Muscarinic Receptor Binding in Rat Bladder Urothelium and Detrusor Muscle by Intravesical Solifenacin

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Solifenacin is an antimuscarinic agent used to treat symptoms of overactive bladder. Pharmacologically significant amounts of solifenacin were excreted in the urine of humans taking a clinical dose of this drug. The aim of this study is to measure muscarinic receptor binding in the bladder urothelium and detrusor muscles of rats following the intravesical instillation of solifenacin. Muscarinic receptors were measured by radioreceptor assay using [N-methyl-3H]scopolamine methyl chloride ([3H]NMS), a selective radioligand of muscarinic receptors. Solifenacin showed concentration-dependent inhibition of specific [3H]NMS binding in the bladder urothelium and detrusor muscle of rats, with no significant difference in K_i values or Hill coefficients between these tissues. Following the intravesical instillation of solifenacin, there was significant muscarinic receptor binding (increase in K_i for specific [3H]NMS binding) in the bladder urothelium and detrusor muscle of rats. Similar bladder muscarinic receptor binding was observed by the intravesical instillation of oxybutynin, but not with trospium. In conclusion, the present study has demonstrated that solifenacin binds muscarinic receptors not only in the detrusor muscle but also in the bladder urothelium with high affinity. These bladder muscarinic receptors may be significantly affected by solifenacin excreted in the urine.

Key words solifenacin; bladder urothelium; muscarinic receptor binding; intravesical administration

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An overactive bladder is characterized by symptoms of urgency and urinary frequency with or without urge incontinence. The condition has a detrimental effect on physiological functioning and psychological well-being and quality of life. Antimuscarinic agents are widely used to treat overactive bladder since parasympathetic innervation is the predominant stimulus for bladder contraction. However, their use is associated with the anticholinergic side effects; dry mouth, constipation, somnolence, blurred vision and cognitive impairment, suggesting the importance of bladder selectivity.

Solifenacin succinate is a muscarinic receptor antagonist intended for the treatment of symptoms of overactive bladder. The inhibitory effect of solifenacin on carbachol-stimulated Ca^2+ mobilization is equipotent with oxybutynin in detrusor cells, but 2 to 10 times weaker in salivary gland cells. In vivo studies in anesthetized rats have shown that solifenacin is 4–7 times more potent in inhibiting bladder contraction than salivation, thereby indicating bladder selectivity.

The bladder urothelium is a multifunctional tissue that acts as a barrier between the vesical contents of the lower urinary tract and the underlying tissues and as a sensory organ by transducing physical and chemical stresses to the associated afferent nerve system and underlying smooth muscle. Muscarinic receptor subtypes are detected in the bladder urothelium of humans. The bladder urothelium was shown to respond to stretch and muscarinic agonist stimulation by releasing mediators such as adenosine triphosphate (ATP), nitric oxide (NO) and acetylcholine (ACh) itself, which may modulate afferent activity through nerve and/or myofibroblasts.

Therefore, urothelial muscarinic receptors could be a site of action for the antimuscarinic agents used to treat overactive bladder.

We have previously shown that solifenacin compared with oxybutynin binds muscarinic receptors persistently in the mouse bladder after oral administration. Kim et al. showed that intravesically infused antimuscarinic agents suppressed the carbachol-induced bladder overactivity. When administered orally, a pharmacologically significant amount of solifenacin was suggested to be excreted in the urine of humans taking clinical dose (5–10 mg) of this agent. Solifenacin succinate has approximately 15% active compound excreted into the urine compared with 3% and less than 1% with darifenacin and tolterodine LA, respectively. These results may indicate that solifenacin blocks the bladder muscarinic receptors partly from the urine. Chuang et al. showed that human urine obtained after taking solifenacin (5 mg) prevented the carbachol-induced detrusor overactivity. Thus, the urine excreted after oral ingestion of solifenacin may provide a localized pharmacological advantage for the treatment of overactive bladder syndrome. The current study aimed to examine such hypothesis by measuring muscarinic receptor binding in the bladder urothelium and detrusor muscles of rats after intravesical instillation of solifenacin.

MATERIALS AND METHODS

Materials [N-Methyl-3H]scopolamine methyl chloride ([3H]NMS, 3.03 TBq/mmol) was purchased from PerkinElmer, Inc., Life Sciences, Inc. (Boston, MA, U.S.A.). Solifenacin succinate was donated by Astellas Pharmaceutical Co., Ltd. (Tokyo, Japan). All other chemicals were purchased from commercial sources.
Animals Male Sprague-Dawley rats (250–300 g) at 8–10 weeks of age were purchased from Japan SLC (Shizuoka, Japan). They were housed in the laboratory with free access to food and water and maintained on a 12-h light–dark cycle in a room with controlled temperature (24 ± 2°C). Animal care and experiments were performed in accordance with the guidelines for the care and use of laboratory animals of the University of Shizuoka (registration number: 136023).

