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Research Paper

Effects of Protoporphyrin on Renal Ischemia/Reperfusion Injury

Veterinary Science

KEYWORDS : Cholelithiasis, hypothyroidism, hyperlipidemia

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ABSTRACT

The present study investigated the effects of CoPP (potent HO-1) inducer on renal dysfunctions in renal ischemia/reperfusion injury. 27 male Sprague Dawley rats were randomized into 3 groups; sham group, ischemic group (left renal ischemia + right nephrectomy), CoPP group (left renal ischemia + right nephrectomy+ CoPP 20 mg/kg 30 min before renal ischemia). It was found that renal ischemia caused significant increase in serum creatinine, uric acid and urea and deteriorations of kidney morphology. Administration of CoPP caused significant improvement in all studied parameters and kidney morphology compared to ischemic group ($p< 0.05$). We concluded that CoPP has renoprotective effect against renal I/R injury.

Introduction

The ischaemia/reperfusion (I/R) injury is a common inflammatory process in living conditions such as shock, organ transplantation and vascular operations in which there a need for clamping of arteries. The renal ischaemia-reperfusion injury in clinical practice occurs as a consequence of both systemic hypoperfusion (shock) with subsequent circulatory resuscitation, and local renal hypoperfusion following aortic cross-clamping or renal transplantation [1].

The renal I/R injury is an important cause of early graft dysfunctions [2] may adversely affect the long-term survival of the allograft [3], so therapeutic strategies capable of ameliorating I/R injury may therefore improve the outcomes of renal transplantation. Renal I/R injury is a complex inflammatory phenomenon in which acute inflammatory response is elicited in kidney tissues and characterized by enhanced oxygen radical production and activation of neutrophils [4]. Reactive oxygen species (ROS), inflammatory cytokines such as TGF_{beta} and heme oxygenase (HO)-1 play important role in this complex inflammatory process. So, in the present study we examined the role of HO-1 inducer cobalt protoporphyrin on the outcome of renal I/R injury (kidney functions and morphology).

Materials and methods

Experimental animals

The material of this work included 27 male Sprague Dawley rats weighing 200-300 gm aging 4-6 months which were bred in the animal research facility in the Nile Center for Experimental Research (NCER) at Mansoura, Egypt.

Study design

Animals were randomly divided into 3 groups each subdivided into 3 subgroups each contain 3 rats **:**

Sham group (9 rats): rats were subjected to right nephrectomy, exposure of left renal pedicle with no ischemia,

Ischemia (c+ve) group (9 rats): rats were subjected to right nephrectomy and left renal ischemia for 45 minutes (definitive ischemia)

Cobalt- protoporphyrine "CoPP" treated group (9 rats): rats were injected intraperitoneally 30 minutes before ischemia by CoPP (20 mg/kg) (Sigma Chemical Company (St.Louis, MO, USA)).

Experimental rat model of renal ischemia

The rats obtained from the cage, kept in a metal container containing a piece of cotton soaked with 10 ml of halothane, (the rat left for 30 seconds) then the animals were maintained on sodium thiopental at a dose of 12 mg/100gm BW which was injected intraperitoneally. All steps of renal ischemia and sham operation were following **[5]**.

Collection of blood sample and measurement of serum creatinine, uric acid and urea

Blood samples were collected from ophthalmic plexus at 24 hrs, 48 hrs and 7 days after renal ischemia under light halothane anesthesia. Blood was centrifuged and serum was used for measurement of serum creatinine, uric acid and urea level. These markers were measured by Kits were purchased from Diamond Diagnostics, Egypt.

Harvesting kidney specimens and histopathological examination

By the end of experiment, the animals were anesthetized by large dose of sodium thiopental, then their necks were dislocated to ensure that they are dead. Then the abdomen was opened again and the kidney was removed rapidly and bisected into two equal halves by a scalpel, one half was rapidly placed in a container containing 10% neutral buffered formalin. The kidney specimen was processed for embedding in paraffin and sectioned in 4 µm thick slices and stained with H&E and examined by light microscopy in a blinded fashion for glomerular sclerosis, tubular dilatation, distal tubular cast, loss of proximal tubular brush border and vascular congestion.

Statistical analysis

One way ANOVA test with Tukey's posthoc test were used to study the statistical significance of the parameters among all groups using SPSS version 16. P-value < 0.05 was considered significant.

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Results

Effect of COPP on renal functions

Compared to sham group, ischemic group showed significant increase in serum creatinine, uric acid and urea at 24 hrs, 48 hrs after renal ischemia ($p< 0.05$). Also, these markers showed significant attenuation in CoPP group compared to ischemic group at 24 hrs and 48 hrs ($p< 0.05$).

