ORIGINAL ARTICLES

The Benefit of the Reperfusion Procedure after Renal Ischaemia in Rabbits Being Initiated with Distilled Water

C Ozturk[1], MI Turan[2], IS Turan[3], E Sener[4], MA Gul[5]

ABSTRACT

Objective: *This was a biochemical and histopathological investigation of the effect of distilled water on oxidative injury induced with ischaemia/reperfusion in rabbit kidneys.*

Methods: *Rabbits were divided into four groups: renal ischaemia (RI), renal ischaemia-reperfusion (RIR), renal ischaemia induced and reperfusion initiated with distilled water (RIRW) and a sham operation (SO) group. With the exception of the SO group, ischaemia was induced with a clamp affixed to the renal arteries in all rabbits. Distilled water was applied to the renal arteries in the RIRW group after clamp removal. Reperfusion was established subsequently in the RIRW and RIR groups. Histopathological and biochemical examinations were performed on renal tissues extracted.*

Results: *Biochemical experiment results from the kidney tissues of SO, RI, RIR and RIRW groups revealed malondialdehyde (MDA) levels of 1.46 ± 0.12, 2.4 ± 0.1, 3.53 ± 0.13 and 1.76 ± 0.06 µmoL/g protein and glutathione (GSH) levels of 6 ± 0.57, 3.5 ± 0.42, 1.75 ± 0.3 and 5.83 ± 0.6 nmoL/g protein, both respectively. Histopathological experiment results revealed haemorrhage and slight swelling in renal tissue of the IR group and significant necrosis in tubular epithelial cells, hyaline cast deposition, interstitial inflammation and large numbers of apoptotic bodies in the RIR group. While mild necrosis was observed in the RIRW group, no apoptotic bodies, interstitial inflammation or hyaline cast deposition were encountered.*

Conclusions: *Our study indicates that the use of distilled water can be beneficial in protecting against injury caused in the kidneys by the ischaemia/reperfusion procedure in surgical interventions.*

Keywords: Injury, ischaemia-reperfusion, rabbit, water

Beneficio del Procedimiento de Reperfusión Después de la Isquemia Renal en Conejos Iniciada con Agua Destilada

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RESUMEN

Objetivo: *Se realizó una investigación bioquímica e histopatológica del efecto del agua destilada en la lesión oxidativa inducida con isquemia/reperfusión en riñones de conejos.*

Métodos: *Los conejos fueron divididos en cuatro grupos: isquemia renal (RI), isquemia-reperfusión renal (RIR), isquemia renal inducida y reperfusión iniciada con agua destilada (RIRW), y un grupo de operación simulada (SO). Con excepción del grupo SO, la isquemia fue inducida por pinzamiento de las arterias renales en todos los conejos. A las arterias renales en el grupo RIRW, se le aplicó agua destilada*

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después del retiro de la pinza. La reperfusión se estableció posteriormente en los grupos RIRW y RIR. Se realizaron exámenes histopatológicos y bioquímicos en los tejidos renales extraídos.
Resultados: *Los resultados del experimento bioquímico con los tejidos de riñón de los grupos SO, RI, RIR y RIRW revelaron niveles de malondialdehido (MDA) de 1.46 ± 0.12, 2.4 ± 0.1, 3.53 ± 0.13 y 1.76 ± 0.06 μmoL/g proteína, y niveles de glutatión (GSH) de 6 ± 0.57, 3.5 ± 0.42, 1.75 ± 0.3 y 5.83 ± 0.6 nmoL/g proteína, ambos respectivamente. Los resultados del experimento histopatológico revelaron hemorragia e hinchazón ligera en el tejido renal del grupo IR y necrosis significativa en las células epiteliales tubulares, deposición de cilindros hialinos, inflamación intersticial, y gran número de cuerpos apoptóticos en el grupo RIR. Si bien se observó necrosis leve en el grupo RIRW, no se encontraron cuerpos apoptóticos, ni inflamación intersticial, ni deposición de cilindros hialinos.*
Conclusiones: *Nuestro estudio indica que el uso de agua destilada puede ser beneficioso en la protección contra lesiones causadas en los riñones por el procedimiento de isquemia/reperfusión en las intervenciones quirúrgicas.*

Palabras claves: Lesión, isquemia-reperfusión, conejo, agua destilada

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INTRODUCTION

The re-establishment of blood flow to an ischaemic tissue is known as reperfusion (1). Ischaemia/reperfusion (I/R) in the kidney may develop following events such as shock, trauma and renal transplantation (2–4). Renal I/R is one of the main causes of acute kidney damage. It may also lead to significant morbidity and mortality in clinical practice. Histologically, ischemia in renal tissue leads to necrosis and apoptotic cell death, as well as cell dysfunction (5). Reperfusion following renal ischaemia can further increase ischaemia-related cell damage and renal dysfunction (6). It has been suggested that reactive oxygen products (ROS), purine metabolites and vasoactive agents may give rise to these negative outcomes resulting from reperfusion in kidney cells (7–9).

