RESEARCH PAPER

Effects by silodosin on the partially obstructed rat ureter *in vivo* and on human and rat isolated ureters

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BACKGROUND AND PURPOSE

α_1-adrenoceptor (-AR) antagonists may facilitate ureter stone passage in humans. We aimed to study effects by the α_{1A}-AR selective antagonist silodosin (compared to tamsulosin and prazosin) on ureter pressures in a rat model of ureter obstruction, and on contractions of human and rat isolated ureters.

EXPERIMENTAL APPROACH

After ethical approval, ureters of male rats were cannulated beneath the kidney pelvis for *in vivo* ureteral intraluminal recording of autonomous peristaltic pressure waves. A partial ureter obstruction was applied to the distal ureter. Mean arterial blood pressure (MAP) was recorded. Approximate clinical and triple clinical doses of the α_1-AR antagonists were given intravenously. Effects by the α_1-AR antagonists on isolated human and rat ureters were studied in organ baths.

KEY RESULTS

Intravenous silodosin (0.1–0.3 mg kg^{-1}) or prazosin (0.03–0.1 mg kg^{-1}) reduced obstruction-induced increases in intraluminal ureter pressures by 21–37% or 18–40% respectively. Corresponding effects by tamsulosin (0.01 or 0.03 mg kg^{-1}) were 9–20%. Silodosin, prazosin and tamsulosin reduced MAP by 10–12%, 25–26% ($P < 0.05$), or 18–25% ($P < 0.05$) respectively. When effects by the α_{1A}-AR antagonists on obstruction-induced ureter pressures were expressed as a function of MAP, silodosin had six- to eightfold and 2.5- to eightfold better efficacy than tamsulosin or prazosin respectively. Silodosin effectively reduced contractions of both human and rat isolated ureters.

CONCLUSIONS AND IMPLICATIONS

Silodosin inhibits contractions of the rat and human isolated ureters and has excellent functional selectivity *in vivo* to relieve pressure-load of the rat obstructed ureter. Silodosin as pharmacological ureter stone expulsive therapy should be clinically further explored.

Abbreviations

-AR, -adrenoceptor; EFS, electric field stimulation; IUP, intra-ureteral pressure; MAP, mean arterial pressure; MaxP, maximum ureter pressure; MinP, minimum ureter pressure

Introduction

Urolithiasis is a multifactorial disease that often is encountered in daily urological practice and that is increasing in Western countries (Stamatelou *et al.*, 2003; Pearle *et al.*, 2005). Mechanical obstruction, ureteral spasms and luminal dilation are causes for significant discomfort in patients with urolithiasis. When calculi are small (≤10 mm), located in the

distal part of the ureter, and with no clinical evidence of infection and pain, conservative pharmacological expulsive therapy may be indicated to accelerate spontaneous passage of ureter stones (Hollingsworth *et al.*, 2006; Seitz *et al.*, 2009).

Several transmitter systems or receptors may be considered to be putative pharmacological targets to modify ureteral peristalsis (Canda *et al.*, 2007). Adrenergic nerve fibres that are similarly distributed in ureters from different species, including humans, exhibit most abundant expression in the distal ureter, (Rolle *et al.*, 2008) localized in the muscular layers, within the perivascular networks, as well as, to a lower extent, in the suburothelial space (Rolle *et al.*, 2008). Alpha$_1$-adrenoceptors (α_1-ARs) have been detected in ureters from both animals and humans (Morita *et al.*, 1994; Sigala *et al.*, 2005; Park *et al.*, 2007; Kobayashi *et al.*, 2009b). The density of α_1-ARs in the ureteral smooth muscle appears to be greater than other adrenoceptors (Morita *et al.*, 1994; Sigala *et al.*, 2005). A rather homogenous distribution of different α_1-AR subtypes along of the human ureter is reported (Park *et al.*, 2007). Various α_1-AR antagonists that are available in clinics, for example, alfuzosin, doxazosin, tamsulosin and silodosin, exhibit inhibitory effects on contractions on isolated ureter of a variety of species (Kobayashi *et al.*, 2009a; 2009b), including humans (Rajpathy *et al.*, 2008; Sasaki *et al.*, 2011) and appear to reduce human ureteral activity *in vivo* (Davenport *et al.*, 2007). Results obtained from randomized controlled trials suggest that α_1-AR antagonists may be used in patients to facilitate passage of ureter stones (Hollingsworth *et al.*, 2006; Seitz *et al.*, 2009). On the other hand, recently presented contradictory results indicate that tamsulosin, an α_1- AR antagonist with some selectivity for α_{1A}- and α_{1D}-AR, is not efficacious as medical ureter stone expulsion therapy (Vincendeau *et al.*, 2010). It is currently not clear if α_1-AR antagonists with different receptor-subtype preferences other than tamsulosin may have better effects on ureteral contractile functions or ureteral peristaltic activity. Even so, silodosin, a highly selective α_{1A}-AR antagonist, was recently proposed as a putative drug for expulsive therapy for ureter stones (Itoh *et al.*, 2011).

