Original Paper

Urologia Internationalis

Urol Int 2011;86:197–203
DOI: 10.1159/000321927

Received: July 20, 2010
Accepted after revision: September 26, 2010
Published online: December 16, 2010

The Beneficial Effects of Tadalafil on Renal Ischemia-Reperfusion Injury in Rats

Mehmet Guzeloglu[e] Fatih Yalcinkaya[b] Soner Atmaca[d] Alper Bagriyanik[d]
Suleyman Oktar[c] Oguz Yuksel[b] Iyad Fansa[a] Eyup Hazan[e]

Departments of [a]Cardiovascular Surgery, [b]Urology and [c]Pharmacology, Tayfur Ata Sokmen Medical Faculty, Mustafa Kemal University, Hatay, and Departments of [d]Histology and Embryology and [e]Cardiovascular Surgery, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

Key Words
Kidney · Ischemia-reperfusion injury · Leukocyte infiltration · Tadalafil · Rats

Abstract
Acute renal failure due to ischemia-reperfusion (I/R) injury is a common complication in cardiovascular surgery. We determined the influence of tadalafil on renal injury in a renal I/R model in rats. For this purpose, 21 male Wistar albino rats were separated into 3 groups: sham, placebo and tadalafil. A right nephrectomy was performed, and the left renal pedicles were occluded for 60 min and reperfused for 60 min in the placebo and tadalafil groups. A single dose of tadalafil (10 mg/kg) through an orogastric tube was administered to the tadalafil group. Tubular atrophy with acute inflammation in renal histology, total oxidant status (TOS) and total antioxidant status (TAS) were determined in tissue homogenates. Compared to the tadalafil group, tubular atrophy and acute inflammation was significant in the placebo group. TAS levels were significantly higher in the tadalafil group compared to the placebo ($p = 0.01$) and sham groups ($p = 0.04$). While TOS levels were significantly higher in the placebo group ($p = 0.03$), tadalafil did not significantly alter the TOS levels. The beneficial effects of tadalafil can be attributed to its protective effects on renal tubular cells and inhibition of leukocyte infiltration in renal tissue. We think that tadalafil treatment has an important role in reducing renal injury resulting from renal I/R.

Introduction

Acute renal failure due to ischemia-reperfusion (I/R) injury is one of the most severe complications which occurs after clamping the aorta above the renal artery level in conditions such as coarctation of the aorta, thoracoabdominal aortic aneurysm and aortic dissection [1, 2].

Renal ischemia results in cell damage and cell death via a decrease in cellular energy; an accumulation in intracellular sodium, calcium and reactive oxygen species; and activation in multiple enzyme systems [3]. Although reperfusion is vital to prevent tissue death, it causes local injury secondary to an acute inflammatory response that involves tissue infiltration by activated polymorphonu-

This study was performed at the Department of Cardiovascular Surgery, Dokuz Eylul University, Izmir.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0042–1138/11/0862–0197$38.00/0

Accessible online at:
www.karger.com/uin

Mehmet Guzeloglu
Department of Cardiovascular Surgery
Faculty of Medicine, Dokuz Eylul University
TR–35340 Izmir (Turkey)
Tel. +90 532 472 0220, E-Mail mehmetguzeloglu@hotmail.com

clear leukocytes and platelets [4]. Tissue damage is mediated by cytokines, a local imbalance in nitric oxide levels, endothelial-cell adhesion molecules, platelet-activating factors and free radicals [5].

Phosphodiesterases (PDEs) are intracellular enzymes that hydrolyze cyclic adenosine monophosphate and guanosine 3′,5′-cyclic phosphate (cGMP) into inactive metabolites (adenosine monophosphate and guanosine monophosphate, respectively). PDE5 effectively decreases the level of cGMP; therefore, inhibition of PDE5 may increase the level of intracellular cGMP [6]. PDE5 inhibitors, including sildenafil, vardenafil and tadalafil (one of the newest members of the group), are routinely used in the treatment of erectile dysfunction [7, 8].