Effect of COPP on histopathology

The kidney specimens obtained from rats of sham group showed normal kidney architecture i.e. normal morphology of renal tubules (PCT, DCT and CD) and normal glomerular structure at different time intervals (2a). On other hand, kidney specimens obtained from ischemic group showed severe renal damage in the form of sever necrosis and desquamation of renal tubular epithelium with formation of hyaline cast in the lumen of renal tubules, severe congestion and hemorrhage in interstitial tissue, cloudy swelling and cystic dilatation of renal tubules (2b). While, CoPP group, kidney specimens showed normal renal architecture with normal renal glomeruli and normal renal tubules with normal lining tubular epithelium (2c).

Discussion

The main findings of the present study can be summarized as follow a) renal I/R injury caused significant deterioration in kidney functions and morphology and d) pretreatment with CoPP caused significant improvement in all studied parameters compared to ischemic group at different time intervals.

In the present study, a well known model of renal warm ischaemia –reperfusion (ischaemia for 45 minutes*)* **[6]** was used because the 45 min ischaemia gives rise to a significant derangements in the measured parameters, while maintaining an acceptability of low mortality rate **[6].** The mortality and renal damage were marked in 60and 90min ischaemia models and the mortality is about 100%after the third day in 90min ischaemia model **[7]**.

The present study showed that, renal ischemia caused significant increase in serum creatinine, uric acid and urea on the early time points of follow up (24 hrs and 48 hrs) suggesting significant impairment of glomerular and tubular functions. Also, histopathological examination in the present study showed severe renal damage in the form of sever necrosis and desquamation of renal tubular epithelium with formation of hyaline cast in the lumen of renal tubules, severe congestion and hemorrhage in interstitial tissue, cloudy swelling and cystic dilatation of renal tubules was in line with the results of kidney functions. These findings are in line with the results of kidney functions. Also, these findings are in agreement with those reported by others **[5,8,9]**.

Also, the present study demonstrated significant improvement in markers of kidney functions (serum creatinine, urea and uric acid) in CoPP group compared to ischemic group. This improvement in kidney functions was associated with improvement of the morphology of kidney. These findings suggest renoprotective effects for CoPP against renal I/R injury and are in agreement with previous studies demonstrating renoprotective effects of CoPP. ***Lui et al.,*** [10] reported that induction of HO-1 with cobalt protoporphyrin reduces the degree of microalbuminuria and endothelial dysfunction in diet-induced obese rats. Moreover, ***Bédard et al.,*** [11] reported that peritransplant up-regulation of HO-1 by CoPP (at a dose of 0.5 mg/kg at days – 5, 0, +5) in renal allograft recipients rats significantly attenuates chronic rejection in rat renal allografts by inhibiting transplant vasculopathy. This was associated with upregulation of HO-1 and downregulation of endothelin -1 in kidney tissues. Also, ***Iwai et al.,*** [12] reported that pretreatment with cobalt protoporphyrin (CoPP, a potent HO-1 inducer; 15 or 50 mg/kg) subcutaneously on the day -6 and -1 before unilateral ureteral obstruction caused marked improvement in kidney function and morphology as well as marked inhibition of T cell infiltration and HO-1 upregulation in kidney tissues. Wagner et al., [13] reported that administration of CoPP induced both HO-1, preserved kidney graft function, and prevented postreperfusion apoptosis after cold preservation. The findings of the present study suggest renoprotective effect of CoPP against renal I/R injury.

Conclusion

We concluded that CoPP has a renoprotective effect against I/R injury in rat kidneys. Further studies are needed to clarify its underlying mechanisms.

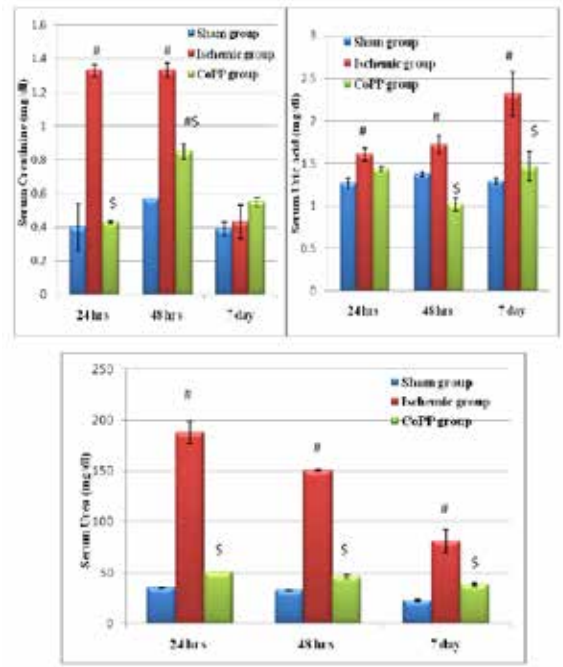

Fig (1): Markers of kidney functions (serum creatinine (a), serum uric acid (b) and serum urea (c)) in different groups at different time intervals. * significant vs sham group and $ significant vs ischemic group.