Intravesical Administration of Solifenacin, Oxybutynin and Trospium For the intravesical instillation of solifenacin, fasted rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally (i.p)), and their bladder was exposed. A 27 G needle connected to a syringe was inserted into the bladder through the bladder dome, and solifenacin (300, 3000 nM/0.2 mL/rat), oxybutynin (30, 300, 3000 nM/0.2 mL/rat) and trospium (3, 30, 300 nM/0.2 mL/rat) were infused into the bladder for 30 min. Control rats received an equivalent amount of saline.

Tissue Preparation After 30 min of drug administration, the anesthetized rats were sacrificed by drawing blood from the descending aorta. The bladder urothelium and detrusor muscle were dissected, and the tissues were minced with scissors and homogenized in a Kinematica Polytron homogenizer (19 volumes of ice-cold 30 mM Na+ piperazine-N'-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES) buffer (pH 7.5). The homogenate was centrifuged at 40000 × g for 20 min. The pellet was suspended in buffer for the binding assay. In the *ex vivo* (intravesical instillation) experiment, there was a possibility that solifenacin might dissociate in part from the receptor sites during the tissue preparation (homogenization and suspension) process after drug administration. Our group has previously shown that the dissociation of antagonists from receptor sites at 4°C was extremely slow.19 Therefore, to minimize the dissociation of solifenacin from the receptor sites, all steps were performed at 4°C. Protein concentrations were measured by BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, U.S.A.).

Muscarinic Receptor Binding Assay The binding assay for muscarinic receptors was performed using [3H]NMS as described previously.19 The homogenates (300–500 µg of protein) of rat tissues were incubated with varying concentrations (0.01–1.5 nM) of [3H]NMS in 30 mM Na+/HEPES buffer (pH 7.5). Incubation was carried out for 60 min at 25°C. The reaction was terminated by rapid filtration (Cell Harvester; Brandel Co., Gaithersburg, MD, U.S.A.) through Whatman GF/B glass fiber filters, and the filters were then rinsed three times with 3 mL of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters overnight by immersion in scintillation fluid [2 L toluene, 1 L Triton X-100, 15 g of 2,5-diphenyloxazole, and 0.3 g of 1,4-bis-[2-(5-phenyloxazolyl)]benzene], and measured with a liquid scintillation counter. Specific [3H]NMS binding was determined experimentally from the difference between counts in the absence and presence of 1 µM atropine. All assays were conducted in duplicate.

Data Analysis The [3H]NMS binding data was subjected to a non-linear regression analysis using Graph Pad PRISM (ver. 5, Graph Pad Software, San Diego, CA, U.S.A.). The apparent dissociation constant \( K_d \) and maximal number of binding sites \( B_{max} \) for [3H]NMS were estimated. The ability of solifenacin to inhibit specific [3H]NMS binding (250 pmol) was estimated from the IC\(_{50}\), which is the molar concentration of antimuscarinic agents necessary to displace 50% of specific [3H]NMS binding. The inhibition constant, \( K_i \), was calculated from the equation, \( \frac{IC_{50}}{L/K_i} \), where \( L \) represents the concentration of [3H]NMS.

The statistical analysis of the receptor binding data was performed with Student’s *t*-test and a one-way ANOVA, followed by Dunnett’s test for multiple comparisons. All data are expressed as the mean ± standard error (S.E.). Statistical significance was accepted at *p* < 0.05.

RESULTS

Inhibitory Effect on Specific [3H]NMS Binding in the Rat Bladder Urothelium and Detrusor Muscle *in Vitro* With increasing ligand concentration (0.01–1.5 nM), significant amount of specific [3H]NMS binding was observed in the homogenates of rat bladder urothelium and detrusor muscle. It was saturable and of high affinity. The estimated \( K_i \) and \( B_{max} \) values were 259 ± 24 pm and 105.7 ± 5 fmol/mg protein (urothelium), respectively, and 289 ± 11 pm and 125 ± 3 fmol/mg protein (mean ± S.E., *n* = 4), respectively.