Several studies have investigated the relationship between oxidative stress and renal I/R injury, and antioxidant therapy has been shown to be beneficial (10–12). Reactive oxygen species form during electron transfer in oxidative phosphorylation (13). They contain superoxide, peroxide and hydroxyl radicals and reactive nitrogen species. Reactive oxygen species oxidize extracellular components, such as nucleotide, protein and polysaccharide. In this way, various products manufactured by ROS form during I/R, and these can be detected from peripheral venous blood (14).

Distilled water is a neutral (pH = 7), colourless, tasteless and odourless fluid, containing no organic or inorganic substances, that forms when the water vapour emitted during boiling is cooled and concentrated (15). Studies performed with water saturated with H_2 have reported a positive effect on oxidative damage induced in the kidney using a variety of techniques (16, 17). But, our scan of the literature revealed no reports regarding the effect of pure distilled water in I/R injury established in the kidney.

The purpose of this study was therefore to perform a biochemical and histopathological investigation of the effects of pure distilled water on oxidative damage induced with I/R in the rabbit kidney.

SUBJECTS AND METHODS

Animals

Twenty-four rabbits weighing 3.7–4.5 kg were used. These were obtained from the Atatürk University Medical Experimental Practice and Research Center. The animals were kept and fed for seven days at normal room temperature (22 ˚C) in the Pharmacology Department where the study would be performed in order to adapt to their environment. All animal procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the institutional Animal Care and Use Committee.

Chemical substances

Of the chemical substances used for the experiments, thiopental sodium was provided by IE Ulagay – Turkey.

General procedure

The rabbits used in the experiment were divided into four groups; unilateral renal ischaemia (RI), unilateral renal ischaemia/reperfusion (RIR), unilateral renal ischaemia reperfused with pure distilled water (RIRW) and a sham operation (SO) group. Surgical procedures were performed in a sterile and appropriate environment with 25 mg/kg intraperitoneal (ip) thiopental sodium.

Surgical procedures

Following thiopental sodium injection, animals were kept until the appropriate time for surgical intervention, regarded as when they remained in the supine position. In this period, the left kidney in all rabbits was accessed through a unilateral opening from the rear. Apart from the SO group, ischaemia was induced for three hours in all rabbits by clamping the renal arteries. One millilitre of distilled water was applied to the RIRW group renal artery, without removal of the clamps, for five minutes to the renal artery area between the kidney and

the clamp. Reperfusion was then performed for six hours in the RIRW and RIR groups. The RI group rabbits were sacrificed with high-dose anaesthesia immediately after an ischaemia, and the RIR and RIRW group animals using the same method six hours after an ischaemia. Extracted renal tissue was examined biochemically and histopathologically. The biochemical and histopathological data obtained from the RIRW group were analysed and compared with those from the RI, RIR and SO groups.

Biochemical procedures

Biochemical analysis of kidney tissues

Homogenates were prepared from kidney tissues in order to measure enzyme activities in the tissues. Total glutathione (tGSH) and MDA levels in the supernatants obtained from these homogenates were determined using appropriate techniques based on the literature.

Specimen preparation

At this stage of the study, 0.2 g was weighed from each kidney. Specimens were homogenized in ice-cold 1.15% potassium chloride solution for MDA assay and in phosphate buffer, pH = 7, made-up to 2 mL, for other measurements. The supernatant part was used as analysis specimen.

Chemical parameter assay

Malondialdehyde (MDA) assay: The absorbance of the pink complex formed by thiobarbituric acid (TBA) and malondialdehyde MDA at high temperature (95 ˚C) was measured spectrophotometrically at 532 nm (17).

*Total glutathione (GSH) assay***:** DTNB [5, 5'-dithiobis (2-nitrobenzoic acid)] is a disulphide chromogen and easily reduced by sulfhydryl group compounds. The resulting yellow colour was measured spectrophotometrically at 412 nm (19).

Creatinine and blood urea nitrogen (BUN) assay: Venous blood samples were collected into tubes without anticoagulant. Serum was separated by centrifugation after clotting and stored at -80 ˚C until assay. Creatinine and urea levels were determined with a Cobas 8000 (Roche) spectrophotometric system using the colorimetric method. Blood urea nitrogen levels were calculated with the formula BUN = Urea × 0.48.

