LABORATORY STUDY

Pretreatment with Pentoxifylline and N-Acetylcysteine in Liver Ischemia Reperfusion-Induced Renal Injury

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Abstract

Background and Aims: Acute hepatic injury causes systematic inflammatory responses which may finally lead to functional disturbances in remote organs. In this study, the effects of an inhibitor of inflammatory cytokines (pentoxifylline, PTX) and a well-known antioxidant, N-acetylcysteine (NAC), were evaluated on renal damage and oxidative stress following liver ischemia reperfusion (IR).

Method: Five groups of six male rats were used. Group 1 was sham operated. In group 2, 90 min liver partial ischemia was induced by a clamp around both hepatic artery and portal vein and then followed by 4 h of reperfusion. In groups 3 and 4, PTX or NAC was injected intraperitoneally before the ischemia, while in group 5 both drugs were co-administered. The levels of alanine amino-transferase (ALT), aspartate amino-transferase (AST), blood urea nitrogen (BUN), and creatinine in serum as well as malonyldialdehyde (MDA) and glutathione (GSH) levels and morphological changes in renal tissues were assessed.

Results: Significant increase in the serum levels of ALT and AST in IR group is indicative of liver functional damages. Elevated BUN and renal tissue MDA, decreased GSH levels, and morphological damages in IR group demonstrate a significant kidney injury and oxidative stress comparing to sham group. Administration of PTX alone and PTX + NAC prevented the IR-induced increase in renal MDA levels. Administration of both drugs and their co-administration prevented the reduction in renal GSH levels and morphological changes.

Conclusion: Pretreatment with PTX and NAC before liver IR may be useful to ameliorate renal oxidative damage by preservation of cellular GSH concentration and a reduction in MDA levels.

Keywords: ischemia reperfusion injury, liver, kidney, oxidative stress, pentoxifylline, N-acetylcysteine

INTRODUCTION

Hepatic ischemia reperfusion (IR) injury occurs in many occasions including liver transplantation, liver trauma, and hepatectomy. Hepatic IR is shown to frequently result in remote organ injury including damages to the kidneys, lung, and heart. In particular, acute kidney injury (AKI) after liver IR is common. The development of AKI after liver injury greatly increases mortality and morbidity in patients. Injury to remote organs has been attributed to oxidative stress mediators and other remotely released factors, including proinflammatory cytokines, tumor necrosis factor (TNF), and interleukin-1. However, the mechanisms underlying this response are poorly understood.

IR injury of the liver has two distinct phases that contribute to hepatocyte damage. The acute phase is characterized by Kupffer cell production of reactive oxygen species (ROS) which results in moderate hepatocyte injury. The later phase includes a cascade of inflammatory events resulting in neutrophil infiltration of the post-ischemic liver. Accumulated neutrophils cause substantial injury to hepatocytes through their release of oxidants and proteases. In addition, acute hepatic damage is caused by hepatic IR, which is known to lead to a systemic inflammatory response that results in impaired function of remote organs. Hepatic ischemia has been documented to cause inflammatory lung injury. In other studies, acute myocardial injury and renal injury was attributed to hepatic ischemia. Pentoxifylline (PTX), a non-specific phosphodiesterase...
inhibitor, has been shown to improve tissue oxygenation and endothelial function as well as inhibiting proinflammatory cytokine production. PTX also inhibits cell proliferation and extracellular matrix accumulation. Human studies have proved its antiproteinuric effect in patients with glomerular diseases. N-Acetylcysteine (NAC) is an antioxidant that acts by increasing intracellular glutathione (GSH) levels and also by the direct scavenging of ROS such as hypochlorous acid, hydrogen peroxide, superoxide, and hydroxyl radical. Thus, in the present study we evaluated the possible involvement of oxidative stress in the liver IR-induced renal injury in rats by examining the effects of PTX, NAC, and their combination.

