The effects of dexketoprofen on renal ischemia-reperfusion injury: an experimental study

Ersin Koksal a,∗, Yasemin Burcu Ustun a, Sezgin Bilgin a, Abdurrahman Aksoy b, Yavuz Kursad Das b, Murat Yarim c, Fatih Ozkan a, Cengiz Kaya a, Burhan Dost a

a Ondokuz Mayis University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Samsun, Turkey
b Ondokuz Mayis University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Samsun, Turkey
c Ondokuz Mayis University, Faculty of Veterinary Medicine, Department of Pathology, Samsun, Turkey

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Dexketoprofen; Ischemia-reperfusion; Kidney; Malondialdehyde; Reactive oxygen species

Abstract
Objective: Ischemia/reperfusion (I/R) may cause irreversible damage to tissues and organs. We evaluated the effects of dexketoprofen on a renal I/R model in rats.
Methods: The study included 30 male rats. Control group received 1 mL of saline. Dexketoprofen group received 1 mL (25 mg) of dexketoprofen intraperitoneally. After 60 minutes renal ischemia, 23 hours reperfusion was applied. In Sham group, laparotomy was performed with a medial line incision without any additional procedure. Changes in the plasma malondialdehyde (MDA), renal tissue MDA, plasma glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), BUN, creatinine and albumin levels, and histopathological changes were evaluated.
Results: CAT values were significantly lower in Control as compared with the Sham group. Plasma levels of MDA in the Control group were significantly higher than in the Dexketoprofen group. BUN and creatinine values were significantly higher in the Dexketoprofen group. The severity of tissue injury in the Dexketoprofen group was significantly higher than in Control and Sham groups
Conclusion: Although dexketoprofen reduces the I/R-induced systemic inflammation, it increases renal tissue damage.
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∗ Corresponding author.
E-mail: ersin.koksal@omu.edu.tr (E. Koksal).

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Introduction

Ischemia is a restriction or reduction of the blood flow into the tissues and reperfusion is the process of achieving renewed blood flow and reoxygenation. Renal ischemia and reperfusion (I/R) damage are among the pathological situations encountered in organ transplantation. The restriction of renal blood flow during ischemia leads to a reduction in oxygen and nutrient supply to the tissues and the pooling of harmful metabolites. As a consequence of reperfusion, reactive oxygen species (ROS) are produced in the tissues and an inflammatory response occurs. These responses may cause irreversible tissue damage via the degradation of the nucleic acids, lipids and proteins in the cells. The cells respond to this damage through various enzymatic (glutathione peroxidase – GPx, superoxide dismutase – SOD, catalase – CAT) and non-enzymatic (alpha-tocopherol, ascorbic acid, melatonin, carotene, transferrin and cerulo- loplasmin) pathways.4,5 These endogenous enzymes (GPx, SOD, CAT) activities increase to protect the tissue against the oxidative injury. The superoxide dismutase, which is synthesized in mamalian cells, is generally considered the first line of defence against ROS. It is a sulphur containing tripeptide (glutine, cysteine, glutamine) that reduces hydrogen peroxide (H₂O₂) to water (H₂O) by using glutathione as a substrate. Superoxide dismutase, which is an oxido-reductase that contains copper, zinc or manganese at the active site, catalyses the dismutation of superoxide (O₂⁻) to oxygen (O₂), and H₂O₂. Catalase is a heme-protein located in peroxisomes that converts H₂O₂ to H₂O and O₂. Malondialdehyde (MDA) is one of the markers for ROS and oxidative stress, and an increase in the MDA level indicates elevated levels of ROS and consequently organ damage.6–8

Dexketoprofen is a cyclooxygenase (COX) inhibitor that has recently come into use as a painkiller. However, its efficacy in reducing ischemia/reperfusion damage has not been confirmed in current studies. In our study, we aimed to examine the biochemical and histopathological effects of dexketoprofen on the degree of ischemia/reperfusion damage in the rat kidney model.

Methods

Animals and experimental design

The study animals were obtained from the Experimental Animal Research Centre of Ondokuz Mayis University in Samsun, Turkey. Thirty male Wistar Albino rats weighing from 200 to 250 g were included in the study. We did not set any inclusion and exclusion criteria before the recent study except animals’ unexpected death. All the subjects were cared for under appropriate conditions (temperature: 21 ± 1 °C, humidity: 40–70%, 12 hours of dark and 12 hours of light, and ad libitum feeding and access to water). The study protocol was approved by the Local Ethics Committee for Experimental Animals at Ondokuz Mayis University (Approval number: 2012/67).