Whereas only few studies have compared the effect of different α-blockers on ureteral activity of isolated human tissues (Rajpathy *et al.*, 2008; Sasaki *et al.*, 2011), several studies have addressed the effect of α_1-AR antagonist in dogs and mice using an α-AR agonist-induced ureteral contraction model (Kobayashi *et al.*, 2009a; 2010). Mechanical and complete unilateral acute ureteral obstruction has been utilized in pigs to evaluate the potential of β-AR agonists as putative new ureter relaxing drugs for the management of stone disease (Wanajo *et al.*, 2011). These models however, do not allow for registration of the endogenously generated ureter contractions that propel urine from the kidney to the bladder. This peristaltic ureter activity occurs spontaneously along a functional and not completely obliterated ureter and can be continuously recorded as regular pressure waves. To better understand how different α_1-AR antagonists may affect ureteral contractions and spontaneous peristaltic propulsive pressure waves, one goal of our project was to further develop a rat model of acute unilateral ureteral obstruction. In this model, the main aim of our study was to compare the effects by intravenous administration of approximate relevant clinical doses of silodosin, tamsulosin and prazosin on intralumi-nal ureter pressure parameters and systemic blood pressures. In separate *in vitro* experiments, we also aimed to compare the effects of the selected α_1-AR antagonists on contractions of isolated rat and human distal ureters.

Methods

Ethical approvals
All experiments involving animals were approved by the Institutional Animal Care and Use Committee, San Raffaele Scientific Institute, Milan, Italy and carried out in accordance with the ARRIVE Guidelines (Kilkenny *et al.*, 2010). Human specimens were obtained after the signature of an informed consent, as specified by the Institutional Ethical Committee approved Protocol n. URI003-2010 (approval date 9 December 2010).

Animals
A total of 55 male Sprague Dawley rats (250–300 g, Charles River, Italy) were used. The animals were maintained under standard laboratory conditions with a 12:12 h light : dark cycle and free access to food pellets and tap water. For surgery, isofluorane (alveolar concentration 2%) was used as inhalation anaesthesia. Forty rats were treated with different α-blockers; (i) silodosin (α_{1A}-AR selective) at 0.1 and 0.3 mg kg^{-1} (n = 6 for both doses), (ii) tamsulosin ('α_{1A-D}-AR selective') at 0.01 and 0.03 mg kg^{-1} (n = 7 for both doses) and (iii) prazosin (non-α-AR selective) at 0.03 and 0.1 mg kg^{-1} (n = 7 for both doses). Fifteen animals were used as controls to record the effect of vehicles. Rats were killed by carbon dioxide asphyxia and ureters were harvested for immediate functional investigations in organ baths.

Human tissues
Macroscopically, normal human ureter was obtained from patients (n = 13, age: 59 ± 4 years) who had undergone surgery for urological malignancies, but not exposed to chemotherapy, radiotherapy or immunotherapy before the surgical procedure. The normal tissue was isolated by an uropathologist distally from the tumour lesion, immediately placed in a chilled Krebs solution and transported to the laboratory. All tissues were immediately used in organ bath experiments.

Partial ureteral obstruction and in vivo *pressure recording in rats*
Under surgical anaesthesia, a midline abdominal incision was performed and the left ureter was visualized from the renal pelvis to the bladder. The proximal ureter was carefully isolated by microsurgical dissection. A small incision was made just below the ureteropelvic junction to allow insertion of a polyethylene catheter (PE-10, Clay-Adams, Parsippany, NJ, USA) into the ureter. The catheter was then attached to a microsyringe pump (CMA 100; Carnegie Medicine AB, Solna, Sweden) and connected to a pressure transducer. While room temperature physiological saline was continuously infused at a speed of 0.4 mL h^{-1} to simulate normal urine production, (Schmidt *et al.*, 2001) ureteral intraluminal pressure oscilla-

tions were constantly recorded. A partial ureteral obstruction was created by suturing the underlying psoas muscle around the distal 1/5 portion of the ureter just before the vesicoureteric junction (Ulm and Miller, 1962). As previously described (Becker *et al.*, 1998), the urinary bladder was incised at the dome to avoid interference on ureter pressures from detrusor contractions.

The partial obstruction was considered optimal when the ureteral reproducibly and stably regained its spontaneous peristaltic activity over time and the minimal ureteral pressure achieved was greater than the maximal pressure before the obstruction.

In order to monitor systemic blood pressure, a heparinized (5 IU mL^{-1}) saline-filled polyethylene catheter (PE-50, Clay-Adams) was positioned in the carotid artery for recording of mean arterial pressure (MAP). An additional saline-filled polyethylene catheter (PE-10, Clay-Adams) was positioned in the femoral vein to allow drugs administration. All pressure data were acquired continuously with Acq Knowledge 3.8.1 software and a MP100 data acquisition system (BIOpac Syst. Inc. Santa Barbara, CA, USA) connected to a Grass polygraph (Model 7E, Grass Technologies, Warwick, RI, USA). The following ureteral parameters were analysed: frequency (*contractions per minute*) and amplitude (*cmH$_2$O*) of autonomous peristaltic pressure waves, minimum pressure (MinP; *cmH$_2$O*), maximum pressure (MaxP; *cmH$_2$O*) and the AUC per second (*cmH$_2$·s^{-1}*). An example of the *in vivo* ureteral pressure recording after partial obstruction is depicted in Figure 1, showing representative original traces for each tested drug.

