Comparative Evaluation of Central Muscarinic Receptor Binding Activity by Oxybutynin, Tolterodine and Darifenacin Used to Treat Overactive Bladder

Tomomi Oki, Aiko Kageyama, Yukiko Takagi, Shinya Uchida and Shizuo Yamada*

From the Department of Pharmacokinetics and Pharmacodynamics and Center of Excellence Program in the 21st Century, School of Pharmaceutical Sciences, University of Shizuoka, Suruga-ku, Shizuoka, Japan

Purpose: We characterized muscarinic receptor binding in the mouse cerebral cortex after oral administration of anticholinergic agents used to treat overactive bladder.

Materials and Methods: Muscarinic receptors in the mouse cerebral cortex and bladder after oral administration of anticholinergic agents were measured using [^{3}H]N-methylscopolamine.

Results: In vitro binding affinities of tolterodine and its metabolite 5-hydroxymethyl metabolite in the mouse cerebral cortex and bladder were considerably greater than those of oxybutynin and darifenacin. Also, muscarinic receptor binding affinity of oxybutynin and its metabolite N-desethyl-oxybutynin in the cerebral cortex compared with that in the bladder was 2 to 3 times higher, whereas that of tolterodine and 5-hydroxymethyl metabolite was approximately 2 times lower. Oral administration of oxybutynin (76.1 μmol/kg), tolterodine (6.31 μmol/kg) and darifenacin (59.1 μmol/kg) showed binding activity that was approximately equal to that of bladder muscarinic receptors. Oral administration of oxybutynin (76.1 μmol/kg) showed significant binding of cerebral cortical muscarinic receptors in mice, as indicated by about a 2-fold increase in K_d values for specific [^{3}H]N-methylscopolamine binding 0.5 and 2 hours later. On the other hand, tolterodine and darifenacin given at oral doses that would exert a similar extent of bladder receptor binding activity as oxybutynin showed only a low level of binding activity of central muscarinic receptors in mice.

Conclusions: Significant binding of brain muscarinic receptors in mice was observed by the oral administration of oxybutynin but not tolterodine and darifenacin.

Key Words: bladder; mice; receptors, muscarinic; cholinergic antagonists; cerebral cortex

Overactive bladder exerts a detrimental effect on physical functioning and psychological well-being as well as significantly decreasing quality of life. Several anticholinergic agents are currently available and developmental for the treatment of OAB, including oxybutynin,[1] tolterodine[2] and darifenacin.[3] While anticholinergic agents have proven efficacy in patients with OAB, they are also associated with the troublesome anticholinergic side effects of dry mouth, constipation, somnolence and blurred vision.[1] Agents that cross the BBB and bind to muscarinic receptors in the brain carry a risk of CNS dysfunction, including cognitive impairment. In particular in elderly patients CNS adverse effects are an important concern with anticholinergic therapy because of the increased BBB permeability associated with normal aging.[4] In fact, case reports of CNS effects with oxybutynin have documented cognitive dysfunction and neuropsychiatric adverse reactions during clinical use for OAB.[5] On the other hand, it was shown that tolterodine and darifenacin may be associated with fewer cognitive effects than oxybutynin.[6–11] However, Diokno et al noted that the incidence in the appearance of CNS side effects was similar in a comparative clinical trial of extended release formulations of oxybutynin and tolterodine in 790 women with OAB.[12] Thus, it is not clear whether there is a significant difference in CNS effects between oxybutynin and tolterodine at pharmacologically relevant oral doses for OAB. To clarify this issue brain muscarinic receptor occupancy after oral administration of anticholinergic agents should be directly compared but to our knowledge such studies have not yet been done.

Our previous studies documented that the time dependent estimation of brain receptor occupancy after systemic administration of centrally acting drugs may be a powerful way to evaluate the permeability of these agents through the BBB.[13] Therefore, we quantified the penetration of oxybutynin, tolterodine and darifenacin into brain tissues by comparing muscarinic receptor binding in the cerebral cortex and bladder from mice orally administered these agents. Mice are an appropriate model species because similar metabolic patterns have been observed in rodents and humans for oxybutynin and tolterodine.[14,15]

MATERIALS AND METHODS

Materials

[^{3}H]NMS (3.03 TBq/mmol) was obtained from PerkinElmer Life Sciences, Boston, Massachusetts. Oxybutynin hydro-

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* Correspondence: Department of Pharmacokinetics and Pharmacodynamics and Center of Excellence Program in the 21st Century, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan (telephone: +81-54-264-5631; FAX: +81-54-264-5635; e-mail: yamada@ys7.u-shizuoka-ken.ac.jp).

