Tadalafil for prevention of renal dysfunction secondary to renal ischemia

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Introduction: *Growing evidence suggests that phosphodiesterase-5 inhibitors may mitigate ischemia-related renal damage through multiple mechanisms. We evaluated the role of tadalafil in renal function preservation during experimentally induced ischemia/reperfusion injury (IRI) in a solitary kidney porcine model.*
Materials and methods: *Ten adult female pigs underwent left laparoscopic nephrectomy followed by a 1 week recovery period. They were then randomized to tadalafil versus no treatment prior to cross-clamping the contralateral renal hilum for 90 minutes. The experimental group received 40 mg tadalafil in two equally divided doses, 12 hours before and just prior to surgery. Serum creatinine for each animal was obtained just prior to ischemia induction (D0) and at days 1, 3 and 7 following hilar occlusion. Median creatinine at each time point was compared between groups using the Kruskal-Wallis test.*
Results: *Median serum creatinine at D0 was significantly lower in the tadalafil group (after two doses of tadalafil) (123.8 µmol/L versus 168.0 µmol/L, p = 0.009). As expected, median creatinine for each group rose significantly on D1 (p = 0.04 for each). Median creatinines following hilar occlusion at D1, D3 and D7, however, were not significantly different between groups.*
Conclusions: *In this porcine model, administration of perioperative tadalafil improves preoperative renal function, but it does not appear to mitigate ischemia/reperfusion injury from hilar occlusion.*

Key Words: phosphodiesterase-5 inhibitors, ishchemia/reperfusion injury, kidney, nephrectomy

Introduction

The renal transplantation literature is ripe with examples of renoprotective agents used during cold ischemia while the donor kidney is awaiting implantation. Such agents include mannitol, enalapril, allopurinol and ATP.[1,2] The transplant model, however, is far different from in situ renal arterial clamping. In the former scenario, the renoprotective agent is continuously delivered during ischemia, either by pump perfusion or passive osmosis in solution, while in the latter the agent is only delivered to the kidney before and after hilar occlusion. Nevertheless, there is growing evidence for several renoprotective systemic agents administered prior to in situ hilar occlusion. Mannitol, well studied in clinical trials, is renoprotective by optimizing reperfusion and inhibiting tubular reabsorption of water and leukocytes.[3,4] Heparin (2000-3000 IU IV) prior to clamping may prevent thrombosis of vessels although there is still no strong evidence for its use.[3,5] Several more contemporary agents have shown good promise including erythropoietin,[6-9] prolyl-hydroxylase,[10] and alpha-melanocyte stimulating hormone.[11] The proposed mechanisms of action for these agents include inhibition of apoptosis, inflammation, and HIF signaling.

Phosphodiesterase-5 (PDE-5) inhibitors are known to improve renal perfusion and may also have anti-apoptotic and anti-inflammatory properties. However, the more clinically relevant outcome of renal function following ischemia has not been tested to date in large animal models or in humans. We investigated the role of tadalafil in preserving early postoperative renal function following temporary renal ischemia in a solitary kidney porcine model, with the hypothesis that it would mitigate the expected reduction in postoperative renal function.

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Material and methods

The study was approved by our Institutional Animal Care and Use Committee (IACUC). Ten adult female pigs underwent unilateral laparoscopic nephrectomy. Following a 1 week recovery period, they were randomized to receive tadalafil versus no treatment prior to inducing temporary renal ischemia in the contralateral kidney. The experimental group received a total of 40 mg of orally administered tadalafil, in two equally divided doses, 12 and 3 hours prior to induction of renal ischemia. The timing of taldalafil administration was based on its known pharmacodynamics, namely good oral bioavailability with maximal serum levels achieved within 2 hours of ingestion and a half-life of 17.5 hours.[12] The supraphysiologic dose of 40 mg was intentionally used to maximize the desired ischemia protective response. Serum creatinine was measured preoperatively (prior to left nephrectomy), 1 week after nephrectomy prior to the second surgery (D0), and on days 1 (D1), 3 (D3) and 7 (D7) postoperatively.

Surgical procedures

Laparoscopic nephrectomy: The initial left nephrectomy was performed via a laparoscopic transperitoneal approach. The pigs were positioned right lateral decubitis after induction of general anesthesia. A 12 mm camera port was placed with lateral to the umbilicus. A 12 mm left lateral port and 5 mm infracostal port were placed. The kidney was mobilized, the hilum transected en bloc with an endovascular stapler, and the ureter was divided. The kidney was removed by extending the umbilical incision.

