Synergistic Effect by Co-Administration of Tamsulosin and Solifenacin on Bladder Activity in Rats

Saori NISHIJIMA,1 Kimio SUGAYA,1* Katsumi KADEKAWA,1 Katsuhiro ASHITOMI,1 Akiyoshi OHTAKE,2 Masao SASAMATA,2 and Hideyuki YAMAMOTO3

1Southern Knights’ Laboratory LLP, Okinawa, Japan, 2Applied Pharmacology Research Labs, Astellas Pharma Inc., Ibaraki, Japan, and 3Department of Biochemistry, University of the Ryukyus, Okinawa, Japan

Objectives: We examined the effects of alpha1-adrenoceptor antagonist (tamsulosin hydrochloride) and antimuscarinic agent (solifenacin succinate) alone or in combination on the urinary adenosine triphosphate (ATP) level and cystometric parameters before and after bladder stimulation.

Methods: Female rats were administered tamsulosin hydrochloride (0.5 or 3 μg/kg/h) and/or solifenacin succinate (20 or 100 μg/kg/h) via a subcutaneously implanted osmotic minipump. Rats receiving distilled water were used as control. After 2 weeks, continuous cystometry with physiological saline or 0.1% acetic acid solution was performed. Urinary ATP level was also measured before and after stimulation by 0.1% acetic acid solution.

Results: During cystometry with bladder stimulation, the interval between voiding became shorter and the maximum voiding pressure (MVP) became higher in the control group. In the high-dose tamsulosin and solifenacin groups, the inhibition of urinary frequency was observed. The MVP also became higher in the high-dose tamsulosin group, but such a change was not seen in the high-dose solifenacin group. In case of low-dose administration, either agent alone did not inhibit the increase of urinary frequency and MVP due to bladder stimulation. However, co-administration of these ineffective low doses of tamsulosin and solifenacin resulted in the inhibition of urinary frequency. The high-dose or low-dose solifenacin group and the co-administration group showed similar inhibition of the increase of urinary ATP after bladder stimulation.

Conclusion: Tamsulosin may have a different effect on the bladder and/or the neuronal pathways that is unrelated to ATP, so the combination of tamsulosin and solifenacin may synergistically inhibit urinary frequency after bladder stimulation.

Key words bladder activity, solifenacin, tamsulosin, urinary adenosine triphosphate (ATP)

1. INTRODUCTION

Benign prostatic hyperplasia (BPH) is an extremely common condition among elderly men, and has been reported to occur in up to 70% of men older than 60 years.1 BPH is thought to cause symptoms due to bladder outflow obstruction and these are often referred to as lower urinary tract symptoms. Such symptoms, especially storage symptoms, are bothersome to the patient, interfere with daily activities and have a negative impact on the quality of life.2,3 Storage symptoms are mainly attributable to detrusor instability, which is thought to occur in up to 40–60% of patients with urethral obstruction due to BPH or bladder outlet obstruction.4 Alpha1-adrenoceptor antagonists are the medications of first choice for the treatment of BPH. These drugs relax the smooth muscle in the prostate/urethra and decrease resistance to urine flow in the prostatic urethra.5,6 Alpha1-adrenoceptor antagonists improve voiding disorders as well as storage disorders.7 Moreover, the combination of alpha1-adrenoceptor antagonists and antimuscarinic agents has been reported to be more effective than an alpha1-adrenoceptor antagonist alone for controlling overactive bladder in BPH patients.8–10

Recently, the role of the bladder epithelium in overactive bladder has attracted attention. It has been reported that several receptors including adrenergic and muscarinic receptors are expressed in the bladder epithelial, that these cells produce acetylcholine, nitric oxide, prostaglandin and adenosine triphosphate (ATP), and that afferent nerve terminals in the bladder have muscarinic and purinergic receptors.11,12 Muscarinic receptors can modulate ATP release, and muscarinic mechanisms may also have a role in urothelial sensory function.11,13,14 Furthermore, it has been reported that ATP secreted from bladder epithelial cells influences bladder activity, and
an alpha1-adrenoceptor antagonist has been shown to inhibit ATP secretion from the bladder epithelium in vitro.\textsuperscript{13} These results suggest that alpha1-adrenoceptor antagonists and anti-muscarinic agents have a dual role in alleviating bladder storage disorders.

