Vardenafil Effect on Ureteric Smooth Muscle: In Vitro Study in Porcine Model

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Abstract

Introduction: Phosphodiesrase type 5 inhibitors have been recently reported to induce a relaxing effect on ureteral smooth muscle. We conducted an in vitro study to elucidate the relaxing effect of various doses of vardenafil on the porcine ureter. Moreover, we propose a porcine ureter model for the evaluation of the effect of different substances on the ureter.

Materials and Methods: A total number of 24 ureters were obtained by domestic pigs. The obtained ureteral specimens were immediately placed in Krebs solution. All specimens were cut into 4- to 5-mm-long tubular segments, which were mounted in 10 mL vertical chambers of an organ bath system. The same specific conditions were set to the organ bath device for all specimens. The tubular segments were connected to a force/pressure transducer device. After automatic ureteral contractions with stable frequency were achieved, different doses of vardenafil (0.1, 1, and 10 μM) were added to the bath chambers. Isometric responses of the tissues throughout the experiment were recorded and statistically analyzed.

Results: The administration of vardenafil resulted in reduction of both rate and tension of the ureteral contraction regardless of the dose of vardenafil. Nevertheless, statistical analysis revealed significantly reduced ureteral contraction rate and tension when vardenafil 1 or 10 μM was administered in comparison to the initial steady state.

Conclusion: Vardenafil concentrations of 1 and 10 μM should be considered as appropriate for ureteral relaxation. The porcine model replicates human ureteral response in vitro at least in the case of phosphodiesrase type 5 inhibitors and probably would be useful for the evaluation of other pharmaceutical agents.

Journal of Endourology, Volume 25, Number 3, March 2011
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Pp. 505-509
DOI: 10.1089/end.2010.0244

Introduction

Acute renal colic represents the most common symptom of urolithiasis in case of ureteral obstruction and has constantly increasing incidence in Western countries.1 Ureteral obstruction observed during renal colic is associated with inhibition of spontaneous passage of ureteral calculi. Several factors have an impact to the severity of the condition: stone size, configuration and location, smooth muscle spasm, as well as the local anatomy of the ureter.2 Medical expulsive therapy (MET) has been proposed as one of the options for the management of urolithiasis in several clinical scenarios. Numerous substances have been proposed for MET. The nonsteroidal anti-inflammatory drugs reduce inflammation and edema while decreasing ureteral contractions. Antimuscarinics (Buscopan, N-butylscopolamine) might induce genitourinary smooth muscle relaxation and subsequently reduce colic pain. Nevertheless, the above two categories of pharmacological agent do not have an impact on the expulsion rate of ureteral stones. On the contrary, methylprednisone has been observed to increase expulsion rates by anti-inflammatory action. Moreover, the calcium channel blockers nifedipine and verapamil reduce spontaneous rhythmic contractions of the human ureter, whereas the α-blockers inhibit ureteral musculature contractions, reduce basal muscle tone, and decrease peristaltic rate. The latter medication reduces colic pain and facilitates ureteral stone expulsion.3

Phosphodiesrase type 5 (PDE5) inhibitors represent a new class of drugs that have been developed for the treatment of erectile dysfunction and include the pharmacological substances of sildenafil, tadalafil, and vardenafil.4,5 Several clinical trials have suggested the potential to use PDE5 inhibitors not only for erectile dysfunction but also to expanded indications in conditions such as lower urinary tract symptoms caused by benign prostatic hyperplasia, urinary incontinence, premature ejaculation, Peyronie’s disease, and symptoms of female sexual dysfunction.6–10 PDE enzymes...
have been identified in human ureter and the inhibition of PDE4 and PDE5 isoenzymes revealed ureteral smooth muscle relaxing effect in a dose-dependent manner.\textsuperscript{11–13} The effect of PDE5 inhibitors was similar to those of tamsulosin, suggesting the potential use of these drugs for the management of renal colic and the possible use as MET.\textsuperscript{3} Further investigation on the effect of PDE5 inhibitors on the ureter, the clarification of the appropriate dose, and the evaluation of the clinical outcome is deemed necessary for the acceptance of these drugs as MET.

In this work we conducted an \textit{in vitro} study to elucidate the relaxing effect of various doses of vardenafil on porcine ureter. Moreover, we propose a porcine ureter model for the evaluation of the effect of different substances on the ureter.

**Materials and Methods**

**Animals and specimen removal**

A total number of 24 ureters were obtained from domestic pigs weighing between 20 and 25 kg that were kept in the facilities of our institution. The protocol was approved by the Institutional Animal Care Committee of our institution. Doses of anesthesia were administered to the pigs for euthanasia. The animals were dissected immediately, and the ureters were obtained.