Solifenacin (10–1000 nM) inhibited concentration-dependently specific [3H]NMS binding in the homogenates of rat bladder urothelium and detrusor muscle (Fig. 1), and there was no significant difference in \( K_i \) values (64.3 ± 7.5, 56.4 ± 2.3 nm, and 38.2 ± 3.6 nm).
respectively) and Hill coefficients (0.95±0.15 and 0.98±0.01, respectively) between these tissues (Table 1). The Hill coefficients were close to unity for this agent in these tissues. Thus, solifenacin displayed extremely high affinity to these bladder muscarinic receptors.

Similarly, oxybutynin (3–300 nm) inhibited specific [3H]NMS binding in the bladder urothelium and detrusor muscle of rats in a concentration-dependent manner (Fig. 1). The $K_i$ value in the bladder urothelium was slightly but significantly higher than that in the detrusor muscle. The Hill coefficients were close to one. Relatively low concentration of trospium (0.3–30 nm) also showed a concentration-dependent inhibition of specific [3H]NMS binding in the rat bladder tissues. There was no significant difference between the bladder urothelium and detrusor muscle in the $K_i$ values and Hill coefficients for trospium (Table 1).

**Effect of Intravesical Instillation of Solifenacin, Oxybutynin and Trospium on Specific [3H]NMS Binding in the Rat Bladder Urothelium and Detrusor Muscle**

Following the intravesical instillation of solifenacin (3000 nm/0.2 mL/rat) for 30 min, there were significant increases in $K_i$ values for specific [3H]NMS binding in the homogenates of rat bladder urothelium and detrusor muscle compared with each control value (Table 2). The increase rates were 25% (urothelium) and 34% (detrusor muscle), respectively. The similar intravesical instillation of lower concentration of solifenacin (300 nm/0.2 mL/rat) for 30 min tended to enhance the $K_i$ value for specific [3H]NMS binding in the rat bladder urothelium. On the other hand, there was little change in $B_{max}$ values for specific [3H]NMS binding in these bladder tissues following the intravesical instillation of solifenacin.

**DISCUSSION**

The present study was undertaken to characterize the bladder muscarinic receptor binding of solifenacin excreted in the urine by measuring specific [3H]NMS binding in the bladder urothelium and detrusor muscle of rats after the intravesical instillation of this drug.

There was a significant amount of muscarinic receptors in the rat bladder urothelium as well as the detrusor muscle, as shown by similar values of $K_i$ and $B_{max}$ of specific [3H]NMS binding in these tissues. Under in vitro conditions, solifenacin, oxybutynin and trospium demonstrated concentration-dependent inhibition of specific [3H]NMS binding in the homogenates of bladder urothelium and detrusor muscle of rats, with $K_i$ values within the nanomolar range, suggesting high affinity of these agents to the muscarinic receptors. The $K_i$ values for solifenacin and trospium were not significantly different between the bladder urothelium and detrusor muscle, while oxybutynin showed slight, but significantly higher affinity to the receptor in the detrusor muscle (Table 1). The reason why oxybutynin but not solifenacin and trospium displayed a higher affinity to the muscarinic receptors in the detrusor than in the urothelium is not clear. These results demonstrate the existence of pharmacologically relevant muscarinic receptors in the bladder urothelium as well as the detrusor muscle of rats. This study was the first to directly identify urothelial muscarinic receptors in the rat bladder by a radioligand binding assay. In addition, solifenacin, as well as oxybutynin and trospium, has been shown to bind the urothelial muscarinic receptors with high affinity.

### Table 1. $K_i$ Values and Hill Coefficients (nH) for in Vitro Inhibition by Solifenacin, Oxybutynin and Trospium of Specific [3H]NMS Binding in the Urothelium and Detrusor Muscle of Rat Bladders

|                | Bladder urothelium | Detrusor muscle |
|----------------|--------------------|----------------|
|                | $K_i$ (nM)         | nH             | $K_i$ (nM)         | nH             |
| Solifenacin    | 64.3±7.5           | 0.95±0.15      | 56.4±2.3           | 0.98±0.01      |
| Oxybutynin     | 11.2±3.4           | 1.24±0.09      | 7.99±0.65*         | 1.19±0.05      |
| Trospium       | 0.82±0.05          | 1.17±0.06      | 0.83±0.08          | 1.13±0.13      |

Each value represents the mean±S.E. of three to six rats. Asterisks show a significant difference from urothelium values, *$p<0.05$.

