Effect of TAK-637, a Tachykinin NK$_1$-Receptor Antagonist, on Lower Urinary Tract Function in Cats

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ABSTRACT—The effect of TAK-637 (\((aR,9R)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-

methyl-5-(4-methylphenyl)-7\H[1,4]diazocino[2,1g][1,7]naphthyridine-6,13-dione\), a tachykinin NK$_1$-recep-
tor antagonist, on lower urinary tract function was investigated in cats. TAK-637 (0.1, 0.3, 1 and 3 mg/kg, i.v.) produced a dose-dependent increase in bladder capacity without any significant reduction in voiding efficiency in decerebrate cats. The maximal increase in bladder capacity was 94%. By contrast, oxybutynin at 1 and 3 mg/kg (i.v.) produced a 18% and 35% increase in bladder capacity, respectively, with a 47% and 45% reduction in voiding efficiency. TAK-637 (3 mg/kg, i.v.) did not inhibit the micturition reflex induced by electrical stimulation of the rostral brainstem near the locus coeruleus, indicating that it does not impair the well-organized micturition reflex (bladder contraction and urethral relaxation). These results suggest that TAK-637 increases bladder storage capability without inhibiting the voiding function of the lower urinary tract, presumably by inhibiting the afferent pathway of the micturition reflex, rather than the efferent pathway.

Keywords: TAK-637, Tachykinin NK$_1$-receptor antagonist, Bladder capacity, Voiding efficiency

The lower urinary tract has two main functions: the storage and periodic voiding of urine. A novel tachykinin NK$_1$-receptor antagonist, TAK-637 (1), increases bladder capacity and decreases the frequency of distension-induced rhythmic bladder contractions (micturition reflex) in normal and spinal guinea pigs under urethane anesthesia (2, 3). It also inhibits the bladder contractions induced by electrical stimulation of the afferent pathway, not the efferent pathway, of the spinal guinea pig pelvic nerve (4). Taken together, these findings suggest that TAK-637 increases bladder volume by inhibiting the afferent pathway of the micturition reflex at the spinal cord level in guinea pigs.

Antimuscarinic and spasmolytic agents, the best known of which is oxybutynin, are regarded as first-line therapy for abnormally frequent micturition and urinary incontinence. However, these drugs lower detrusor muscle contractility and have the potential to cause voiding difficulty when used clinically (5). Because TAK-637 does not affect bladder contractile force (3, 4), it has been assumed to be free of such adverse effects. This assumption has never been confirmed, however, because of the low voiding efficiency of urethane-anesthetized guinea pigs.

The present study addressed two issues: the effect of TAK-637 on voiding efficiency in decerebrate cats with a minimal effect of anesthesia and whether TAK-637 impairs the coordinated micturition reflex or causes dyssynergia between the detrusor and urethral sphincter. The effect of TAK-637 on the micturition reflex induced by electrical stimulation of the cat rostral brainstem was studied to address the second issue.

MATERIALS AND METHODS

This study was conducted in compliance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society, and the experimental protocols were approved by Takeda’s Experimental Animal Care and Use Committee.

Effect on bladder capacity and voiding efficiency in cats decerebrated at the supracollicular level

Adult cats (Keari Co., Ltd., Ibaragi; and Liberty Research, Inc., Waverly, NY, USA) of both sexes, weighing 2.2 – 4.9 kg, were used. Anesthesia was induced with ketamine hydrochloride (40 mg/kg, i.m.). After removing the overlying calvaria, the animals were decerebrated at the precollicular postmammillary level (sparing Barrington’s pontine micturition center). The bladder was exposed extraperitoneally using a ventral approach. Both ureters were cut, and their distal ends were ligated. A catheter (PE-50) was inserted into the bladder dome to allow infusion of warmed (37°C) saline. To minimize the influence of anesthesia, the experiment was started at least 5 h after the

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induction of anesthesia. After emptying the bladder by suction with visual confirmation, saline was infused into the bladder with an infusion pump (Model SP-100; JMC, Hiroshima). The infusion rate was adjusted so that micturition was elicited about 5 min after the start of infusion. Bladder capacity was calculated from the infusion rate and the time required to fill the bladder before voiding. The voided fluid was collected with cotton wool swabs, and the weight (g) of fluid was taken as the volume voided (ml). Voiding efficiency was calculated by using the following formula:

\[ \text{voiding efficiency (％)} = \frac{\text{voided volume}}{\text{bladder capacity}} \times 100 \]

After confirming the reproducibility of the bladder capacity values, increasing doses of TAK-637 or oxybutynin were injected intravenously every 25 min, and their effect was assessed 10 min after each dose.

