Functional protection of heme-oxygenase-1 enzyme in ischemic and toxic acute kidney injury*

Proteção funcional da enzima heme-oxigenase-1 na lesão renal aguda isquémica e tóxica

Protección funcional de la enzima heme-oxigenasa-1 en la lesión renal aguda isquémica y tóxica

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ABSTRACT

Objective: To investigate the functional protection of heme-oxygenase-1 enzyme (HO-1) when using its inducer (Hemin) and inhibitor (zinc protoporphyrin-ZnPP) in ischemic and toxic acute kidney injury by Polymixin B in mice. Materials: Adult male Wistar mice divided into 8 groups were used: SHAM (control), Ischemic (Isq), Isq+Hemin (Inducer of HO-1), Isq+ZnPP (inhibitor of HO-1), SALINA (control), Polimyxin B (PmbX), PmbX+Hemin, PmbX+ZnPP. Method: Analysis consists of Jaffé (creatinine clearance [CrCl]) and FOX-2 (urinary peroxides [Up]). Results: Thirty minutes renal ischemia and its treatment with PmbX reduced the CrCl and maintained urinary output. Urinary peroxide levels increased in both injuries. The administration of the inducer of HO-1 resulted in improvement in renal function and reduction in the levels of urinary peroxide. Conclusions: Findings indicated that ischemia and PmbX induce LAR (acute kidney injury [AKI]) by elevating the levels of urinary peroxide. The HO-1 inducer ameliorated the injury in both animal models through redox mechanism.

Keywords: kidney/injuries; Heme-oxygenase-1

RESUMO

Objetivos: Verificar a proteção funcional da heme-oxigenase (HO-1), por meio do uso do seu indutor (Hemin) e seu inibidor químico (protoporfirina de zinco-ZnPP) na lesão renal aguda isquémica e tóxica pela Polimixina B (PmbX) em ratos. Material: Foram utilizadas ratas Wistar, adultos e machos divididos em 8 grupos: SHAM (controle), Isquemia (Isq), Isq+Hemin (indutor de HO-1), Isq+ZnPP (inibidor de HO-1), SALINA (controle), Polimixina B (PmbX), PmbX+Hemin, PmbX+ZnPP. Métodos: Jaffé (clearance de creatinina, Clcr) e FOX-2 (peróxidos urinários). Resultados: A isquemia (30’) dos pedículos reais e a administração de PmbX reduziu o Clcr e manteram a output urinária. Os peróxidos urinários se elevaram em ambas as lesões. A administração do Indutor de HO-1 determinou melhora da função renal e redução dos níveis de peróxidos urinários. Conclusão: Os resultados deste estudo demonstraram que a isquemia e a PmbX induzem LRA oxidativa. O indutor de HO-1 atenuou a lesão em ambos os modelos por atenuação do mecanismo redox.

Descritores: Rim/lesões; Heme-oxigenase-1

RESUMEN

Objetivos: Verificar la protección funcional de la heme-oxigenasa (HO-1), por medio del uso de su induktor (Hemin) y su inhibidor químico (protoporfirina de zinc-ZnPP) en la lesión renal aguda isquémica y tóxica producida por la Polimixina B (PmbX) en ratos. Material: Fueron utilizadas ratas Wistar, adultos y machos divididos en 8 grupos: SHAM (control), Isquemia (Isq), Isq+Hemin (inductor de HO-1), Isq+ZnPP (inhibidor de HO-1), SALINA (control), Polimixina B (PmbX), PmbX+Hemin, PmbX+ZnPP. Métodos: Jaffé (clearance de creatinina, Clcr) y FOX-2 (peróxidos urinarios). Resultados: La isquemia (30’) de los pedículos reales y la administración de PmbX redujo el Clcr con manutención del fluxo urinario. Los peróxidos urinarios se elevaron en ambas lesiones. La administración del Inductor de HO-1 determinó mejora de la función renal y reducción de los niveles de peróxidos urinarios. Conclusión: Los resultados de este estudio demuestran que la isquemia y la PmbX inducen AKI por la elevación de los peróxidos urinarios. El induktor de HO-1 atenuó la lesión en ambos modelos por atenuación del mecanismo redox.