Isolated ureteral tissue

Tissue strips ($3 \times 3 \times 6$ mm) were dissected from the proximal part of ureters obtained from surgical human specimens and naïve rats never exposed before to pharmacological treatments. Silk ligatures were applied at both ends of the preparations and then mounted in a 5 mL aerated (95% O2 and 5% CO2) organ bath chamber, containing Krebs solution (37°C, pH 7.4). The working Krebs solution was routinely replaced every 30 min. Isometric tension was registered with a Grass Polygraph model 7E (Grass Technologies). The human preparations were stretched to a tension approximately 5 mN and left to equilibrate for 30 min to attain a stable resting tone of 1.82 ± 0.2 mN ($n = 13$). The rat ureteral preparations were stretched to a tension approximately 2 mN and left to equilibrate for 30 min to obtain a stable tension of 0.358 ± 0.02 mN ($n = 18$). The viability of the preparations was verified by addition of high K$^+$ solution (60 mM KCl) to the organ baths to induce force displacement of 0.42 ± 0.07 mN ($n = 13$) and 0.15 ± 0.01 mN ($n = 18$) for human and rat ureteral preparations respectively. Electrical field stimulation (EFS) was performed with two platinum electrodes, placed in parallel to the tissue strips. A Grass S48 stimulator delivered single 0.5 ms square-wave pulses at supra-maximum voltage. The train duration was 5 s and the train interval 120 s. Scouting frequency-response (0.5–40 Hz) experiments were preliminarily carried out on separate ureter specimens to evaluate the optimal stimulation frequency needed to obtain the 75% of maximal contractile effect (data not shown). Effects by cumulative addition of single concentrations of silodosin (range 10^{-7} to 10^{-4} M), tamsulosin (10^{-7} to 10^{-4} M) or
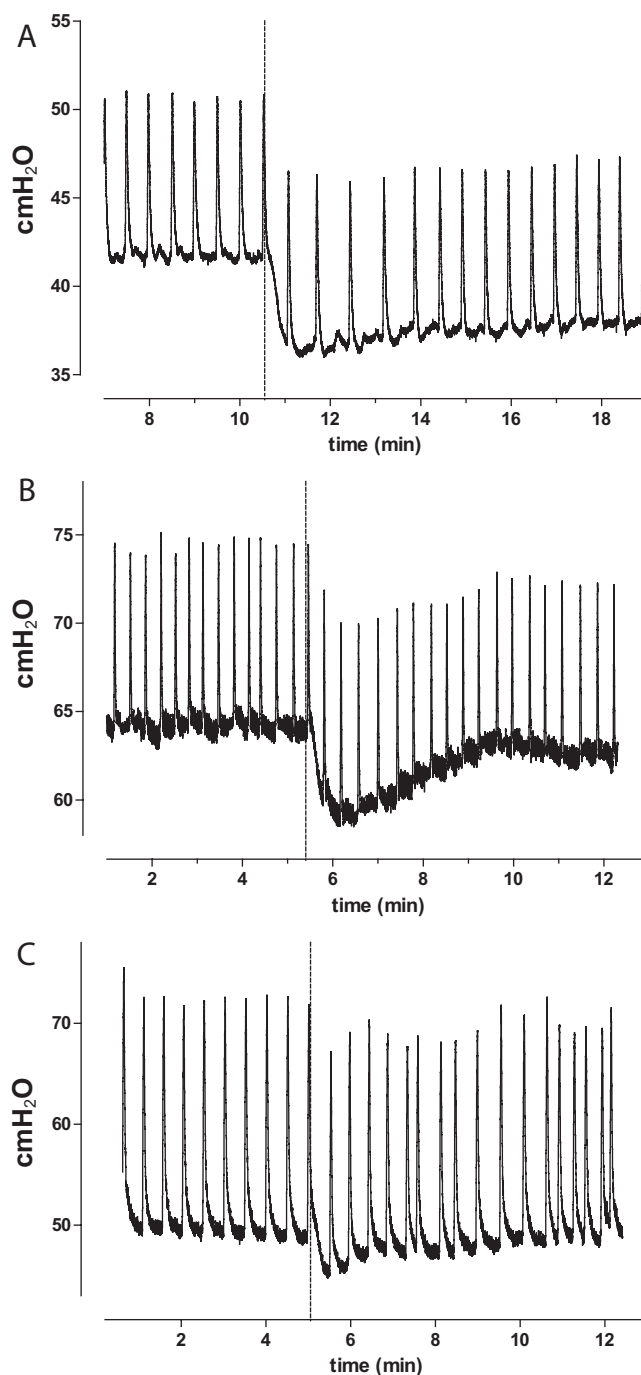

Figure 1

Representative original ureteral pressure traces recorded after partial obstruction and administration of silodosin (A), tamsulosin (B) and prazosin (C) given at the maximal tested dose. Vertical dashed line indicates the timing of intravenous drug injection.

prazosin (10^{-7} to 10^{-4} M) were studied on continuous EFS contractions (20 Hz). The –logIC$_{30}$ values were calculated by graphical interpolation. In separate experiments, vehicles for silodosin, tamsulosin and prazosin were tested on EFS-induced contractions (each $n = 5$).