**Effect on bladder contraction and urethral relaxation induced by electrical stimulation of the rostral brainstem in cats under urethane anesthesia**

Adult female cats (Liberty Research, Inc.) weighing 2.5 – 3.4 kg were used after anesthetizing them with urethane (0.8 – 1.2 g/kg, i.p.). The bladder was exposed extraperitoneally by a ventral approach. Both ureters were cut, and their distal ends were ligated. To measure intravesical pressure, a catheter (PE-90) was inserted into the bladder through the urethral orifice, and the external end of the catheter was connected to a pressure transducer (P23XL; Sanei, Tokyo) and the infusion pump via a three-way stopcock. Saline was infused through the catheter at a constant rate of 1.4 ml/h, and while continuing to monitor the infusion pressure, the catheter was then slowly withdrawn and repositioned at the site of maximum pressure. The infusion pressure at this site was taken as the intravesical pressure. Another catheter (PE-50) was inserted into the bladder dome and connected to a pressure transducer to allow measurement of intravesical pressure. The bladder neck was ligated so that intravesical pressure and intrarectal pressure could be recorded separately, and the bladder was filled with warmed physiological saline to half of the threshold volume of micturition. A concentric bipolar stimulating electrode was stereotaxically inserted into the locus coeruleus (P:2.0, L:2.0, H:–2.0) (6 – 8). Test stimuli (1-ms width pulse; 125, 250, 500 or 1000 μA; 10 Hz) were applied for 10 s, and the resulting changes in intravesical and intrarectal pressure were recorded with a polygraph system (Sanei). Drug effects were assessed by applying an electrical current with an intensity twice the threshold value to obtain reproducible responses. After confirming stable responses, TAK-637 (3 mg/kg) or its vehicle, dimethylsulfoxide (DMSO), was injected intravenously. An anodal current was passed at the end of the experiment to confirm the site of stimulation.

**Chemicals**

TAK-637 was synthesized in Takeda’s Pharmaceutical Research Laboratories. Oxybutynin hydrochloride (oxybutynin) was extracted from commercially available tablets (Kodama, Tokyo) in Takeda’s Pharmaceutical Research Laboratories. Urethane (Aldrich, Milwaukee, WI, USA), and ketamine hydrochloride (Sankyo, Tokyo) and DMSO (Wako, Osaka) were purchased from the suppliers stated. TAK-637 was dissolved in DMSO and administered at a volume of 0.05 ml/kg. Oxybutynin was dissolved in saline and administered at a volume of 0.1 ml/kg.

**RESULTS**

**Effect on bladder capacity and voiding efficiency in decerebrate cats**

The mean bladder capacity (± S.E.M.) of the decerebrate cats (n = 20) was 7.5 ± 1.4 ml, and their mean voiding efficiency (± S.E.M.) was 86.2 ± 2.6%. Cumulative administration of DMSO (0.05 ml/kg, i.v., each time) had no consistent effect on either bladder capacity or voiding efficiency (% increase in bladder capacity: −6.8 ± 7.7%, 11.6 ± 21.8%, −20.5 ± 11.9% and −12.6 ± 22.9%; % change in voiding efficiency: 2.7 ± 2.8%, 3.2 ± 5.4%, 17.0 ± 18.2% and −1.6 ± 4.4%; values for the first, second, third and fourth doses, respectively; n = 4). As shown in Table 1, increasing the dose of TAK-637 from 0.1 to 3 mg/kg, i.v. produced dose-dependent increments in bladder capacity, and the increases were statistically significant at doses of 0.3 mg/kg and above. Although TAK-637 reduced voiding efficiency by about 20% at doses of 1 mg/kg and above, the decreases were not statistically significant (Table 1). Increasing the dose of oxybutynin (1 and 3 mg/kg, i.v.) produced 18% and 35% increases, respectively, in bladder capacity, with 47% and 45% reductions in voiding efficiency (Table 1). Because the effect of saline (the vehicle for oxybutynin) alone was not tested, no statistical analysis of the effects of oxybutynin was allowed.

