

Tadalafil combined with remote ischemic preconditioning in the prevention of renal ischemia/reperfusion injury

Tadalafil combinado com pré-condicionamento isquêmico remoto na prevenção de lesão por isquemia/reperfusão renal

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ABSTRACT

PURPOSE: This study aimed to evaluate whether the combination of tadalafil treatment and remote ischemic preconditioning may provide protection against ischemia reperfusion injury (I/R) in kidneys of rats. **METHODS:** Twenty-four male Wistar rats weighing 280-300g were randomly divided into 3 groups: Group 1 - (control), only right nephrectomy was performed. No treatment with tadalafil. Group II - Left renal ischemia, right nephrectomy, without remote preconditioning, treatment with tadalafil (1.0 mg/kg, oral). Group III - Left renal ischemia, right nephrectomy, with remote preconditioning, treatment with tadalafil. **RESULTS:** 24 h after, urea and creatinine dosage and histopathology of the ischemic kidneys were performed. In the left kidney of control group rats, a higher degree of cellular necrosis was observed when compared to the tadalafil group, with and without remote preconditioning. On the preconditioning + tadalafil group rats, there was a significant reduction in histological parameters when compared to the Ischemia + tadalafil group rats ($p < 0.05$). In group III, comparing to other groups, a significant improvement of the left kidney function was observed, through the lower values of the urea and creatinine dosages.

CONCLUSION: This study demonstrated protective effects of remote preconditioning + tadalafil in an experimental model of renal I/R in the rat. The results demonstrated that a single dose of tadalafil prior to renal I/R attenuated the histopathological lesions, especially cellular necrosis, and renal function tests.

Keywords: Ischemia. Reperfusion. Kidney. Tadalafil. Ischemic Preconditioning.

RESUMO

OBJETIVO: Estudo com o objetivo de avaliar se a combinação de tratamento com tadalafil e pré-condicionamento isquêmico remoto pode fornecer proteção contra lesão por isquemia e reperfusão renal (I/R) em ratos. **MÉTODOS:** Vinte e quatro ratos Wistar machos pesando 280- 300g foram divididos aleatoriamente em 3 grupos: Grupo 1 - (controle), foi realizada apenas a nefrectomia direita. Sem tratamento com tadalafil. Grupo II - Isquemia renal esquerda, nefrectomia direita, sem pré-condicionamento remoto, tratamento com tadalafil (1,0 mg/kg, via oral). Grupo III - Isquemia renal esquerda, nefrectomia direita, com pré-condicionamento remoto, tratamento com tadalafil. Após 24 horas de observação foi feita dosagem de ureia e creatinina e histopatologia do rim isquemiado. **RESULTADOS:** Nos rins esquerdos dos animais do grupo controle foi observado um maior grau de necrose celular quando comparados com o grupo tratado com o tadalafil, com e sem condicionamento. Quando foi acrescentado o condicionamento + tadalafil houve significativa redução dos parâmetros histológicos comparando-se com o grupo de animais Isquemia + tadalafil ($p < 0,05$). No grupo III, comparando-se os demais grupos, observou-se significativa melhora da função do rim esquerdo, através dos valores mais baixos das dosagens de ureia e creatinina. **CONCLUSÃO:** Em conclusão, este estudo demonstrou efeitos protetores do pré-condicionamento remoto + tadalafil em modelo experimental de lesão renal por I/R no rato. Os resultados demonstraram que uma dose única de tadalafil antes da I/R renal atenuou as lesões histopatológicas, especialmente a necrose celular, e provas de função renal observadas após I/R.

Descritores: Isquemia. Reperusão. Rim. Tadalafil. Precondicionamento Isquêmico.

INTRODUCTION

Due to the high energy demand for the functions it performs, and an important microvascular network, the kidney is highly sensitive to ischemia/reperfusion injury (I/R), which is a major cause of acute renal failure, in renal artery stenosis, in

transplantation and renal surgery¹. In addition, renal I/R damage is associated with delayed graft function after transplantation, complicates shock, cardiac and aortic surgery and is a major cause of cardiovascular morbidity and mortality^{2,3}.

Although I/R renal injury is an important and common clinical problem, current strategies to reduce it are ineffective and new therapies are needed. I/R kidney damage is a common and important cause of acute kidney failure. It is inevitably associated with transplantation, involves both the adaptive immune response, and causes subsequent inflammation. The attraction and transmigration of cells from the immune system to the interstitium is associated with increased vascular permeability and loss of endothelial cells and integrity of the tubular epithelial cell^{3,4}.