Drugs and solutions

The composition of the Krebs solution was (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 5.5. A K^+ solution (60 mM) was used, in which the NaCl in the normal Krebs solution was replaced by equimolar KCl. Silodosin (Recordati, Milan, Italy), tamsulosin (Sigma-Aldrich, Milan, Italy) and prazosin (Sigma Aldrich) were used. Silodosin and tamsulosin were dissolved in ethanol (Merck Chemicals, Darmstadt, Germany), prazosin in methanol (Merck Chemicals) and kept as stock solutions (10^{-2} M). Silodosin (0.1 or 0.3 mg kg^{-1}), tamsulosin (0.01 or 0.03 mg kg^{-1}) or prazosin (0.03 or 0.1 mg kg^{-1}) were given i.v. as a single dose as soon as the ureter recovered a stable, post obstruction, spontaneous peristaltic activity for at least 5 min. The dosing range in rats was estimated according to the allometric scaling calculation of metabolic rate and body mass using the clinical dosage in humans (Sharma and McNeill, 2009). In separate experiments, control animals ($n = 5$ for each drug) were injected i.v. with the appropriate vehicles.

Calculations

Values are given as mean ± SEM. For multiple comparisons, Student Newman–Keuls analysis of variance was used. Pairwise and non-pairwise comparisons were made by Student's t-test. All statistical calculations were based on the number of individual animals. Differences were considered significant when $P < 0.05$.

Results

In vivo *ureteral pressures at baseline and after obstruction*

For each animal, baseline autonomous peristaltic ureteral activities were recorded before obstruction ($n = 55$). MinP and MaxP amounted to 18.7 ± 0.9 cmH_2O and 36.2 ± 1.6 cmH_2O respectively. The amplitude and frequency of the peristaltic pressure waves were 16.1 ± 1.2 cmH_2O and 3.8 ± 0.2 contractions per minute, and the AUC was 22.5 ± 0.9 $cmH_2O \cdot s^{-1}$. Upon obstruction of the ureter ($n = 50$), MinP and MaxP increased to 41.8 ± 1.9 cmH_2O ($P < 0.001$) and 57.2 ± 2.1 cmH_2O ($P < 0.001$), respectively and the AUC increased by approximately 50% to 45.3 ± 1.8 $cmH_2O \cdot s^{-1}$ ($P < 0.001$). Obstruction did not affect the amplitude of peristaltic pressure waves (14.6 ± 1.3 cmH_2O, $P = 0.1$), and no changes were noted for frequency (3.8 ± 0.2 contractions per minute). Baseline mean arterial blood pressure amounted to 120.6 ± 3.4 cmH_2O and 120.4 ± 3.5 cmH_2O after obstruction.

In vivo *effects of α_1-AR antagonists on the obstructed ureter*

None of the α_1-AR antagonists altered the amplitude or frequency of peristaltic pressure waves after obstruction (data not shown). The results of the different α-blockers on ureteral MinP, MaxP, AUC and MAP are shown in Figure 2.

Silodosin (i.v.) reduced the obstruction-induced increases in MinP (Figure 2A) by 27.7 ± 7.6% (0.1 mg kg^{-1}; $n = 6$; $P < 0.05$ vs. obstruction) and 20.8 ± 3.3% (0.3 mg kg^{-1}; $n = 6$;

$P < 0.01$ vs. obstruction). Similarly, prazosin reduced MinP by 28.8 ± 6.3% (0.03 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction) and 30.8 ± 6.3% (0.1 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction). Corresponding effects by tamsulosin on MinP were 8.6 ± 2.4% (0.01 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction) and 14.9 ± 3.4% (0.03 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction). Effect by 0.1 mg kg^{-1} of silodosin on MinP was similar to prazosin (0.03 mg kg^{-1}; $P = 0.9$) but larger that tamsulosin (0.01 mg kg^{-1}; $P < 0.05$). When comparing the inhibitory effects on MinP by the highest investigated doses of the α_1-AR antagonists, no differences were noted.

Silodosin treatment appeared to dose-dependently reduce the obstruction-induced increases in MaxP (Figure 2B) by 28.2 ± 4.3% (0.1 mg kg^{-1}; $n = 6$; $P < 0.01$ vs. obstruction) and 37.2 ± 4.4% (0.3 mg kg^{-1}; $n = 6$; $P < 0.001$ vs. obstruction). Prazosin reduced MaxP by 40.4 ± 6.8% (0.03 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction) and 18.4 ± 3.6% (0.1 mg kg^{-1}; $n = 7$; $P = 0.01$ vs. obstruction). The effects by tamsulosin on MaxP were 10.9 ± 2.7% (0.01 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction) and 20.49 ± 3.9% reduction (0.03 mg kg^{-1}; $n = 7$; $P < 0.001$ vs. obstruction). Overall, the effect of 0.1 mg kg^{-1} of silodosin on MaxP was similar to prazosin (0.03 mg kg^{-1}; $P = 0.2$) but better than tamsulosin (0.01 mg kg^{-1}; $P < 0.01$). Nonetheless, at the highest investigated doses, silodosin exerted a greater inhibitory effect on MaxP than prazosin and tamsulosin (all $P < 0.05$).