Laparoscopic contralateral hilar occlusion: One week after left laparoscopic nephrectomy, the pigs were again placed under general anesthesia and positioned in a left lateral decubitus fashion. Animals in the tadalafil group were given 20 mg of tadalafil 12 hours before surgery and again 2 hours prior to anesthesia. All pigs received a 1 liter fluid bolus intraoperatively prior to induction of renal ischemia. After insufflation with a veress needle, three ports were placed (12 mm umbilical trocar, 12 mm trocar right lateral quadrant and 5 mm trocar below the costal margin). The right kidney and hilum was dissected and a satinsky clamp was placed on the renal vessels for 90 minutes. Port sites were closed and the pigs were recovered and monitored for 7 days.

Ninety minutes of hilar occlusion, longer than typical clamp times during clinical partial nephrectomy, was chosen because porcine kidneys are less prone to ischemic damage than human kidneys. Prior study has demonstrated that after 60 minutes of ischemia, all porcine kidneys suffer a temporary renal insult,[13] but 90 minutes ensured a measureable difference in creatinine.

Statistical methods

We analyzed all data using STATA version 11.0. We report descriptive data for all variables of interest. Because our data were skewed due to small sample size, we report median and interquartile range (IQR). We used non-paramatric statistics to ascertain differences at baseline between groups (i.e., placebo versus tadalafil). To determine whether a statistically significant difference existed between pig weights, we used a Kruskal-Wallis test. To determine whether a statistically significant difference existed in median creatinine between the tadalafil compared with the placebo group, we used a Kruskal-Wallis test at each time point (i.e., D0, D1, D3 and D7). We also compared the change in creatinine from baseline between groups using the same method. To illustrate the relationship of median creatinine by group over time, we plotted a line graph of medians and IQRs.

Results

Each animal successfully underwent both operations without any intraoperative or postoperative complications. Median animal weight did not differ at baseline between tadalfil and placebo groups (63 kg versus 68 kg, p = 0.53). Median pre-left nephrectomy serum creatinine was statistically similar for both groups (97.2 µmol/L versus 123.8 µmol/L, p = 0.117). On D0 median serum creatinine for the tadalafil group, measured after the full dose of tadalafil, was significantly lower than that for controls (123.8 µmol/L versus 168.0 µmol/L, p = 0.009). As expected, median creatinine for each group rose significantly on D1 after renal ischemia (p = 0.04 for each). Figure 1 plots the creatinine as a function of time around the ischemic

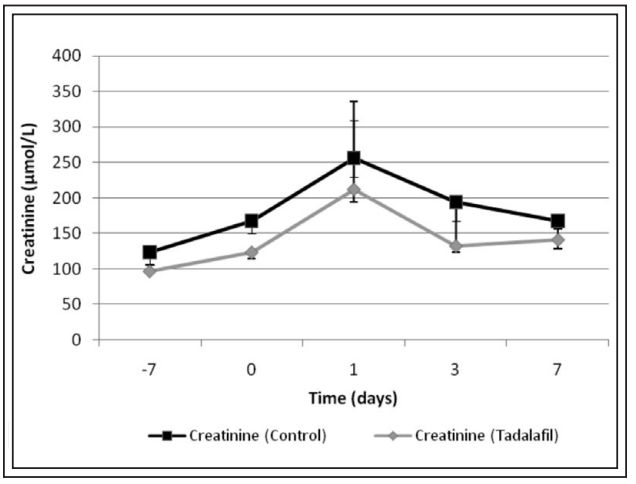

Figure 1. Line graph of median creatinine levels from the time of renal ischemia, with interquartile ranges.

TABLE 1. **Median creatinine levels from the time of renal ischemia**

	Median serum creatinine (µmol/L)		p value
Time (days)	**Control (IQR)**	**Tadalafil (IQR)**	
-7	123.8 (106.1-123.8)	97.2 (97.2-106.1)	0.117
0	168.0 (150.3-168.0)	123.8 (114.9-123.8)	0.009
1	256.4 (229.8-309.4)	212.2 (194.5-336.0)	0.347
3	194.5 (168.0-194.5)	132.6 (123.8-203.3)	0.347
7	168.0 (168.0-176.8)	141.4 (132.6-159.1)	0.142

IQR = interquartile range

event, with IQR ranges included. Median creatinine levels on D1, D3 and D7 were not significantly different between the groups, Table 1.

The median creatinine in the control group decreased to DO baseline levels after 7 days (168 µmol/L in both D0 and D7), but in tadalafil treated pigs creatinine remained elevated above baseline after 7 days (123.8 µmol/L at D0 versus 141.4 µmol/L at D7), Table 2. This translates to a 0% versus 14% increase in creatinine from D0 to D7 for the control and tadalafil group respectively, although the difference was not statistically significant (p = 0.317), Table 2.