The consequences of I/R injury are restriction of blood supply to an organ, followed by restoration of blood flow and re-oxygenation. The inevitable lesions can occur after infarction, sepsis and organ transplantation, and this phenomenon exacerbates tissue damage, initiating an inflammatory cascade^{4,5}. In the kidney, I/R injury contributes to the pathological condition called acute renal injury, which is a clinical syndrome with rapid renal dysfunction and high mortality rates⁶. The pathophysiology of renal I/R is complex, and some important pathological changes such as neutrophil activation, release of reactive oxygen species and other inflammatory mediators, including adhesion molecules and a variety of cytokines, are involved. Surveys have shown beneficial effects of different agents in the fight against I/R damage, for example, doxycycline by reducing the level of proinflammatory cytokines^{7,8}, leptin by reducing levels of tumor necrosis factor alpha (TNF- α) and increased levels of nitrites⁹, levosimendan as antioxidant and mechanisms related to nitrous oxide (ON)¹⁰, iloprost by suppression of lipid peroxidation¹¹ and ascorbic acid by elimination of free radicals (antioxidant)¹², and others.

Data on the types of cells involved typically vary¹³⁻¹⁵. It is well known that increased influx of neutrophils, T and B lymphocytes, as well as monocytes/macrophages, which contributes significantly to the pathogenesis of acute kidney injury^{16,17}.

In 1993, the phenomenon of remote ischemic preconditioning (RIPC) was first demonstrated in a canine model of myocardial infarction, where the preconditioning of

a vascular territory at a distance conferred protection to the vascular bed in the heart¹⁹. Other models has been described²⁰. In rats, hind limb ischemia by brief clamping of the abdominal aorta reduced oxidative stress after 45 minutes of renal ischemia in rats²¹. Remote ischemic limb preconditioning (which has been shown to be effective for the heart and skeletal muscle) has great clinical advantages because the limb is easy to manipulate and relatively resistant to injury by I/R. The underlying mechanisms of RIPC and its signaling pathways remain obscure¹⁹. Some neurogenic factors and the release of biochemical messengers have been implicated²²⁻²⁴.

Inhibitors of forfodiesterases (PDEs) are compounds that inhibit or antagonize the biosynthesis or actions of PDEs. They are widely used for the treatment of pulmonary arterial hypertension and erectile dysfunction in men²⁵. These vasodilatory and inotropic agents have protective effects on vascular structures and myocardial muscles²⁶⁻²⁸. The effects of these drugs have been studied especially in I/R injury. Tadalafil and sildenafil citrate have been widely used to treat erectile dysfunction and also several other diseases such as hypertension, prostatic hyperplasia and coronary heart disease in the clinic^{28,34}. On the other hand, in experimental studies, the protective effects of PDE5 inhibitors on I/R injury have been individually proven in several tissues, such as myocardium³¹, spinal cord³², brain²⁹ and kidney³³.

Based on these informations, we hypothesized that remote ischemic preconditioning associated with tadalafil may influence kidney ischemia and reperfusion in an experimental model in rats.

METHODS

Animals

The protocol was submitted for analysis and approved by the Institutional Ethics Committee on Animal Use (CEUA/HUOL) (Protocol 02/2018). All experiments in this study were performed according to the animal research guidelines of Brazilian Law 11,497/2008. Male Wistar rats were housed in individual polypropylene cages receiving food for rodents and water *ad libitum* at the Nucleus of Experimental Surgery-UFRN, Brazil.

Surgical Procedures

All surgical procedures were performed using aseptic surgical techniques. Anesthesia was performed with ketamine 80 mg/kg and xylazine 7 mg/kg intraperitoneal. Remote ischemic preconditioning (RIPC) for short periods of ischemia and reperfusion of the right hind limb was induced by application of tourniquet with pressure higher than arterial pressure around the proximal portion of the thigh of the animals in three cycles of 10 min/10 min.

After the last 10 min of the RIPC, the rats were subjected to laparotomy and the left renal artery was isolated and clamped for 30 minutes. After 30 min of renal ischemia the vascular microclamp was removed and renal reperfusion will be observed. The right kidney was nephrectomized. In the immediate postoperative period analgesia was performed with meperidine, 10 mg / kg body weight administered s.c. On the 1st postoperative day, euthanasia was performed with anesthetic overdose (thiopental 100 mg / kg associated with lidocaine, i.p).