In the present study, therefore, in order to examine synergistic effect of tamsulosin hydrochloride (an alpha1-adrenoceptor antagonist) and solifenacin succinate (an antimuscarinic agent), we compared the effects of these two agents alone or in combination on the urinary ATP level and cystometric parameters before and after bladder stimulation in rats.

2. METHODS

Eighty-four female Sprague–Dawley rats weighing 205–238 g were used in this study. We performed two experiments. The study protocol was approved by the Institute for Animal Experiments, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan.

2.1. Experiment 1 (high-dose administration)

The rats were divided into three groups, which were a control group (n = 12), a high-dose tamsulosin group (n = 12), and a high-dose solifenacin group (n = 12). Rats in the high-dose tamsulosin group and high-dose solifenacin group received an infusion of tamsulosin hydrochloride (3 \( \mu \)g/kg/h) or solifenacin succinate (100 \( \mu \)g/kg/h), while those in the control group received distilled water. Drugs were infused via a subcutaneous Alzet osmotic minipump that was implanted under 2% isoflurane anesthesia, because that oral administered solifenacin is immediately metabolized to active metabolite M1 but subcutaneous administration of solifenacin can keep the AUCu (area under the plasma concentration curve for the unbound compound) of clinical dosage. All rats were treated for 2 weeks, and had free access to diet and tap water during that period. After 2 weeks, 21 rats (7 per group) were anesthetized with urethane (0.8 g/kg subcutaneously and 0.4 g/kg intraperitoneally) and a small-bore polyethylene catheter (PE50; Clay-Adams, Parsippany, NJ, USA) was inserted into the bladder through the urethra for continuous cystometry. Physiological saline was infused into the bladder (0.05 mL/min) and bladder activity was monitored via the catheter, which was connected to a pressure transducer and a saline infusion pump through a three-way stopcock. Cystometry was continued for at least 60 min, and the interval between voiding, the baseline intravesical pressure, and the maximum voiding pressure were measured during the final 30 min. After continuous cystometry with physiological saline had been completed, the rats also underwent continuous cystometry with 0.1% acetic acid solution. Cystometry was again continued for at least 60 min, and the interval between voiding, the baseline intravesical pressure, and the maximum voiding pressure were measured during the final 30 min. Residual urine volume was also measured after the last bladder contraction.

Urine samples were collected from the remaining 15 rats (5 per group). Each rat was placed on a clean board and spontaneously voided urine was collected carefully. Then the rats were anesthetized with 2% isoflurane, and 1 mL of 0.1% acetic acid solution was infused into the bladder via a catheter for 10 min to provide stimulation. Rats received 100 mg of cefazolin sodium hydrate (Astellas, Tokyo, Japan) subcutaneously to prevent urinary tract infection. Spontaneously voided urine was also collected at 4–6 h (day 0) after recovery from isoflurane anesthesia, as well as on day 1, day 2, day 3, and day 7 after bladder stimulation. The urinary ATP level was measured with an ATP Hygiene Kit HS (Bio Thema AB, Haninge, Sweden) and Gene Light 55 (Microtec Nition, Funabashi, Japan), and the results were corrected by the urinary creatinine (CRE) level.