Creatinine + picric acid → (alkaline solution pH) creatinine-picric acid complex

In alkaline solution, creatinine forms a yellow-orange complex with a picrate. The colour intensity is directly proportional to the creatinine concentration and can be measured photometrically (505 nm). Assays using rate-blanking minimize interference by bilirubin. Serum and plasma samples contain proteins which react non-specifically in the Jaffe method. Serum and plasma results must be corrected by 0.3 mg/dL (26 µmol/L) to obtain accurate values. This correction causes a measurement error of ≤ 1% in urine specimens because these do not contain non-specific proteins.

Urea is hydrolyzed by urease to form CO_2 and ammonia.

Urea + H_2O → (urease) 2 NH_4^+ + CO_2

The ammonia formed then reacts with α-ketoglutarate and NADH in the presence of GLDH to yield glutamate and NAD^+.

α-ketoglutarate + NH_4^+ + NADH→ (GLDH) L-glutamate + NAD^+ + H_2O

The decrease in absorbance due to consumption of NADH is measured kinetically. The NADH complex to decrement is determined photometrically (340 nm).

Histopathological analysis

Specimens were fixed in formalin solution with 10% neutral buffer. Sections 5 µm in thickness were obtained from paraffin blocks prepared after routine preparation. The sections were stained with haematoxylin-eosin (H&E) and periodic acid-schiff (PAS). Histopathological evaluation was carried out under a light microscope (Olympus BX 51, Tokyo, Japan) by a pathologist blind to which procedure had been performed on the animals.

In terms of ischaemic injury, we evaluated the cortex and the medulla, and particularly the outer medulla, the most sensitive region in the kidney. Histological kidney sections were evaluated for tubular necrosis, intratubular cast formation, interstitial inflammation, the presence of apoptotic bodies and loss of brush border.

Statistical analysis

All data were subjected to one-way analysis of variance using Statistical Package for Social Sciences 18.0 (Armonk, NY, USA) software. Differences among groups were analysed using the Tukey option, and significance was set at $p < 0.05$. Results are expressed as mean ± SEM.

RESULTS

Biochemical results

Malondialdehyde (MDA) and tGSH analysis results

Data for the mean MDA levels in the study groups are shown in (Fig. 1). There was no statistically significant difference between the RIRW and SO groups in terms of MDA levels ($p > 0.05$), but the difference between the other groups were significant ($p < 0.05$).

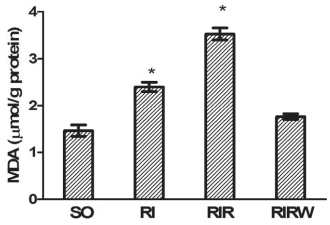

Fig. 1: Comparison of group in terms of malondialdehyde (MDA) levels.

Notes: MDA levels defined in µmol/g protein. Bars are means ± standard error mean. SO, sham operation; RI, renal ischaemia, RIR, renal ischaemia reperfusion; RIRW, induced renal ischaemia and reperfusion initiated with distilled water.

Data for the GSH levels obtained from the groups' kidney tissues are shown in (Fig. 2). There was no statistically significant difference between the RIRW group and the SO group in terms of GSH levels ($p > 0.05$), although a significant difference was determined between the other groups ($p < 0.05$).

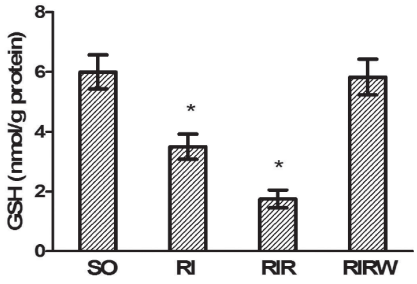

Fig. 2: Comparison of group in terms total glutathione (GSH) levels.

Notes: GSH levels defined in nmol/g protein. Bars are means ± standard error mean. SO, sham operation (SO); renal ischaemia (RI), renal ischaemia-reperfusion (RIR), induced renal ischaemia and reperfusion initiated with distilled water (RIRW).

Creatinine and blood urea nitrogen analysis results

Creatinine levels in the SO, RI, RIR and RIRW group blood specimens were 0.41 ± 0.01, 0.79 ± 0.03, 2.05 ± 0.12 and 0.57 ± 0.04 mg/dL, respectively, while BUN levels were 23.3 ± 1.6, 117.3 ± 4, 198.8 ± 5.5 and 27.7 ± 2.6 mg/dL, respectively. There was no statistically significant difference between the RIRW group and the SO group in terms of creatinine or BUN levels ($p > 0.05$), although a significant difference was determined between the other groups ($p < 0.05$).

