaptive immune systems in postischemic kidneys play an important role in the pathogenesis of renal I/R injury. Inflammation, which is triggered by hypoxic cell injury starts in ischemia period, then accelerates with leukocytes recruitment, upregulation of cytokines and chemokines during reperfusion [21]. According to our data, MPO levels, a marker of neutrophil infiltration, were significantly greater in I/R-mediated renal injury mice in comparison with the sham-operated mice. Several pathways in parallel, including release of the pro-inflammatory cytokine TNF- α , can lead to neutrophil activation and infiltration. One of the early steps is neutrophil-endothelium interaction, which is facilitated by adhesion molecules such as ICAM-1 [22]. Increased adhesion molecules is recognized as an important component of renal I/R. It has been reported that the expression of ICAM-1 increases after I/R in kidney, blockade of ICAM-1 downstream signalling pathway protected against renal I/R injury in normal mice but not in neutrophil-depleted mice [23]. Infiltration of neutrophils contributed to renal injury by releasing reactive oxygen species and proteases. [24] These findings confirm the crucial role of neutrophil in I/R injury. There are also studies suggesting neutrophil depletion or blockade fails to provide protection from renal I/R injury [21]. In this study, quantitative analysis of mRNA and protein expression both indicated the increased ICAM-1 expression in kidney with I/R injury and decreased ICAM-1 expression by treatment of minocycline, suggesting minocycline administration may reduce the inflammation induced by I/R injury.

In addition to strong inflammatory response, renal tubular cell apoptosis is thought to be a primary and major contributor to the

pathophysiology of renal I/R injury, which is also a predictor of the outcome of renal damage independently of inflammation [25]. Apoptosis is increasingly recognized as a major form of cell death during I/R and believed to occur through a cascade of events involving the activation of specific caspases that cleave intracellular proteins and ultimately leads to the destruction of the DNA. Evidence also implicates caspases as an important mediator of cell death. In the present study, renal expression of caspase-3, caspase-8 and caspase-9 in mRNA and protein levels showed a dramatic decrease after minocycline treatment, indicating that inhibiting the expression of caspases may be one of the antiapoptotic mechanisms of minocycline. TUNEL staining of kidney sections may demonstrate tubular cell apoptosis more directly. Initial experiments demonstrated high background staining, a potential limitation of this study and the methods used.

It is generally accepted that reactive oxygen species, including free radicals, oxygen ions and peroxides, play a fundamental role in the pathogenesis of renal I/R injury [26]. It is also widely believed that antioxidants or free radical scavengers might prove useful for therapeutic intervention in alleviating I/R injury in various organs and tissues [27-30]. Minocycline administration could significantly not only enhanced SOD activity but also reduced MDA content in postischemic mice kidney--an action which appears to be one of the mechanisms of minocycline to ameliorate the renal I/R injury.

In conclusion, our data demonstrate that minocycline administration protects renal function and limits tubular damage after I/R through the suppression of apoptosis, inflammatory response and oxidative stress. These data suggest that minocycline should be considered for the prevention of acute renal failure occurring in major cardiovascular surgery and renal transplantation.

Acknowledgments

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