129

Kuraoka S[1], Xuan H[1], Ito Y[1], Yamada S[1]
1. *School of Pharmaceutical Sciences, University of Shizuoka*

CHARACTERIZATION OF BLADDER MUSCARINIC RECEPTORS BY A NOVEL RADIOLIGAND, [3H]IMIDAFENACIN

Hypothesis / aims of study

Imidafenacin, a potent and selective antagonist of M_1 and M_3-muscarinic receptor subtypes, is now used clinically in Japan for the treatment of overactive bladder (OAB) [1-3]. Pharmacological studies of this agent showed selectivity in the bladder over salivary gland and brain. Oral administration of imidafenacin at low doses caused a more selective and longer-lasting binding to muscarinic receptors in the bladder than at other tissues such as the salivary gland, heart, colon, lung and brain, suggesting preferential muscarinic receptor binding in the bladder [2,3]. Pharmacokinetic data showed that the orally administered imidafenacin distributed at a higher concentration in the bladder than the serum or submaxillary gland of rats. The current study aimed to characterize further muscarinic receptor binding sites of currently synthesized [^{3}H]imidafenacin with high specific activity, in the rat bladder under the comparison with other tissues. Also, the muscarinic receptor binding of [^{3}H]imidafenacin in rat tissues was simultaneously compared with that of [*N*-methyl-^{3}H] scopolamine methyl chloride ([^{3}H]NMS), a widely used selective radioligand of muscarinic receptors.

Study design, materials and methods

The homogenates of rat tissues (bladder, submaxillary gland, heart, colon, lung, prostate, brain) were incubated with various concentrations of [^{3}H]imidafenacin at 25℃ for 60min. The receptor binding assay was conducted by rapid filtration. The relative density of M_2 and M_3 receptor subtype labeled by [^{3}H]imidafenasin was quantitatively estimated by the selective inactivation by 4-DAMP mustard, irreversible alkylating agent of M_3 receptor subtype. The competitive inhibitory effects of antimuscarinic agents on specific [^{3}H]imidafenacin binding were examined. Similarly, specific [^{3}H]imidafenacin binding was measured in rat tissue homogenates. Binding parameters of apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [^{3}H]imidafenacin and [^{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 binding and the concentration of radioligand, respectively.

Results

Specific binding of [^{3}H]NMS and [^{3}H]imidafenacin at relatively low concentrations was detected at significant amount in the bladder (Fig. 1) and other tissues of rats, and it was saturable and of high affinity. The dissociation constant (K_d) of [^{3}H]imidafenacin was lower in tissues (0.4-0.7 nM) (submaxillary gland, prostate, cerebral cortex) containing predominantly M_1 and M_3 receptors than M_2 receptor-dominating tissues (2.2-4.5 nM) (heart, cerebellum), intermediate (1.2-1.4 nM) in the bladder, lung and colon. K_d values of specific [^{3}H]NMS binding displayed no significant difference among these tissues. Antimuscarinic agents (imidafenacin, tolterodine, oxybutynin, solifenacin, atropine) inhibited concentration-dependently specific [^{3}H]imidafenacin binding in the bladder and other tissues (Fig. 2). Based on the K_i values, imidafenacin and tolterodine and atropine displayed relatively higher affinity to the bladder [^{3}H]imidafenacin binding than oxybutynin and solifenacin. The 4-DAMP mustard treatment reduced significantly maximal numbers of binding sites (B_{max}) in the bladder, submaxillary gland and colon, indicating a significant amount of M_3 subtype.

Interpretation of result

[^{3}H]Imidafenacin was shown to bind pharmacologically relevant muscarinic receptors (predominantly M_1 and M_2 subtypes) in rat tissues including the bladder with high affinity.

Concluding message

It is concluded that [^{3}H]imidafenacin labels selectively bladder muscarinic receptors. Thus, the present study may provide a rationale for the pharmacological usefulness of imidafenacin as therapeutic agent of overactive bladder. Also, this novel radioligand may be useful to characterize muscarinic receptors in tissues such as the bladder.