The method of Altintas et al. was utilized to perform renal I/R.2 The rats were anesthetized with ketamine – xylazine (100–20 mg.kg⁻¹). Animals were block randomized by random numbers (Microsoft Office 365 Excel, Microsoft, Redmond, WA, http://www.microsoft.com) and allocated to 3 treatment groups, with 10 rats in each group, as follows:

- Group C (Control group): 1 mL of intraperitoneal saline was injected 40 minutes before the surgical procedure. Laparotomy was performed with a medial line incision. The right and left renal veins and arteries were clamped to induce 60 minutes of ischemia. At the end of the ischemia period, reperfusion was provided for 24 hours. After the reperfusion period the left kidney was removed for pathological evaluation and the right kidney was removed for biochemical analysis.

- Group D (Dexketoprofen group): A total of 1 mL (25 mg) of dexketoprofen was injected 40 minutes before the surgical procedure. The rest of the procedure was as per that for Group C.

- Group S (Sham group): Laparotomy was performed with a medial line incision, which was then closed without any additional procedures.

Three different investigators were involved as follows for each animal: a first investigator performed the anaesthetic and surgical procedure based on the randomization table. This investigator was the only person aware of the treatment group allocation. A second investigator was responsible for the biochemical analyses, and the third investigator performed the histopathological evaluation.

For postoperative pain management all rats received a subcutaneous injection of 0.1 mg.kg⁻¹ buprenorphine (Temgesic; Schering-Plough, Brussels, Belgium). After the procedures were completed, blood was drawn via a cardiac puncture. Rats have immediately been euthanized at the end of cardiac puncture by intraperitoneal sodium thiopental (40 mg.kg⁻¹ body weight) (Pental Sodium, Ibrahim Etem, Istanbul, Turkey). The plasma MDA (MDAp), renal tissue MDA (MDAr), plasma GPx, CAT, SOD, blood urea nitrogen (BUN), creatinine (Cr), albumin levels and histopathological changes were evaluated.

Sample preparation and analyses

The extraction method of plasma malondialdehyde (MDAp) The extraction method of plasma malondialdehyde was conducted according to the method described by Yoshioko et al.11

The extraction method of renal tissue malondialdehyde (MDAr) The renal tissue samples were prepared according to the method of Celik and Suzek.12 The tissues were homogenized for five minutes in 50 mL of cold KH₂PO₄ solution (1:5 w/v) by using a homogenizer (Dounce homogenizer Sigma-Aldrich Co. LLC, St. Louis, MO, USA). The homogenates were centrifuged at 3000 g for 20 minutes at 4 °C. To 0.5 mL of each sample; 2.5 mL of 20% TCA solution and 1 mL of 0.67% TBA solution were added. The MDA level was then determined by HPLC (High Performance Liquid Chromatography) method.

Instrumentation and chromatographic conditions

Renal tissue and plasma MDA levels were measured according to the method described by Agarwal and Chase.13 The HPLC system consisted of multichannel pumps (LC 20AT), an
auto sampler (SIL 20 ACHT) and fluorescence detector (FLD) (RF-10A XL) (Shimadzu, Kyoto, Japan). A reverse-phase C18 column (Inertsil® ODS-3V, 5 μm, 4.6 x 250 mm, GL Science, Tokyo, Japan) was used for the MDA determination. The mobile phases consisted of a 40:60 ratio (v/v) of methanol to 50 mM of potassium monobasic phosphate at pH 6.8, adjusted with potassium hydroxide. All the chemicals were purchased from Merck, Darmstadt, Germany. The flow rate of the mobile phases was 1 mL·min⁻¹, and the column oven temperature was set at 30 °C. The FLD was set at an excitation wavelength of 515 nm and an emission wavelength of 533 nm. The injection volume was 20 μL and the run time was 10 minutes per analysis.

Biochemical analyses

Glutathione peroxidase
The analysis of the GPx level was conducted with the GPx assay kit, according to the instructions provided by the manufacturer (Cayman Chemical Company, USA). The absorbance values were read at the wavelength of 340 nm.

