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The role of heme oxygenase 1 in rapamycin-induced renal dysfunction after ischemia and reperfusion injury

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Ischemia and reperfusion injury (IRI) is the main etiology of acute renal failure in native and transplanted kidneys. In the transplantation field, immunosuppressive drugs may play an additional role in acute graft dysfunction. Rapamycin may impair renal regeneration post IRI. Heme oxygenase 1 (HO-1) is a protective gene with anti-inflammatory and anti-apoptotic actions. We investigated whether HO-1 played a role in rapamycin-induced renal dysfunction in an established model of IRI. Rapamycin (3 mg/kg) was administered to mice before being subjected to 45 min of ischemia. Animals subjected to IRI presented with impaired renal function that peaked at 24 h (2.05 ± 0.23 mg/dl), decreasing thereafter. Treatment with rapamycin caused even more renal dysfunctions (2.30 ± 0.33 mg/dl), sustained up to 120 h after reperfusion (1.54 ± 0.4 mg/dl), when compared to the control (0.63 ± 0.09 mg/dl, $P < 0.05$). Rapamycin delayed tubular regeneration that was normally higher in the control group at day 5 (68.53 ± 2.30 vs 43.63 ± 3.11%, $P < 0.05$). HO-1 was markedly upregulated after IRI and its expression was even enhanced by rapamycin (1.32-fold). However, prior induction of HO-1 by cobalt protoporphyrin improved the renal dysfunction imposed by rapamycin, mostly at later time points. These results demonstrated that rapamycin used in ischemic-injured organs could also negatively affect post-transplantation recovery. Modulation of HO-1 expression may represent a feasible approach to limit rapamycin acute toxicity.

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The role of heme oxygenase 1 (HO-1) in rapamycin-induced renal dysfunction after ischemia and reperfusion injury (IRI) was studied. Long-term graft survival is mainly limited by chronic allograft nephropathy.[1] Its natural history tells us that the acute injury is a factor in the early fibrotic damage that ultimately leads to graft loss.[2] IRI is an inflammatory event that causes distinct degrees of cell dysfunction and death, being the most important antigen-independent negative factor associated with chronic allograft nephropathy.[3–8]

New data support the concept that innate and adaptive arms of the immune response participate in different extensions in the pathogenesis of IRI.[9–12] However, it is worthy to emphasize that renal transplantation recipients are exposed to additional detrimental factors besides interruption of blood flow. Donor antigen recognition and side effects of immunosuppressive drugs might elicit additional insults to a graft already dysfunctional.

Rapamycin is a macrocyclic lactone antibiotic with immunosuppressive properties able to suppress IL-2 receptor signaling via the mammalian target of rapamycins pathway.[13] Clinical data have demonstrated its role in preventing acute renal rejection, preservation of renal function in cyclosporine-induced chronic nephrotoxicity patients, and in reducing tumoral growth. However, some authors have reported side effects with its use such as proteinuria and renal function impairment, even in the native kidney.[14–19] Lieberthal *et al*.[19] have just demonstrated that rapamycin could be related to renal tubular regeneration impairment after acute injury through its ability to inhibit cell cycle and to induce apoptosis.

It is well documented that the functional and histological organ outcomes depend not only on the nature and intensity of the aggressor, but also on the ability of the tissue to respond in a protective way. Many molecules have intrinsic cytoprotective properties that include anti-apoptotic, anti-inflammatory, and anti-oxidant actions. HO-1 (EC 1.14.99.3) comprises three distinct genes, that is, HO-1 (hmox1,

P09601), HO-2 (hmox2, P30519) (reviewed in Camara and Soares[20]), and HO-3 (hmox3, rat; O70453) that probably does not encode a protein. HO-1 and HO-2 are the rate-limiting enzymes in the catabolism of heme, a reaction that yields equimolar amounts of biliverdin, Fe^{2+}, and carbon monoxide (CO). HO-2 is expressed in most cell types whereas HO-1 is not. However, expression of HO-1 is readily increased upon organ IRI becoming the rate-limiting factor in the generation of biliverdin, Fe^{2+} and CO.[21–23] In this sense, HO-1 acts as a protective protein expressed by endothelial cells that promote better graft viability.

In this study, we investigated whether HO-1 may also play a role in the additional renal function impairment imposed by the immunosuppressive drugs, especially rapamycin. In an established mouse model of acute renal failure, through vessel pedicle occlusion, we observed that rapamycin worsened the renal function and histology. However, upregulation of HO-1 was associated with the improvement of this dysfunction and normalization of tissue morphology.

