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# Effect of a single treatment with tadalafil on blood flow in lower urinary tract tissues in rat models of bladder overdistension/emptying and abdominal aorta clamping/release

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ABSTRACT

Impaired blood flow in lower urinary tract (LUT) tissues is a pathophysiological cause of LUT symptoms. We investigated the effects of the phosphodiesterase 5 (PDE5) inhibitor tadalafil on the sustained decrease in bladder blood flow (BBF) and time-dependent changes in BBF and prostate blood flow (PBF) resulting from ischemia/reperfusion in two rat models. In a rat model of bladder overdistension/emptying (O/E), the bladder was overdistended by saline infusion and emptied after 2 h. Tadalafil was administered intraduodenally immediately after emptying. In a rat model of clamping/release (C/R), the abdominal aorta was clamped for 2 h after a single oral dose of tadalafil and then the clamp was released. BBF in O/E and C/R rats and PBF in C/R rats were measured by laser Doppler flow imaging. BBF decreased on overdistension and partially recovered after emptying. A progressive decrease in BBF was observed after O/E, and this was prevented by tadalafil treatment. Both BBF and PBF decreased during clamping of the abdominal aorta and partially recovered after clamp removal. Oral pretreatment with tadalafil partially or completely prevented the decreases in BBF and PBF not only after clamp removal but also during clamping. PDE5 mRNA was highly expressed in the bladder and the supporting vasculature. Tadalafil inhibited the O/E-induced decrease in BBF and the C/R-induced time-dependent decreases in BBF and PBF. PDE5 inhibition by tadalafil may improve both BBF and PBF.





## 1. Introduction

Benign prostatic hyperplasia (BPH) is a clinically common disease that often causes lower urinary tract symptoms (LUTS) in older men. Generally, male LUTS are considered to be a result of mechanical bladder outlet obstruction (BOO) caused by a hypertrophic prostate gland. BOO induces a decrease in bladder blood flow (ischemia phase) with a resulting decrease in oxygen tension (hypoxia) in the thick bladder wall, followed by an increase in blood flow and oxygen tension after micturition (reperfusion phase). Cyclical ischemia/reperfusion in the bladder may be an important mechanism of obstructive bladder dysfunction (Levin et al., 1997).

Recent attention has been focused on impaired blood flow in lower urinary tract (LUT) tissues, including the bladder and prostate, as a common pathophysiological cause of LUTS regardless of the presence or absence of prostate enlargement and the degree of BOO. Clinical studies have revealed a markedly lower bladder blood flow in patients with LUTS in comparison with asymptomatic younger controls (Pinggera et al., 2008a, 2008b), suggesting that the reduction in blood flow in LUT tissues is a pathogenic factor in the development of LUTS. Therefore both chronic ischemia in the bladder and prostate, as well as repeated ischemia/reperfusion during the micturition cycle in the bladder, may be partly responsible for LUTS associated with BPH (Levin et al., 1997; Yamaguchi et al., 2014).

Tadalafil (once-daily use) has been approved by the US Food and Drug Administration and the European Medical Agency as a new treatment option for men with BPH/LUTS as well as for men with a combination of BPH and erectile dysfunction. In January 2014, tadalafil was also approved in Japan for the treatment of BPH/LUTS. Recent systematic reviews and meta-analyses of the efficacy and safety of PDE5 inhibitors alone or in combination with α-adrenergic blockers in LUTS/BPH patients have consistently found a significant improvement in storage and voiding symptoms (Gacci et al., 2012).

While the precise mechanisms of LUTS improvement by tadalafil remain unclear, several LUT tissues—prostate, urethra, and bladder, as well as their vasculatures—are thought to represent potential targets for this drug (Anderson et al., 2011; Giuliano et al., 2013). Tadalafil has multiple effects, including smooth muscle relaxation, increased pelvic blood perfusion in LUT tissues (Anderson et al., 2011) and afferent nerve activity (Minagawa et al., 2012). Concerning tissue perfusion, tadalafil has been shown to improve penile oxygenation (Vignozzi et al., 2006) and prostate hypoxia in rat models (Morelli

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et al., 2011). However, a few reports explore in detail the effect of tadalafil on bladder blood flow (BBF) in animal models. Therefore this study was undertaken to investigate how tadalafil affects bladder perfusion in two rat models of ischemia/reperfusion. In one model, we investigated the effect of tadalafil on the progressive decrease in BBF when the bladder was subjected to overdistension followed by emptying (O/E model). In the other model, we investigated the effect of pretreatment with tadalafil on the changes in both BBF and PBF throughout the ischemia and reperfusion induced by clamping/release of the abdominal aorta (C/R model).

## 2. Materials and methods

### 2.1. Animals

Female 14- or 15-week-old and male 14-week-old Sprague–Dawley rats (Japan SLC, Hamamatsu, Japan) were housed three or four per cage in a room maintained at 20–26 °C and 35–75% relative humidity with an alternating 12-h light/dark cycle (the lights on automatically at 8:00 a.m.). They were allowed free access to food pellets (F-2; Funabashi Farm, Funabashi, Chiba, Japan) and tap water. The study was conducted in compliance with the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as revised on 1 June 2006).

