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Hepatorenal protection in renal ischemia/reperfusion by celecoxib and pentoxifylline

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ABSTRACT

Background: Renal ischemia/reperfusion (I/R) is a major clinical problem. Its pathogenesis is multifactorial involving oxidative stress, cytokine overproduction, and inflammatory responses in the kidney and remote organs. This study was performed to evaluate the effects of celecoxib (CEB) and pentoxifylline (PTX) on kidney and liver changes after renal I/R in rats.
Materials and methods: Renal ischemia was induced by clamping renal pedicles for 1 h followed by reperfusion for another 1 h. The rats were assigned to five groups: sham control, untreated I/R, CEB + I/R, PTX + I/R, and (CEB + PTX)+I/R. Drug treatment was given for 7 d before I/R. Serum and tissue biochemical and histomorphologic changes were evaluated after reperfusion.
Results: Renal I/R caused changes in kidney and liver histology with a significant reduction in the function of both organs. An increase in tumor necrosis factor-alpha, myeloperoxidase, and malondialdehyde levels with a decrease in glutathione content and superoxide dismutase activity was observed in kidney and liver tissues. Pretreatment with CEB, PTX, or CEB + PTX attenuated all these changes and the extent of improvement was similar in all drug-treated groups.
Conclusions: This study is the first experimental work demonstrating the simultaneous nephroprotective and hepatoprotective effects of CEB and PTX after renal I/R. It seems likely that both drugs protect the kidney and liver by reducing oxidative stress, attenuating tumor necrosis factor-alpha production and inhibiting neutrophil tissue infiltration. No additive protective effects were observed in rats received the combined treatment. Thus, our results may imply a promising therapeutic approach by using CEB or PTX to protect the kidney and liver against the hazardous consequences of renal I/R.

Introduction

Renal ischemia/reperfusion (I/R) injury is frequently encountered in many clinical conditions such as kidney transplantation and cardiac bypass surgery.[1,2] It is a major cause of renal failure that leads to significant morbidity and mortality.[3,4] The kidney is very vulnerable to ischemic injury as the high rate of baseline oxygen use by renal cells,

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especially the metabolically active proximal tubule cells, renders the kidney incapable of increasing oxygen transport in response to hypoxia, thus leading to tubular cell injury.[5] After ischemia, reperfusion is undoubtedly essential for the survival of ischemic tissues as the reestablishment of blood flow in the ischemic region brings indispensable substances to tissue repair. Paradoxically, reperfusion may augment tissue injury in excess of that produced by ischemia alone.[6,7] In addition, injury to organs remote from the site of ischemia has been observed after reperfusion of ischemic tissues, which suggests that circulating humoral and/or cellular mediators originating from the ischemic tissues are responsible for mediating remote organ injuries.[8,9]

The mechanisms of renal I/R injury appear to be multifactorial and interdependent involving hypoxia, excessive reactive oxygen species (ROS) production with a resultant oxidative stress, cytokine overproduction, and inflammatory responses with eventual cell death.[10,11] Several experimental studies have focused on the role of ROS and oxidative stress in I/R-induced injury. The result of oxygen radical overproduction is an oxidative damage to tissue biomolecules including cellular lipids, proteins, and nucleic acids.[12] Also, I/R may initiate a damaging inflammatory response characterized by induction of proinflammatory cytokines and neutrophil infiltration. Tumor necrosis factor-alpha (TNF-α), a pleiotropic cytokine, is an initial mediator of the inflammatory reaction and is thought to play a pivotal role in I/R-induced injury not only to the ischemic organ but also to remote organs.[13] In addition, the presence of neutrophils in the ischemic region and their adhesion to vascular endothelial cells and infiltration into inflamed tissues after reperfusion contribute to the development of I/R-induced tissue damage.[14]

Furthermore, several observations have indicated that cycloooxygenase-2 (COX-2), an enzyme involved in inflammatory processes, plays a critical role in the progression and worsening of ischemic tissue injury.[15] It has been demonstrated that COX-2 expression is upregulated in the ischemic kidney[16] and arachidonic acid metabolites may be involved in I/R-induced tissue injury through stimulating neutrophil aggregation and recruitment, causing vasoconstriction and increasing microvascular permeability.[17]

Therefore, this study was designed to evaluate the effects of the selective COX-2 inhibitor celecoxib (CEB), the potent TNF-α production inhibitor pentoxifylline (PTX) and their combination against renal I/R-induced kidney and liver changes in rats using biochemical and histomorphologic parameters indicative of organ function, inflammation and oxidative stress.

Materials and methods

Animals

Adult male albino rats (200-250 g body weight) were used in this study. All rats were housed under conditions of controlled temperature and a 12-h lighting cycle and were given free access to standard rat chow and water. All experimental animal procedures were approved by the Animal Care Committee of the Medical Research Institute, Alexandria University.

Drug administration

CEB (Pfizer Pharma, Cairo, Egypt) and PTX (Sanofi Aventis Pharma, Cairo, Egypt) were dissolved in 2% (w/v) gum acacia solution and administered orally by gavage in a constant volume of 0.5 mL per 100 g body weight. At the beginning of each experiment, fresh drug solutions were prepared, and drug treatment was given once daily and continued for 7 d.

Experimental protocol

In this study, the rats were assigned to one of five groups of seven rats each:

- Sham-operated control group: control rats were treated daily with 0.5 mL gum acacia (2% solution) per 100 g body weight by oral gavage for 7 d. On the eighth day, the rats of this group were subjected to a sham operation, which was identical to the surgical procedure used for the I/R group but without clamping the renal pedicles.
- I/R group: the rats, in this group, were treated with 0.5 mL gum acacia (2% solution) per 100 g body weight daily by oral gavage for 7 d. Thereafter, the rats were subjected to renal I/R. Under pentobarbital sodium anesthesia (30 mg/kg, i.p.), each rat was placed under a heating lamp to maintain the body temperature, a midline incision was performed, and both kidneys were exposed. Renal ischemia was induced by clamping both renal pedicles using smooth vascular clamps for 1 h followed by reperfusion initiated with the removal of clamps and continued for another 1 h. Renal ischemia was confirmed by observing the paleness of the kidney, and reperfusion was confirmed by the restoration of color.

In the following three groups, drug treatment was continued for 7 d, and thereafter, all rats were anesthetized and operated on as in the I/R group:

- CEB + I/R group: the rats were treated with CEB at a dose of 10 mg/kg/day. This dose was previously shown to significantly reduce the elevated kidney COX-2 activity in rat models of I/R injury.[18,19]
- PTX + I/R group: the rats were treated with PTX at a dose of 200 mg/kg/day. This dose was previously shown to provide effective anti-inflammatory and antioxidant effects in several animal studies.[20,21]
- (CEB + PTX) + I/R group: the rats were treated with both CEB and PTX as in the previous two groups.

Blood samples were drawn from the abdominal aorta of each rat at the end of the reperfusion (1 h) period. Immediately after collection, blood samples were centrifuged at 4000 rpm for 15 min, and serum samples were separated and stored at −20°C until they were assayed. After blood collection, the two kidneys and liver of each rat were removed. Tissue specimens were taken and fixed in 10% phosphate-buffered formalin and