The renoprotective effect of oral Tadalafil pretreatment on ischemia/reperfusion injury in rats[1]

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Abstract

Purpose: To evaluate the effect of tadalafil in renal ischemia/reperfusion (I/R) injury in rats.
Methods: Group I/R saline rats (n=6) were subjected to 45 minutes of left renal ischemia and treated with saline; the I/R tadalafil rats (n=6) received oral 10mg/kg tadalafil microemulsion one hour before ischemia. In both groups, 8 hours after ischemia, laboratory analysis were performed.
Results: Better tissue perfusion was lower in ischemic left/kidney than in right/kidney in saline group, suggesting reduced kidney clearance. Fluorescence in left/kidneys of tadalafil treated rats was lower than in right/kidneys (difference not significant). The fluorescence signal intensity in kidneys of tadafil treated rats was higher than in saline rats. TNF-α levels were significantly lower in I/R tadalafil group rats compared to I/R saline group (154±10.3 *vs* 391.3±12.3), as well as IL-1β (163.4±13.2 *vs* 279±11.5pg/dL), and IL-6 (122.9±8.1 *vs* 173.7±6.3 respectively; p=0.0001). Urea, creatinine and C-reactive protein were significantly lower in tadafil treated rats then in saline group.
Conclusion: Tadalafil therapy decreased the expression of circulating pro-inflammatory cytokines in a renal I/R rodent model, while improving kidney function proofs.
Key words: Ischemia. Reperfusion. Kidney. Tadalafil. Fluorescence. Cytokines. Rats.

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■ Introduction

Ischemia and reperfusion renal injury (I/R) is one of the main causes of acute renal failure, which may be accompanied by acute inflammation and secondary tissue injury[1]. I/R is frequently observed during and in the postoperative period of partial nephrectomies and in surgical repair of traumatic renal lesions[2]. Death of renal epithelial cells and delayed recovery of kidney function in the posttransplantation period can lead to interstitial fibrosis and may aggravate chronic kidney disease[3]. Renal I/R has been analyzed in experimental models using rodents[4,5], in which many of allopathic and phytotherapeutic drugs have already been examined. Some of them were effective and others ineffective[6,7].

Renal ischemia is unavoidable during transplantation, but its reperfusion is more damaging and causes injury to the renal tissue by several mechanisms, such as free radical release, apoptosis stimulation, inflammation, glomerular necrosis, leukocyte infiltration into the renal graft, Being able to arrive at the bankruptcy of multiple organs and systems[8,9]. At the same time, impairment of microcirculation following renal graft ischemia affects the function of the early transplanted organ[10]. In this regard, renal arterial flow should be evaluated whenever possible by Doppler or fluorescence examination[11].

Various drugs have been used in experimental studies, supposed to protect the organs from the effects of I/R[12-15]. Some the attention has turned to phosphodiesterase type-5 inhibitors such as sildenafil, which increase the concentration of cyclic guanosine 3,5-monophosphate (cGMP) resulting in release of nitric oxide, and subsequent arterial vasodilation[16]. Sildenafil has proved protective against endothelial dysfunction in the transplanted heart, and hemodynamic improvement in self-transplanted kidneys[17,18].

I/R has been studied in several experimental models, and some synthetic or plant extracts have been used, supposedly to protect the ischemic and reperfused organs[12-15]. Inhibitors of type 5 phosphodiesterase, such as sildenafil, which have well-characterized effects in the arterial flow, increase the production of cyclic guanosine 3,5-monophosphate (cGMP), consequently of nitric oxide, resulting in vasodilation[16]. Sildenafil has been shown to protect cardiovascular endothelium after heart transplantation, and has improved renal function in I/R model[17,18].

In a previous study we demonstrated that the pretreatment with sildenafil has a positive effect on I/R of kidneys of rats[19]. There have been few studies on the effects of tadalafil on kidney I/R[20-22]. The present study aims to examine the effects of tadalafil on the prevention of renal damage after normothermic renal ischemia/reperfusion in rats.

■ Methods

This protocol was approved by the institutional Ethics Committee on Animal Use (Protocol nº 028/2012).