MATERIALS AND METHODS

Animals
Male Sprague–Dawley rats (250–300 g) were housed in standard conditions (12 h light/day cycle with 20–22°C temperature and 40–50% humidity) and had free access to commercial chow and water. Animal care was in compliance with the guidelines of the Animal and Human Ethical Committee of Tehran Medical Sciences University.

Surgical Protocol
Five groups of rats (n = 6) were included in this study and randomly assigned into one of the experimental groups: sham-operated group (group 1); 90 min liver ischemia and 4 h reperfusion (group 2); in groups 3 and 4, PTX (40 mg/kg) or NAC (400 mg/kg) was injected intraperitoneally before the ischemia, while in group 5 both drugs were co-administered. The levels of alanine amino-transferase (ALT), aspartate amino-transferase (AST), blood urea nitrogen (BUN), and creatinine (Cr) in serum as well as malonyldialdehyde (MDA) and GSH levels in renal tissues were measured. Morphological changes in the kidneys were evaluated by light microscopy.

Rats were placed on a warming pad and were anesthetized by a combination of ketamine (75 mg/kg; Rotex Medica, Trittau, Germany) and xylazine (10 mg/kg; Alfasan, Woerden, The Netherlands). A tracheotomy was performed to facilitate free breathing. After tracheotomy, tail vein was cannulated (using Venflon 22GA, 0.98IN, ID 0.8 mm, Helsingborg, Sweden) and 0.9% normal saline was infused to maintain euvolement. Systolic blood pressure was measured by the tail-cuff method connected to a pneumatic transducer using a PowerLab/4sp data acquisition system (software Chart, version 5, AD Instruments, Castle Hill, Australia). Body temperature was maintained at 37 ± 1°C. A midline laparotomy was performed and liver artery and portal vein was carefully separated from the surrounding tissues. To induce partial ischemia, portal vein and liver artery perfusing left and median lobes of the liver were occluded by a non-traumatic microvascular clip (Beimer-Clip, Aesculap, Germany).

Sham-operated animals underwent identical surgical treatment, including isolation of the liver artery. However, artery occlusion was not performed. After completion of the surgery, rats were allowed to stabilize for 30 min.

Ninety minutes of liver ischemia was induced and rats were killed after 4 h of reperfusion. Blood samples were collected and centrifuged at 4000 × g for 10 min at 4°C, and serum was collected for chemical analysis. Kidney tissues were fixed in formalin (10% phosphate-buffered, pH = 7.4) for histological evaluations. Remainders of renal tissues were washed in cold phosphate-buffered saline and snap-frozen in liquid nitrogen. The samples were stored at −70°C until further studies.

Biochemical Assay
BUN and Cr were used as renal functional indices. Blood concentrations of AST and ALT were determined by commercially available kits to confirm IR-induced injury to the liver.

Measurement of Renal Oxidative Stress Markers
The tissue MDA level was determined by the method of Esterbauer and Cheeseman based on its reaction with thiobarbituric acid (TBA) at 90–100°C and the measurement of the absorbance at 532 nm. MDA reacts with TBA and produces a pink pigment which has a maximum absorption at 532 nm. The value of each sample was obtained from the standard curve and was expressed as nmol/g tissue. Renal GSH concentration was assessed as a measure of nonenzymatic antioxidant. Total GSH concentration of kidney tissues was measured according to the modified method of Tietze by Griffith which was based on the conversion of 5,5′-dithiobis-2-nitrobenzoic acid to 5-thio-2-nitro benzoate by nicotinamide adenine dinucleotide phosphate in the presence of GSH reducer. Formation of 5-thio-2-nitrobenzoate was measured by spectrophotometry at 412 nm.

Histological Analysis
After formalin fixation (10% phosphate-buffered) and dehydration, paraffin-embedded renal sections (4 μm) were stained by hematoxylin and eosin. Tubules were evaluated for the presence of degenerative changes (vacuolization), tubular dilatation, luminal debris, cast formation, and loss of brush borders from proximal tubules.