**Effect on bladder contraction and urethral relaxation induced by electrical stimulation of the rostral brainstem in urethane-anesthetized cats**

Before starting the experiment proper, the dose-response effect of TAK-637 on bladder capacity was confirmed in cats anesthetized with urethane to identify the optimal dose of TAK-637. Cumulative administration of DMSO (0.05 ml /kg, i.v., each time) did not affect bladder capacity: the mean ± S.E.M. of the percentage increases were 5.0 ± 4.9%, 2.3 ± 7.9%, 4.9 ± 9.5% and 5.6 ± 10.7%, after the first, second, third and fourth dose, respectively (n = 6). Increasing doses of TAK-637 produced successive increments in
Effect of TAK-637 on Micturition in Cats

Table 1. Effect of TAK-637 and oxybutynin on bladder capacity and voiding efficiency in decerebrate cats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg, i.v.)</th>
<th>Bladder capacity (ml)</th>
<th>Voiding efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAK-637</td>
<td>pre (n = 9)</td>
<td>8.5 ± 2.3</td>
<td>87.1 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>9.2 ± 2.4</td>
<td>87.8 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>12.3 ± 3.8</td>
<td>84.2 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15.0 ± 4.8</td>
<td>71.5 ± 11.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16.4 ± 5.7</td>
<td>67.4 ± 13.2</td>
</tr>
<tr>
<td>Oxybutynin</td>
<td>pre (n = 7)</td>
<td>8.1 ± 2.5</td>
<td>88.0 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9.4 ± 3.2</td>
<td>47.5 ± 13.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.0 ± 2.9</td>
<td>50.9 ± 13.5</td>
</tr>
</tbody>
</table>

The drugs were administered intravenously in increasing doses. All data are expressed as means ± S.E.M. The number of animals injected with each drug is shown in parentheses. The statistical analysis of the effect of TAK-637 on bladder capacity was performed by comparing the % increase by the paired t-test (P<0.05). Voiding efficiency was not significantly affected by TAK-637 (P>0.05). Since the effect of saline (the vehicle for oxybutynin) alone was not tested, no statistical analysis of the effects of oxybutynin could be performed.

The rostral brainstem near the locus coeruleus of urethane-anesthetized cats was electrically stimulated for 10 s (1-ms width pulse; 125, 250, 500 or 1000 µA; 10 Hz). Figure 1 shows the sites of stimulation in a representative animal used in this experiment. Figures 2 and 3 show the responses of the urinary bladder and the urethra to electrical stimulation of the brainstem, and the stimulus intensity in Fig. 3 is expressed as the ratio of the current intensity to the threshold current that induced clear bladder contraction and urethral relaxation. The actual current intensities of 1/2, 1 and 2 in Fig. 3 were 196 ± 67, 393 ± 134 and 786 ± 267 µA, respectively (mean ± S.D.; n = 7). In most cats, the threshold currents for bladder contraction were higher than for urethral relaxation. The effect of TAK-637 was assessed by applying an electrical current at stimulus intensity 2, which induced reproducible responses in both the bladder and the urethra.

Since a comparison of pre-drug values with post-drug values showed that DMSO had a slight but significant inhibitory effect on bladder contraction induced by electrical stimulation (P = 0.0147, paired t-test), the statistical analysis of the effects of TAK-637 was performed by comparison between TAK-637-treated and DMSO-treated groups. There was no significant effect of TAK-637 on bladder contraction induced by electrical stimulation (Table 2). TAK-637 did not affect either basal intraurethral pressure or the fall in urethral pressure following electrical stimulation (Table 2).
DISCUSSION

In our previous study in guinea pigs, TAK-637 and related compounds increased bladder capacity and decreased the frequency of the distension-induced rhythmic bladder contractions, and these effects were well correlated with its tachykinin NK₁-receptor antagonist activity (1). The structurally different tachykinin NK₁-receptor antagonist (±)-CP-99,994 had the same effect on the micturition reflex (simultaneous bladder contraction and urethral relaxation). Since TAK-637 does not affect bladder contractile force in urethane-anesthetized guinea pigs (3, 4), it has been assumed not to suppress voiding of urine from the bladder. In the present study, the effect of TAK-637 on urinary voiding function was assessed by comparing it with oxybutynin in decerebrate cats, whose voiding efficiency was about 85%.