Male Wistar (*Rattus norvegicus*) rats weighing 285±25g were used. All animals were distributed in individual cages with water and rodent feed (Presence®) *ad libitum* and acclimatized in the laboratory for 7 days. They were kept under temperature control (22°C), humidity (60-70%), 12/12 hours light/dark cycle, and handled in accordance with the precepts of ethics in animal experiments required by the Brazilian Law no. 11794/08. The rats were randomly allocated into 02 groups of 06 each and anesthetized with Xylazine (10mg/kg) and Ketamine (70mg/kg) intraperitoneally (i.p.). All surgical procedures were performed by experienced surgeon in experimental surgery using aseptic technique. The postoperative pain was prevented with meperidine (10mg/kg s.c.).

Study design

I/R saline group (n=6): one hour before renal ischemia, rats received 1 mL of saline 0.9% orally by gavage;

I/R tadalafil group (n=6): one hour before subjecting the rat to ischemia, 1 mL of 10 mg/kg tadalafil microemulsion was injected orally by gavage.

An abdominal median laparotomy was performed. The left renal vascular pedicle was occluded with a nontraumatic vascular clamp for 45 minutes, during which time the rats were kept warm using a thermal pad (Insight, Ribeirão Preto, SP, Brazil). The clamp was then removed, and the kidney was inspected for immediate color change, indicating successful reperfusion; the incision was then sutured. The body temperature was maintained normothermic during and after surgery using a thermal pad. After 8 hours of reperfusion, blood was collected for dosages.

Fluorescence imaging

Immediately after blood collection, rats were injected into the femoral vein with indocyanine green 10mg/Kg, (Ophthalmos, São Paulo, Brazil). Ten minutes after, rats were sacrificed using anesthetic overdose (thiopental 100 mg/Kg i.p.) and both kidneys were removed and imaged *ex-vivo*. The uninjured right kidney served as a control for each animal. Optical imaging was performed using a FX In Vivo fluorescence reflectance imaging device (Carestream Molecular Imaging). Adequate filters for excitation and emission were used. The kidneys were placed at the equipment chamber. An imaging protocol (exposure time 20 seconds, 2x2 binning, f-stop 2.8, field of view 120 mm, and focal plane 10 mm) was maintained for all images, and comparative images were taken comparing groups. The optical images of the ex-vivo study were evaluated qualitatively by assessing the presence or absence of visibly increased fluorescence in the kidneys. Fluorescence grayscale images were colored for depiction purposes according to a color scale set to the highest and lowest levels of fluorescence intensity (red and purple indicated maximum and minimum light intensity, respectively).

Measurement of plasma inflammatory cytokines and biochemistry

Blood samples were collected by cardiac puncture into separate ethylenediamine-tetraacetic acid (EDTA) tubes, stored immediately on ice and centrifuged at 3,000 rpm for 10 minutes. The plasma was separated and stored at -40ºC until analysis. Plasma expression of TNF-α, IL-1β and IL-6 were quantified using ELISA assay kits (PeproTec, USA). Plasma samples were used to measure urea, creatinine and C-reative protein using commercially available colorimetric assay kits. The biochemical analysis were performed using autoanalyzer (Weiner Lab BT Plus 3000) and spectrophotometer Konelab 60i, (kit da Weiner, São Paulo, Brazil). The rats were euthanased with thiopental overdose (100mg/Kg i.p.), associated with lidocaine.