Silodosin (i.v.) also reduced the obstruction-induced increases in AUC (Figure 2C) by 28 ± 7.3% (0.1 mg kg^{-1}; $n = 6$; $P < 0.01$ vs. obstruction) and 20.1 ± 3.1% (0.3 mg kg^{-1}; $n = 6$; $P < 0.01$ vs. obstruction). This effect was comparable to prazosin that diminished AUC by 27.7 ± 7.3% (0.03 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction) and 24.7 ± 5.5% (0.1 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction). Corresponding effects by tamsulosin on AUC were a reduction of 10.4 ± 2.4% (0.01 mg kg^{-1}; $n = 7$; $P < 0.01$ vs. obstruction) and 15.1 ± 2.5% (0.03 mg kg^{-1}; $n = 7$; $P < 0.001$ vs. obstruction). Effect by 0.1 mg kg^{-1} of silodosin on AUC was similar to prazosin 0.03 mg kg^{-1} ($P = 0.9$) but greater than tamsulosin 0.01 mg kg^{-1} ($P < 0.05$). When comparing the inhibitory effects on AUC by the highest investigated doses of the different α1-AR antagonists, no differences were noted.

Silodosin 0.1 mg kg^{-1} reduced the MAP (Figure 2D) to 108.7 ± 5.5 cmH_2O (11.7 ± 4.4%, $n = 6$; $P = 0.07$ vs. obstruction) and to 97.3 ± 9.7 cmH_2O (10.2 ± 2.5%, 0.3 mg kg^{-1}; $n = 6$; $P < 0.05$ vs. obstruction). Prazosin 0.03 mg kg^{-1} diminished MAP to 90.1 ± 7 cmH_2O (25.4 ± 4%; $n = 7$; $P = 0.001$ vs. obstruction) and to 95.9 ± 11.5 cmH_2O (26.4 ± 3.9% 0.1 mg kg^{-1}; $n = 7$; $P < 0.001$ vs. obstruction). Similarly, tamsulosin 0.01 mg kg^{-1} lowered MAP to 105.7 ± 4.7 cmH_2O (25.1 ± 3.1% $n = 7$; $P < 0.01$ vs. obstruction) and to 100.4 ± 3.3 cmH_2O (17.5 ± 1.6%, 0.03 mg kg^{-1}; $n = 7$; $P < 0.001$ vs. obstruction). At the investigated doses, effects by silodosin on MAP were less than those of prazosin and tamsulosin (all $P < 0.05$). When expressed as a function of MAP (percent effect per cmH_2O reduction of MAP; Figure 3), silodosin demonstrated at the investigated doses significantly better inhibition of obstruction-induced increases in ureter pressure parameters than did tamsulosin or prazosin. The gain of effect for silodosin (Figure 3) ranged from six to eightfold higher than tamsulosin and from 2.5- to eightfold higher than prazosin for MaxP, MinP and AUC.
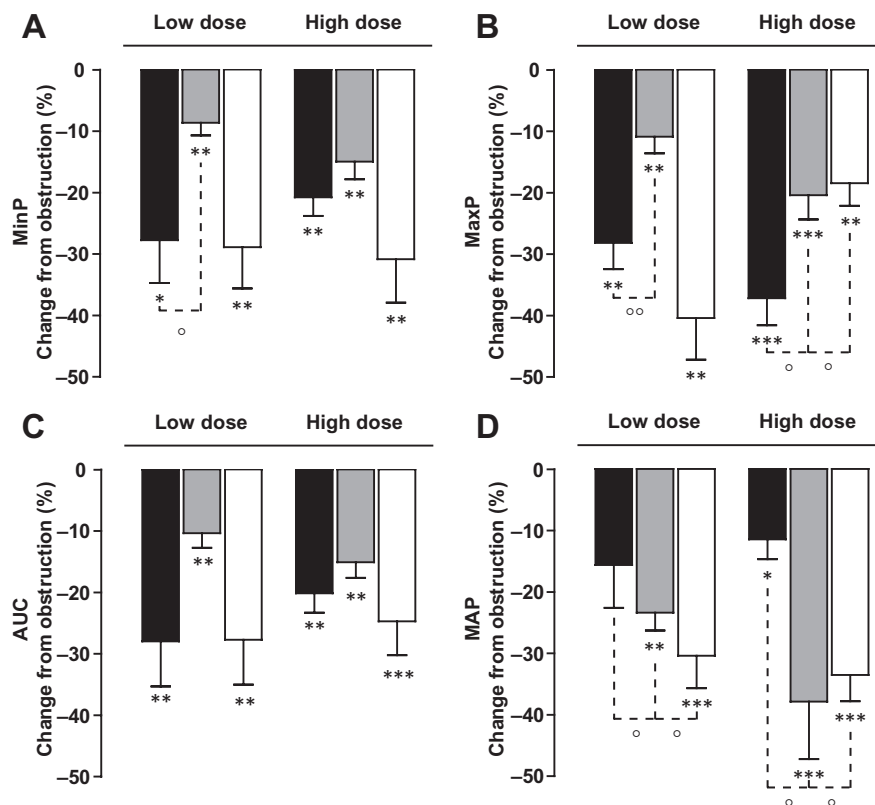
Figure 2

Ureter pressure parameters *in vivo*. Inhibitory effects of different doses of silodosin (black bars), tamsulosin (grey bars) or prazosin (white bars) on minimal (A) and maximal (B) ureter pressure, and AUC (C). Data are expressed as percent reduction of the increase in intraluminal ureter pressure due to obstruction. Panel D shows the effect of the investigated α_1-AR antagonists on mean arterial pressure (MAP) expressed as percent reduction of MAP before drug administration. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus obstruction alone. °$P < 0.05$ °°$P < 001$ versus silodosin, two-way Student's *T*-test.