Superoxide dismutase
The analysis of the SOD level was conducted with the GPx assay kit, according to the instructions provided by the manufacturer (Cayman Chemical Company, USA). The absorbance values were read at 440 nm.

Catalase
The analysis of the SOD level was conducted with the GPx assay kit, according to the instructions provided by the manufacturer (Cayman Chemical Company, USA). Absorbance values were read at a wavelength of 540 nm.

The blood samples were centrifuged (Shimadzu UV160A, S. No: 28006648, Japan) at 3000 g for 10 minutes and the serums were stored at -80 °C. The stored samples were thawed at room temperature on the day of analysis. All assays were conducted according to the manufacturer’s instructions.

The concentrations of BUN in the serums were analysed with by using commercially available kinetic UV assay kits (Roche Diagnostics GmbH, Ref.No.11729691, Mannheim, Germany).

The concentration of creatinine in the serum was analysed with Jaffe colorimetric assay kits (Roche Diagnostics GmbH, Ref.No.06407137, Mannheim, Germany). This kinetic colorimetric assay is based on the Jaffé method.

The concentration of albumin in the serum was analysed with a Roche Hitachi Cobas 8000 device and by using a commercially available colorimetric assay kit (Roche Diagnostics GmbH Ref. No.05166861 Mannheim, Germany).

After the kidney tissues had been fixed for 24 hours in a 10% formaldehyde solution, they were dehydrated in ethanol and embedded in paraffin wax. Sections of 5 μm thickness were cut and stained with haematoxylin and eosin for histopathological evaluation. The kidney sections obtained from rats were examined under a light microscope (Nikon, Eclipse E600). Kidney damage was graded semi-quantitatively on a scale of 0–4, with: 0 (zero), normal histology; 1, tubular dilatation; 2, degeneration only without necrosis; 3, tubular necrosis with adjacent structure intact; 4, when tubular necrosis with total loss of structure.

Sample size and statistical analysis
A power analysis was performed considering SOD values as a primary endpoint. The sample size was estimated in 10 rats per group in order to determine a mean difference of 2.11 ± 1.12 units/mL, with a 95% confidence interval and power of 95%.

Statistical analyses were performed with SPSS 21.0 for windows. Data were presented as mean ± standard deviation (SD), or as median and interquartile range (IQR). The Shapiro–Wilk test was used to analyze the normal distribution of the quantitative outcomes. One-Way ANOVA followed by Tamhane test were used to analyze parametric data. Kruskal–Wallis analysis of variance was used to compare non parametric data, and then Mann Whitney U test with Bonferroni correction was used for pairwise comparison. A p-value less than 0.05 was considered statistically significant.

Results

Glutathione peroxidase, superoxide dismutase, and catalase levels in the blood

There was no statistically significant difference between groups regarding SOD and GPx levels (p ≥ 0.05) (Table 1). The CAT values were significantly lower in Group C than in Group S (p = 0.018) (Table 1).

Malondialdehyde levels in kidney tissues

There was no statistically significant difference between groups regarding the tissues MDA levels (p ≥ 0.05) (Table 1). The HPLC chromatogram for MDA for the kidney tissue samples is shown in Figure 1.

Plasma malondialdehyde levels

The plasma MDA levels were significantly higher in Group C than in Group D (p = 0.001) (Table 1). The HPLC chromatogram for MDA for plasma samples is shown in Figure 2.

Blood urea nitrogen, creatinine, and albumin levels

The BUN and Cr values were significantly higher in Group D than in Groups C and S (p < 0.001). These values were significantly higher in Group C than in Group S (p < 0.001). In addition, there were no significant differences among the groups regarding their albumin levels (p ≥ 0.05) (Table 2).

Histopathological analysis

When the changes in the kidney tissues were histopathologically analysed under a light microscope in terms of necrosis, degeneration, tubular dilatation, protein cylinders and interstitial, lymphocyte infiltration, no pathological changes were observed in Group S. In Groups C and D which were subjected to I/R, degeneration, tubular dilatation, and
protein cylinders were detected; these pathological changes were more prominent in Group D than in Group C (Fig. 3).