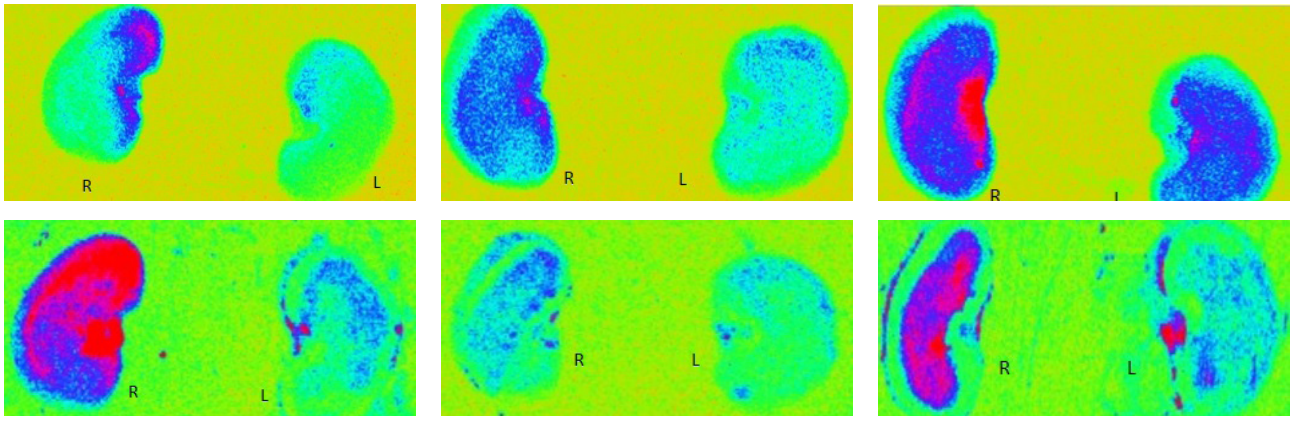
The statistical analysis were carried out using BioEstat 5.0 software. The Student t test was used to compare laboratory data. A value of $p<0.05$ indicated statistical significance.

■ Results

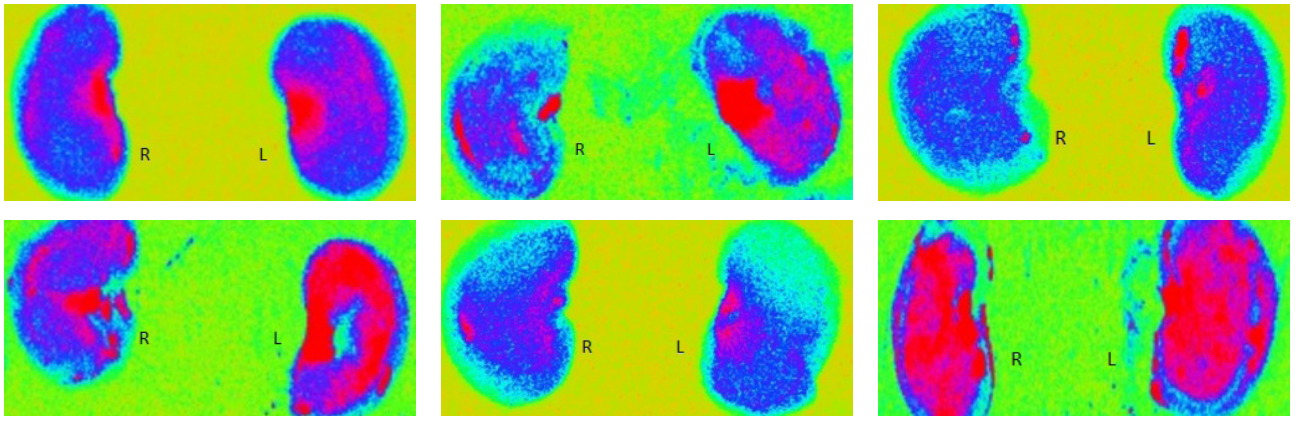
Fluorescence imaging of kidneys in rats using indocyanine green (ICG)

We examined *ex-vivo* kidneys fluorescence images of each group. Topographic representation of the *ex-vivo* kidneys images of I/R saline treated rats (I/R saline group) demonstrated lower ICG signal in the left ischemic kidneys in contrast to fluorescence

signal in the contralateral kidney (Figure 1A). As can be observed in Figure 1B, the I/R tadalafil treated group rats expressed renal ICG fluorescence signals higher than in saline I/R group rats. Comparing the fluorescence signals of right and left (ischemic) kidneys under the effect of tadalafil, no difference was observed (Figure 1B).