In vitro *activities of α_1-AR antagonists on rat and human isolated ureteral tissues*

All α_1-AR antagonists exhibited concentration-dependent inhibitory effects on EFS-induced contractions of isolated rat (Figure 4A) and human (Figure 4B) ureters. Inhibitory effects by silodosin on EFS-induced contractions of the rat ureter amounted to $7 \pm 5\%$ (10^{-7} M), $27 \pm 15\%$ (10^{-6} M), $74 \pm 8\%$ (10^{-5} M, $P < 0.01$ vs. tamsulosin and prazosin), with a maximal effect of $88.9 \pm 7\%$ obtained at the highest investigated concentration of 10^{-4} M ($P < 0.05$ vs. tamsulosin). Tamsulosin showed no inhibitory effect on EFS-induced contractions of the rat ureter at 10^{-7} M, whereas the drug inhibited contractions by $12 \pm 6\%$, $16 \pm 6\%$ and $42.6 \pm 12\%$ at 10^{-6} M, 10^{-5} M and 10^{-4} M respectively. Prazosin inhibited EFS-induced contractions of the rat ureter only at 10^{-5} M and 10^{-4} M by $31 \pm 12\%$ and $76 \pm 9\%$ ($P < 0.05$ vs. tamsulosin) respectively. The calculated $-\log IC_{30}$ values of the different α_1-AR antagonists amounted to 5.98 ± 0.30 (silodosin, $P < 0.05$ vs. tamsulosin), 4.61 ± 0.46 (tamsulosin) and 5.39 ± 0.41 (prazosin). The order of potency and efficacy at the currently investigated concentrations for the drugs on the rat ureter may be summarized as silodosin = prazosin > tamsulosin.

In isolated human ureteral preparations (Figure 4B), silodosin inhibited contractions by $17 \pm 7\%$ (10^{-7} M), $24 \pm 8\%$ (10^{-6} M; $P = 0.05$ vs. prazosin), $38 \pm 9\%$ (10^{-5} M) and $74 \pm 6\%$ (10^{-4} M; $P < 0.05$ vs. prazosin and tamsulosin). Tamsulosin (Figure 4B) exhibited inhibitory effects of $17 \pm 5\%$ (10^{-7} M), $27 \pm 5\%$ (10^{-6} M), $37 \pm 9\%$ (10^{-5} M) and $43 \pm 10\%$ (10^{-4} M). Prazosin, inhibited contractions by $5 \pm 2\%$ (10^{-7} M), $6.1 \pm 6\%$ (10^{-6} M), $14.8 \pm 3\%$ (10^{-5} M) and $38.5 \pm 9\%$ (10^{-4} M). The $-\log IC_{30}$ values amounted to 6.22 ± 0.53, 5.64 ± 0.49 and 4.32 ± 0.23 for silodosin, tamsulosin and prazosin ($P = 0.06$ vs. silodosin) respectively. For the human ureter, the order of potency at the currently investigated drug concentrations was silodosin = tamsulosin > prazosin, and the order of efficacy was silodosin > tamsulosin = prazosin. All together, silodosin appeared to perform better than tamsulosin and prazosin in reducing the EFS-induced ureteral contractions, both in rat and human isolated preparations. Vehicles did not exhibit any effects on EFS-induced contractions of the isolated rat or human ureters (not shown).

Discussion and conclusions

Medical expulsive therapy represents a valuable alternative to interventional approaches. Several clinical trials have investigated clinically the use of the $\alpha_{1A/D}$-selective α-blockers such
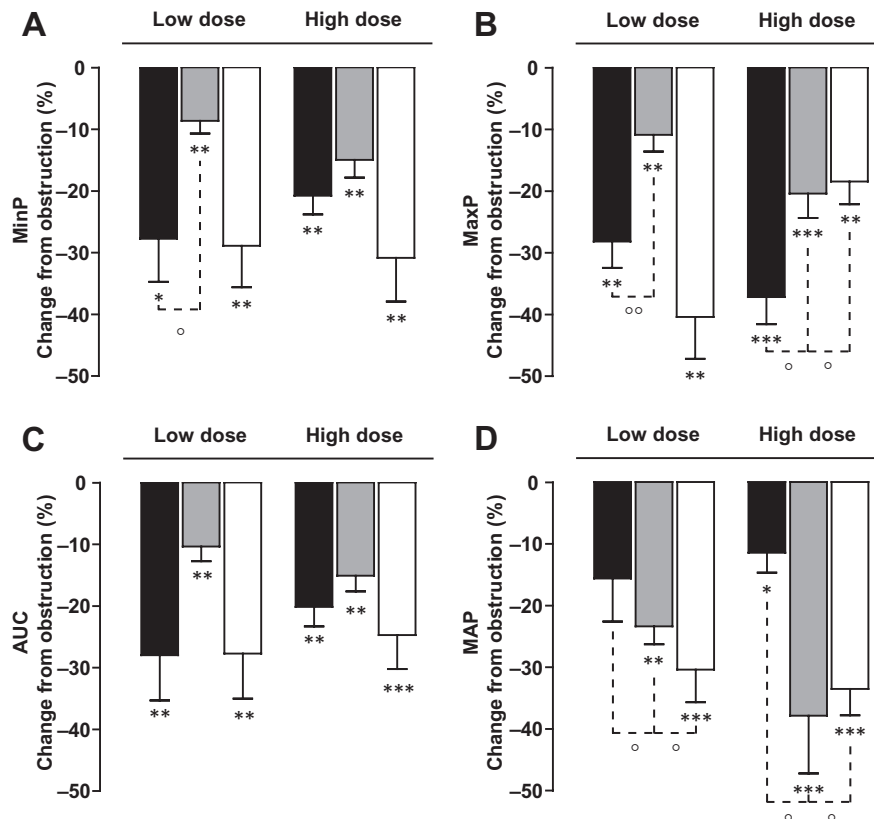
Figure 3