Experimental design

Twenty-four male Wistar rats weighing 280-300g were randomly divided into 3 groups, each with six animals. Tadalafil (Eli Lilly origin from Brazil) was dissolved in saline solution and administered according to the groups as a single dose (1mg/kg) through gavage 60 min prior to surgical procedures.

Under anesthesia, an incision was made in the midline of the upper abdomen and the left renal pedicle vessels were isolated using minimal dissection.

Group I – Left renal ischemia and right nephrectomy were performed. No treatment with tadalafil.

Group II - Left renal ischemia, right nephrectomy, without remote preconditioning, tadalafil treatment

Group III - Left renal ischemia, right nephrectomy, with remote preconditioning, treatment with tadalafil.

Biochemical analysis

Whole blood (5 mL) was collected by cardiac puncture for dosages. Serum levels of urea and creatinine were measured. The blood samples were collected in dry tubes, centrifuged and the serum stored in a -20°C freezer until the tests were carried out. The parameters were measured using commercial Labtest kits, Belo Horizonte, MG, Brazil, according to the manufacturer's recommendations, using the Labmax240 equipment. The left kidney was removed for examination.

Histopathological evaluation

The histopathological evaluation was performed in the kidneys for the quantitative analysis of the histological pattern of tissue changes after ischemia, and were graded by scores, as shown in table 1. The score rank was applied for microscopic changes compatible with acute tubular necrosis: tubular light dilatation, tubular cell vacuolization, intratubular cylinders and tubular cell necrosis

Table 1 – Scores used for histopathological evaluation

SCORE	HISTOPATHOLOGICAL PATTERN
0	Normal
0.5	Small injured focal areas
1	<10% of the injured cortical area
2	10 to 25% of the damaged cortical area
3	25 to 75% of the damaged cortical area
4	> 75% of injury

Each kidney had correspondent scores determined in duplicate by the same observer, who was not aware of which groups the slides belonged to. The experiment was completely randomized.

Statistical treatment

Statistical analysis was done using the Statistical Package for Social Sciences, version 20.0 for Windows, (SPSS, IBM, USA). Histological damages between the groups

were compared by the Tukey and Mann-Whitmann tests. The p value <0.05 was considered statistically significant.

RESULTS

Histopathological data

In the left kidney of the ischemia group (24 hours post-ischemia), a higher degree of cellular necrosis (CN) was observed when compared to the Tadalafil group, with and without preconditioning. When preconditioning was added to tadalafil treatment, there was a significant reduction of histological scores when compared with the group of animals Ischemia + tadalafil. ($p < 0.05$). These data are summarized in table 2.

Table 2 – Descriptive data and respective statistical test of the histopathological changes in the left kidney. Right nephrectomy was done in all rats; comparative data between the analyzed groups.

Histomorphometric parameter	Left kidney		
	Ischemia	Ischemia + Tadalafil No preconditioning	Ischemia + tadalafil with preconditioning
TLD	1.51 ■ 0.09 ^a	2.55 ■ 0.07 ^a	1.9 ■ 0.04 ^a
CV	3.29 ■ 0.43 ^a	1.77 ■ 0.25 ^a	1.38 ■ 0.11 ^a
IC	3.82 ■ 0.33 ^a	2.18 ■ 0.38 ^a	1.25 ■ 0.09 ^a
CN	3.39 ■ 1.29 ^a	1.27 ■ 0.04 ^a	1.10 ■ 0.03 ^a

Average Standard Deviation*

Repeated equal letters mean significant difference between groups (significance level of 5%). TLD - Tubular Light Dilatation; CV - Cellular Vacuolization; IC - Intra-tubular cylinders; CN - Cell Necrosis.

Regarding cell vacuolization (CV) and intratubular cylinders (IC), there was a greater renal impairment in the ischemia group rats, with a statistically significant difference comparing with the other groups. A greater dilation of the tubular lumen in tadalafil treated animals was demonstrated when compared to the controls, characterizing a significant difference ($p < 0.05$).

Table 3 – Data described and respective statistical test of urea and creatinine dosages, comparing the groups.