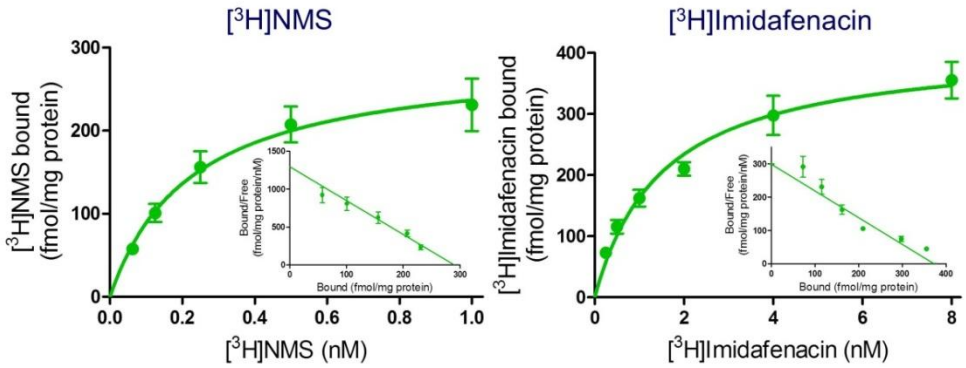

Fig. 1. Specific binding of various concentrations of [^{3}H]NMS and [^{3}H]imidafenacin in homogenates of rat bladder

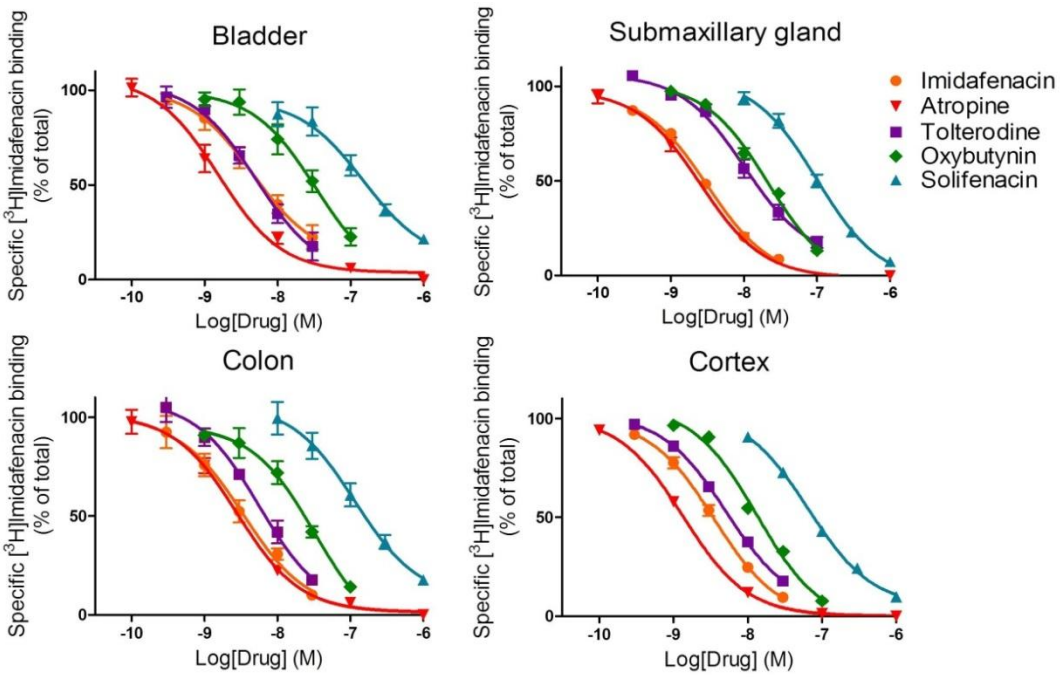

Fig. 2 Competitive inhibition by various concentrations of antimuscarinic agents of specific [3H]imidafenacin binding in homogenates of rat tissues (bladder, submaxillary gland, colon, cerebral cortex).

References
1. Arzneimittelforschung 57: 92-100, 2007.
2. J Pharmacol Sci 112: 142-50, 2010.
3. J Pharmacol Exp Ther 336: 365-371, 2011.

Disclosures
Funding: No **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** the guidelines for the care and use of laboratory animals of the University of Shizuoka.