Descritores: Rínón/lesión; Hemo-oxigenase-1

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INTRODUCTION

Ischemia and nephrotoxicity are the most common causes of acute kidney injury (AKI). AKI is characterized by an increase in plasma levels of protein metabolism products (creatinine and urea), a reduction in glomerular filtration rate (creatinine clearance) and a reduction in urinary flow, and is associated with prolonged hospitalization and increased mortality(1-2).

Various mechanisms play a role in the physiopathology of AKI, such as vasoconstriction of the renal microvasculature involving reactive oxygen species, inflammatory response and activation of cellular protection mechanisms such as heme oxygenase-1 (HO-1)(1,3). The mechanisms underlying AKI are found in models of ischemia and models of toxicity at different levels, which lead to less severe patterns in clinical practice for injuries due to nephrotoxicity in situations in which other pathological conditions are absent. It should be pointed out that the observation of AKI alone in the absence of other morbid conditions is rare.

The present study focuses on polymyxin B (PmxB), a cationic antibiotic exerting a surfactant action on the bacterial cell membrane and therefore acting against Gram-negative microorganisms, because of its recent re-emergence in clinical practice. PmxB exerts a significant nephrotoxic effect which, although the drug is not commonly used, is relevant since PmxB might be the only therapeutic option.

The HO system breaks down the heme group, yielding biliverdin, ferritin and carbon monoxide. The subproducts have shown a cellular protective action: biliverdin converted into bilirubin by biliverdin reductase confers an antioxidant action, ferritin is the inactive form of iron and does not participate in redox imbalance, and carbon monoxide exerts a vasodilatating effect(4-5).

The hypothesis of this study is that HO may exert a protective effect on renal function in rats with AKI induced by PmxB toxicity or ischemia, and that this effect is due to interference with the oxidative mechanism described for this syndrome.

OBJECTIVE

The objective of the present study was to investigate the protective function of HO-1 in ischemic and toxic (PmxB) AKI in rats using an inducer (hemin) and a chemical inhibitor (zinc protoporphyrin, ZnPP) of this enzyme.

METHODS

All procedures were conducted in accordance with the Ethical Guidelines for Animal Experimentation adopted by the Brazilian College of Animal Experimentation and the study was approved by the Ethics Committee on Animal Experimentation of the Institute of Biological Sciences IV, University of São Paulo.

Adult male Wistar rats were divided according to the protocols of ischemia- or toxicity (PmxB)-induced AKI.

Ischemia protocol
1. Sham (control): rats submitted to surgical treatment without clamping of the renal pedicles.
2. Ischemia: rats submitted to clamping of the renal pedicles for 30 minutes.
3. Ischemia + hemin: rats submitted to renal ischemia and intraperitoneal (ip) administration of hemin (1 mg/kg) 24 hours before the surgical procedure.
4. Ischemia + ZnPP: rats submitted to renal ischemia and ip administration of ZnPP (50 mMol/kg) one hour before the surgical procedure.

Toxicity protocol
5. Saline (control): rats receiving 0.9% saline, ip, for 5 days.
6. PmxB: rats receiving PmxB (4 mg/kg/days), ip, for 5 days.
7. PmxB + hemin: rats receiving PmxB for 5 days and hemin (1 mg/kg), ip, once a day.
8. PmxB + ZnPP: rats receiving PmxB for 5 days and ZnPP (50 mMol/kg), ip, once a day.

The animals were kept in metabolic cages (individual cage to permit the measurement of biological variables) for 24 hours for the collection of urine, with free access to water and chow. After removal from the metabolic cage, the animals were anesthetized with 20-30 mg/kg sodium thiopental, ip, for puncture of the abdominal aorta and collection of plasma samples. The animal was sacrificed at the end of the experiment according to ethical guidelines on the handling of laboratory animals.

The urine and blood samples were used for the measurement of creatinine clearance (CCr/100 g) by the Jaffe method(6), which was adopted in this study as a marker of renal function. Oxidative injury was evaluated by the measurement of urinary peroxides using the FOX-2 method(7).

RESULTS

The results are shown in Table 1 Ischemia of the renal pedicles for 30 minutes and administration of PmxB for 5 days in rats confirmed the model of AKI, with a reduction in the glomerular filtration rate. Analysis of urinary peroxides confirmed the participation of a redox imbalance in ischemia and toxicity models of renal injury,
with the observation of an increase in urinary peroxide levels.

