Pharmacological Characterization of a New Antimuscarinic Agent, Solifenacin Succinate, in Comparison with Other Antimuscarinic Agents

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Solifenacin succinate [YM905; (3R)-1-azabicyclo[2.2.2]oct-3-yl(1S)-1-phenyl-3,4-dihydroisoquinoline-2(1H)-carboxylate monosuccinate] is a new muscarinic receptor antagonist developed for the treatment of overactive bladder. The aim of the present study was to evaluate the antimuscarinic properties of solifenacin and to compare the results with those obtained for tolterodine, oxybutynin, darifenacin, propiverine and atropine. In radioligand receptor binding assay, \( K_i \) values of solifenacin for human muscarinic M1, M2, M3, M4 and M5 receptors were 26, 170, 12, 110 and 31 nM, respectively. In isolated rat urinary bladder, solifenacin competitively antagonized carbachol-induced contractions, with a pA2 value of 7.44±0.09. In these in vitro studies, the antimuscarinic action of solifenacin was more potent than that of propiverine and less potent than those of tolterodine, oxybutynin, darifenacin and atropine. In anesthetized rats, solifenacin and oxybutynin increased the maximum bladder capacity in a dose-dependent manner and also decreased the maximum intravesical pressure. The dosages required to produce a 30% increase in maximum bladder capacity (ED30 values) of solifenacin and oxybutynin were 0.35 and 0.30 mg/kg i.v., respectively, indicating approximately equal efficacies. These results support the fact that solifenacin, similarly to currently used antimuscarinic agents, is an effective agent in the treatment of overactive bladder symptoms such as urinary frequency and urge incontinence.

Key words solifenacin; muscarinic receptor; urinary bladder; bladder capacity

Urinary bladder smooth muscle is innervated by both sympathetic and parasympathetic nerves. Acetylcholine released from postganglionic parasympathetic nerve terminals activates postjunctional muscarinic receptors in urinary bladder, which modulate urinary bladder contraction during the voiding phase and control detrusor tone during the filling phase. Five muscarinic receptor subtypes (M1—M5) have been identified by both molecular biological and pharmacological investigations.1) The urinary bladder smooth muscle contains a mixed population of muscarinic M1 and M2 receptors.2) Although muscarinic M2 receptors are numerically predominant, muscarinic M1 receptors are considered to predominate in the mediation of bladder contraction.3) An important functional role of the muscarinic M1 receptor in mediating bladder contraction has also been suggested in experiments using mutant mice lacking the muscarinic M1 receptor gene.3

Overactive bladder is characterized by symptoms of urgency and urinary frequency with or without urge incontinence. It has a profoundly negative effect on the quality of life of those affected. Muscarinic receptor antagonists are the most widely used therapy for overactive bladder.6–8) Solifenacin succinate [YM905, (3R)-1-azabicyclo[2.2.2]oct-3-yl(1S)-1-phenyl-3,4-dihydroisoquinoline-2(1H)-carboxylate monosuccinate] is a new muscarinic receptor antagonist developed for the treatment of overactive bladder. Affinity constants (\( K_i \) values) of this drug for human muscarinic M1, M2 and M3 receptors only have been reported, along with its antagonism of the contractile effect of carbachol in isolated guinea pig urinary bladder.9) The present study was therefore undertaken to investigate the affinity of solifenacin for all human muscarinic receptor subtypes (M1—M5) and its functional muscarinic M3 receptor antagonism in rats, and to compare the results with those for tolterodine, oxybutynin, darifenacin, propiverine and atropine. Additionally, we also investigated the effect of solifenacin on voiding function in anesthetized rats.

MATERIALS AND METHODS

Materials Solifenacin succinate (YM905, Vesicare®), tolterodine tartrate, darifenacin and propiverine hydrochloride were prepared by Astellas Pharma Inc. (Tokyo, Japan). Oxybutynin chloride, atropine sulfate and carbachol (carbamylcholine chloride) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Darifenacin was dissolved in dimethyl sulfoxide and the others were dissolved in dimethyl sulfoxide, Krebs–Henseleit solution or physiological saline.