In experimental studies performed with PDE5 inhibitors, it has been demonstrated that these substances improve endothelial function [9] and reduce the size of the ischemic area after myocardial infarction [10–12]. The protective effect of tadalafil against myocardial infarction in rats reported by Sesti et al. [10] has received considerable attention and is one of the latest contributions demonstrating the cardioprotective effects of PDE5 inhibitors, which are commonly used to treat erectile dysfunction in males. The study provides further support to the original hypothesis that PDE5 inhibitors have anti-infarct effects against I/R injury [13].

The minimum operation duration of thoracoabdominal vascular surgery that requires the clamping of the aorta above the renal arteries is 4–5 h. As the drug has to be administered orally during the preoperative period, tadalafil, which has the longest half-life, is the best alternative among the PDE5 inhibitors for use in clinical practice to protect the kidney against renal I/R injury.

In light of this information, we aimed to investigate the effects of tadalafil, one of the newest members of PDE5 inhibitors, on histopathological changes and oxidative status in rats with renal injuries using an experimental renal I/R model.

Materials and Methods

All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by the Dokuz Eylul University Animal Ethics Committee.

Experimental Groups

Twenty-one male Wistar albino rats, weighing 250–300 g, were randomly divided into 3 groups, each consisting of 7 animals. After the rats were anesthetized with 75 mg/kg of ketamine and 8 mg/kg of xylazine intraperitoneally, an upper abdominal midline incision was made and renal blood vessels were isolated bilaterally with minimal dissection. A right nephrectomy was performed in group I (sham group). In group II [I/R group (placebo group)] and group III (tadalafil group), the rats underwent a right nephrectomy, followed by occlusion of the left renal pedicle for 60 min and reperfusion for 60 min. Additionally, the rats in group III were administered tadalafil dissolved in saline solution as a single dose (10 mg/kg) through an orogastric tube 60 min before the operation. Physiological saline was administered to the rats in Group II at the same doses. Tadalafil was administered at a dose of 10 mg/kg as previously described [14]. At the end of the experimental procedure, the rats underwent a left nephrectomy. The left kidneys were cut in half and immediately fixed by immersion in 10% neutral buffered formalin and Zenker's fluid for histological evaluation, or stored at –80°C for subsequent biochemical analyses.

Histopathological Evaluation

The fixed kidneys were dehydrated through graded alcohols. 5-mm thick sections were cut, embedded in paraffin wax by standard methods and stained with hematoxylin and eosin, Masson's trichrome and periodic acid-Schiff according to standard protocols, and photographed using a Leica DM6000 microscope fitted with a DC280 digital camera (Leica, Wetzlar, Germany).

