Modulation of Bladder Afferent Activity by Propiverine and its Active Metabolites (M-1 and M-2) in Rats

Yoshihiro MATSUMOTO,1,2 Kazumasa TORIMOTO,2 Yukio HAYASHI,3 Masato NANRI,3 Mamoru KINIWA,3 Yoshihiko HIRAO,2 and Naoki YOSHIMURA1

1Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA, 2Department of Urology, Nara Medical University, Nara, Japan, and 3Taiho Pharmaceutical Co. Ltd., Tokyo, Japan

Objectives: To evaluate the effects of propiverine and its active metabolites (M-1 and M-2) on bladder function through modulation of afferent activity in rats.

Methods: Cystometry was performed in urethane anesthetized female rats. We examined the effects of intravesical administration of propiverine, M-1 and M-2 on bladder overactivity induced by oxotremorine-M (Oxo-M; non-selective mAChR agonist).

Results: Intravesical administration of Oxo-M (200 μM) elicited bladder overactivity as evidenced by decreased intercontraction interval (ICI) and pressure threshold (PT) without changing maximum voiding pressure or baseline pressure. These effects were blocked by intravesical administration of propiverine (30 μM) or M-2 (300 μM). Intravesical administration of M-1 (30 μM) alone increased ICI and PT, but did not prevent Oxo-M-induced decreases in ICI and PT.

Conclusion: These results suggest that propiverine and M-2 have anticholinergic effects on bladder afferent activity and that M-1 has an inhibitory effect through the mechanism other than muscarinic receptor modulation. Thus, clinical benefits of propiverine in patients with overactive bladder could be mediated by multiple actions of propiverine and its active metabolites.

Key words afferent pathway, metabolite, propiverine, rat, urinary bladder

1. INTRODUCTION

Propiverine hydrochloride (1-methyl-4-piperidyl diphenylprooxyacetate hydrochloride) is commonly used as a muscarinic acetylcholine receptor (mAChR) antagonist for the treatment of patients with overactive bladder (OAB).1,2 Propiverine is rapidly absorbed after oral administration and is extensively metabolized in the liver to several active metabolites such as M-1 (1-methyl-4-piperidyl diphenylprooxyacetate N-oxide) and M-2 (1-methyl-4-piperidyl benzilate N-oxide).3–6 The concentration of these metabolites in plasma of rats and human after oral administration of propiverine has been shown to be relatively high.3,4 It has also shown in in vitro and in vivo pharmacological studies that these metabolites exert anticholinergic and calcium channel antagonistic effects in the urinary bladder.5,6 However, it has not been fully characterized how propiverine and its active metabolites exert their therapeutic effects in OAB patients.

Antimuscarinics are considered to act on mAChRs in the detrusor that are stimulated by ACh released from parasympathetic nerves, thereby inhibiting voiding contractions. However, antimuscarinics can relieve symptoms such as frequency and urgency during the storage phase, suggesting the effects on other sites, including bladder afferent pathways. Recently, the changes in afferent signaling from the bladder have been considered to be an important etiology of bladder overactivity. mAChRs are present not only in the detrusor, but also in the urothelium of different species, including humans.7,8 Recent data showed that intravesical administration of mAChR agonists alters reflex bladder contractions by modulation of bladder afferent activity.9–11

In the present study we investigated the effects of propiverine and its active metabolites (M-1 and M-2) on baseline bladder activity and bladder overactivity induced by local mAChR stimulation in rats.

2. METHODS

2.1. Animals

Adult female Sprague–Dawley rats weighing 250–300 g (Hilltop; Scottsdale, PA, USA) were used. All experiments

Received 30 December 2011; revised 11 February 2012; accepted 5 March 2012.

© 2012 Wiley Publishing Asia Pty Ltd
were conducted in accordance with institutional guidelines and approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

2.2. Drugs

In the present study we used oxotremorine methiodide (Oxo-M; Sigma, St Louis, MO, USA) as a non-selective mAChR agonist. Propiverine hydrochloride and its metabolites (M-1 and M-2) were provided by Taiho Co., Ltd. All drugs were dissolved in saline to a final concentration and administered intravesically.

2.3. Cystometry

Rats were anesthetized with urethane anesthesia (1.0 g/kg, subcutaneously; Sigma). A midline abdominal incision was made and a catheter (PE-50) with a fire-flared tip was inserted into the urinary bladder from the dome and secured with a silk thread for bladder filling and pressure recording. A three-way stopcock was connected to the transvesical catheter to monitor bladder pressure. During cystometry, intercontraction interval (ICI), pressure threshold (PT), maximum voiding pressure (MVP), and baseline pressure (BP) were measured with a PowerLab unit and Chart software (AD Instruments, Colorado Springs, CO, USA).