2.2. Experiment 2 (low-dose administration)

The rats were divided into four groups, which were a control group (n = 12), a low-dose tamsulosin group (n = 12), a low-dose solifenacin group (n = 12), and a co-administration group. Rats in the low-dose tamsulosin group and low-dose solifenacin group received an infusion of tamsulosin hydrochloride (0.5 \( \mu \)g/kg/h) or solifenacin succinate (20 \( \mu \)g/kg/h), while those in the co-administration group received both tamsulosin hydrochloride (0.5 \( \mu \)g/kg/h) and solifenacin succinate (20 \( \mu \)g/kg/h). Rats from the control group received distilled water. Drugs were infused via a subcutaneous Alzet osmotic minipump by the same procedure as experiment 1. After 2 weeks, 28 rats (7 per group) were performed continuous cystometry under urethane anesthesia. Cystometry was continued for at least 60 min, and the interval between voiding, the baseline intravesical pressure, and the maximum voiding pressure were measured during the final 30 min. After continuous cystometry with physiological saline had been completed, the rats also underwent continuous cystometry with 0.1% acetic acid solution. Cystometry was again continued for at least 60 min, and the interval between voiding, the baseline intravesical pressure, and the maximum voiding pressure were measured during the final 30 min. Residual urine volume was also measured after the last bladder contraction.

Urine samples were collected from the remaining 20 rats (5 per group). Each rat was placed on a clean board and spontaneously voided urine was collected carefully. Then the rats were anesthetized, and 1 mL of 0.1% acetic acid solution was infused into the bladder via a catheter for 10 min to provide stimulation. Rats received 100 mg of cefazolin sodium hydrate subcutaneously to prevent urinary tract infection. Spontaneously voided urine was also collected at 4–6 h (day 0) after recovery from isoflurane anesthesia, as well as on day 1, day 2, day 3, and day 7 after bladder stimulation. The urinary ATP level was measured, and the results were corrected by the urinary CRE level.

Results were reported as the mean ± standard error of mean. Student’s paired or unpaired t-test was used.
3. RESULTS

In the experiment with high-dose administration, there were no significant differences of any of the cystometric parameters among the three groups during continuous cystometry with physiological saline. When continuous cystometry was done with 0.1% acetic acid solution, the maximum voiding pressure was significantly higher (9–14%, \( P = 0.005–0.010 \)) in control group and high-dose tamsulosin group compared with that during cystometry with physiological saline (Table 1). The interval between voiding was also significantly shorter (36%, \( P < 0.001 \)) than during cystometry with physiological saline in the control group, but not in the high-dose tamsulosin group. In the high-dose solifenacin group, however, there was no significant difference of any of the cystometric parameters before and after infusion of 0.1% acetic acid solution. There was no significant difference of baseline intravesical pressure before and after infusion of 0.1% acetic acid solution in all groups. There was a minimal amount of residual urine (<0.05 mL) in all rats.

The urinary ATP level corrected for CRE (urinary ATP/CRE ratio) showed no differences among the three groups before infusion of 0.1% acetic acid solution into the bladder (Fig. 1). After infusion of acetic acid, the urinary ATP/CRE ratio was significantly increased on day 0 (484 ± 203 mol/mg CRE × E-10, \( P = 0.005 \)), day 1 (131 ± 65 mol/mg CRE × E-10, \( P = 0.014 \)), day 2 (80 ± 39 mol/mg CRE × E-10, \( P = 0.014 \)), and day 3 (40 ± 1 mol/mg CRE × E-10, \( P < 0.001 \)) in the control group compared with that before infusion. In the high-dose tamsulosin group and high-dose solifenacin group, the ATP/CRE ratio was also significantly increased on day 0 (543 ± 112 mol/mg CRE × E-10, \( P = 0.001 \); 121 ± 51 mol/mg CRE × E-10, \( P = 0.002 \); respectively), day 1 (142 ± 54 mol/mg CRE × E-10, \( P = 0.018 \); 40 ± 9 mol/mg CRE × E-10, \( P < 0.001 \); respectively), and day 2 (63 ± 40 mol/mg CRE × E-10, \( P = 0.001 \); 27 ± 8 mol/mg CRE × E-10, \( P = 0.012 \); respectively) after bladder stimulation. However, the increase of urinary ATP/CRE ratio of the high-dose solifenacin group was significantly lower (\( P = 0.045 \) and \( P = 0.004 \); about one fourth to one fifth) on day 0 after acetic acid infusion compared with the control group and high-dose tamsulosin group. The urinary ATP/CRE ratio of each group returned to baseline after 7 days, and no significant differences were observed among the three groups.