Histopathological findings

Sham operation (SO) rabbit group kidney tissue

No pathological findings were encountered in the SO kidney tissue. Tubules had a brush border and microvillus structures had a natural appearance (Fig. 3).

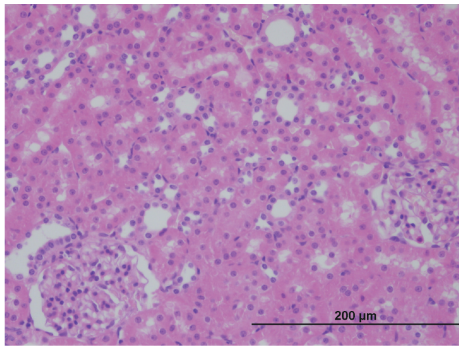

Fig. 3: Normal renal histology (cortex) in healthy animals (H&E).

Ischaemia-reperfusion (IR) group rabbit tissue

Histopathological examination of IR group rat kidneys revealed haemorrhage in the tubular lumens and interstitial areas and swelling in tubular epithelial cells, but no interstitial inflammation, tubular necrosis or apoptotic bodies. The brush border (microvillus) structure was preserved in the tubules (Fig. 4a).

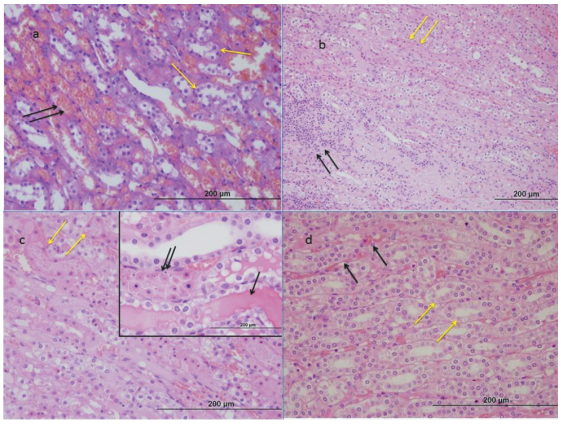

Fig. 4: a) Haemorrhage in the tubular lumens and interstitial haemorrhage (black arrows) swelling in tubular epithelial cells (yellow arrows) were observed in the kidneys of rats administered ischaemia only, but no interstitial inflammation, tubular necrosis or apoptotic bodies were encountered. (H&E) b) Pronounced necrosis (yellow arrows) in tubular epithelial cells, particularly in the outer part of the renal medulla, and inflammation (black arrows) in the interstitial area in rats exposed to ischaemia/reperfusion. (H&E) c) A large number of apoptotic bodies (yellow arrows) and hyaline cast deposition (black arrows) were observed at higher magnification in rabbits exposed to ischaemia/reperfusion. (H&E) d) Mild interstitial haemorrhage (black arrows) and mild swelling in tubular epithelial cells (yellow arrows) was seen in the kidneys of rabbits exposed to ischaemia/reperfusion and given distilled water. (H&E)

Renal ischaemia induced (RIR) group rabbit kidney tissue

As shown in (Fig. 4b), there was significant necrosis (yellow arrows) in tubular epithelial cells in RIR group kidney tissue, particularly in the outer medulla (outer stripe), and interstitial inflammation [black arrows] (H&E). Figure 4c, at higher magnification, shows numerous apoptotic bodies (yellow arrows) and hyaline cast deposition (black arrows) in dilated tubules (H&E).

Renal ischaemia induced and reperfusion initiated with distilled water (RIRW) group rabbit kidney tissue

Examination of the RIRW group kidneys revealed moderate interstitial haemorrhage (black arrows) and mild swelling (yellow arrows) in tubular epithelial cells (Fig. 4d). Necrosis was rarely seen. No apoptotic bodies, inflammation in the interstitium or hyaline cast deposition were observed (Figs. 4d−5). The brush borders in the renal proximal tubules was partially preserved in the RIRW group. Figure 5 shows mild swelling (arrows) in tubular epithelial cells. Necrosis was rare in tubular epithelial cells, and no apoptotic bodies, interstitial inflammation or hyaline cast deposition were observed (H&E).