Ureter pressure parameters *in vivo*. Inhibitory effects of silodosin (black bars), tamsulosin (grey bars) or prazosin (white bars) on minimal ureter pressure (MinP) and maximal ureter pressure (MaxP) and AUC. Data are expressed as percent reduction of the increase in intraluminal ureter pressure due to obstruction per reduction of mean arterial pressure (MAP; cmH_2O) at the lowest (silodosin 0.1, tamsulosin 0.01, prazosin 0.03, A) and highest (silodosin 0.3, tamsulosin 0.03, prazosin 0.1, B) investigated doses ($mg \cdot kg^{-1}$). $°P < 0.05$ $°°P < 001$ versus silodosin, two-way Student's *T* test.

as tamsulosin, naftopidil and silodosin, demonstrating an overall benefit in enhancing stone expulsion. Given the evidence that generally α-blockers are comparably effective in treating ureteral obstructions, more studies are required to address the uroselectivity and the potential adverse effects that may help to select the most appropriate molecule. To this respect, appropriate animal modelling is critical towards achievement of meaningful results. Initial models of ureteral obstruction were specifically designed to mimic congenital hydronephrosis, not to evaluate *in vivo* ureter pharmacology (Ulm and Miller, 1962; Wen *et al.*, 1998) Different approaches in various species developed experimental models to study the ureteral peristalsis either in absence of obstruction (Becker *et al.*, 1998) or in a pharmacologically induced increase of intraluminal ureter pressure to the goal of evaluating the relaxant effects of selective $α_{1A\ D}$-AR antagonists (Kobayashi *et al.*, 2010). Even though previous studies demonstrated effective action of various α-blockers on ureteral relaxation and differential side effects at the circulatory level, we sought to develop an original rat model in which, while inducing a mechanical partial obstruction of the ureter, the spontaneous ureteral peristalsis activity were maintained and measurable. In the present study indeed, the ureter activity

was modulated only by a non-complete obstruction obtained by psoas muscle ligation, without pharmacological induction of ureteral contractions. We believe that this model better recapitulates the physio-dynamics of an obstructed ureter towards the evaluation of effective treatments for expulsive therapy in stone disease, which is after all a mechanically induced dysfunction. We definitely consider that this approach better simulates the *in vivo* pathophysiology of urolithiasis. Also, in order to avoid any interference on the peristaltic cycles or intraluminal pressures by retrograde pressure inclines originating from the bladder during micturitions, the bladder dome of the rats was notched open to equilibrate the internal back pressure. This procedure allows the measure of the genuine intra-ureteral pressure (IUP) and the spontaneous ureteral dynamics as previously described (Becker *et al.*, 1998).

While tamsulosin and alfuzosin have been reported to increase stones expulsion rate and reduce stone expulsion time in patients with urolithiasis (Agrawal *et al.*, 2009), some controversial data on the real-life efficacy of $α_1$-AR antagonists in ureteral stone disease have been more recently reported in a multicentre placebo-controlled clinical trial that demonstrated no significant effects by tamsulosin treatment
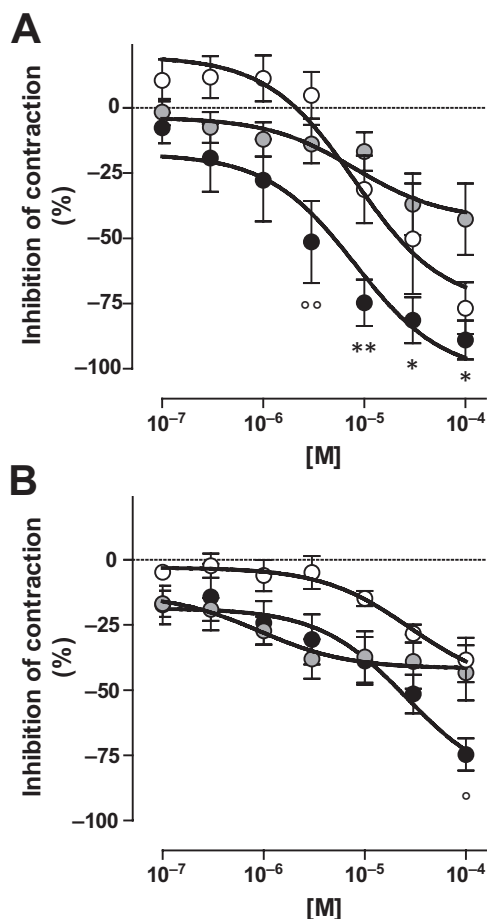
Figure 4