A - Ischemia/reperfusion kidneys of saline treated rats (I/R saline)



B - Ischemia/reperfusion of tadalafil treated rats (I/R tadafil)

Figure 1 - *Ex-vivo* contrast enhanced fluorescence imaging of kidneys dissected from rats subjected to unilateral left kidney I/R, and treated with saline **(A)** or tadalafil **(B)**. Fluorescence grayscale images were colored according to a color scale set to the highest and lowest levels of fluorescence intensity. Red and purple indicated maximum and minimum light intensity, respectively. **A**- I/R saline; **B**- I/R tadalafil (R, right; L, left).

Plasma inflammatory cytokines

Pro-inflammatory cytokines were decreased in rats treated with tadalafil, when compared to the saline-treated rats (Table 1). TNF-α was significantly reduced compared to saline-control (254±10.3 vs 391.3±12.3), as well as IL-1β (163.4±13.3 vs 279±11.5 pg/dL, respectively; p=0.0001). The cytokine IL-6 was decreased in the tadalafil (122.9±8.1 pg/dL) treated rats as compared to saline-treated (173.7±6.3), (p<0.001, n = 6/group; Table 1).

Table 1 - Data of comparative cytokines in renal I/R rats treated and untreated with tadalafil.

Parameters	Groups		
	I/R Saline	I/R tadalafil	p-value
TNF (pg/dL)	391.3±12.3	254±10.3	0.001
IL-1β (pg/dL)	279±11.5	163.4±13.3	0.001
IL-6 (pg/dL)	173.7±6.3	122.9±8.1	0.001

Values in mean±standard deviation.

Plasma urea, creatinine and C-reactive protein levels were measured at the end of experiment. The tadalafil treated rats showed a significant decrease in plasma urea, creatinine and C-reactive protein, compared to I/R saline rats (Table 2).

Table 2 - Data of comparative tests of renal function and C-reactive protein in renal I/R rats treated and untreated with tadalafil.

Parameters	Groups		
	I/R Saline	I/R tadalafil	p-value
Urea (mg/dL)	57.2±8.05	44.1±4.1	0.01
Creatinine (mg/dL)	0.62±0.08	0.47±0.07	0.001
C-reactive Protein (mg/dL)	6.3±0.12	4.02±0.19	0.001

Values in mean±standard deviation.

■ Discussion

Tadalafil is a vasoactive agent used to treat erectile dysfunction that has a different chemical structure when compared with sildenafil and vardenafil[23]. Tadalafil reaches maximum plasma concentration in 2 hours, and its plasma half-life is four-fold longer (17.5 hours) than those of sildenafil and vardenafil (4 hours)[16]. This prolonged effect is considered advantageous for lowering vascular resistance and treating ischemia. In view of this fact, we observed reperfusion for 8 hours, which is within the range from zero to 17 hours of tadalafil action.

The histopathological and antioxidant effects of tadalafil was studied on renal I/R damages, and tadalafil exerted a protective effect on tissues by increasing the antioxidant capacity 24. In other study the blood total antioxidant capacity levels decreased significantly in the I/R group, and tadalafil was administration 1 hour before I/R[16]. Following this method, we decide the inject tadalafil one hour before the I/R induction .

During ischemic periods, renal leukocyte infiltration is activated and increased[26]. Activated neutrophils can release cytokines[27]. In the present study, we showed that circulating levels of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 were significantly reduced after tadalafil treatment. There was a trend towards a reduction in pro-inflammatory cytokines in tadalafil treated rats

compared to I/R saline group. Besides an overall decreased inflammatory profile, we observed that tadalafil improved fasting plasma urea, creatinine and C-reactive protein levels, and our results correlate with previously published data and known physiological pathways[19,25].

According to the literature, renal ischemia duration of 45 to 75 minutes is most commonly chosen in experimental models, because this ischemia time allows for intermediate survival. We chose 45 minutes of renal ischemic time, based on a known survival rate of 100% at 7 days and 85% at 30 days in rats[28].

■ Conclusions

Tadalafil therapy ameliorates renal fluorescence, and decreases the expression of circulating pro-inflammatory cytokines in renal I/R rodent model, while improving renal function proofs levels. These results suggest that pretreatment with tadalafil may be a promising therapy for renal protection in kidney I/R. Further trials may lead to potential application of tadalafil in clinical practice.

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