2.4. Experimental protocols

Saline was continuously infused at a room temperature for at least 2 h at a rate of 0.04 mL/min to record cystometrograms during a control period. After baseline cystometry, vehicle (saline), propiverine, M-1, or M-2 was instilled intravesically for 30 min. Thereafter, 200 μM Oxo-M was added to the infusate to examine whether intravesical application of Oxo-M with vehicle, propiverine, M-1, or M-2 induced bladder overactivity. We selected this dose of Oxo-M because 200 μM Oxo-M elicited significant and consistent bladder overactivity as shown by a reduction in ICI and PT without apparent effects on the detrusor function (MVP or BP) in our previous experiments.10 We also used doses ranging 30–300 μM for propiverine, M-1 and M-2 because intravesical application at these concentrations had a significant effect while those over 300 μM directly affected detrusor function shown by reduced MVP and BP in our preliminary experiments (data not shown).

2.5. Statistical analysis

All data values are expressed as the mean ± standard error of the mean. P < 0.05 was considered significant. The overall comparisons among groups were performed using Prism software (GraphPad Software, San Diego, CA, USA). For each cystometric parameter, two or three measurements were averaged. A paired t-test was used to compare the cystometric parameters, before and after drug administration.

3. RESULTS

Intravesical administration of Oxo-M (200 μM) produced bladder overactivity (Fig. 1a) as evidenced by decreased ICI and PT (Table 1, 788.6 ± 74.1 to 469.0 ± 43.0 sec, 6.93 ± 0.40 to 5.75 ± 0.35 cmH2O, respectively). MVP or BP was not altered during Oxo-M instillation (Table 1).

Intravesical administration of propiverine alone (30 μM) had no effect on any cystometric parameters. Thereafter, concomitant application of propiverine and Oxo-M (200 μM) did not produce any changes in cystometric parameters, indicating that propiverine can suppress Oxo-M-induced bladder overactivity (Fig. 1b, Table 1).

Intravesical application of M-1 (30 μM) alone increased PT and ICI, although this effect was observed during two or three voiding cycles after administration of M-1. MVP or BP was not altered (Fig. 1c, Table 1). When Oxo-M was added to M-1 thereafter, PT and ICI were significantly decreased without affecting MVP or BP (Fig. 1c).

Intravesical application of M-2 (30 μM) alone had no effect on cystometric parameters, and concomitant application with Oxo-M significantly decreased PT and ICI.
(data not shown). Therefore, we used a high concentration (300 μM) of M-2 to confirm the effect of intravesical administration. No significant change was observed after administration of the high dose M-2 alone. However, concomitant application with Oxo-M did not produce any effect on cystometric parameters, indicating that the high concentration of M-2 suppressed Oxo-M-induced bladder overactivity (Fig. 1d, Table 1).

### 4. DISCUSSION

After oral administration, propiverine is rapidly metabolized to several active metabolites. Plasma concentrations of main active metabolites, M-1 and M-2, in rats and human after oral administration of propiverine are reportedly higher than the concentration of propiverine itself. Forty-eight hours after oral application of propiverine, the concentrations of these metabolites recovered in human urine were 12–17%. In agreement with these observations, it is plausible that these metabolites excreted in the urine may have some local effects on bladder activity and contribute to the clinical effects of propiverine in the treatment of OAB patients.

The present study demonstrated that intravesical administration of propiverine or M-2 suppressed bladder overactivity induced by intravesical administration of a non-selective mAChR agonist (Oxotremorine-M; Oxo-M). Our previous study demonstrated that Oxo-M induces bladder overactivity due to activation of mAChR because it was completely blocked by atropine and tolterodine, which are pure mAChR antagonists, despite that Oxo-M also reportedly has affinity for nicotinic ACh receptors. Therefore, it is likely that propiverine and M-2 have antagonistic activity against mAChR stimulation in the bladder. Meanwhile, local application of M-1 alone exhibited inhibitory effects on bladder activity. When Oxo-M was added intravesically to the M-1 infusate thereafter, bladder overactivity occurred, indicating that M-1 exerts inhibitory effects on baseline bladder activity with the mechanism other than that mediated by mAChR inhibition.

A previous study showed that muscarinic receptor binding activity of the M-2 metabolite was equipotent to that of propiverine, whereas muscarinic receptor binding activity of the M-1 metabolite was much less in rats. Thus, it was suggested that M-2 could also be responsible for the clinical effects of antimuscarinic actions of propiverine. In addition, it was reported that after oral application, although the concentration of M-2 was smaller than that of M-1 and propiverine in human plasma, M-2 concentration in urine was more than propiverine and M-1 concentrations. As shown in our previous and current studies, intravesical administration of Oxo-M decreased pressure threshold (PT) and intercontraction interval (ICI) without affecting baseline and voiding bladder contractility. Moreover, Oxo-M, propiverine or M-2 by itself had no effect on MVP or BP, which reflect detrusor and efferent nerve activity. Therefore, it is plausible that these drugs do not penetrate to the detrusor muscle layer to affect bladder contractility at least at the concentrations used in this study, and rather exert their effects through mAChR expressed in the urothelium and/or bladder afferent nerves located in the suburothelial layer to modulate afferent activity in the bladder. The current study thus suggests that, if M-2 contributes to the therapeutic effect of propiverine, M-2 could exert its antimuscarinic action locally through urothelially or suburothelially expressed mAChR, thereby suppressing bladder activity.