### Table 2. $K_i$ and $B_{max}$ Values of Specific [3H]NMS Binding in the Bladder Urothelium and Detrusor Muscle of Rats after Intravesical Instillation of Solifenacin, Oxybutynin and Trospium

|                | Bladder urothelium | Detrusor muscle |
|----------------|--------------------|----------------|
|                | $K_i$ (pM)         | $B_{max}$ (fmol/mg protein) | $K_i$ (pM)         | $B_{max}$ (fmol/mg protein) |
| Control        | 283±3              | 105±2          | 279±2              | 117±2          |
| Solifenacin    | 324±26             | 86±9           | 294±21             | 111±19         |
| 3000 nm        | 355±15 (1.25)***   | 91±8           | 375±21 (1.34)***   | 119±14         |
| Oxybutynin     | 240±11             | 104±12         | 294±38             | 110±28         |
| 3000 nm        | 230±3              | 88±8           | 266±24             | 107±16         |
| Trospium       | 449±35 (1.59)***   | 102±7          | 349±36 (1.25)*     | 106±7          |
| 3 nm           | 294±24             | 102±8          | 255±33             | 104±11         |
| 30 nm          | 324±27             | 116±9          | 268±28             | 114±12         |
| 300 nm         | 275±37             | 117±22         | 279±15             | 110±12         |

Rats received solifenacin (300, 3000 nm/0.2 mL), oxybutynin (30–3000 nm/0.2 mL) or trospium (3–300 nm/0.2 mL) intravesically for 30 min, and then the bladder tissues were dissected for the radioligand assay of muscarinic receptors. Each value represents the mean±S.E. of three to fifteen rats. Asterisks show a significant difference from control values, *$p<0.05$, ***$p<0.001$. Values in parentheses represent the fold-increase in $K_i$ values relative to control.
After oral administration of solifenacin (5–10 mg) in healthy volunteers, approximately 11% of the dose was excreted into urine as the parent compound and the urine concentration was estimated as 500–1500 nM. The muscarinic receptor binding affinity ($K_i$) of solifenacin was shown as 64.3 nM (bladder urothelium) and 56.4 nM (detrusor muscle) (Table 1). Therefore, it was assumed that the binding of bladder muscarinic receptors by oral administration of solifenacin might be caused partly by the parent compound excreted into the urine.

Following the intravesical instillation of solifenacin, there was a significant increase in $K_i$ for specific [3H]NMS binding in the bladder urothelium and detrusor muscle of rats, compared with control values (Table 2). The dose of injected solifenacin was given at the pharmacologically relevant dose. The increase in $K_i$ values for radioligands in drug-pretreated tissues in the radioreceptor assay refers generally to competition with the radioligand for the same binding sites, suggesting that the intravesically injected solifenacin binds to muscarinic receptors in the rat bladder urothelium and detrusor muscle. Taken together, these results indicate that solifenacin excreted in the urine after the oral administration may be transferred directly from the urine to the bladder tissue by simple diffusion.

In the in situ simultaneous measurement of bladder contraction and salivary secretion in anesthetized rats, Ikeda et al. have shown that intravenous solifenacin was 2 and 6.5 times, respectively, weaker than oxybutynin in inhibitory potency on the bladder contractile and salivary secretory response to carbachol, indicating the bladder selectivity of solifenacin. Additionally, Ohtake et al. reported essentially similar pharmacological selectivity of solifenacin for bladder over salivary gland in rats. Our previous study has shown that orally administered solifenacin exerts relatively long-lasting binding of muscarinic receptors in the bladder. These data suggest that binding of bladder muscarinic receptors by the excreted urinary solifenacin may contribute partly to the long-lasting binding of bladder muscarinic receptors. The current study has suggested that a bladder selectivity of solifenacin after the oral administration may be attributable partly to the excreted urinary parent compound.

The intravesical instillation of oxybutynin displayed significant binding of muscarinic receptors in the rat bladder urothelium and detrusor muscle. This finding may corroborate pharmacological results that intravesically infused antimuscarinic agents suppressed the carbachol-induced bladder overactivity. On the other hand, the intravesical instillation of trosiprium showed little significant binding of muscarinic receptors in the bladder urothelium and detrusor muscle. In general, the passive penetration of drugs through physiologic barrier such as the bladder urothelium depends on physicochemical factors such as high lipophilicity, low degree of ionization (neutral charge) and small molecular size. The characteristics of chemical properties of oxybutynin with high lipophilicity ($\log K_{ow}$: 4.68) and neutral polarity ($pK_a$: 6.44) may make it the most likely to cross the urothelial barrier. No significant muscarinic receptor binding in the rat bladder following the intravesical instillation of trosiprium may be attributable to the poor permeability due to the quaternary ammonium group in the chemical structure.