The administration of an HO-1 inducer conferred functional protection in the two models, with the observation of improvement of renal function and a reduction in urinary peroxide levels. On the other hand, the administration of an HO-1 inhibitor did not show significant differences between the ischemia and PmxB groups.

DISCUSSION

The etiology of AKI is multifactorial, with the most common causes being ischemic and nephrotoxic injury. The underlying physiopathological mechanism involves a complex cascade of events, in which the inflammatory response and redox imbalance play an important role\(^2,8\).

The present study confirmed the involvement of redox imbalance in ischemic and toxic (induced by PmxB) AKI, with the observation of increased urinary peroxide levels and reduced renal function using creatinine clearance as a marker, which was significantly reduced in these situations.

The administration of an HO-1 inducer (hemin) demonstrated the protective function of this enzyme in models of ischemia- and toxicity-induced AKI. The inducible isoform HO-1 has been widely reported to be an effective cytoprotective system in models of ischemia and toxicity\(^9,12\). HO-1 plays an important role in the maintenance of renal function and in the protection of tubular cells, especially in the segment of the nephron susceptible to ischemic or toxic injury, as demonstrated by its protective function in \textit{in vivo} and \textit{in vitro} experimental models\(^{21,15}\). The protective function of HO-1 is believed to be associated with the action of one or more of the subproducts resulting from heme degradation: bilirubin, ferritin and carbon monoxide\(^{16}\).

Curiously, administration of ZnPP, a highly specific and selective HO-1 and HO-1 inhibitor, did not result in alterations in renal function or in interference with the redox mechanism. In fact, studies using animal models of ischemia-reperfusion and administering high doses of ZnPP demonstrated significant improvement of renal function. This suggests that this inhibitor might be involved in other routes of cellular protection, irrespective of HO activity, or that inhibition of HO-1, if satisfactory, does not cause functional alterations detectable by creatinine clearance\(^{10}\).

In summary, the present study demonstrated the protective function of HO-1 in animal models of ischemia and nephrotoxicity, with improvement of renal function and a reduction in urinary peroxide levels after the administration of an HO-1 inducer.

CONCLUSION

The results of this study demonstrated that ischemia and PmxB induced renal injury characterized by an increase in urinary peroxide excretion. Administration of an HO-1 inducer attenuated renal injury in both models, whereas the administration of an inhibitor had no significant effect on renal injury. The findings confirm the participation and protective function of HO-1 in ischemic and nephrotoxic renal injury.

REFERENCES

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Table 1 – Overall renal function (CCr) and excretion of urinary peroxides (UP) in the groups submitted to ischemia- and toxicity (PmxB)-induced acute kidney injury (São Paulo, 2008)

<table>
<thead>
<tr>
<th>Ischemia groups</th>
<th>UP (nmol/mg creatinine)</th>
<th>CGr (ml/min)</th>
<th>Toxic groups</th>
<th>CGr (ml/min)</th>
<th>UP (nmol/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>5.6±0.4</td>
<td>0.60±0.03</td>
<td>Saline</td>
<td>0.70±0.01</td>
<td>5.05±0.10</td>
</tr>
<tr>
<td>Ischemia</td>
<td>13.6±0.6*</td>
<td>0.21±0.01*</td>
<td>PmxB</td>
<td>0.28±0.03*</td>
<td>35.32±0.60*</td>
</tr>
<tr>
<td>Ischemia+hemin</td>
<td>4.9±0.4**</td>
<td>0.46±0.02**</td>
<td>PmxB+hemin</td>
<td>0.50±0.09**</td>
<td>10.78±0.90**</td>
</tr>
<tr>
<td>Ischemia+ZnPP</td>
<td>11.9±0.5*</td>
<td>0.37±0.01**</td>
<td>PmxB+ZnPP</td>
<td>0.31±0.13**</td>
<td>29.65±0.04*</td>
</tr>
</tbody>
</table>

Results are reported as the mean ± standard error of the mean. *p < 0.05 versus sham and saline; **p < 0.05 versus ischemia and PmxB.