Animals Male Wistar rats and male Sprague-Dawley rats were purchased from Charles River Laboratories Japan (Kanagawa, Japan) and Japan SLC (Shizuoka, Japan), respectively. In in vitro studies, rats were sacrificed by exsanguination under ether anesthesia. All animal experiments were performed in compliance with the regulations of the Institutional Animal Ethical Committee of Astellas Pharma Inc.

Radioligand Receptor Binding Assay Membranes of Chinese hamster ovary (CHO)-K1 cells expressing human muscarinic receptors were purchased from PerkinElmer Life Science (Wellesley, MA, U.S.A.). Briefly, 50 µl of drug solution and 100 µl of \( [\text{H}]\)N-methyl scopolamine (final concentration of 0.25 nM) were mixed with 50 µl of membrane suspension in 50 mM Tris/HCl (pH 7.4) buffer containing 1 mM EDTA and 10 mM MgCl2 in a final volume of 200 µl. This mixture was incubated at room temperature for 120 min. Reactions were terminated by filtration through UniFilter GF/C (Packard Instrument Co., Meriden, CT, U.S.A.) and the filter was washed with ice-cold 50 mM Tris/HCl (pH 7.4) buffer

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containing 0.9% NaCl. Radioactivity retained on the filter was counted with a TopCount™ microplate scintillation counter (Packard Instrument Co.) using a scintillation cocktail (MicroScint-20™, Packard Instrument Co.). Nonspecific binding was determined with the reaction mixture including 5 μM atropine, and total binding was determined with the reaction mixture without test drugs, respectively. A total of 10 drug concentrations were used, appropriately chosen from the range $3 \times 10^{-11}$ to $1 \times 10^{-7}$M using a common ratio of approximately 3. Further, we performed saturation binding studies to yield the dissociation constants ($K_d$) of $[^{3}H]$N-methyl scopolamine for each muscarinic receptor under the same conditions as above.

**Carbachol-Induced Contraction of Urinary Bladder Strips**

The urinary bladder was isolated from male Wistar rats weighing 420—510 g and its dome and base were excised to make a cylindrical preparation. The tissue was then cut into strips of 0.3 cm width and 1 cm length. These strips were vertically suspended with 1.0 g tension in a 10 ml organ bath containing Krebs–Henseleit-buffered solution (NaCl, 118.4 mM; KCl, 4.7 mM; KH$_2$PO$_4$, 1.2 mM; MgSO$_4$, 1.2 mM; CaCl$_2$, 2.5 mM; NaHCO$_3$, 25.0 mM; glucose, 11.1 mM), maintained at 37 °C and gassed with a mixture of 95% O$_2$ and 5% CO$_2$. The strips were attached to isometric force-displacement transducers (TB-611T; Nihon Kohden, Tokyo, Japan) connected to a recorder. After equilibration, bladder contractions were elicited by 10 μM carbachol three times and a cumulative-concentration–response curve for carbachol was constructed by increasing bath concentration of the agonist approximately 3-fold. After the strips were allowed to equilibrate again, the concentration–responses to carbachol were reconstructed by serially increasing the concentration of the agonist in the absence or presence of antagonists following 30 min incubation. Each antagonist was examined at three different concentrations in the same preparation.

**Cystometry in Anesthetized Rats**

Male Sprague-Dawley rats weighing 350—490 g were anesthetized intraperitoneally (i.p.) with urethane (1.2 g/kg). A midline abdominal incision was made, and then a catheter (PE-50; Becton, Dickinson & Co., NJ, U.S.A.) was inserted into the bladder through its superior aspect. A three-way tap was attached to the other end of the catheter, and a pressure transducer (TP-400; Nihon Kohden, Tokyo, Japan), for the measurement of intravesical pressure, and an infusion pump (STCS25; Terumo, Tokyo, Japan), for the infusion of physiological saline, were also attached. Intravesical pressure was recorded continuously on a recorder. A catheter was placed in a femoral vein for the administration of the test drugs. Following a stabilization period after surgery of at least 30 min, physiological saline warmed to about 37 °C was infused continuously into the bladder at the rate of 4.2 ml/h in order to induce the micturition reflex. The volume of physiological saline infused until micturition was defined as maximum bladder capacity. Cystometry was performed four times, with the test drugs intravenously (i.v.) administered 5 min before the fourth cystometry. The mean values of the parameters measured in the second and third cystometrogram were designated the initial values. The difference between the initial values and the post-administration values was used in the evaluation of pharmaceutical efficacy.