Statistical Analyses
Data are expressed as the mean ± SEM. Comparisons between groups were made by one-way analysis of variance followed by the post hoc Tukey test. The SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc., Chicago, IL, USA) was used for data analysis. p-Values <0.05 were considered significant.
RESULTS

Liver Functional Changes
Liver ischemia for 90 min followed by 4 h reperfusion resulted in significant increase in liver injury demonstrated by an increase in serum concentrations of ALT (9630 ± 196 vs. 309 ± 36.3 U/L, p < 0.05) and AST (6740 ± 690 vs. 410 ± 43.4 U/L, p < 0.05) compared to sham-operated rats.

Renal Functional Changes
Liver ischemia for 90 min followed by 4 h reperfusion resulted in a significant rise in BUN but no change in serum Cr concentrations compared to sham-operated rats (Figure 1). There were no significant changes in serum Cr and BUN in PTX or NAC alone and PTX + NAC combination groups compared to IR group (Figure 1). There were also no significant differences in serum Cr and BUN between PTX and NAC alone and PTX + NAC combination groups (Figure 1).

Renal Oxidative Status Changes
Liver ischemia for 90 min followed by 4 h reperfusion resulted in significant changes in renal oxidative stress markers including increase in renal MDA (Figure 2A) and decrease in renal GSH levels (Figure 2B) compared to sham-operated rats. Administration of PTX alone and PTX + NAC prevented the IR-induced increase in renal MDA levels (Figure 2A). Administration of both drugs and their co-administration prevented the reduction in renal GSH levels (Figure 2B). There were no significant differences in the above indices between PTX and NAC alone and PTX + NAC combination groups (Figure 2).

Histological Evaluations
As shown in Figure 3, there are no detectable changes using light microscopy in the kidney of sham group (Figure 3A). Cells are healthy, nuclei are normal, the brush borders are intact, and tubular lumens are open. In liver ischemia for 90 min followed by 4 h reperfusion (Figure 3B), there is significant alteration in the kidney histology, including formation of luminal debris, flattening of tubular cells, cellular vacuolization, irregularities in cytoplasm and nuclei of epithelial cells, and loss of brush borders. In NAC group, there was partial recovery in tubular cells compared to IR group (Figure 3D). Similarly, in PTX alone and PTX + NAC combination groups (Figure 3C and E), there were less structural differences.
damage compared to IR group. This was demonstrated by less cytoplasmic vacuolation and lower tubular debris in a way that most of the tubules are open.

DISCUSSION

The present study demonstrates that hepatic ischemia for 90 min followed by 4 h reperfusion results in partial impairment of renal function as well as morphological changes and oxidative stress of the renal tissues. Pretreatment with PTX, NAC, and their combination significantly reduced renal oxidative stress.

Hepatic IR is known to induce remote organ dysfunction in the heart and lung.

The incidence of renal dysfunction following liver transplantation is high and secondary renal dysfunction occurs commonly in chronic hepatic failure. Acute hepatic damage due to hepatic IR is also known to cause a systemic inflammatory response that results in the damage of remote organ systems.

The results of this study as well as similar findings of Behrends et al. demonstrate moderate changes in BUN concentrations but no change in serum Cr concentrations in this model of hepatic IR. We can only speculate that the kidney has a higher tolerance for systemic cytokines, resulting in only minimal effects of remote organ ischemia on the kidneys. Cytokines such as TNF-α are known to be the mediators of remote myocardial dysfunction and remote lung injury. In support of this hypothesis, the cytokine concentrations seen in septic shock, which are considerably higher than those seen after hepatic IR, rarely result in acute tubular necrosis. An explanation for this resistance against remote renal injury was presented in a study by Tanaka et al. which demonstrated that hepatic IR resulted in upregulation of renal heme oxygenase-1 (HO-1). The known cytoprotective effects of HO-1 might be responsible for the prevention of massive reduction in renal function.

