HEPATORENAL PROTECTION IN RENAL ISCHEMIA/ REPERFUSION BY CELECOXIB AND PENTOXIFYLLINE

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INTRODUCTION

- Renal ischemia/reperfusion (I/R) injury is a major cause of renal failure that leads to significant morbidity and mortality. The kidney is very vulnerable to ischemic injury as the high rate of baseline oxygen use by renal cells, 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. 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. 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.
- 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. The result of oxygen radical overproduction is an oxidative damage to tissue biomolecules including cellular lipids, proteins, and nucleic acids. Also, I/R may initiate a damaging inflammatory response characterized by induction of proinflammatory cytokines such as Tumor necrosis factor-alpha (TNF-α), and neutrophil infiltration. Tumor necrosis factor-alpha plays a pivotal role in I/R-induced injury not only to the ischemic organ but also to remote organs. The neutrophils infiltration and their adhesion to vascular endothelial cells in the ischemic region after reperfusion contribute to the development of I/R-induced tissue damage (Figure 1).
- Cyclooxygenase-2 (COX-2), an enzyme involved in inflammatory processes, plays a critical role in the progression and worsening of ischemic tissue injury. COX-2 expression is upregulated in the ischemic kidney 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.
- This study was designed to evaluate the effects of the selective COX-2 inhibitor celecoxib (CEB), the potent TNF-a 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. Further investigations are recommended to be performed to detect the effect of renal I/R on other remote organs including the brain. That may assist in discovering new applications in the neuropharmacology field.

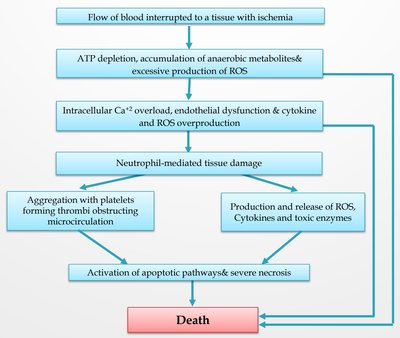

Figure (1): Summarized pathophysiologic cascade in ischemia/reperfusion-induced cell injury.

MATERIALS & METHODS

- The present study was designed to investigate the effects of celecoxib and pentoxifylline, given alone and in combination, on kidney damage induced by bilateral renal I/R. In addition, the effects of these two drugs on the changes induced by renal I/R in the liver, as a remote organ, were evaluated.
- Thirty five male albino rats weighing 200-250 g were included in this study and randomly assigned into the following five experimental groups (7 rats per group):

Group 1

- Control (sham-operated) group: The rats of this group received 1ml of gum acacia by oral gavage daily for 7 days. Thereafter, the rats of this group were anesthetized and operated but were not subjected to any renal ischemia/reperfusion (I/R).

Group 2

- (I/R group): The rats of this group received 1m of gum acacia (prepared as 2% solution) by oral gavage daily for 7 days. Thereafter, the rats in this group were exposed to renal ischemia for 1 hour followed by reperfusion for another 1 hour.
- The rats in this group were exposed to renal I/R. All rats of this group were anesthetized with an intraperitoneal (i.p) injection of sodium thiopental (30mg/kg). A midline incision was made and renal ischemia was induced by bilateral renal pedicle clamping for one hour with smooth vascular clamps followed by reperfusion, initiated with the removal of clamps and continued for another one hour. Occlusion was approved visually by color change of the kidney to a paler shade and reperfusion by blushing.

Group 3

- (CEB+I/R group): The rats of this group were treated with celecoxib (CEB) at a dose of 10 mg/kg given by oral gavage daily for 7 days. Thereafter, all rats of this group were anesthetized and operated as in I/R group.

Group 4

- (PTX+I/R group): The rats of this group were treated with pentoxifylline at a dose of 200 mg/kg by oral gavage daily for 7 days. Thereafter, all rats of this group were anesthetized and operated as in I/R group.

Group 5

- ((CEB+PTX) +I/R group)): The rats in this group were treated with the two drugs as in groups 3 and 4 for 7 days. Thereafter, all rats of this group were anesthetized and operated as in I/R group.

- At the end of experimental were drawn from the abdominal aorta of each rat at the end of the reperfusion (1 h) period. After centrifugation and serum separation, serum was used for the determination of:
  - Urea concentration and Creatinine concentration.
  - Alanine aminotransferase (ALT) activity and Aspartate aminotransferase (AST) activity.
  - Tumor necrosis factor-alpha (TNF-α) level.
- Tissue specimens were taken and fixed in 10% phosphate-buffered formalin and the rest of kidney and liver tissues were rinsed with ice-cold physiological saline, blotted dry and kept frozen at -80oC until analysis for the determination of:
  - TNF-α level.
  - Myeloperoxidase (MPO) level.
  - Reduced glutathione (GSH) level.
  - Superoxide dismutase (SOD) activity.
  - Malondialdehyde (MDA) level.
  - Total protein content.
  - Histological examination.