In the experiment with low-dose administration, there were no significant differences of any of the cystometric parameters among the four groups during continuous cystometry with physiological saline. When continuous cystometry was done with 0.1% acetic acid solution, the maximum voiding pressure was significantly higher (6–12%, \( P = 0.002–0.046 \)) in all groups compared with that during cystometry with physiological saline.
The urinary ATP/CRE ratio showed no differences among the four groups before infusion of 0.1% acetic acid solution into the bladder (Fig. 2). After infusion of acetic acid, the urinary ATP/CRE ratio was significantly increased on day 0 (407 ± 209 mol/mg CRE × E-10, $P = 0.045$) in the control group compared with that before infusion. In the low-dose tamsulosin group, low-dose solifenacin group, and co-administration group, the urinary ATP/CRE ratio was also significantly increased on day 0 (443 ± 191 mol/mg CRE × E-10, $P = 0.034$; 231 ± 92 mol/mg CRE × E-10, $P = 0.021$; 219 ± 85 mol/mg CRE × E-10, $P = 0.039$; respectively) after bladder stimulation. However, the increase of urinary ATP/CRE ratio of the low-dose solifenacin group and co-administration group were lower (about one half, although not significant) on day 0 after acetic acid infusion compared with the control group and low-dose tamsulosin group. The urinary ATP/CRE ratio of each group returned to baseline after 3–7 days, and no significant differences were observed among the four groups.

### 4. DISCUSSION

In the present study, administration of high-dose or low-dose tamsulosin and/or solifenacin without bladder stimulation did not influence cystometric parameters or the urinary ATP level, suggesting that the dosages of these drugs had no effect on normal bladder function. The doses of tamsulosin (1 μg/kg/h, subcutaneous administration) and solifenacin (100 μg/kg/h, subcutaneous administration) were comparable to the clinically effective doses (tamsulosin; 0.2 mg/man, solifenacin; 10 mg/man) from the perspective of the plasma concentration AUCu (data not shown).

During cystometry with bladder stimulation by 0.1% acetic acid, the interval between voiding became shorter and the maximum voiding pressure became higher in the control group. The maximum voiding pressure also became higher in the high-dose tamsulosin group, but such a change was not seen in the high-dose solifenacin group. In case of low-dose administration, either agent alone did not inhibit the increase of urinary frequency and the maximum voiding pressure due to bladder stimulation. However, co-administration of these ineffective low

**TABLE 2.** Change of cystometric parameters by bladder stimulation in the control group, low-dose tamsulosin group, low-dose solifenacin group, and co-administration group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Interval (min)</th>
<th>MVP (cmH2O)</th>
<th>Baseline (cmH2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal saline</td>
<td>Acetic acid</td>
<td>Normal saline</td>
</tr>
<tr>
<td>Control</td>
<td>7.2 ± 1.8</td>
<td>5.3 ± 1.5x</td>
<td>35.3 ± 2.1</td>
</tr>
<tr>
<td>Low-dose tamsulosin</td>
<td>11.2 ± 2.3</td>
<td>6.7 ± 1.6x</td>
<td>37.2 ± 4.1</td>
</tr>
<tr>
<td>Low-dose solifenacin</td>
<td>7.1 ± 1.1</td>
<td>4.5 ± 0.8x</td>
<td>45.1 ± 6.2</td>
</tr>
<tr>
<td>Co-administration</td>
<td>8.6 ± 2.5</td>
<td>8.2 ± 1.5</td>
<td>43.2 ± 4.5</td>
</tr>
</tbody>
</table>

Mean ± standard error of the mean. $*P < 0.05$, $**P < 0.01$ compared with cystometry by normal saline. Interval, interval between voiding; MVP, maximum voiding pressure.
doses of tamsulosin and solifenacin resulted in the inhibition of urinary frequency. With regard to the urinary ATP level, the high-dose or low-dose solifenacin group and the co-administration group showed similar inhibition of the increase of urinary ATP after bladder stimulation. Therefore, tamsulosin may have a different effect on the bladder and/or the neuronal pathways controlling the bladder that is unrelated to ATP, so that the combination of tamsulosin and solifenacin may synergistically inhibit urinary frequency after bladder stimulation.