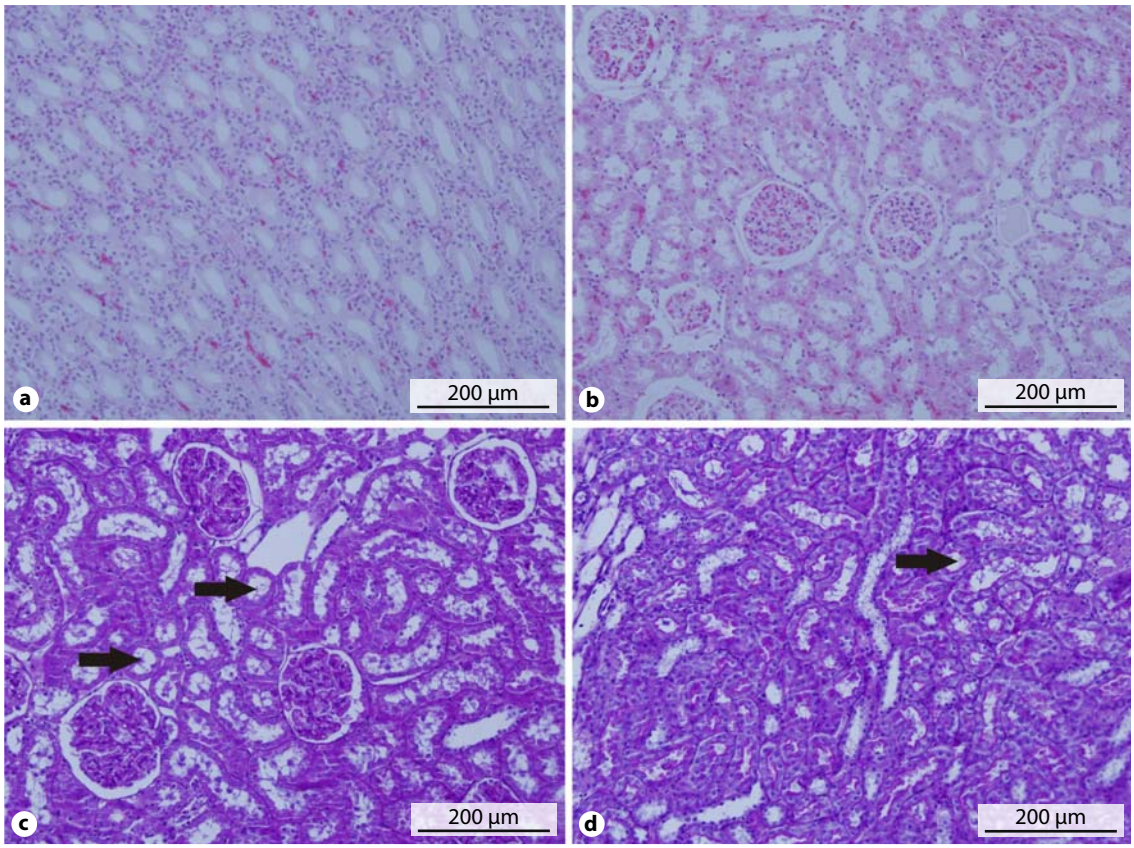
For the histopathological analysis, the sections were examined by 2 histologists who were blinded to the treatment groups. 25 tubules, each from 4 different areas of the kidney (outer cortex, inner cortex, outer stripe of the outer medulla and medullary rays), were randomly evaluated. 100 tubules were scored for each section. The mean score was calculated for each of the 4 regions. A mean overall renal tubular score that included all 4 regions was also calculated for each rat. Tubular atrophy was graded as follows: 0 = no atrophy, 1 = <25%, 2 = between 25% and 50% and 3 = >50% [15]. Additionally, acute inflammation was assessed using a 4-point scoring system: 0 = no detectable inflammation, 1 = minimal focal inflammation, 2 = multifocal polymorphonuclear leukocytes and 3 = moderate patchy-form polymorphonuclear leukocytes.

Biochemical Examination

Tissue samples were kept at –80°C, then homogenized in 50 mmol phosphate buffer (pH = 7.4) when ready for use. Total antioxidant status (TAS) and total oxidant status (TOS) levels were determined with spectrophotometric kits (Rel Assay Diagnostics, Gaziantep, Turkey) as previously described [16].

Statistical Analysis

Data were analyzed using SPSS, version 15.0 for Windows (SPSS Inc., Chicago, Ill., USA). For the evaluation of biochemical data, the groups were analyzed with the 1-sample Kolmogorov-Smirnov test. Since the TAS values of all groups were normally distributed, a 1-way analysis of variance (ANOVA) test was performed and post hoc multiple comparisons were performed with least-squares differences. Since other data were not normally distributed, a nonparametric Mann-Whitney U test was used. The results are presented as the mean ± standard deviation (SD). The significance level was set at $p < 0.05$.



Color version available online

Fig. 1. a, **b** Hematoxylin and eosin staining of the cortex and medullary regions of the sham group. **c**, **d** Periodic acid-Schiff staining of the cortex and medullary regions of the sham group (20×).