Kidney damage scores were higher in the Group D than in Group C

Table 1  Results of CAT, SOD, GPx' and MDA analyses for Wistar albino rats.

|                          | Group D         | Group C         | Group S         | p    |
|--------------------------|-----------------|-----------------|-----------------|------|
| CAT (nmol.min⁻¹.mL⁻¹)    | 158.4 ± 75.9    | 106.3 ± 39.4ᵇ   | 176.1 ± 57.4ᵇ   | 0.037|
| Mean ± SD                |                 |                 |                 |      |
| SOD (units.mL⁻¹)         | 37.6 (32.3–44.4)| 31.5 (23.9–43.1)| 36.8 (29.5–63.3)| 0.179|
| Median (Min–Max)         |                 |                 |                 |      |
| GPX (nmol.min⁻¹.mL⁻¹)    | 30.5 (23.4–49.9)| 30.1 (24.4–42.7)| 32.6 (29.5–39.7)| 0.212|
| Median (Min–Max)         |                 |                 |                 |      |
| MDAt (µM.mg⁻¹)           | 0.08 (0.04–0.30)| 0.10 (0.07–0.14)| 0.09 (0.05–14)  | 0.067|
| Median (Min–Max)         |                 |                 |                 |      |
| MDAp (µM.mL⁻¹)           | 0.87 (0.72–1.1)ᵇ| 1.22 (0.95–1.67)| 0.94 (0.78–1.94)| 0.003|
| Median (Min–Max)         |                 |                 |                 |      |

CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDAt, renal tissue malondialdehyde; MDAp, plasma malondialdehyde.

ᵃ p < 0.05 versus Group S.
ᵇ p < 0.05 versus Group C.
Table 2  Results of BUN, Cr and albumin analyses for *Wistar albino* rats.

|                      | Group D          | Group C          | Group S          | p     |
|----------------------|------------------|------------------|------------------|-------|
| **BUN (mg.dL⁻¹)**    | 119.6 (36.4–146.6)ᵃᵇ | 29.7 (21.8–115.8)ᵃᶜ | 18.9 (14.9–26.3)ᵇᶜ | < 0.001 |
| **Cr (mg.dL⁻¹)**     | 1.98 (0.51–2.96)ᵃᵇ | 0.47 (0.28–1.89)ᵃᶜ | 0.28 (0.25–0.32)ᵇᶜ | < 0.001 |
| **Albumin (gr.dL⁻¹)**| 2.31 ± 0.58      | 2.42 ± 0.37      | 2.59 ± 0.65      | 0.527  |

BUN, blood urea nitrogen; Cr, creatinine.
ᵃ p < 0.05 versus Group S.
ᵇ p < 0.05 versus Group C.
ᶜ p < 0.05 versus Group D.

Figure 3  Appearance under light microscopy of kidney sections from the rats in Group C, S and D: (a) normal histological appearance in Group S, (b) mild tubular dilatation (arrowed) in Group C, (c) diffuse protein cylinders (arrowed) in tubule lumens in Group D, (d) diffuse necroses (arrowed) in tubule lumens in Group D (Haematoxylin&Eosin staining, a, b, d x240; c x120).

Discussion

This study has demonstrated that renal I/R caused an increase in plasma levels of MDA, BUN, and Cr and decrease in CAT plasma levels. However, plasma levels of GPx, SOD, albumin, and tissue MDA were not affected by renal I/R. The administration of dexketoprofen, in association with I/R, reduced plasma levels of MDA and increased BUN and Cr plasma levels. I/R caused major histopathological changes in the kidney tissues and dexketoprofen increased this damage.

The acute ischemia that occurs in situations such as kidney transplantation, trauma, surgery, and cardiopul-
monary bypass is among the major causes of acute kidney failure.\textsuperscript{15,16} Ischemia initiates cellular damage because there is insufficient blood flow to sustain the cell's normal metabolic activities. Reperfusion leads to a higher rate of formation of ROS and increases the amount of tissue damage by triggering inflammation.\textsuperscript{17} In the current study, we aimed to investigate the potential protective effects of dexketoprofen, which is an NSAID, against renal I/R damage.