ROS are critical mediators of the early phase of ischemic (IR) injury. Overproduction of these free radicals induces an increase in lipid peroxidation (MDA) by destroying unsaturated fatty acids in the cell membrane, causing a decrease in endogenous antioxidants such as GSH in renal tissues. In this study, administration of PTX and NAC to rats resulted in the preservation of GSH concentration. PTX administration also caused a significant reduction in MDA levels compared to IR.
group. Nitescu et al. showed that NAC improves kidney function and reduces renal interstitial inflammation in rats subjected to renal IR injury. These effects were associated with increased renal GSH levels and decreased plasma ascorbyl concentrations, suggesting that NAC is able to attenuate renal as well as systemic oxidative stress in this model.10

In the present study, GSH levels decreased during IR while administration of NAC preserved GSH levels. The contribution of antioxidants, such as NAC, in ameliorating the parenchymal lesions, inflammatory parameters, and functional variables in renal IR is still controversial. The protective effect of NAC was better observed at high concentrations and early period of times.20 In addition, beneficial effects of combination of NAC and a scavenger of peroxynitrite on renal ischemia/reperfusion injury were observed in a recent study by Kizilgun et al. in 2011.21

IR injury is a biphasic phenomenon. The first phase is ischemia that initiates the tissue injury by causing energy deprivation, which may ultimately lead to cellular dysfunction and death. Reperfusion, as the second phase, is more important but may exacerbate this damage by inflammatory reactions, production of oxygen free radicals, and release of endothelial factors.22 Previous studies have shown that NAC, PTX, and/or multi-drug administration prevented hepatic reperfusion injury.23,24

PTX is a hemorheologic agent used for patients with vascular disease and other conditions caused by defects in regional microcirculation.25 PTX prevents increases of microvascular permeability and formation of edema.26 In addition, PTX prevents ischemic vasoconstriction.27,28 NAC also promotes vasodilatation and improves blood flow in microcirculation.29,30

The lower morphological damage in PTX-treated kidneys suggests a reduction in oxidative stress in this group. PTX, a xanthine derivative, which is a well-known suppressor of TNF-α production from inflammatory cells, has also been shown to inhibit the growth of hepatic stellate cells and inhibit collagen synthesis in these cells.31 Recent reports suggest that PTX can enhance the chemotactic response of neutrophils and may inhibit phagocytosis and superoxide production by neutrophils and monocytes.32 PTX has been shown to improve tissue oxygenation and endothelial function and to inhibit proinflammatory cytokine production.8 In the present study, PTX improved tissue oxygenation by a reduction in oxidative stress but this was not sufficient for preservation of renal function. Although downregulation of proinflammatory cytokines seems to be important for the protection or attenuation of injury it is possible that mitochondria were already highly damaged by the ischemic process. Previous studies have shown a beneficial effect of 50 mg/kg PTX supplementation to correct the overproduction of oxidative stress and inflammatory indices in IR-induced spinal cord injury and fatty liver disease13,34 and a preventive effect for 400 mg/kg NAC was demonstrated in the rat organs against the oxidative damages.35,36

Results of this study also demonstrated that beneficial effects of PTX were more than NAC. This suggests the importance of inflammatory factors among other mediators in the induction of renal damage. Previous studies have demonstrated that glomerular filtration rate was significantly improved by PTX pretreatment in renal IR and this protective effect of PTX on functional changes was supported by morphological studies. The expression of TNF-α mRNA was increased after reperfusion, which was inhibited by PTX pretreatment. These results suggest that PTX may exert a protective effect against ischemic acute renal failure by inhibiting the production of TNF-α in rabbits.37

In conclusion, the significant increase in MDA levels, decrease in GSH, and destructive appearance in histology accompanied by partial impaired function in the kidney suggested that hepatic IR-induced renal injury may be mediated through oxidative reactions. In addition, PTX and NAC administrations protected kidney tissue against this oxidative damage caused by hepatic IR injury.

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

REFERENCES


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