**Data Analysis**

All data are expressed as the mean±S.E.M. or the mean with 95% confidence interval. In radioligand binding experiments, specific binding was calculated as total binding minus nonspecific binding. The concentration of each test drug required to reduce the specific binding of $[^{3}H]$N-methyl scopolamine by 50% (IC$_{50}$ value) was obtained by non-linear regression analysis. A $K_d$ value of $[^{3}H]$N-methyl scopolamine for each muscarinic receptor subtype was yielded by Scatchard plot analysis. $K_i$ values were calculated from the formula $K_i=IC_{50}(1+[^{3}H]\text{N-methyl scopolamine}/K_d)$. In the isolated rat bladder experiments, the 50% effective concentration (EC$_{50}$) of carbachol was estimated by non-linear regression. For calculation of p$_A$ values, Schild plot analysis was performed in each preparation using these EC$_{50}$ values, and the result was expressed as an apparent p$_A$ when the Schild slopes were different from unity using Student’s t-test. In in vivo study, ID$_{50}$ values, the doses produce a 30% increase in maximum bladder capacity, was determined by linear regression analysis. Statistical differences were analyzed using Dunnett’s multiple comparison test.

All data analyses were performed using the SAS statistical software (SAS Institute, NC, U.S.A.). Differences with a $p<0.05$ were considered statistically significant.

**RESULTS**

**Radioligand Receptor Binding Assay**

Solifenacin, tolterodine, oxybutynin, darifenacin and propiverine inhibited the specific binding of $[^{3}H]$N-methyl scopolamine to each muscarinic receptor subtype in a concentration-dependent manner (Fig. 1). $K_i$ values are summarized in Table 1. The affinity of solifenacin for the muscarinic M$_3$ receptor was 2.2-, 15-, 9.1-, and 2.6-fold higher than those for M$_1$, M$_2$, M$_4$ and M$_5$ receptors, respectively. The affinities of oxybutynin, darifenacin and propiverine for the muscarinic M$_3$ receptor were 1.8- to 5.9-, 4.2- to 53-, and 1.4- to 3.8-fold higher than those for other muscarinic receptor subtypes, respectively, but tolterodine was devoid of any M$_3$ receptor subtype selectivity. The affinities of oxybutynin and tolterodine for muscarinic M$_1$—M$_4$ receptors and those of darifenacin for M$_3$—M$_5$ receptors were higher than those of solifenacin. On the other hand, propiverine showed about one order lower affinities for all muscarinic receptor subtypes compared with solifenacin.

**Carbachol-Induced Contraction of Urinary Bladder Strips**

In isolated rat urinary bladder strips, solifenacin (30—300 nM), tolterodine (3—30 nM), oxybutynin (30—300 nM), darifenacin (3—30 nM), propiverine (1—10 μM) and atropine (3—30 nM) shifted the concentration–contraction curves of carbachol to the right in a concentration-dependent manner (Fig. 2). In addition, darifenacin at 30 nM and propiverine at 10 μM depressed the maximum contraction to a value less than 80%. Affinity estimates (p$_A$ values) and Schild slopes are summarized in Table 2. Schild slopes of solifenacin and tolterodine were not significantly different from unity, whereas those of oxybutynin, darifenacin, propiverine and atropine were significantly different from unity.

**Cystometry in Anesthetized Rats**

When physiological saline was infused continuously at 4.2 ml/h into the bladders in anesthetized rats, intravesical pressure increased gradually and micturition was observed about 10 min after start of the
infusion. The initial values of maximum bladder capacity and maximum intravesical pressure were approximately 700 ml and 40 cm H\textsubscript{2}O, respectively. There were no significant differences among the all groups in these initial values. Both solifenacin (0.1—1 mg/kg i.v.) and oxybutynin (0.1—1 mg/kg i.v.) increased the maximum bladder capacity in a dose-dependent manner and also decreased maximum intravesical pressure (Fig. 3). The ED\textsubscript{30} values of solifenacin and oxybutynin (95% confidence interval) were 0.35 (0.17—0.83) and 0.30 (0.21—0.43) mg/kg i.v., respectively.