Bladder urothelium is a multifunctional tissue that acts not only as barrier between vesical contents of lower urinary tract and underlying tissues, but also as sensory organ by transducing physical and chemical stresses to the attendant afferent nervous system and underlying smooth muscle. Muscarinic receptor subtype was detected in the human bladder urothelium. The urothelium was shown to respond to stretch and muscarinic agonist stimuli by releasing ATP, nitric oxide, and acetylcholine itself, which may, in turn, modulate afferent activity through nerves and/or myofibroblasts. Therefore, urothelial muscarinic receptors may be the site of action for antimuscarinic agents such as solifenacin.

In conclusion, the current study demonstrated that solifenacin, as well as oxybutynin and trosiprium, exert high affinity to the muscarinic receptors in the rat detrusor muscle and bladder urothelium. In addition, intravesical injection of solifenacin at the pharmacologically relevant concentration, binds significantly to these receptors. The bladder selectivity of solifenacin may be attributed to a direct blockade of muscarinic receptors in the bladder urothelium and detrusor muscle by the excreted urinary parent compound.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1. Bulmer P, Abrams P. The overactive bladder. Rev. Contemp. Pharmacother., 11, 1–11 (2000).
2. Wein AJ, Rovner ES. Definition and epidemiology of overactive bladder. Urology, 60 (Suppl. 1), 7–12, discussion, 12 (2002).
3. Abrams P, Andersson KE. Muscarinic receptor antagonists for overactive bladder. BJU Int., 100, 987–1006 (2007).
4. Thind P, Lose G, Colstrup H, Andersson KE. The effect of pharmacological stimulation and blockade of autonomic receptors and of pudendal blockade on urethral stress relaxation in healthy women. Br. J. Urol., 74, 86–92 (1994).
5. Ikeda K, Kobayashi S, Suzuki M, Miyata K, Takeuchi M, Yamada T, Honda K. M3 receptor antagonism by the novel antimuscarinic agent solifenacin in the urinary bladder and salivary gland. Naunyn Schmiedebergs Arch. Pharmacol., 366, 97–103 (2002).
6. Kobayashi S, Ikeda K, Miyata K. Comparison of in vitro selectivity profiles of solifenacin succinate (YM905) and current antimuscarinic drugs in bladder and salivary glands: a Ca2+ mobilization study in monkey cells. Life Sci., 74, 843–853 (2004).
7. Ohtake A, Ukai M, Hatanaka T, Kobayashi S, Ikeda K, Sato S, Miyata K, Sasamata M. In vitro and in vivo tissue selectivity profile of solifenacin succinate (YM905) for urinary bladder over salivary gland in rats. Eur. J. Pharmacol., 492, 243–250 (2004).
8. Bischleipfer T, Schukowski K, Weidner W, Grando SA, Schwantes U, Kummer W, Lips KS. Expression and distribution of cholineric receptors in the human urothelium. Life Sci., 80, 2303–2307 (2007).
9. Mansfield KJ, Liu L, Mitchelson FJ, Moore KH, Millard RJ, Burcher E. Muscarinic receptor subtypes in human bladder detrusor and mucosa, studied by radioligand binding and quantitative competitive RT-PCR: changes in ageing. Br. J. Pharmacol., 144, 1089–1099 (2005).
10. Tyagi S, Tyagi P, Vanle S, Yoshimura N, Chancellor MB, de Miguel F. Qualitative and quantitative expression profile of mus-
carinic receptors in human urothelium and detrusor. *J. Urol.*, **176**, 1673–1678 (2006).

11) Olsen SM, Stover JD, Nagatomi J. Examining the role of mechanosensitive ion channels in pressure mechanotransduction in rat bladder urothelial cells. *Ann. Biomed. Eng.*, **39**, 688–697 (2011).

12) Andersson KE, Persson K. Nitric oxide synthase and nitric oxide-mediated effects in lower urinary tract smooth muscles. *World J. Urol.*, **12**, 274–280 (1994).

13) Yoshida M, Inadome A, Maeda Y, Satoji Y, Masunaga K, Sugiyama Y, Murakami S. Non-neuronal cholinergic system in human bladder urothelium. *Urology*, **67**, 425–430 (2006).

14) Oki T, Sato S, Miyata K, Yamada S. Muscarinic receptor binding, plasma concentration and inhibition of salivation after oral administration of a novel antimuscarinic agent, solifenacin succinate in mice. *Br. J. Pharmacol.*, **145**, 219–227 (2005).

