545

Fuchihata Y[1], Ogoda M[1], Ito Y[1], Yamada S[1]
1. *School of Pharmaceutical Sciences, University of Shizuoka*

ANTIMUSCARINIC AGENTS EXCRETED IN THE URINE BIND TO MUSCARINIC RECEPTORS IN THE RAT BLADDER UROTHELIUM AND DETRUSOR MUSCLE

Antimuscarinic agents such as imidafenacin and propiverine to treat overactive bladder have been reported to be excreted in urine as unchanged forms or active metabolites in human [1,2]. Our current study has shown that muscarinic receptors are present in the bladder urothelium and detrusor muscles, and antimuscarinic agents bind these receptors with high affinity [3]. Such *in vitro* observations may be pharmacologically relevant under the *in vivo* condition after the oral administration of antimuscarinic agents. In the current study, such hypothesis was investigated by measuring directly muscarinic receptor binding in the urothelium and detrusor muscles of rats after the intravesical instillation of antimuscarinic agents.

Study design, materials and methods

The bladder tissues of rats were dissected in the urothelium and detrusor. Muscarinic receptors were measured by radioreceptor assay using [N-methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS), a selective radioligand of muscarinic receptors. Specific [^{3}H]NMS binding was measured in the presence of various concentrations of antimuscarinic atgents. Furthermore, rats were intravesically instilled antimuscarinic agents for 30 min, and then muscarinic receptors in the urothelium and detrusor were simultaneously measured. Binding parameters of apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [^{3}H]NMS were estimated by nonlinear regression analysis using Graph Pad Prism. The inhibition constant, K_i, was calculated from the equation, $K_i=IC_{50}/(1+L/K_d)$, where IC_{50} and L represent the molar concentration of antimuscarinic agents necessary to displace 50 % of specific [^{3}H]NMS binding and the concentration of [^{3}H]NMS, respectively.

Results

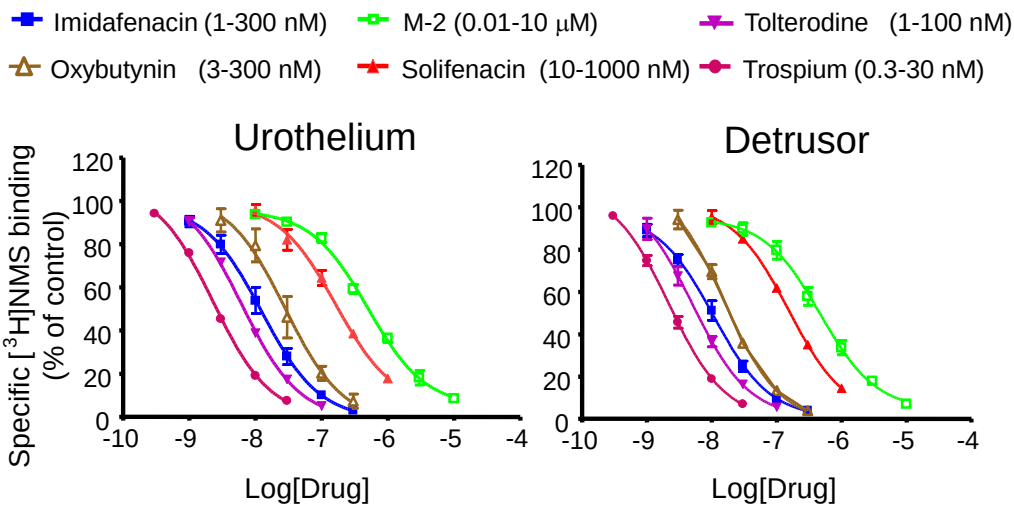
Antimuscarinic agents (oxybutynin, imidafenacin, active metabolite of propiverine (DPr-P-4(N→O)) (M-2), tolterodine, solifenacin and trospium) inhibited concentration-dependently specific [^{3}H]NMS binding in the urothelium and detrusor of rats (Fig. 1), and their K_i values were little significantly different between these tissues. Following the intravesical instillation of imidafenacin (30, 300 nM), DPr-P-4(N→O)(M-2) (3, 30 □M), tolterodine (30, 300 nM), oxybutynin (3 □M)and solifenacin (0.3, 3 □M), there was a significant muscarinic receptor binding (increase in K_d for specific [^{3}H]NMS binding) in the bladder urothelium and detrusor of rats, compared with control values (Table 1). Interestingly, intravesical instillation of trospium showed little significant binding of muscarinic receptors in the urothelium and detrusor of rats.

Interpretation of result

Antimuscarinic agents bind muscarinic receptors in the rat urothelium and detrusor muscle of rats, and their binding affinities were similar in these bladder tissues. Antimuscarinic agents except trospium excreted in the urine, may bind significantly to bladder muscarinic receptors, exerting significant effect on bladder function. No significant muscarinic receptor binding of intravesical trospium may be attributable to the poor permeability due to the quaternary ammonium group in the chemical structure.

Concluding message

Antimuscarinic agents bind muscarinic receptors not only in the detrusor muscle but also in the urothelium with high affinity. The bladder muscarinic receptors may be significantly affected by antimuscarinic agents excreted in the urine, thereby associating with the bladder selectivity of these agents.



Each point shows mean ± S.E. of 3 to 6 rats.

Fig.1 *In vitro* inhibition by antimuscarinic agents of specific [^{3}H]NMS binding in rats bladder urothelium and detrusor muscle

Table.1 Kd and Bmax for specific [^{3}H]NMS binding in the urothelium and detrusor muscle of rats after intravesical instillation of antimuscarinic agents for 0.5 hr

Tissue		Urothelium		Detrusor	
Drug		K_d (pM)	B_{max} (fmol/mg protein)	K_d (pM)	B_{max} (fmol/mg protein)
	Control	273 ± 5	107 ± 4	268 ± 6	118 ± 4
Imidafenacin	30 nM	307 ± 9 (1.12)*	113 ± 9	283 ± 12	124 ± 11
	300 nM	348 ± 15 (1.27)**	120 ± 10	301 ± 12 (1.12)*	126 ± 9
M-2	3 μM	316 ± 12 (1.16)**	118 ± 9	289 ± 11	120 ± 11
	30 μM	341 ± 20 (1.25)**	115 ± 11	253 ± 16	116 ± 8
Tolterodine	30 nM	348 ± 4 (1.27) **	108 ± 14	333 ± 26 (1.24) **	120 ± 16
	300 nM	319 ± 5 (1.17) **	105 ± 10	329 ± 25 (1.23) **	124 ± 11
Oxybutynin	3 μM	449 ± 35 (1.64) ***	102 ± 7	349 ± 36 (1.30) ***	106 ± 7
Solifenacin	300 nM	324 ± 26 (1.19) **	86 ± 9	353 ± 40 (1.32) **	111 ± 19
	3 μM	355 ± 15 (1.30) **	91 ± 8	375 ± 21 (1.40) **	119 ± 14
Trospium	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

Values are means ± S.E. of 4-7 rats.
Values in parentheses represent the fold-increase in K_d values relative to control.
Asterisks show a significant difference from the control values, *P<0.05, **P<0.01, ***P<0.001.

References
1. Jpn Pharmacol Ther 34: 569-875, 2006
2. Biomed Chromatogr 21: 940-948, 2007
3. Urol 78: 721.e7-721.e12, 2011

Disclosures
Funding: None **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** the guidelines for the care and use of laboratory animals of the University of Shizuoka.