Oxybutynin is widely used for the treatment of overactive bladder, but is well known to occasionally cause voiding difficulty by inhibiting detrusor muscle contraction (5). In the present study, oxybutynin was found to decrease voiding efficiency in decerebrate cats, a finding that is consistent with its reported adverse effects (5). By contrast, at doses of 0.1 and 0.3 mg/kg, i.v., TAK-637 had hardly any effect on voiding efficiency, and it actually increased bladder capacity to the same extent as oxybutynin. At a dose of 3 mg/kg, i.v., TAK-637 produced an approximately twofold increase in bladder capacity with only a slight and insignificant decrease in voiding efficiency. These results confirmed that TAK-637 increases bladder capacity without any substantial effect on voiding efficiency, and that its mode of action on urinary bladder capacity may be different from that of oxybutynin.

The finding that TAK-637 does not affect voiding efficiency implies that it has no effect on either bladder contraction or urethral relaxation during micturition and that it does not cause detrusor-sphincter dyssynergia. To confirm this, the effect of TAK-637 on the micturition reflex induced by stimulation of the pontine micturition center (which includes the locus coerules) was investigated. During voiding, the pontine micturition center activates the sacral parasympathetic preganglionic neurons, which stimulate bladder contraction, and inactivates the spinal
motoneurons in Onuf’s nucleus, resulting in relaxation of the striated urethral sphincter muscle (9, 10). In the present study, electrical stimulation of the brainstem near the locus coeruleus evoked a well-coordinated micturition reflex, consisting of simultaneous bladder contraction and urethral relaxation, as described by Mallory et al. (8). TAK-637 showed no significant effect on the bladder response and did not affect urethral relaxation at all. Therefore, it is reasonable to conclude that TAK-637 does not affect the micturition reflex induced by electrical stimulation of the pontine micturition center, at least at the dose that produces an approximately twofold increase in bladder capacity, and that it does not cause detrusor-sphincter dyssynergia.

In rats, it has been reported that substance P-containing neurons are present in the pelvic nerve, which innervates the urinary bladder (11), that substance P binding sites are found in the dorsal horn of the sacral spinal cord where the afferent fibers of the pelvic nerve terminate (12, 13), and that intrathecal administration of non-peptide tachykinin NK₁-receptor antagonists inhibits the micturition reflex (14). These findings suggest that substance P and its receptors in the sacral spinal cord may play an important role in transmission of the afferent input from the bladder to the brain. As TAK-637 has low affinity for tachykinin NK₁ receptors in rats (1), its effects on the lower urinary tract functions were investigated in guinea pigs, in which TAK-637 has high antagonistic activity (1). TAK-637 inhibited the bladder contractions induced by electrical stimulation of the afferent pathway, but not the efferent pathway, of the guinea pig pelvic nerve (4). Taken together with the results of this study, these findings strongly suggest that the effect of TAK-637 on bladder capacity is attributable to inhibition of the afferent pathway of the micturition reflex in cats, rather than the efferent pathway.

Electrophysiological studies in cats have shown that the afferents from the urinary bladder are either thinly myelinated Aδ-fibers or unmyelinated C-fibers and that the mechanosensitive input from the bladder, which signals the fullness of the bladder, is mediated chiefly by Aδ-fibers, not C-fibers (15 – 17). As most substance P-containing neurons are C-fibers (18), there seems to be some inconsistency with our own findings in this study. This discrepancy remains to be elucidated.

The results of the present study suggest that TAK-637 may be useful for the treatment of urinary storage disorders, especially detrusor hyperreflexia, being free of possible adverse effects such as voiding difficulties.

REFERENCES

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