It is known that several receptors including adrenergic and muscarinic receptors are expressed in the bladder epithelium, that these cells produce acetylcholine and ATP, and that afferent nerve terminals in the bladder have both muscarinic and purinergic receptors.\(^{11,16}\) Therefore, autocrine activation may occur, in which acetylcholine produced by bladder epithelial cells activates these cells via their muscarinic receptors. When bladder epithelial cells are stimulated, it is thought that these cells produce acetylcholine and ATP, with autocrine activation by acetylcholine inducing further ATP production, after which acetylcholine and ATP activate afferent nerve terminals in the bladder and finally induce bladder over-activity. In this context, it has been reported that an alpha1-adrenoceptor antagonist naftopidil decreases ATP release from the bladder epithelium in vitro,\(^{15}\) and that both naftopidil and antimuscarinic agent propiverine (especially propiverine) inhibit the increase of urinary ATP after bladder stimulation.\(^{17}\) In the present study, solifenacin inhibited the increase of urinary ATP after bladder stimulation, but tamsulosin did not, suggesting that muscarinic receptors are highly related to ATP release compared with alpha1-adrenoceptors. Therefore, the ameliorative effect of solifenacin on urinary frequency may be partly due to blocking ATP release from the bladder epithelium, while the ameliorative effect of tamsulosin would not be related to ATP.

Generally, bladder stimulation affects the voiding frequency rather than the amplitude of micturition contraction,\(^{15,18}\) because ordinary micturition contraction can be evoked by a stimulus with an intensity just above the threshold level evoking the micturition reflex, while high-amplitude of micturition contraction probably require a more intense stimulus that activates many afferent and efferent components of the micturition reflex pathway. In the present study, co-administration of low (ineffective) doses of tamsulosin and solifenacin inhibited an increase in the voiding frequency, but did not prevent an increase of contraction amplitude. Tamsulosin inhibits the afferent limb of the micturition reflex pathway in the lumbosacral spinal cord, and increases the interval between voiding.\(^{19}\) Therefore, the lack of any change in the interval between voiding after bladder stimulation in rats receiving co-administration of low-dose tamsulosin and solifenacin might be explained by synergy between the spinal actions of tamsulosin and the bladder epithelial actions of solifenacin. However, it seems unlikely that tamsulosin acts at the spinal cord level, because its transfer to the brain is very low.\(^{20}\) Further investigations are thus required to clarify the mechanism.

ATP is a major neurotransmitter for both efferent and sensory neurons in the peripheral nervous system.\(^{21}\) Recent findings have supported the notion that epithelial tissues (including the urothelium) may be involved in bladder sensory mechanisms.\(^{22,23}\) Activation of the urothelium by pressure, stretch, or hypo-osmotic stimulation leads to the release of ATP from urothelial cells, both in culture and in vitro bladder system.\(^{23,24}\) It has been reported that assay of urinary ATP by the firefly luciferin-luciferase method allows rapid and simple assessment of renal function (especially tubular function), and that diabetic patients often demonstrate unusually high free ATP levels in the urine.\(^{25}\) In the present study, the urinary ATP level was increased by bladder stimulation with acetic acid, while solifenacin inhibited this increase of ATP. Although we need to exclude an increase of ATP due to bacterial infection or renal dysfunction,\(^{26}\) an increased urinary ATP level can possibly be used as a marker for activation of the bladder urothelium.

In conclusion, high-dose solifenacin and co-administration of tamsulosin and solifenacin (at low doses where each agent administered alone was ineffective) inhibited the afferent pathway from the bladder, presumably due to synergy between the inhibitory effect of ATP released by solifenacin, and the mechanism of tamsulosin which remains to be defined.

**DISCLOSURE**

We have no disclosure or financial support.

**REFERENCES**

9. Lee KS, Choo MS, Kim DY et al. Combination treatment with propiverine hydrochloride plus doxazosin controlled release gastrointestinal therapeutic system formulation for


14. de Groat WC. The urothelium in overactive bladder: passive bystander or active participant? *Urology* 2004; **64**: 7–11.