**Organ bath conditions**

The obtained ureteral specimens were immediately placed in Ringer-Krebs solution, pH 7.4, composed of 120 mM NaCl, 25.6 mM NaHCO\textsubscript{3}, 4.7 mM KCl, 2.5 mM CaCl\textsubscript{2}, 1.2 mM NaH\textsubscript{2}PO\textsubscript{4}, 1.2 mM MgCl\textsubscript{2}, 22 mM glucose, and 0.1 mM 2Na\textsuperscript{+} (Ca\textsuperscript{2+}) ethylenediaminetetraacetic acid. The ureters were transported to the laboratory facilities within 5 minutes. The smooth muscle of the ureters was dissected free of fat and connective tissue. All specimens were cut into 4- to 5-mm-long tubular segments, which were mounted in 10 mL vertical chambers of an ML870B5/10 Panlab organ bath system (Fig. 1). Ureteral rings were obtained from proximal, middle, and distal segments of the ureter. The organ bath contained four chambers, and four samples from each ureter were evaluated simultaneously: one ring from proximal ureter, one ring from middle, and two rings from distal. The conditions of the organ bath were set according previous investigators.\textsuperscript{13,14} The tubular segments were mounted between two hooks. One of the hooks was connected by a string to a force/pressure transducer device (Fig. 2). The solution contained in the chambers was continuously gassed with 95% O\textsubscript{2} to 5% CO\textsubscript{2} while the temperature was 37°C. The pretension of the specimens was set at 4 g. The latter tension was found optimal for this preparation in preliminary experiments of other investigators.\textsuperscript{13} The musculature was then exposed to KCl (80 mM). The ureter was left to equilibrate in the above stable conditions for 60 minutes and to achieve a steady rate of automatic contractions. The isotonic motor activity (contractions) of the ureteral segments were recorded by pressure transducers and recorded by a computer with Labchart software (ADInstruments Ltd., Oxfordshire, United Kingdom). After stable contraction frequency was reached, one of the three available doses of vardenafil was added to the bath chambers. Each concentration was evaluated on eight ureters. Isometric responses of the tissues were recorded and analyzed.

**Vardenafil solution**

Vardenafil provided by Bayer Vital GmbH, Pharmaceutical Business Unit (Leverkusen, Germany), and made up as stock solution (10 mM) using saline and dimethylsulfoxide and further diluted with Krebs solution. Three doses of vardenafil solution were used: 0.1, 1, and 10 \textmu M.

**Statistical analysis**

LabChart software was used for the analysis of the tension recordings. Amplitude (tension) mean values and standard

![FIG. 1. The organ bath device in full function.](image1)

![FIG. 2. A tubular ureteral segment placed between hooks during the experiments.](image2)
deviations were calculated with the aid of Spike Histogram module, whereas activity rates (contraction rate) were calculated using the intrinsic Cyclic Measurements functions. Peak recognition was fine-tuned for each specimen and/or phase of the experiment so as to avoid counting erratic behavior.

The mean value and SD of peak amplitude was calculated for each specimen and substance concentration, both before and after vardenafil administration. Accordingly, one activity rate value, calculated as the number of peaks divided by the duration of activity and expressed in beats per minute, was registered for each phase of the experiment.

The comparisons were performed using a 5% significance level, as follows:

(i) With regard to the presence of substance: Within the same specimen, the tension amplitude registered after the administration was compared with that under clean solution, using t-test.

(ii) With regard to the substance concentration: For tension amplitudes, the difference between mean amplitude in a clean solution and in the presence of substance was calculated for each concentration. Then, the mean of differences for each concentration was tested using one-sample t-test and Wilcoxon’s signed rank test.

Mean activity rates across concentrations were compared using analysis of variance and its nonparametric form (Kruskal–Wallis test).

Results

The spike histograms obtained by LabChart software revealed almost uniform effect of vardenafil in ureteral contractile function. All specimens initially reached a steady contraction rate. The administration of vardenafil resulted in reduction of both rate and tension of the ureteral contraction regardless of the dose of vardenafil (Figs. 3 and 4). Nevertheless, statistical analysis revealed significantly reduced ureteral contraction rate and tension when vardenafil 1 or 10 μM was administered in comparison to the initial steady state. The results of the study are summarized in Table 1.