Table 1. TAS and TOS levels in rat renal tissues

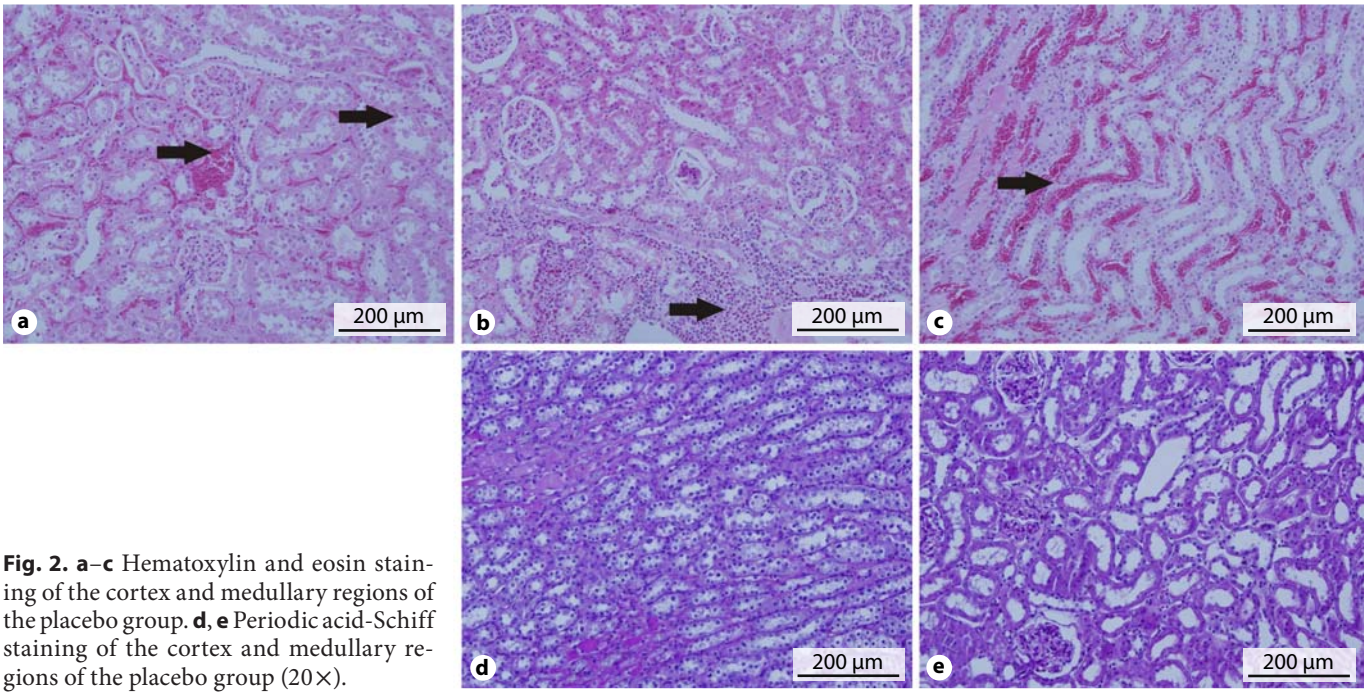
	Sham (n = 7)	Placebo (n = 7)	Tadalafil (n = 7)
TAS[1], mmol Trolox Eq/l	0.682 ± 103	0.648 ± 0.152	0.821 ± 0.087*, #
TOS[2], mmol H_2O_2 Eq/l	8.98 ± 3.01	15.51 ± 4.99#	12.65 ± 6.17

Values are represented as mean ± SD. * $p < 0.05$ compared with placebo group; # $p < 0.05$ compared with sham group.
[1] One-way ANOVA and post hoc LSD test was used. [2] Mann-Whitney U test was used.

Results

The TAS and TOS levels of the groups are summarized in table 1. The TAS levels were significantly higher in tissues of the tadalafil group compared with the placebo ($p = 0.01$) and sham groups ($p = 0.04$). There was no difference between the placebo and sham groups in terms of TAS levels. The tissue levels of TOS were significantly higher in the placebo group as compared to the sham group ($p = 0.03$). Although the TOS levels were lower in the tadalafil group when compared with the placebo group, the change was not significant.