Discussion

The incidence of small renal masses meeting the criteria for nephron-sparing surgery (NSS) is increasing[14,15] and minimally invasive surgical (MIS) treatment approaches are becoming more common as expertise develops. Instituting cold ischemia, the most effective renoprotective strategy known,[16,17] is more challenging during an MIS case than placing the kidney in ice slush during an open partial nephrectomy. In lieu of ice slush, several renoprotective strategies have been proposed for MIS approaches including early unclamping,[18] intravascular cold perfusion[19] and retrograde cooling,[20] although each method has its downsides. While the importance of maximal nephron preservation has garnered recent attention,[21] surgeons will always be limited by the size of the mass. Fortunately, pharmacological renoprotective agents can be easily administered and offer the potential for both short and long term renal preservation.

Tadalafil is a long-acting PDE-5 inhibitor that inhibits the breakdown of cGMP resulting in smooth muscle relaxation and vasodilation. PDE-5 inhibitors are clinically approved for the treatment of erectile dysfunction and pulmonary hypertension,[22] but there is evidence they may also mitigate renal damage from ischemia. Ischemic injury results from a complex and multifactorial sequence of events. An initial inflammatory response leads to vasoconstriction and vascular spasm, resulting in endothelial injury. This results in an ongoing cycle of cytokine activation with increased renal vascular resistance, loss of self vascular regulation, ATP depletion and disruption of cellular osmotic gradients. Injury is compounded by reperfusion injury when the clamp is released.[3] PDE-5 inhibitors may target several of these mechanisms such as decreasing renal vascular resistance, inhibiting apoptotic pathways, and decreasing leukocyte infiltration.[22]

One rationale for the evaluation of PDE-5 inhibitors is the known physiological effects it exerts on the kidney. In a small case series, impotent human male transplant patients who took sildenafil had an increase in their GFR of 14 +/- 5mL/min/1.73 m^2 ($p < 0.05$)

TABLE 2. **Change in median creatinine levels from baseline prior to renal ischemia**

Time (days)	Control		Tadalafil		p value
	Median creatinine change (µmol/L)	Percent change	Median creatinine change (µmol/L)	Percent change	
0-1	88.4	52.6	88.4	71.4	1.000
0-3	26.5	15.8	8.8	7.1	0.317
0-7	0	0	17.7	14.3	0.317

and a decrease in renal vascular resistance 2 hours after administration.[23] In addition, PDE-5 receptors have been identified in renal tissue during histological studies on mice. In the same study, glomerular levels of cGMP were doubled after 6 days of PDE-5 inhibitor administration.[24] In a study of pigs undergoing autotransplantation, preoperative PDE-5 inhibitor treatment resulted in improved vascular parameters post transplantation, including improved vascular flow and decreased resistance.[22]

There is increasing evidence that PDE-5 inhibitors may protect the kidney from ischemic injury. Zaprinast, another PDE-5 inhibitor,[25] was given to rats 24 hours after bilateral renal artery clamping for 60 minutes. Forty-eight hours following renal ischemia, the GFR was 0.14 +/- 0.04 (mL/min/100 g body wt) in the control versus 0.94 +/- 0.29 (mL/min/100 g body wt) in the zaprinast treated animals. Using laser doppler flowmeters, cortical and medullary blood flow was also observed to significantly increase by 17% and 40% respectively.[26] In a more recent study of sildenafil, rats underwent unilateral nephrectomy followed by 35 minutes of contralateral hilar occlusion. Sildenafil was administered intraperitoneally at a dose of 0.5 mg/kg, 1 hour preoperatively. Postoperative creatinine levels were significantly lower in the sildenafil group at 24 and 48 hours. The sustained effect on renal function beyond the half-life of sildenafil suggested a possible renoprotective benefit, although the statistical difference was lost by days 3, 5 and 7.[27]

In this study, tadalafil treated uni-nephric pigs had improved GFR over controls pre-ischemia induction, consistent with other studies showing that PDE-5 inhibitors increase GFR.[23,28] However, GFR following hilar occlusion, was not significantly different after tadalafil compared with controls. These findings suggest that perioperative taldalafil administration may potentially create a renal preconditioning effect, given the temporarily increased tubular filtration, although this was not sustained postoperatively despite its long half-life. There are a number of possible explanations for our observed findings. Despite the use of a maximal dose for humans (40 mg), adequate serum levels sufficient for ischemia protection may not have been achieved in the pig model. This dose is similar to the 0.5 mg/kg dose given in the rat trials, although there may be species differences in metabolism and pharmacodynamics. Future studies in which serum levels are quantitatively measured may be useful in further exploring this possibility. Secondly, we appreciate that statistical significance is difficult to demonstrate with our sample size. Nevertheless, if the magnitude of the effect was large, we would have expected a greater trend towards a postoperative improvement in GFR with tadalafil in a solitary kidney model which was not observed.