**DISCUSSION**

Overactive bladder is a condition which encompasses a number of urinary symptoms, including urgency, excessive frequency of micturition, nocturia and urge incontinence. The generation of abnormal bladder contractions in disease states as well as in normal physiological voiding is critically dependent on acetylcholine-induced stimulation of muscarinic receptors in the bladder smooth muscle. Antimuscarinic therapy is thus the mainstay of pharmacological treatment for the symptoms of overactive bladder. The aim of the present study was to evaluate the antimuscarinic properties of solifenacin, a new muscarinic receptor antagonist developed for the treatment of overactive bladder, and to compare the results with those obtained for other muscarinic antagonists, namely tolerodine, oxybutynin, darifenacin and propiverine.

The results of the *in vitro* studies demonstrate that solifenacin is a potent and competitive muscarinic receptor antagonist. In radioligand receptor binding assay, solifenacin showed the highest affinity for the muscarinic M\textsubscript{3} receptor, which mediates the urinary bladder contraction, but its affinity for the M\textsubscript{1} receptor was only marginal over those for the M\textsubscript{1} and M\textsubscript{5} receptor subtypes. The affinity of solifenacin for the muscarinic M\textsubscript{1} receptor was 30-fold higher than that of propiverine, but 2.7- to 6.0-fold lower than those of tolerodine, oxybutynin and darifenacin. The affinities of oxybutynin and propiverine for the muscarinic M\textsubscript{3} receptor were at most 5.9-fold higher than those for other muscarinic receptor subtypes, and tolerodine was devoid of any muscarinic M\textsubscript{3} receptor subtype selectivity. On the other hand, the affinity of darifenacin for the muscarinic M\textsubscript{3} receptors was 4.2- to 53-fold higher than those for other muscarinic receptor subtypes. These results are markedly similar to those reported previously\textsuperscript{9,12,13}.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>K\textsubscript{i} (nM)</th>
<th>M\textsubscript{1}</th>
<th>M\textsubscript{2}</th>
<th>M\textsubscript{3}</th>
<th>M\textsubscript{4}</th>
<th>M\textsubscript{5}</th>
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<tbody>
<tr>
<td>Solifenacin</td>
<td>26±2.0</td>
<td>170±37</td>
<td>12±4.4</td>
<td>110±45</td>
<td>31±6.3</td>
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<tr>
<td>Tolerodine</td>
<td>2.7±0.23</td>
<td>4.2±0.51</td>
<td>4.4±0.45</td>
<td>6.6±1.7</td>
<td>2.5±0.49</td>
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<tr>
<td>Oxybutynin</td>
<td>6.1±1.5</td>
<td>21±3.6</td>
<td>3.4±0.65</td>
<td>6.6±2.7</td>
<td>18±4.0</td>
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</tr>
<tr>
<td>Darifenacin</td>
<td>31±2.6</td>
<td>100±14</td>
<td>2.0±0.21</td>
<td>52±15</td>
<td>8.2±1.7</td>
<td></td>
</tr>
<tr>
<td>Propiverine</td>
<td>490±110</td>
<td>1400±220</td>
<td>350±53</td>
<td>900±200</td>
<td>490±120</td>
<td></td>
</tr>
</tbody>
</table>

*Each value represents the mean±S.E.M. of four separate experiments performed in duplicate.*
In isolated rat urinary bladder, solifenacin shifted the concentration–contraction curves of carbachol to the right in a concentration-dependent manner, and the Schild slope was not significantly different from unity, suggesting that the antagonistic effect on the muscarinic M3 receptor is competitive. The antimuscarinic action of solifenacin was more potent than that of propiverine, and less potent than those of tolterodine, oxybutynin and darifenacin. These results were considered to nearly reflect the rank order of affinity for the muscarinic M3 receptor. However, the antagonistic potencies of solifenacin, oxybutynin and propiverine were weaker than their affinities for the muscarinic M3 receptor. This discrepancy was probably caused by restricted drug diffusion into structured tissues, which hinders equilibrium conditions. Schild slopes for oxybutynin, darifenacin and propiverine