Normal morphological findings were observed in renal tissues in the sham group on histopathological evaluation (fig. 1). Diffuse tubular necrosis, intracytoplasmic vacuolization, congestion and mononuclear cell infiltra-



Color version available online

Fig. 2. a–c Hematoxylin and eosin staining of the cortex and medullary regions of the placebo group. **d**, **e** Periodic acid-Schiff staining of the cortex and medullary regions of the placebo group (20×).

tion was observed in the medullary regions and cortex of the placebo group (fig. 2). In addition to the minimal congestion and tubular necrosis, a slight mononuclear cell infiltration was observed in the tadalafil group (fig. 3). The histopathological findings are presented in table 2.

Renal artery occlusion caused significant acute inflammation and tubular atrophy in renal tissues of the placebo group ($p < 0.001$). Pretreatment with tadalafil reduced renal injuries significantly in the tadalafil group ($p < 0.001$).

Table 2. Histopathological results based on the study groups

	Sham (n = 7)	Placebo (n = 7)	Tadalafil (n = 7)
Tubular atrophy[1]	0 ± 0	3 ± 0#	1.85 ± 0.69*, #
Acute inflammation[1]	0 ± 0	2 ± 0#	1 ± 0*

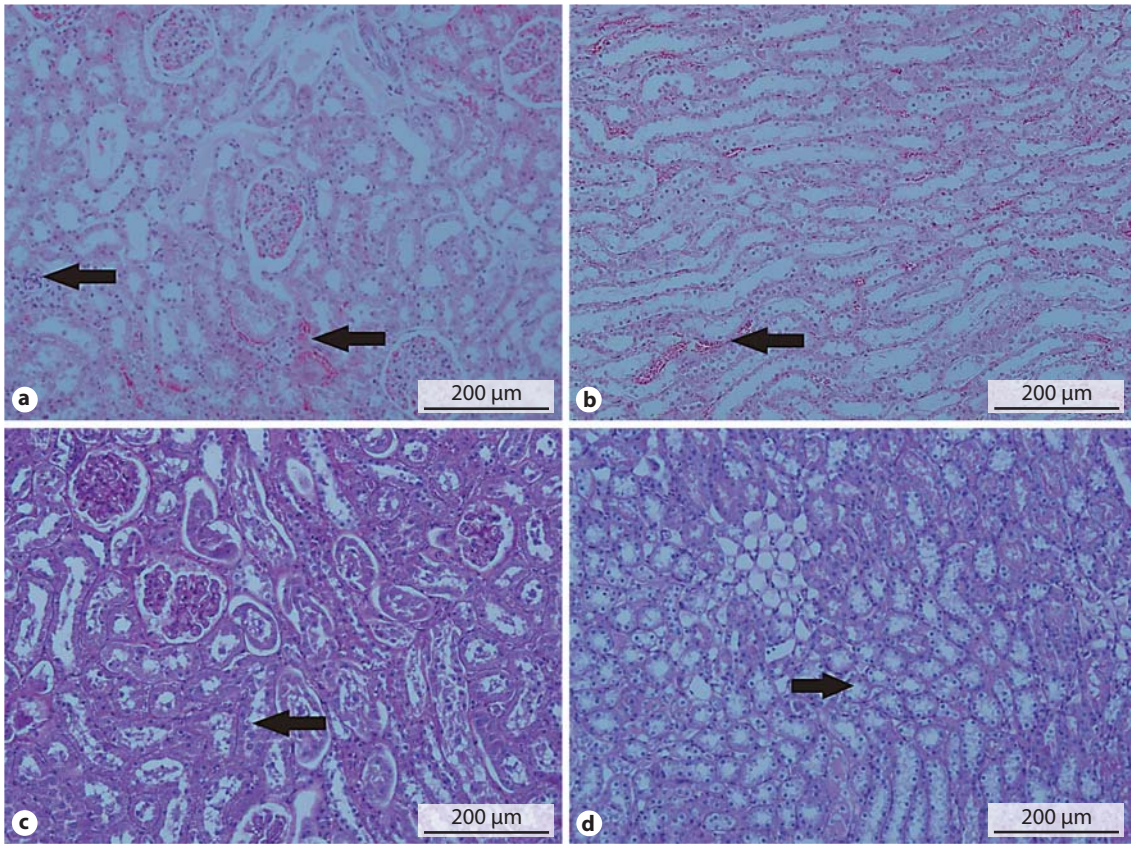
Values are represented as mean ± SD. * $p < 0.05$ compared with placebo group; # $p < 0.05$ compared with sham group.
[1] Mann-Whitney U test.