Discussion

Urolithiasis affects ~15% of men and 7% of women, whereas renal colic is the most common presentation of urolithiasis at the emergency department. The pathophysiology of renal colic is directly related to ureteral obstruction by a stone that leads to a cascade of events such as edema, inflammation, increased intraluminal pressure proximal to the obstruction, and stimulation of ureteric smooth muscle contraction to overcome the obstructing stone.16 The incident of acute pain is usually managed and controlled by analgesia. Nevertheless, further therapeutic approaches include conservative or interventional management to relieve the obstruction. The conservative approach is based on pharmaceutical agents that possess the potential to relax the ureteral smooth muscle, temporarily control the pain, and facilitate the spontaneous stone passage without major systemic complications. Recent studies have reported the benefits to manage ureteral stones using MET.3 These studies have shown a significant increase in the spontaneous stone passage, decreased pain, and shorten the time to stone passage.

PDE5 inhibitors are considered as first-line treatment option of erectile dysfunction. PDE enzymes of at least 11 types have been detected throughout the body and are responsible for a variety of functions. Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are intracellular messenger molecules that regulate cellular response to extracellular stimulation. Intracellular high levels of cAMP and cGMP lead to a complex cascade of events that
result in smooth muscle relaxation. PDE isoenzymes are responsible for the degradation of intracellular cyclic nucleotides. PDE inhibitors increase the intracellular concentration of cyclic nucleotides and cause smooth muscle relaxation. PDE isoenzymes 4, 7, and 8 are specific for cAMP, whereas PDE 5, 6, and 9 are specific for cGMP. The remaining isoenzymes of the PDE family have action on both cyclic nucleotides. PDE inhibitors increase the intracellular concentration responsible for the degradation of intracellular cyclic nucleotides and cause smooth muscle relaxation.

The presence of PDE1, 2, and 4 in the human ureter has been detected. The inhibition of the above enzymes led to relaxation of the ureter. Rolipram, which is a PDE4 inhibitor, and forskolin, an adenylate cyclase activator, induced pig ureter relaxation. Moreover, Kuhn et al. revealed that rolipram and E 4021 (a PDE5 inhibitor) reduced pig ureter relaxation. Kuhn et al. proposed that the use of selective PDE inhibitors especially for isoenzymes 4 and 5 might be favorable in the management of ureteral stones and ureteral colic. Gratzke et al used sildenafil, vardenafil, and tadalafil on human ureteral strips inserted in the chambers of organ bath device. The strips were pretensioned, PDE5 inhibitors were added incrementally to the specimens, and ureteral relaxation was observed. The reversion of tissue tension was dose dependent, whereas the tissue cGMP levels were increased threefold or fourfold by the use of PDE5 inhibitors. The authors concluded that PDE5 inhibitors provide relaxation of the human ureter by cGMP-mediated pathway.

In the current study, we attempted to confirm the effect of PDE5 inhibitor vardenafil in the contractile function of the ureter and to determine the appropriate dose for the latter effect. We selected to propose a porcine model for the evaluation of vardenafil in an attempt to standardize a method for further evaluation of effect of different pharmaceutical substances to the ureter. Although Gratzke et al. evaluated PDE5 inhibitors directly on the human ureteral tissue, the porcine model provides wide tissue availability and could be used for extensive evaluation of ureteral response in several other substances. The current results are in accordance with those obtained by the administration of PDE5 inhibitors to the human ureters.

Table 1. Statistical Analysis of Tension and Contraction Rate Results Between the Different Concentrations of Vardenafil Used

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean ± SD</th>
<th>t-test (p-value)</th>
<th>Wilcoxon test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vardenafil 0.1 μM</td>
<td>0.0144 ± 0.2271</td>
<td>0.9431</td>
<td>1.00</td>
</tr>
<tr>
<td>Vardenafil 1 μM</td>
<td>2.238 ± 1.399</td>
<td>0.0112</td>
<td>0.0313</td>
</tr>
<tr>
<td>Vardenafil 10 μM</td>
<td>1.760 ± 1.651</td>
<td>0.0082</td>
<td>0.048</td>
</tr>
</tbody>
</table>

The same concentrations (1 and 10 μM) of the vardenafil were observed to have the highest impact on the ureter. Thus, it could be advocated that the porcine model replicates human ureteral response in vitro at least in the case of PDE5 inhibitors and probably would be useful for the evaluation of other pharmaceutical agents on the ureteral contractile function. Vardenafil concentrations of 1 and 10 μM should be considered as appropriate for ureteral relaxation and further evaluation in a clinical setting could also be considered.

Disclosure Statement

No competing financial interests exist.

References


Abbreviations Used

cAMP = cyclic adenosine monophosphate
cGMP = cyclic guanosine monophosphate
MET = medical expulsive therapy
PDE5 = phosphodiesterase type 5

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