Dosing parameter	GROUPS		
	Ischemia	Ischemia + Tadalafil No preconditioning	Ischemia + tadalafil With preconditioning
UREA	53.2 ± 7.3 ^b	49.18 ± 4.75 ^a	35.2 ± 4.31 ^{a,b}
CREATININE	3.04 ± 0.36 ^a	1.52 ± 0.25 ^a	1.14 ± 0.15 ^a

Mean Standard Deviation

*Repeated equal letters mean significant difference between groups (significance level of 5%).

In the renal ischemia + tadalafil + preconditioning group, a significant improvement of the left kidney function was observed, as shown by the urea and creatinine values (table 3).

DISCUSSION

Phosphodiesterases (PDE) represent a family of enzymes that regulate the cellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP)^{34,35}. PDE-1 is found in high concentration in vascular smooth muscle cells of the cavernous bodies of the penis, in the smooth muscle cells of peripheral arterial and venous vessels, as well as in the coronary and pulmonary circulation and in the platelets³⁴.

Remote ischemic preconditioning has been shown to protect the organs and tissues of IR injury. It involves complex signaling and interactions that activate ATP and adenosine-dependent potassium channels, release adenosine and bradykinin, generate neurohormones, nitric oxide and chemokines, which attenuate the opening of the transition pores of mitochondrial permeability³⁹. In this study, we showed that remote ischemic preconditioning protected the kidneys against acute injuries. Renal function and histological integrity of the kidneys were better preserved in preconditioning + treated with tadalafil rats compared to other treatments.

Increased vascular resistance is produced by loss of renal vascular endothelial capacity to release nitric oxide (NO), or an imbalance between NO and endothelin-1 production. At this point, PDE-1 inhibitors can increase the production of cGMP by removing PDE away⁴⁰. They increase NO release and improve endothelial dysfunction⁴¹.

In the literature, few studies have examined the effects of PDE inhibitors on renal I/R damage. Chintala et al⁴² found that a selective cGMP PDE-1 inhibitor, Zaprinast, had anti-platelet effects after I/R in rats. Lledo-Garcia et al⁴⁰ reported that sildenafil improved the outcome of ischemic kidneys in the immediate posttransplant period. Oruc et al³³ revealed that pre-ischemic treatment with sildenafil citrate can significantly attenuate I/R-induced renal injury, thereby decreasing leukocyte infiltration. Although the protective effects of Sildenafil and vardenafil on renal I/R injury have been examined and accepted in several studies, there are rare studies on the effect of tadalafil on renal I/R injury. Tadalafil is a class of mild vasoactive drugs developed for the treatment of erectile dysfunction²⁷. The half-lives of sildenafil and vardenafil are 4 h and that of tadalafil is 17.5 h⁴³. So, this molecule is a good option in the treatment of regulation of the decrease of vascular resistance and ischemia. According to Sesti et al²⁶, tadalafil served as a cardioprotector in the configuration of an experimental myocardial infarction.

The effect of tadalafil can be attributed to its effect as a protector in renal tubular cells and the inhibition of leukocyte infiltration in renal tissue⁴⁴. The damage of the renal tubular epithelium plays an active role in the initiation of the inflammatory response⁴⁵. During ischemic periods, renal leukocyte infiltration is activated and increased. Activated neutrophils can release cytokines, reactive oxygen species, proteases, myeloperoxidase, and other enzymes to cause further damage⁴⁶. In our laboratory we demonstrated that Sildenafil had a protective effect in rat kidneys subjected to normothermic I/R, demonstrated by scintigraphy and histomorphometry⁴⁷. Regarding to preconditioning in an other study we concluded in rodent model that morphologically, ischemic preconditioning with short times promoted greater intestinal protection against the IR lesion in rats⁴⁸. Continuing in this field of research, in the present study, the tubular morphology in the tadalafil pre-treated ischemia group and the pre-treated tadalafil + preconditioning I/R group was significantly better than in the only ischemic group. There was also statistical significance when the groups with and without preconditioning were compared.

CONCLUSION

This study demonstrated the protective effects of remote preconditioning + tadalafil in an experimental model of renal injury by I/R in the rat. The results of our study demonstrated that a single dose of tadalafil prior to renal I/R attenuated the histopathological lesions and enhanced the renal function after I/R. Cellular necrosis, which can be considered as an important issue in the development of renal injury, has also attenuated with remote preconditioning and administration of tadalafil.

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