Discussion

Acute renal failure is a common problem in the context of cardiac surgery. In addition to vascular surgical interventions in which the aorta is clamped above the renal artery level, postoperative renal failure is frequently encountered in patients who undergo open heart surgery and also in patients who remain hypotensive in the course of surgery; acute renal failure negatively affects the prognosis of these patients. Acute renal failure due to I/R injury may appear in various forms, ranging from prerenal azotemia without remarkable tissue damage to severe acute renal failure from tubular or cortical necrosis [17–19].

Renal ischemia triggers a series of complex biochemical events and results in cellular damage and death [3]. Reperfusion following renal ischemia paradoxically leads to secondary tissue damage due to acute inflammation. The formation of reactive oxygen species at this stage is one of the causes of tissue damage. Endogenous antioxidant enzymes protect the cells from the devastating effects of reactive oxygen species. The amounts of these enzymes represent the severity of oxidative stress that develops during I/R [20].

It was demonstrated in previous studies that free oxygen radicals are mostly produced within the first few minutes of reperfusion, thus reperfusion injury is the highest during the early period [21]. Different measures,



Color version available online

Fig. 3. a, **b** Hematoxylin and eosin staining of the cortex and medullary regions of the tadalafil group. **c**, **d** Periodic acid-Schiff staining of the cortex and medullary regions of the tadalafil group (20×).

including the use of antioxidant agents, have been used to reduce the devastating effects of reperfusion in tissues [5, 22–25].

PDE5 is widely expressed in the vasculature, platelets, kidneys and other tissues in the body. In a study conducted by Dousa [26], PDE5 was shown to localize in the glomeruli, mesangial cells, cortical tubules and inner medullary collecting duct cells in the rat kidney. PDE5 induced cGMP breakdown and the inhibition of PDE5 increased intracellular cGMP levels [27]. PDE5 inhibitors, including sildenafil, vardenafil and tadalafil are routinely used in the treatment of erectile dysfunction [7]. In experimental studies performed with PDE5 inhibitors, it has been demonstrated that these substances reduce the ischemic area after myocardial infarction [10–12]. However, the degree of reduction in infarct size was shown to be lower with tadalafil in comparison with sildenafil or vardenafil [10]. Additionally, in an experimental study performed with sildenafil, which is a short-acting PDE5 inhibitor, it was demonstrated that treatment with sildenafil prior to ischemia can substantially reduce the renal injury that occurs due to renal I/R [5].

To the best of our knowledge, the effects of tadalafil treatment on renal I/R and TAS and TOS levels have not been reported yet. In the current study we determined the acute effects of tadalafil, a long-acting PDE5 inhibitor and a cGMP-specific hydrolytic enzyme, on the histopathological features and TAS and TOS levels of tissues in a rat renal I/R injury model. Previous experimental studies have shown that cGMP has an important role in the regulation of platelet functions and intracellular Ca^{2+} level during renal I/R injury [28].

Tadalafil differs from sildenafil and vardenafil with its pharmacokinetic profile; a half-life of 17.5 h, reaching a maximum plasma concentration 2 h after being administered orally, and an ongoing efficacy for 36 h are other differences between tadalafil and other PDE5 inhibitors [7]. Although sildenafil and tadalafil have a similar onset of action, tadalafil has a considerably longer duration of activity as compared to sildenafil [29].

Our results demonstrated that TAS was increased in the tadalafil pretreated group, with an insignificant change in the total TOS levels compared with the placebo group. Accordingly, the cause of the positive histological effect observed in tadalafil-administered rats is the protective effect of tadalafil on tissues, which is characterized by the increased antioxidant capacity.