### 2.2. Preparation of tadalafil

Tadalafil was kindly provided by Eli Lilly and Company (Indianapolis, IN, USA). The purity of tadalafil was 99.7%. Tadalafil was suspended in 0.5% (weight per volume) methylcellulose solution (MC–water) with an agate mortar. Tadalafil was administered intraduodenally in a volume of 1 mL/kg or orally in a volume of 5 mL/kg. In both cases, the final dose was 10 mg/kg.

### 2.3. Bladder overdistension/emptying (O/E)

Surgery for bladder O/E was carried out as described by Kawai et al. (2013). Female rats were anesthetized intraperitoneally with pentobarbital (50 mg/kg). With the rat in the supine position, a midline suprapubic incision was made and the bladder was exposed. A PE-50 catheter (Becton Dickinson, Franklin Lakes, NJ, USA) was inserted into the bladder through the urethra and secured with a tight ligature around the external urethral orifice. After the residual urine had been drained, the catheter was connected to an infusion pump and 2 or 3 mL of saline was infused at a rate of 0.1 mL/min for 20 or 30 min, respectively, to overdistend the bladder. After completion of the infusion, overdistension was maintained for 2 h and then the bladder was emptied. We measured BBF at the point of overdistension for 2 h (that is, before the bladder was emptied after overdistension had been maintained for 2 h) and again 1 h after emptying. For the experiment on the effect of tadalafil on BBF, rats were divided into three groups. Rats in group 1 underwent a sham operation. Rats in groups 2 and 3 underwent bladder overdistension by infusion of 3 mL of saline as described above, overdistension was maintained for 2 h, and then the bladder was emptied. Just after emptying, rats were treated intraduodenally with the vehicle (MC–water; groups 1 and 2) or tadalafil 10 mg/kg (group 3). BBF was measured before and during overdistension and 0, 1, 2, 3 and 4 h after emptying.

### 2.4. Clamping/release of abdominal aorta (C/R)

Male rats were divided into three groups. Rats in group 1 were treated orally with the vehicle, MC–water, and underwent a sham operation. Rats in groups 2 and 3 were treated orally with vehicle and 10 mg/kg tadalafil, respectively, and then underwent surgery for C/R of the abdominal aorta as described previously (Saito and Miyagawa, 1999) with minor modifications. Rats were anesthetized with pentobarbital (50 mg/kg intraperitoneally). With the rat in the supine position, the abdominal aorta was clamped just above its bifurcation with a metal vessel clip for 2 h, after which the clip was removed. Both BBF and PBF were measured in each rat before clamping, during clamping and 1 and 2 h after removal of the clip.

### 2.5. Measurement of BBF and PBF

BBF and PBF were determined with a laser speckle blood flow imager (Omegazone OZ-1; Omegawave, Tokyo, Japan) as described previously (Kawai et al., 2013). With the rat under pentobarbital anesthesia, the ventral prostate and bladder were exposed through an abdominal midline incision to allow measurement. Image pixels were analyzed to produce average perfusion values.

### 2.6. PDE5 expression by RT-PCR

A total of 20 intact male rats were used. The vesical and iliac arteries, and bladder were removed, rapidly soaked in RNAlater RNA stabilization reagent (Life Technologies, Carlsbad, CA, USA), and kept until required for RNA extraction. For tissue collection, the rats were divided into five groups of four. Each tissue from each group of four rats was pooled to give five pooled samples of each tissue. Total RNA was extracted using the SV Total RNA isolation system (Promega, Madison, WI, USA) and reverse transcription (RT) was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. RT-PCR was performed with the LightCycler 480® Real-Time PCR System (Roche Diagnostics, Rotkreuz, Switzerland) by using SYBR® *Premix EX Taq*™ II (Tli RNaseH Plus) (Takara Bio, Shiga, Japan). PDE5 mRNA levels were normalized to mRNA levels of the ribosomal protein L19 (RPL19) housekeeping gene and expressed relative to the PDE5 mRNA levels of the bladder. Primer sequences for the PDE5 and RPL19 genes were obtained from Filippi et al. (2007). Q4

### 2.7. Statistical analysis

Data were expressed as the mean ± S.E.M. and analyzed with SAS® version 9.1.3 (SAS Institute, Cary, NC, USA). The data in Fig. 1 were analyzed by Dunnett's multiple-comparison test against the sham-operated group. Otherwise, differences between the sham-operated and vehicle groups were analyzed for statistical significance by Student's *t*-test (for equal variance) or the Welch test (for unequal variance). For the investigation of the effects of tadalafil in this model, when a significant difference was noted, differences between the vehicle and tadalafil groups were analyzed by Student's *t*-test (for equal variance) or the Welch test (for unequal variance). $P < 0.05$ was considered to indicate a statistically significant difference.

## 3. Results

### 3.1. The effect of infused saline on BBF in the bladder of female rats

Bladder distension produced by infusion of 3 mL of physiological saline reduced BBF compared with the sham group (Fig. 1A), and after emptying of the bladder a significant reduction in BBF was maintained (Fig. 1B). In contrast, in the 2-mL saline-infusion group no significant reduction in BBF was observed at either time-point. Therefore, an infusion volume of 3 mL was used in subsequent studies.



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