Excessive production of ROS and oxidative stress play a major role in the pathophysiology of renal I/R damage because they negatively affect proteins, membrane lipids and nucleic acids in kidney tissues.\textsuperscript{2-4} However, this harmful process is counteracted by GPx, SOD and CAT enzymes that reduce the kidney damage induced by I/R.\textsuperscript{2} GPx, SOD and CAT, and that the administration of antioxidant and anti-inflammatory drugs provides relative protection of the levels of these enzymes by minimizing inflammation and reducing kidney damage.\textsuperscript{2,3,18,19} Altıntaş et al.\textsuperscript{1} showed in a renal I/R model that the SOD and CAT levels in rats that had been administered dexamethasone were higher than those in the control group. Furthermore, Tasdemir et al.\textsuperscript{2} demonstrated in a renal I/R model for rats that the immunomodulator, infliximab, increased both SOD and GSH levels. In addition, Süleyman et al.\textsuperscript{3} administered the selective COX-2 inhibitor, etoricoxib, in a rat I/R model. In comparison with the control group, etoricoxib better maintained tGSH, SOD and CAT levels and hence reduced renal damage via its antioxidant, anti-inflammatory and anti-apoptotic effects.

MDA is an important indicator of oxidative stress. The blood and tissue levels of MDA increase in response to lipid peroxidation caused by I/R damage. Several studies have shown an increase in MDA levels due to lipid peroxidation caused by renal ischemia, and that this increase can be reduced by the administration of anti-inflammatory and antioxidant agents.\textsuperscript{2,3,20,21} In our study, the plasma MDA levels increased in Group C but the administration of dextroprofen significantly decreased the plasma MDA levels in Group D by suppressing lipid peroxidation.

NSAIDs have been commonly used for a long period but their side effects are frequently encountered. NSAID-related renal dysfunction is dependent on dosage and exposure time. Even their short-term usage may disrupt renal function in sensitive individuals. This disruption in renal function is evidenced by increases in serum BUN and Cr levels.\textsuperscript{22-24} NSAIDs cause renal damage by inhibiting the synthesis of prostaglandin (PG).\textsuperscript{25} Altintaş et al.\textsuperscript{2} demonstrated that renal I/R increases both BUN and Cr levels. Bolat et al.\textsuperscript{21} demonstrated that diclofenac, which is an NSAID, increased BUN levels to a dose-dependent degree. Similarly, in the current study, BUN and Cr levels increased in response to I/R. Additionally, dexketoprofen caused higher BUN and Cr levels by increasing renal damage. In addition to I/R damage, the reduction of vasodilator PG synthesis due to the effects of dexketoprofen also increases renal damage by reducing glomerular capillary pressure.\textsuperscript{24}

NSAIDs inhibit COX-1 and COX-2 activity which affects the renal vascular tone.\textsuperscript{26} The extent of renal damage depends on the accumulation of the reactive metabolites that are formed in response to I/R. The usage of NSAIDs may increase this damage by causing vasospasm in renal vascular structures, because they reduce the activity of reactive metabolites through COX inhibition.\textsuperscript{24-26} In our study, I/R caused renal damage. The administration of dexketoprofen further increased the renal damage. This damage appears to have increased due to renal vascular spasms that occurred because of COX inhibition by dexketoprofen. The diffuse protein cylinders in the tubule lumens and diffuse necroses in the tubules of the kidney tissues in Group D also support this view.

There are a few limitations in this study. First, we could not test the immunohistochemistry of kidneys due to a lack of funds and equipment. Second, analyzing acute kidney injury biomarkers could have been beneficial.

Conclusions

In the present study, dexketoprofen reduced systemic inflammation related to a renal ischemia/reperfusion animal model, but it caused significant renal tissue damage. Therefore, we consider that its usage is not appropriate in the presence of renal hypoperfusion and ischemia. However, it may be useful to conduct further studies with different doses of dexketoprofen to better understand its role in the ischemia/reperfusion scenario.

Ethical approval

The experimental protocol of this study was reviewed and approved by the Animal Care and Use Committee of Ondokuz Mayis University, Samsun, Turkey (Ethical Committee Approval Number: 2012/67) and carried out according to the Animals in Research: Reporting In Vivo Experiments (ARRIVE) statement. Also this manuscript adheres to the Enhancing the Quality and Transparency of Health Research (EQUATOR) guidelines.

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Conflicts of interest

The authors declare no conflicts of interest.

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