Verit et al. [30] confirmed that tadalafil revealed a beneficial effect on the cardiovascular system by reducing serum levels of oxidative stress. It has also been demonstrated that oral administration of tadalafil (10 mg/kg) 2 h before a 30-min coronary artery occlusion results in a smaller infarct size (42 ± 2%) compared with vehicle-treated animals (54 ± 3%; p = 0.01). This infarct-limiting protection by tadalafil is only associated with a mild decline in mean arterial blood pressure [10].

In a previous study on spinal cord injuries, Serarslan et al. [31] demonstrated that the administration of tadalafil decreased the level of MDA as the level of SOD and GSH-px were increased. The outcomes of this study in which a single dose of tadalafil was administered support the findings in our study.

Normal morphological findings were observed in renal tissue in the sham group based on histopathological evaluation. Diffuse tubular necrosis, intracytoplasmic vacuolization, congestion and mononuclear cell infiltration were observed in the medullary regions and cortex of the placebo group. In addition to minimal congestion and tubular necrosis, a slight mononuclear cell infiltration was noted in the tadalafil group. While occlusion of the renal artery caused a significant acute inflammation and tubular atrophy in renal tissue in the placebo group, pretreatment with tadalafil significantly reduced the degree of renal injury.

Taking into consideration the time involved in vascular surgery procedures, which requires clamping of the aorta above the renal artery level, tadalafil is the best alternative among the PDE5 inhibitors used in clinical practice to protect the kidney from renal I/R.

Conclusion

The results of this study in a rat model of renal I/R injury suggests that pretreatment with tadalafil can reduce renal injury by its protective effects on renal tubular cells and inhibition of leukocyte infiltration to renal tissue. However, further studies also evaluating functional parameters are required to confirm this finding and to elucidate the exact mechanism of action before clinical use.

References

1 Torsello G, Reinecke P, Szabo Z, Sandmann W: The role of pharmacologic kidney protection in preventing post-ischemic renal failure in animal experiment. Zentralbl Chir 1993;118:412–419.
2 Ellenberger C, Schweizer A, Diaper J, et al: Incidence, risk factors, and prognosis of changes in serum creatinine early after aortic abdominal surgery. Intensive Care Med 2006;32:1808–1816.
3 Lien YH, Lai LW, Silva AL: Pathogenesis of renal ischemia/reperfusion injury: lessons from knockout mice. Life Sci 2003;74:543–552.
4 Riera M, Torras J, Herrero I, et al: Neutrophils accentuate renal cold ischemia-reperfusion injury. Dose-dependent protective effect of a platelet-activating factor receptor antagonist. J Pharmacol Exp Ther 1997;280:786–794.
5 Oruc O, Inci K, Aki FT, et al: Sildenafil attenuates renal ischemia reperfusion injury by decreasing leukocyte infiltration. Acta Histochem 2010;112:337–344.
6 Sandner P, Hütter J, Tinel H, et al: PDE5 inhibitors beyond erectile dysfunction. Int J Impot Res 2007;19:533–543.
7 Rosen RC, Kostis JB: Overview of phosphodiesterase 5 inhibition in erectile dysfunction. Am J Cardiol 2003;92:9M–18M.
8 Raheem AA, Kell P: Patient preference and satisfaction in erectile dysfunction therapy: a comparison of the three phosphodiesterase-5 inhibitors sildenafil, vardenafil and tadalafil. Patient Prefer Adherence 2009;3:99–104.
9 Rosano GM, Aversa A, Vitale C, et al: Chronic treatment with tadalafil improves endothelial function in men with increased cardiovascular risk. Eur Urol 2005;47:214–222.
10 Sesti C, Florio V, Johnson EG, et al: The phosphodiesterase-5 inhibitor tadalafil reduces myocardial infarct size. Int J Impot Res 2007;19:55–61.
11 du Toit EF, Rossouw E, Salie R, et al: Effect of sildenafil on reperfusion function, infarct size, and cyclic nucleotide levels in the isolated rat heart model. Cardiovasc Drugs Ther 2005;19:23–31.
12 Salloum FN, Ockaili RA, Wittkamp M, et al: Vardenafil: a novel type 5 phosphodiesterase inhibitor reduces myocardial infarct size following ischemia/reperfusion injury via opening of mitochondrial K(ATP) channels in rabbits. J Mol Cell Cardiol 2006;40:405–411.
13 Kukreja RC, Ockaili R, Salloum F, et al: Cardioprotection with phosphodiesterase-5 inhibition – a novel preconditioning strategy. J Mol Cell Cardiol 2004;36:165–173.
14 Sesti C, Florio V, Johnson EG, et al: The phosphodiesterase-5 inhibitor tadalafil reduces myocardial infarct size. Int J Impot Res 2007;19:55–61.
15 Onal A, Astarcioğlu H, Ormen M, et al: The beneficial effect of L-carnitine in rat renal ischemia-reperfusion injury. Ulus Travma Acil Cerrahi Derg 2004;10:160–167.
16 Erel O: A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004;37:112–119.
17 Weight SC, Bell PR, Nicholson ML: Renal ischemia-reperfusion injury. Br J Surg 1996;83:162–170.
18 Woolfson RG, Millar CG, Neild GH: Ischaemia and reperfusion injury in the kidney: current status and future direction. Nephrol Dial Transplant 1994;9:1529–1531.
19 Döşlüoğlu HH, Aktan AO, Yeğen C, et al: The cytoprotective effects of verapamil and iloprost (ZK 36374) on ischemia/reperfusion injury of kidneys. Transpl Int 1993;6:138–142.