As expected from the typical course of acute tubular necrosis, renal function in both cohorts approached baseline levels 7 days following hilar occlusion. Percent median creatinine change from D0 to D7 in the control and tadalafil cohorts were 0% and 14.3% respectively ($p = 0.317$). Although the tadalafil arm did not fall all the way to baseline like the control arm, there appeared to be more rapid recovery of renal function in tadalfil treated pigs as the percent median creatinine change from D0 to D3 was less pronounced in the tadalafil arm (7.1% versus 15.8% respectively). One of the limitations of this study is the short follow up period in which we are only able to evaluate short term renal injury as a surrogate for long term renal function, an association which is supported by epidemiological studies.[29,30] Another limitation of this study is that histological analysis was not performed, which may have captured differences in the magnitude of acute renal injury between cohorts.

Although a clinically significant renoprotective effect of PDE-5 inhibitors has not been established, several potential renoprotective mechanisms have been elucidated in addition to its effects on renal vascular physiology. The most promising renoprotective agents, including mannitol, erythropoietin,[6-9] prolyl-hydroxylase,[10] and alpha-melanocyte stimulating hormone, function through anti-apoptotic and anti-inflammatory pathways rather than renal vascular dynamics. Indeed, pure vasodilatory agents have had limited success, perhaps because they have no effect during hilar clamping, the most crucial time of the ischemic insult. Enalapril, for example, induces vasodilation that may increase renal blood flow and prevent vasospasm.[3,5] While it has shown renoprotective properties in donor kidney preservation solution, there is no consistent evidence for benefit in a kidney that is clamped in situ.[2] Dopamine and diltiazem increase renal blood flow after injury but are also not renoprotective and offer no clinical benefit.[31,32] These findings imply that if PDE-5 inhibitors are in fact renoprotective, the mechanism of action is unlikely to be improved renal vascular flow. Several studies have demonstrated that PDE-5 inhibitors do affect apoptosis and inflammatory pathways. Apoptosis is an important mechanism of cell death in cultured renal tubular cells, and is associated with the upregulation of Bax, a proapoptotic protein, and downregulation of Bcl-2, an antiapoptotic protein. Choi and colleagues showed that sildenafil treatment in rats following renal ischemia resulted in a decreased Bax/Bcl-2 ratio. In addition, there were lower levels of other apoptotic markers, including decreased activation

of caspase-3 and decreased TUNEL-+ve cells.[27] Sildenafil also induces ERK activation, a cascade that mediates cell growth and survival in many cell types and protects against oxidative injury.[27] In a similar study, the kidneys of tadalafil treated rats showed decreased tubular atrophy, decreased leukocyte infiltration and increased total antioxidant status levels. This lead the authors to conclude that tadalafil is renoprotective primarily through its anti-inflammatory and renal tubular effects.[33] Histological effects were not evaluated in this study and differences in markers of apoptosis and inflammation may have been missed. Whether such markers can predict renal functional outcomes, or simply provide information regarding mechanism of action, is still uncertain.

Based on the studies performed to date, tadalafil may temporarily improve GFR around the time of an ischemic insult. Such a role could have potential utility in preventing temporary dialysis following partial nephrectomy in a solitary kidney. Prolonged dosing of tadalafil following the ischemic insult may provide sustained GFR improvements although this requires further testing. Despite the potential benefits of PDE-5 inhibitors, strong evidence for sustained renal function preservation is still lacking. Larger studies with longer follow up are required to allow an accurate assessment of long term renal preservation. Evaluation of other PDE subtypes also warrants further investigation. Tadalafil was chosen because of its ready availability and familiarity to urologists which would facilitate its clinical application, but targeting a different PDE receptor may prove more effective. The renoprotective role of both PDE-3 and PDE-4 inhibitors have been studied in rats, and both agents reduced the magnitude of creatinine elevation following hilar occlusion.[34,35] PDE-4 inhibitors may mitigate renal dysfunction through more potent anti-inflammatory effects by decreasing the release of cytokines such as interleukin-1β and tumor necrosis factor-α.[36]

Conclusion

Effective renoprotective agents are likely to have complex and potentially multifactorial mechanisms of action. Targeting of inflammatory, apoptotic and other signaling pathways, rather than vascular physiology, appear to yield the best results. Tadalafil increased GFR prior to ischemia, suggesting a potential role in preconditioning, but failed to preserve GFR after hilar occlusion. Currently there is insufficient evidence to begin human trials with phosphodiesterase inhibitors. Instead, larger studies with longer follow up in large animal models are needed to evaluate the long term impact on renal function. □

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