While propiverine exerted inhibitory effect with the dose of 30 μM, a high concentration (300 μM) of M-2 was required to completely inhibit the effect of Oxo-M. Recently, Yamada et al. showed that the binding activity of M-2 was roughly equipotent to that of propiverine, whereas M-1 was considerably less active than propiverine. We are not certain why a higher dose of M-2 was needed to inhibit Oxo-M-induced bladder overactivity. It is well known that propiverine has a calcium channel antagonistic activity in addition to its anticholinergic effect, although other antimuscarinic agents, such as darifenacin, solifenacin, tolterodine and trospium chloride are pure antimuscarinics. Propiverine suppresses L-type calcium channels in bladder smooth muscle from various species including human. It is therefore possible that this calcium antagonistic activity of propiverine is related to our results that Oxo-M-induced bladder overactivity was suppressed by propiverine with a lower dose than that of M-2. Another explanation could be that M-2 might need a higher concentration to penetrate the urothelial layer compared to propiverine or M-1. Further studies are needed to clarify these points.

---

**TABLE 1. Effects of intravesical administration of Oxo-M with or without propiverine or its metabolites (M-1, M-2) on cystometric parameters (n = 6)**

<table>
<thead>
<tr>
<th>Control</th>
<th>Propiverine</th>
<th>Oxo-M + propiverine</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (cmH₂O)</td>
<td>BP (cmH₂O)</td>
<td>BP (cmH₂O)</td>
</tr>
<tr>
<td>Control</td>
<td>6.93 ± 0.40</td>
<td>6.93 ± 0.40</td>
</tr>
<tr>
<td>Propiverine</td>
<td>8.79 ± 0.86</td>
<td>8.79 ± 0.86</td>
</tr>
<tr>
<td>M-1</td>
<td>5.20 ± 0.51</td>
<td>5.20 ± 0.51</td>
</tr>
<tr>
<td>M-2</td>
<td>7.60 ± 0.40</td>
<td>7.60 ± 0.40</td>
</tr>
</tbody>
</table>

*P < 0.05 versus saline; **P < 0.05 versus saline or M-1 alone. Values are presented as mean ± SEM. BP, baseline pressure; ICI, intercontraction interval; MVP, maximum voiding pressure; PT, pressure threshold.
In the present study, intravesical administration of M-1 alone induced a reduction in voiding frequency as evidenced by increased PT and ICI, and concomitant application of Oxo-M still caused bladder overactivity, indicating that M-1-mediated inhibition of baseline bladder activity is not induced by the modulation of mACHr. It has been reported that M-1, but not M-2, has significant binding affinity to L-type calcium channels in bladder smooth muscle. After oral administration of propiverine, M-1 was detected in human plasma at 3–10 times higher concentrations, as compared to the original concentration of propiverine. Thus, M-1 could contribute to the inhibitory effect of propiverine on bladder overactivity with calcium channel antagonistic action rather than antimuscarinic effects although Wuest et al. reported that M-1 did not block L-type calcium channels in human detrusor even at the highest concentration studied (100 μM) while it reduced electrical field stimulation-induced force completely at 1 mM. Because, in our study, intravesical application of Oxo-M alone reduced voiding frequency without affecting MVP or BP, this inhibitory effect of M-1 seems to be mediated through the suppression of bladder afferent activity rather than detrusor or efferent function. We have previously reported that L-type calcium channels are expressed in L6-S1 dorsal root ganglion neurons innervating the bladder in rats. In addition, De Wachter and Wyndaele showed that intravesical administration of oxybutynin, which is another antimuscarinic agent that has calcium channel antagonistic action, can suppress C-fiber afferent activity originating from the bladder wall in rats, suggesting that oxybutynin has a direct inhibitory effect on bladder afferent fibers. Overall, it is possible that M-1 might contribute to the therapeutic effect of propiverine by reducing bladder afferent activity, possibly through the inhibition of L-type calcium channels expressed in bladder afferent pathways. In conclusion, the present study implies that propiverine and M-2 inhibit bladder overactivity induced by intravesical administration of Oxo-M through the blockade of mACHRs by locally modulating bladder afferent activity, and that M-1 has an inhibitory effect on bladder function through the mechanism other than muscarinic receptor modulation, possibly due to calcium channel antagonistic activity. Taken together, clinical benefits of propiverine in OAB patients could be attributable to the multiple mechanisms of propiverine and its active metabolites such M-1 and M-2.

Disclosure
Yukio Hayashi, Masato Nanri and Mamoru Kiniwa are employees of Taiho Pharmaceutical Co., Ltd.

REFERENCES