Table 2. Potency Estimates and Schild Slopes for Solifenacin and Other Muscarinic Receptor Antagonists in Carbachol-Induced Contraction in Isolated Rat Urinary Bladder

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>pA2</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solifenacin</td>
<td>7.44±0.09</td>
<td>1.11±0.06</td>
</tr>
<tr>
<td>Tolterodine</td>
<td>8.61±0.13</td>
<td>1.03±0.07</td>
</tr>
<tr>
<td>Oxybutynin</td>
<td>7.84±0.06</td>
<td>1.22±0.07*</td>
</tr>
<tr>
<td>Darifenacin</td>
<td>8.50±0.03</td>
<td>1.50±0.15*</td>
</tr>
<tr>
<td>Propiverine</td>
<td>6.03±0.04</td>
<td>2.14±0.12*</td>
</tr>
<tr>
<td>Atropine</td>
<td>8.47±0.06</td>
<td>1.73±0.16*</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. of five preparations. \( a \) Schild slope is significantly different from unity (Student’s \( t \)-test).
were significantly different from unity. A steep Schild plot is thought to be attributable to the presence of a saturable antagonist removal process in the tissue. Further, since propiverine is reported to have Ca²⁺ channel blocking action, it is likely that its action contributed to the observed decrease in maximum contraction, resulting in the steep slope.

In anesthetized rats, solifenacin and oxybutynin increased the maximum bladder capacity in a dose-dependent manner and also decreased the maximum intravesical pressure. These effects are considered to be based on the muscarinic M₃ receptor antagonizing action in urinary bladder. At highest dose of solifenacin, the increase in maximum bladder capacity was 47%, and the decrease in maximum intravesical pressure was only 16%. In conscious cerebral infarcted rats, solifenacin was demonstrated to increase the maximum bladder capacity and voided volume without influencing maximum intravesical pressure. The present study was conducted under urethan anesthesia. Since urethan anesthesia is considered to induce a weak inhibition for neural reflex, the influence of the anesthesia on micturition reflex could not be role out. In fact, urethan anesthesia inhibited somewhat micturition reflex. It was thus likely that solifenacin was more easy to decrease maximum intravesical pressure in the present study compared with previous study under conscious condition.

In the previous studies, solifenacin exhibited greater selectivity for urinary bladder over salivary gland than tolterodine, oxybutynin, darifenacin and atropine. Since the muscarinic M₃ receptor mediates both bladder contraction and salivary secretion, the bladder selectivity demonstrated for solifenacin can clearly be attributable to selectivity for a single muscarinic M₃ receptor subtype. Recently, muscarinic M₁ and M₂ receptors as well as M₃ receptors have been reported to play a partial albeit important role in salivary gland, and mutant mice lacking the muscarinic M₁ and M₂ receptor gene have reduced pilocarpine-induced salivary secretion. However, solifenacin showed only 2.2- and 2.6-fold higher affinity for the muscarinic M₁ receptor than for the M₂ and M₃ receptors, respectively. On the other hand, oxybutynin showed 1.8- and 5.1-fold higher affinity for the muscarinic M₁ receptor than for the M₂ and M₃ receptors. Darifenacin showed 16- and 4.2-fold higher affinity for the muscarinic M₁ receptor than for the M₂ and M₃ receptors, respectively. Interestingly, however, these two antagonists showed no bladder selectivity. The bladder selectivity demonstrated for solifenacin is thus not considered to be attributable to its low affinity for the muscarinic M₁ and M₂ receptors. Therefore, selectivity for the other non-M₁ muscarinic receptor subtypes is not considered to contribute to the bladder selectivity. Thus, a great deal of investigation would be required to clarify the mechanism of bladder selectivity of solifenacin.

In conclusion, solifenacin is a potent and competitive muscarinic receptor antagonist and increases the maximum bladder capacity. These findings support the fact that solifenacin is useful in the treatment of overactive bladder, similarly to currently used antimuscarinic agents. In this regard, we note with interest that solifenacin at 5 and 10 mg/man has been shown in clinical studies to be more effective than placebo in improving overactive bladder symptoms.

REFERENCES