20 Singh D, Chander V, Chopra K: Protective effect of catechin on ischemia-reperfusion induced renal injury in rats. Pharmacol Rep 2005;57:70–76.
21 Kaminski KA, Bonda TA, Korecki J, et al: Oxidative stress and neutrophil activation – the two keystones of ischemia/reperfusion injury. Int J Cardiol 2002;86:41–59.
22 Degirmenci B, Acar M, Albayrak R, et al: Effects of sildenafil citrate (Viagra) on renal arteries: an evaluation with Doppler ultrasound. Urol Int 2006;77:170–172.
23 Choi DE, Jeong JY, Lim BJ, et al: Pretreatment of sildenafil attenuates ischemia-reperfusion renal injury in rats. Am J Physiol Renal Physiol 2009;297:F362–F370.
24 Teixeira CE, Priviero FB, Webb RC: Differential effects of the phosphodiesterase type 5 inhibitors sildenafil, vardenafil, and tadalafil in rat aorta. J Pharmacol Exp Ther 2006; 316:654–661.
25 Eherer AJ, Schwetz I, Hammer HF, et al: Effect of sildenafil on oesophageal motor function in healthy subjects and patients with oesophageal motor disorders. Gut 2002;50: 758–764.
26 Dousa TP: Cyclic-3′,5′-nucleotide phosphodiesterase isozymes in cell biology and pathophysiology of the kidney. Kidney Int 1999;55:29–62.
27 Kotera J, Fujishige K, Omori K: Immunohistochemical localization of cGMP-binding cGMP-specific phosphodiesterase (PDE5) in rat tissues. J Histochem Cytochem 2000;48: 685–693.
28 Chintala MS, Bernardino V, Chiu PJ: Cyclic GMP but not cyclic AMP prevents renal platelet accumulation after ischemia-reperfusion in anesthetized rats. J Pharmacol Exp Ther 1994;271:1203–1208.
29 Seftel AD: Phosphodiesterase type 5 inhibitor differentiation based on selectivity, pharmacokinetic, and efficacy profiles. Clin Cardiol 2004;27:I14–I19.
30 Verit A, Savas M, Ciftci H, Aksoy N, Taskin A, Topal U: Assessment of the acute effects of tadalafil on the cardiovascular system based on examination of serum oxidative status and paraoxonase activity in men with erectile dysfunction: a preliminary study. Int J Impot Res 2010;22:115–119.
31 Serarslan Y, Yönden Z, Ozgiray E: Protective effects of tadalafil on experimental spinal cord injury in rats. J Clin Neurosci 2010;17: 349–352.