DISCUSSION

This study was a biochemical and histopathological investigation of whether initiating reperfusion with distilled water in rabbits exposed to renal ischaemia would be useful in pre-

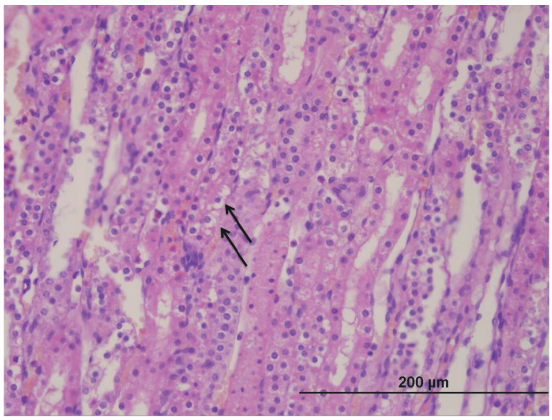

Fig. 5: Mild swelling (arrows) was seen in the tubular epithelial cells in rabbits exposed to ischaemia/reperfusion and given distilled water. Necrosis in tubular epithelial cells was rare, and no apoptotic bodies, interstitial inflammation or hyaline cast deposition were observed. (H&E)

venting renal I/R injury. Our biochemical and histopathological findings show that initiating reperfusion with distilled water significantly prevented oxidative damage in renal tissue compared to the RI and RIR groups.

Data for biochemical parameters such as MDA and GSH in RIRW group kidney tissue were similar to those from the SO group. The level of MDA, an oxidant parameter, in RIR group kidney tissue was higher than that in the RI group, while that of GSH was lower. This suggests that renal injury increases still further with a reperfusion. Tok *et al*, revealed biochemically and histopathologically that kidney damages arising from ischaemia intensifies with the reperfusion (10). This suggests that procedures performed during reperfusion can be useful in reducing the severity of the renal injury. Our scan of the literature showed that various models of I/R damage have been established and that various drugs and techniques have been used in order to prevent this (14, 18–20). Although there are several methods intended to prevent renal ischaemia damage, renal injury and the complications resulting from it are still major problems. Some studies have reported the use of distilled water enriched with H_2 in order to prevent I/R injury. Distilled water enriched with H_2 has been reported to reduce renal oxidative damage caused by nephrotoxicity induced with cisplatin, chronic kidney damage and chronic allograft nephropathy (16, 17, 21). No intervention using distilled water H_2 molecule was performed in our study.

Creatinine and BUN levels were significantly elevated in blood specimens of animal groups with high MDA and low GSH levels. It has been suggested that the rise in creatinine and BUN levels stems from permanent damage to the renal tubules (22). This information from the literature is compatible with our biochemical and histopathological study results. A significant necrotic injury was seen in the tubule epithelial cells in the RIR group, which had high MDA, creatinine and BUN levels.

Histopathological findings such as haemorrhage in the renal tubular lumen and interstitial area and swelling in tubular epithelial cells were encountered in the RI group. The O_2 pressure and ATP levels decrease in tubular epithelial cells in ischaemic tissue. Emptying of ATP stores leads to intracellular Na^+ deposition concentration, increased hyperosmolarity and swelling of the tubular epithelial cells (23). Antioxidant activity has been reported to be important in the prevention of severe necrosis seen in the tubular epithelial cells (24, 25). Severe necrosis in the renal outer medulla, loss of microvillus structures, inflammation, numerous apoptotic bodies and hyaline cast deposition in dilated tubules were observed in the RIR group. However, the initiation of reperfusion with distilled water prevented inflammation and the formation of apoptotic bodies in kidney tissue and reduced loss of microvillus structures and the severity of necrosis. The prevention of oxidative stress-related microvillus loss with antioxidant activity is known to suppress the development of tubular necrosis and apoptosis (26). Histopathological damage was more pronounced in kidney tissue with high MDA and low GSH in our study. Antioxidant therapy applied in ischaemic renal injury has been reported to be effective in reducing both the amount of oxidant parameters and histopathological damages (27). These data from the literature are compatible with our results and show that distilled water is a significant agent against oxidative stress.

Renal ischaemia leads to oxidative stress, and that stress is further intensified with reperfusion. Initiation of post-ischaemic reperfusion with distilled water significantly suppressed oxidative stress. Ischaemia-reperfusion injury being reduced with distilled water may be attributed to products of anaerobic metabolism that accumulate in kidney tissue during ischaemia being diluted with distilled water or else being washed out mechanically. Our study indicates that distilled water may be beneficial in preventing renal damage caused by the I/R procedure in the kidneys. Additionally, it shows that distilled water can be used after surgical procedures in addition to isotonic solution, 5% dextrose and other fluid therapies.

AUTHORS' NOTE

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