Contractions of isolated ureter preparations. Inhibition of contractions induced by electrical field stimulation (EFS; 0.5 ms, 50 V, 20 Hz) of rat (A) and human (B) isolated ureter preparations upon exposure to 10^{-7} to 10^{-4} M of silodosin (●), tamsulosin (◉) or prazosin (○). Effects by the investigated drugs on contractions induced by EFS are expressed as percent inhibition of contractions before drug treatment. (*$P < 0.05$ **$P < 0.01$ versus tamsulosin, °$P < 0.05$ °°$P < 001$ vs. prazosin; two-way ANOVA, Bonferroni post test). Non-linear regression calculation and statistical comparisons were made by using Graphpad Prism 5.0 software.

on acceleration of stone expulsion in patients with ureteral colic (Vincendeau *et al.*, 2010). Although these last findings show a not significant numerical superiority of tamsulosin compared to placebo in facilitating stone expulsion, successive clinical reports suggest that α_{1A}-AR antagonists, such as silodosin, perform better than α_{1D}-specific blockers such as naftopidil in the medical expulsion therapy for ureteral stone in a Japanese population (Itoh *et al.*, 2011; Tsuzaka *et al.*, 2011). There seem to be some discrepancies in the efficacy rates of α_1-AR antagonists in the therapy of ureteral stone, which deserve further clinical investigations and possibly more accurate patient stratification (Vincendeau *et al.*, 2010). Even so, our present results are supportive of a beneficial effect of α_1-AR antagonists in improving peristaltic dynamics in obstructed ureters *in vivo* and that α_1-AR antagonists modify contractile functions of the isolated rat and human

ureter. Our findings show that, at doses approximately corresponding to normal and triple human therapeutic use, each tested drug significantly reduced *in vivo* the obstruction-induced increase of IUP, without affecting contraction frequency or wave amplitude. Hence, following systemic administration of α_1-AR antagonists, a main effect seems to be reduction of intraluminal pressure burden without altering spontaneous peristaltic work by the ureter. It may be speculated if this reduces the metabolic demand on the obstructed ureter with improved propulsive capacity. If translatable to the clinical situation, this might lead to an easier and less painful passage of the ureteral stone.

In the current investigation, the α_{1A}-AR selective antagonist silodosin seemed to display a better efficacy-to-safety ratio compared to tamsulosin and prazosin. Significant reductions of MAP were always observed immediately after i.v. administration of prazosin and tamsulosin, while silodosin exerted a smaller hypotensive effect. Indeed, when the effect of the investigated drugs on ureteral pressures is reported as a function of the effect on MAP, silodosin exerted significantly larger inhibitory effect on IUP than prazosin and tamsulosin. These findings confirm the results obtained previously in anesthetized dogs (Kobayashi *et al.*, 2010) and strongly suggest that the α_{1A}-AR antagonist silodosin has a better *in vivo* uroselectivity than other α_1-AR antagonists. Several other preclinical reports also demonstrate that silodosin has a high uroselectivity, as shown by, for example, the tissue discrimination of its binding on liver and aortic α_{1A} and α_{1B} receptors versus prostate α_{1A}-ARs (Russo *et al.*, 2011). Previous data demonstrated a clear reduced affinity and selectivity of silodosin towards human vascular structures expressing α_{1B}-ARs, accounting for an approximately 200-fold less affinity compared to prostatic tissue. In addition, functional studies carried out on noradrenaline-induced tissue contraction also demonstrated that silodosin inherently possesses a very high affinity and selectivity for the prostate, mainly mediated by α_{1L}-AR subtype (Murata *et al.*, 2000), a functional phenotype of α_{1A}-AR. The reference drug tamsulosin also displayed good affinity for the prostate but a comparatively higher affinity for the mesenteric aorta was reported (Tatemichi *et al.*, 2006), suggesting relatively higher cardiovascular systemic side effects in comparison with silodosin.

Functional selectivity by silodosin towards the urinary system has also been described in other animal models, and upon systemic administration, silodosin has been reported to display little cardiovascular effects when compared to other α_1-AR antagonists. In addition, as previously reported in isolated ureteral tissues from animals (Kobayashi *et al.*, 2009b) and humans (Sasaki *et al.*, 2011), where silodosin and prazosin exerted similar effects in inhibiting phenylephrine-induced contraction on isolated ureter preparations, we further verify that silodosin has indeed a higher efficacy on EFS-induced contractions of isolated human and rat ureter than tamsulosin or prazosin.

As a limitation remark, however, we cannot completely exclude that, although human ureter specimens were evaluated by a pathologist as normal, any undiagnosed kidney or ureter dysfunctions might have influenced the ureter function.

Taken together, our data suggest that the α_{1A}-AR subtype, without interfering with the autonomous peristaltic activity,

plays an important role in the contractile function of the ureter. Since ureteral relaxation and maintenance of spontaneous contraction are main physiological requirements for enhancing stone passage, a pharmacological approach that selectively blocks α_{1A}-AR activity may represent a valid treatment option for patients with distal ureteral stones. Moreover, by targeting the α_{1A}-AR subtype, a minimal hypotensive systemic effect is possibly anticipated.

In our in vivo rat model of ureteral obstruction, silodosin reduces ureteral pressure with less systemic side effects than tamsulosin and prazosin. Silodosin exhibits better inhibitory efficacy on EFS-induced contraction of human and rat isolated ureters than tamsulosin and prazosin. Selective inhibition of the α_{1A}-AR subtype with silodosin should be further considered and evaluated clinically as an option for pharmacological expulsive therapy in patients with distal ureteral stones.

Acknowledgements

Conflicts of interest

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