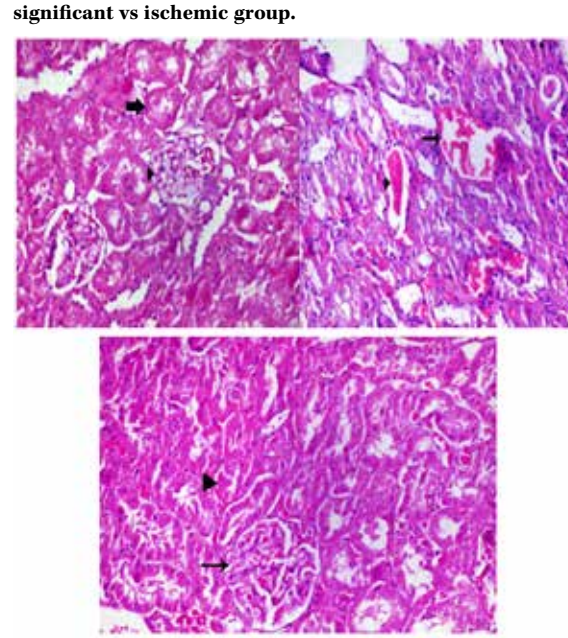

Fig. (2): Kidney specimen from sham group showing (a) normal renal glomeruli (arrow head) and normal renal tubules

(arrow), (b) necrosis and desquamation of renal tubular epithelium with formation of hyaline cast in the lumen of renal tubules (arrow head), in addition severe congestion and hemorrhage in interstitial tissue (arrow) and (c) normal renal glomeruli (arrow) and normal renal tubules with normal lining tubular epithelium (arrow head) at 7 days (H&E, 400x).

References

1. Jefaryl MK, Grace PA, and Mahte RT (2000): Attenuation of reperfusion injury by renal ischemic preconditiong: the role of nitric oxide. BJU international 85:1007-1013.
2. Shoskes DA, Halloran PF, etal., (1996): Delayed graft function in renal transplantation: etiology, management and long-term significance. J. Urol. 155:1837-1840.
3. Waaga AM, Rocha AM, Tilney NL (1997): Early risk factors contributing to the evolution of long –term allograft dysfunction. Transplant. Rev. 11:208-216.
4. Sehirli A, Sener G, Satiroglu H (2003): Protective effect of N-acetyl cysteine on renal ischaemia-reperfusion injury in the rat .J. Nephrol. 16:75-80.
5. Shokeir AA, Hussein AM, Awadalla A, Samy A, Abdelaziz A, Khater S, and Barakat N. 2012. Protection against renal ischaemia/reperfusion injury: A comparative experimental study of the effect of ischaemic preconditioning vs. post-conditioning. *Arab Journal of Urology*. 2012 10(4): 418-24.
6. Weight SC, Furness PN, Nicholson ML (1998): New model of renal warm ischaemia-reperfusion injury for comparative functional, morphological and pathophysiological studies. BJS 85:1669-1673.
7. Mehta A, Sekhon CP, Giri S, Orak JK, Singh AK (2002): Attenuation of ischaemia-reperfusion induced MAP kinases by N-acetyl cysteine, sodium nitroprusside and phosphoramidon. Mol. Cell Biochem. 240(1-2): 19-29.
8. Shokeir AA, Hussein AM, Barakat N, Abdelaziz A, Elgarba M, Awadalla A. 2014 Activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and Nrf-2-dependent genes by ischaemic pre-conditioning and post-conditioning: new adaptive endogenous protective responses against renal ischaemia/reperfusion injury. *Acta Physiol (Oxf)*. 210(2): 342-353.
9. Abdelaziz M. Hussein, , Ahmed Harraz, Amira Awadalla, Nashwa Barakat Shery Khater, Kamel Hemmaid, Ahmed A. Shokeir,. (2016). Remote limb ischemic preconditioning (rIPC) activates antioxidant and antiapoptotic genes and inhibit proinflammatory cytokine genes in renal ischemia/reperfusion injury. Gen. Physiol. Biophys. (2016), 35, 77–86
10. Liu X, Zang P, Han F, Hou N, Sun X. 2015. Renal protective effects of induction of haem oxygenase-1 combined with increased adiponectin on the glomerular vascular endothelial growth factor–nitric oxide axis in obese rats. Exp Physiol.;100(7):865-76. doi: 10.1113/EP08511
11. Bédard EL, Jiang J, Parry N, Wang H, Liu W, Garcia B, Kim P, Chakrabarti S, Buelow R, Zhong R. 2005. Peritransplant treatment with cobalt protoporphyrin attenuates chronic renal allograft rejection. Transpl Int. 2005 18(3):341-9.
12. Iwai T, Kitamoto K, Teramoto K, Machida Y, Tamada S, Yukimura T, Iwao H, Nakatani T, Miura K. 2008. Cobalt protoporphyrin attenuates rat obstructive nephropathy: role of cellular infiltration. Urology. 72(2):432-8
13. Wagner, M., Cadetg, P., Ruf,R., Mazzucchelli, L., Ferrari P., and Redaelli C.A. 2003. Heme oxygenase-1 attenuates ischemia/reperfusion-induced apoptosis and improves survival in rat renal allografts. *Kidney International* 63, 1564–1573