15) Kim Y, Yoshimura N, Masuda H, de Miguel F, Chancellor MB. Antimuscarinic agents exhibit local inhibitory effects on muscarinic receptors in bladder afferent pathways. *Urology*, **65**, 238–242 (2005).

16) Doroshyenko O, Fuhr U. Clinical pharmacokinetics and pharmacodynamics of solifenacin. *Clin. Pharmacokinet.*, **48**, 281–302 (2009).

17) Guay DR. Clinical pharmacokinetics of drugs used to treat urge incontinence. *Clin. Pharmacokinet.*, **42**, 1243–1283 (2003).

18) Chuang YC, Thomas CA, Tyagi S, Yoshimura N, Tyagi P, Chancellor MB. Human urine with solifenacin intake but not tolterodine or darifenacin intake blocks detrusor overactivity. *Int. Urogynecol. J. Pelvic Floor Dysfunct.*, **19**, 1353–1357 (2008).

19) Yamada S, Yamamura HI, Roeske WR. Characterization of alpha-1 adrenergic receptors in the heart using [3H]WB4101: effect of 6-hydroxydopamine treatment. *J. Pharmacol. Exp. Ther.*, **215**, 176–185 (1980).

20) Krauwinkel WJ, Smulders RA, Mulder H, Swart PJ, Taekema-Roelvink ME. Effect of age on the pharmacokinetics of solifenacin in men and women. *Int. J. Clin. Pharmacol. Ther.*, **43**, 227–238 (2005).

21) Yamada S, Kusaka T, Urayama A, Kimura R, Watanabe Y. In vitro and ex vivo effects of a selective nociceptin/orphanin FQ (N/OFQ) peptide receptor antagonist, CompB, on specific binding of [3H]N/OFQ and [35S]GTPgammaS in rat brain and spinal cord. *Br. J. Pharmacol.*, **139**, 1462–1468 (2003).

22) Oki T, Kimura R, Saito M, Miyagawa I, Yamada S. Demonstration of bladder selective muscarinic receptor binding by intravesical oxybutynin to treat overactive bladder. *J. Urol.*, **172**, 2059–2064 (2004).

23) Scheife R, Takeda M. Central nervous system safety of anticholinergic drugs for the treatment of overactive bladder in the elderly. *Clin. Ther.*, **27**, 146–153 (2005).

24) Abrams P. Evidence for the efficacy and safety of tolterodine in the treatment of overactive bladder. *Expert Opin. Pharmacother.*, **2**, 1685–1701 (2001).

25) Watanabe T. The present conditions and the prospects of a skin application drug. *Drug Deliv. Syst.*, **22**, 450–457 (2007).

26) Yokoyama O, Ishiura Y, Nakamura Y, Ohkawa M. Urodynamic effects of intravesical oxybutynin chloride in conscious rats. *J. Urol.*, **155**, 768–771 (1996).

27) Bschleipfer I, Schukowski K, Weidner W, Grando SA, Schwantes U, Kummer W, Lipt S. Expression and distribution of cholinergic receptors in the human urothelium. *Life Sci.*, **80**, 2303–2307 (2007).

28) Mansfield KJ, Liu L, Mitchelson PJ, Moore KH, Millard RJ, Burcher E. Muscarinic receptor subtypes in human bladder detrusor and mucosa, studied by radioligand binding and quantitative competitive R1-PCR: changes in ageing. *Br. J. Pharmacol.*, **144**, 1089–1095 (2005).

29) Tyagi S, Tyagi P, Van-le S, Yoshimura N, Chancellor MB, de Miguel F. Qualitative and quantitative expression profile of muscarinic receptors in human urothelium and detrusor. *J. Urol.*, **176**, 1673–1678 (2006).

30) Olsen SM, Stover JD, Nagatomi J. Examining the role of mechanosensitive ion channels in pressure mechanotransduction in rat bladder urothelial cells. *Ann. Biomed. Eng.*, **39**, 688–697 (2011).

31) Andersson KE, Persson K. Nitric oxide synthase and nitric oxide-mediated effect in lower urinary tract smooth muscles. *World J. Urol.*, **12**, 274–280 (1994).

32) Yoshida M, Inadome A, Maeda Y, Satoji Y, Masunaga K, Sugiyama Y, Murakami S. Non-neuronal cholinergic system in human bladder urothelium. *Urology*, **67**, 425–430 (2006).