RESULTS

Rapamycin worsens acute renal function

IRI is a relevant negative factor in early and late renal graft outcomes. Immunosuppressive drugs can also be a negative factor by retarding graft recovery and sustaining the extension phase of acute renal failure. We initially investigated the impact of rapamycin treatment in a renal IRI mouse model. The drug was administered 1 day before and immediately before the surgery. IRI induced a severe renal dysfunction assessed by serum creatinine levels that peaked at 24 h (2.05 ± 0.23 mg/dl), decreasing thereafter (48 h: 1.05 ± 0.09 mg/dl; 120 h: 0.63 ± 0.09 mg/dl) (Figure 1). Interestingly, rapamycin administration was associated with more accentuated impairment in renal function that was significantly higher at 48 (2.17 ± 0.6 mg/dl vs ischemic injured only animals $P < 0.05$) and 120 h (1.54 ± 0.4 mg/dl $P < 0.05$) than those observed in animals subjected only to IRI. Although, we observed that 24 h after reperfusion, serum creatinine was slightly higher (2.30 ± 0.33 mg/dl) than those observed in animals subjected only to IRI, the difference did not reach a statistical significance. It is noteworthy to say that the sham and animals treated only with the drug had similar serum creatinine levels compared with the normal animals (0.50 ± 0.08 mg/dl) (Figure 1a).

However, in clinical setting organ transplant recipients are under continuous use of immunosuppressive drugs. Then, we questioned whether a daily treatment of rapamycin could impair renal function else well. As seen in Figure 1b, the exposure of rapamycin daily was associated with worse renal function, compared to controls animals. Although the levels were not statistically different from those seen in animals just pre-treated with rapamycin (days −1 and 0), they were considerably higher than controls (24 h: 2.14 ± 0.28 vs 2.05 ± 0.23 mg/dl; 48 h; 2.69 ± 0.05 vs 1.05 ± 0.09 mg/dl; 120 h: 1.91 ± 0.11 vs 0.63 ± 0.09 mg/dl).

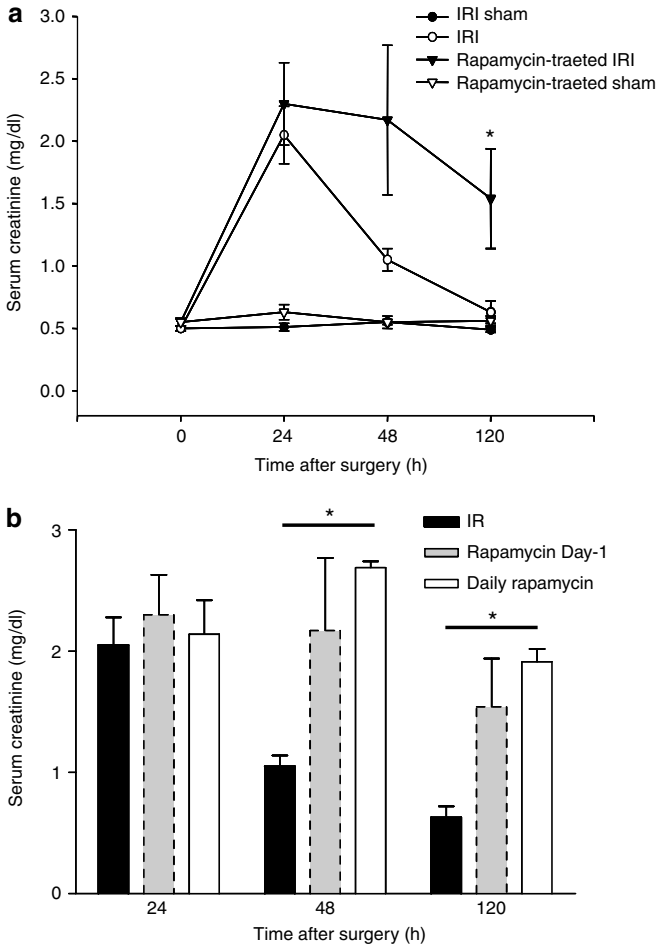

Figure 1 | Mean serum creatinine (s.d.) of animals subjected to IRI ($N = 9$) or not (sham, $N = 5$), pre-treated with rapamycin (days −1 and 0), daily treated ($N = 8$) or not ($N = 12$). (**a**) Ischemic and reperfusion injured animals pre-treated with rapamycin presented a sustained renal dysfunction, more prominent at 48 (*$P < 0.05$) and 120 h (*$P < 0.05$), compared to IRI animals. Sham and rapamycin-treated sham animals had similar levels of serum creatinine. (**b**) Ischemic and reperfusion injured animals daily treated with rapamycin presented a sustained renal dysfunction, more prominent at 48 (*$P < 0.05$) and 120 h (*$P < 0.05$), compared to IRI animals.

Morphometric analyses confirmed the functional data described above. Indeed, the pretreatment with rapamycin (days −1 and 0) was associated with higher levels of acute tubular necrosis (ATN) in those animals subjected to IRI, more important at day 1 after reperfusion (54.07 ± 2.56 vs 23.68 ± 1.95, respectively, $P < 0.05$). The regeneration percentages observed in renal tubules were prominent in IRI animals at 120 h after reperfusion, but severely decreased in those animals treated with rapamycin (68.53 ± 2.30 vs 43.63 ± 3.11, respectively, $P < 0.05$) (Figure 2).

Rapamycin induces early expression of HO-1 expression

HO-1 is a stress-induced enzyme related to cytoprotection through heme degradation end-products. We were interested in evaluating the expression of HO-1 after IRI by quantitative