RESULTS

- The results of this study demonstrated that renal ischemia for 60 minutes followed by 60 minutes reperfusion caused local responses in the kidney and remote effects on the liver as reflected by detrimental changes in kidney and liver histology, function, inflammatory status and oxidant-antioxidant balance. Also, the present study showed that treatment of rats with CEB, PTX or simultaneously with both drugs before renal I/R induction provided protection to the kidney and liver against the consequences of renal I/R as evidenced by the following observations:

1. Drug pretreatment, in the present study, protected the functions of the kidney and liver as shown by the changes in serum levels of Creatinine and urea (as indices of kidney function) and serum activities of ALT and AST (as indices of liver function). These parameters, in drug-pretreated I/R groups, were very close to the corresponding values in the sham-operated control group. [Figure 2]
2. Drug pretreatment in I/R groups reversed the changes in kidney and liver tissue levels of TNF-α and MPO (as indices of inflammation and neutrophil tissue infiltration), and MDA and GSH levels and SOD activities (as indices of the oxidant and antioxidant status) as compared to the untreated I/R group. [Figure 3, 4, 5]
3. Drug pretreatment in I/R groups attenuated the I/R-related histopathological changes in both the kidney and liver. [Figure 6]

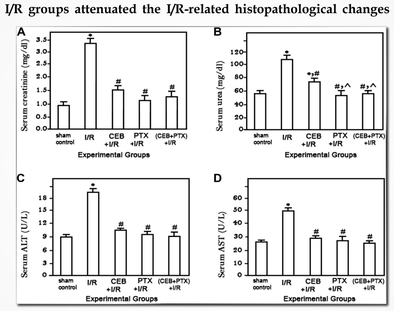

*Figure (2): Serum creatinine (A) and urea (B) levels, and ALT (C) and AST (D) activities in sham-operated control, I/R, CEB, PTX, and CEB D PTX pretreated I/R groups. Values shown are mean ± standard error of the mean (n [7 per group). *P < 0.05, compared with the sham-operated group. #P < 0.05, compared with the I/R group. ^P < 0.05, compared with the CEB D I/R group.*

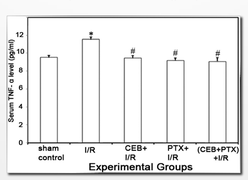

*Figure (3): Serum TNF-a levels in sham-operated control, I/R, CEB, PTX, and CEB D PTX pretreated I/R groups. Values shown are mean ± standard error of the mean (n [7 per group). *P < 0.05, compared with the sham-operated group. #P < 0.05, compared with the I/R group.*

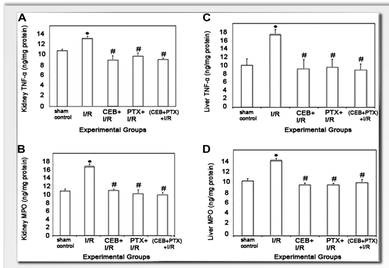

*Figure (4): TNF-a and MPO levels in kidney (A and B, respectively) and liver (C and D, respectively) of sham-operated control, I/ R, CEB, PTX, and CEB+PTX pretreated I/R groups. Values shown are mean ± standard error of the mean, n [5 (for TNF-α) or 7 (for MPO) per group. *P < 0.05, compared with the sham-operated group. #P <0.05, compared with the I/R group.*

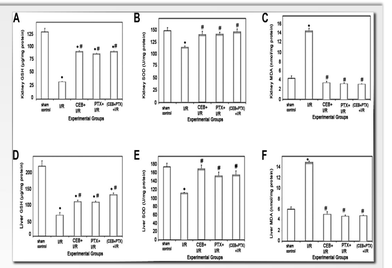

*Figure (5): GSH content, SOD activity and MDA level in kidney (A-C, respectively) and liver (D-F, respectively) of sham-operated control, I/R, CEB, PTX, and CEB D PTX pretreated I/R groups. Values shown are mean ± standard error of the mean (n[7 per group). *P < 0.05, compared with the sham-operated group. #P < 0.05, compared with the I/R group.*

Correlation coefficients (r values) between serum indices of kidney and liver function and TNF-a level and tissue

Parameters	Serum indices			
	Kidney function		Liver function	
	Creatinine	Urea	ALT	AST
Serum TNF-α	0.709*	0.659*	0.520[b]	0.616*
Kidney tissue				
GSH	−0.798*	−0.785*		
SOD	−0.633*	−0.573*		
MDA	0.852*	0.801*		
Liver tissue				
GSH			−0.597*	−0.535*
SOD			−0.622*	−0.594*
MDA			0.830*	0.875*

*P < 0.001; [b]P ~ 0.01.

** The results of all experimental groups were used in these correlations (n ¼ 30-35).*

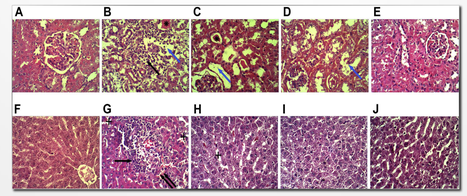

Figure (6): Photomicrographs of representative kidney (A-E) and liver (F-J) sections (hematoxylin and eosin, 403 magnifications). A and F: Sections from the sham-operated control group show normal kidney and liver morphology, respectively. B and G: Sections from the vehicle-treated I/R group show histomorphologic alterations in the kidney (leukocytic infiltration [a single black arrow], tubular dilatation [a single blue arrow], and cast formation [a star]) and liver (areas of necrotic hepatocytes [double arrows], leukocytic infiltration [a single black arrow], and dilated sinusoids [a plus sign]), respectively. C-E and H-J: Sections from CEB D I/R, PTX D I/R and (CEB D PTX)DI/R groups show marked reduction of the histologic changes in the kidney and liver.

CONCLUSIONS

- This study demonstrated clearly that pretreatment of rats with CEB or PTX significantly attenuated the renal I/R induced inflammation and oxidative stress locally in the kidney and remotely in the liver. Thus, our results may imply a promising therapeutic approach by using CEB or PTX to protect against the local and remote hazardous consequences of renal I/R that may be encountered in many clinical conditions such as kidney transplantation or suprarenal procedures of the aorta. Moreover the same model can be used to detect the effects of the renal ischemic injury on the brain function as a distant organ.

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