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chloride and DEOB were provided by Meiji Milk Products, Odawara, Japan. Tolterodine tartrate and 5-HM were provided by Pharmacia, Kalamazoo, Michigan. Darifenacin hydrobromide was provided by Pfizer, Tokyo, Japan.

Animals

Male ddY strain mice (Japan SLC, Shizuoka, Japan) at ages 9 to 13 weeks were used in this study. They were housed with a 12-hour light-dark cycle and had free access to laboratory food and water.

Administration of Anticholinergic Agents

Mice were fasted for 16 hours and then orally administered oxybutynin (76.1 μmol/kg), tolterodine (6.31 and 21.0 μmol/kg) or darifenacin (59.1 μmol/kg) dissolved in distilled water. The doses of tolterodine and darifenacin were based on the difference relative to the in vitro muscarinic receptor binding potency of oxybutynin (table 1). Control animals received vehicle alone. The study was done in accordance with the guidelines of the Experimental Animal Ethical Committee of the University of Shizuoka.

Tissue Preparation and Muscarinic Receptor Binding Assay

At 0.5 to 6 hours after drug administration the mice were exsanguinated by taking the blood from the descending aorta under light anesthesia. The tissues were then perfused with cold saline from the aorta. The bladder and cerebral cortex were removed and each tissue was homogenized in a Kinematica Polytron® homogenizer in 19 volumes of ice-cold 30 mM Na^+/HEPES buffer (pH 7.5). The homogenates were centrifuged at 40,000 × gravity for 20 minutes. The resulting pellet was resuspended in buffer for the binding assay. All steps for the preparation were performed at 4C.

The mouse tissue homogenates (70 to 370 μg protein) were incubated with different concentrations (0.03 to 1.0 nM) of [^{3}H]NMS in 30 mM Na^+/HEPES buffer. Incubation was done for 60 minutes at 25C. The reaction was terminated by rapid filtration using a cell harvester (Brandel, Gaithersburg, Maryland) through a Whatman® GF/B glass filter. The filters were then rinsed 3 times with 3 ml ice-cold buffer. Tissue bound radioactivity was extracted from the filters overnight in scintillation fluid and 0.3 gm 1,4-*bis*[2-(5-phenyloxazolyl)]benzene). Radioactivity was determined by a liquid scintillation counter. Specific [^{3}H]NMS binding was determined experimentally from the difference between counts in the absence and presence of 1 μM atropine. All assays were done in duplicate.

Data Analysis

K_d and B_{max} for [^{3}H]NMS (0.03 to 1.0 nM) were estimated by Rosenthal analysis of the saturation data. The ability of muscarinic receptor antagonists to inhibit specific [^{3}H]NMS binding (125 pM) was estimated from IC_{50} values and K_i was calculated using the equation, $K_i = IC_{50}/(1+L/K_d)$, where L represents the concentration of [^{3}H]NMS. Statistical analysis of data was performed by 1-way ANOVA, followed by Dunnett's test for multiple comparison. Statistical significance was considered at $p < 0.05$.

RESULTS

In Vitro Muscarinic Receptor Binding

Each anticholinergic agent inhibited specific [^{3}H]NMS binding in the mouse bladder and cerebral cortex in a concentration dependent manner (fig. 1). The K_i value for oxybutynin in the cerebral cortex was significantly lower than that in the bladder (1/2.6), while the K_i value for DEOB was not different between these tissues (table 1). On the other hand, K_i values for tolterodine and 5-HM in the cerebral cortex were approximately 2 times higher than those in the bladder.

The K_i value for oxybutynin in the bladder was significantly higher than the values for DEOB, tolterodine and 5-HM (2.8, 12 and 18 times, respectively) and it was equivalent to the value for darifenacin. The K_i value for oxybutynin in the cerebral cortex was significantly higher than the values for tolterodine and 5-HM (2.1 and 3.4 times, respectively), and significantly lower than that for darifenacin (1/5.1). Thus, K_i value ratios in the cerebral cortex relative to the bladder were 0.39 for oxybutynin, 0.68 for DEOB, 2.13 for tolterodine, 1.99 for 5-HM and 1.31 for darifenacin (table 1).

Effects of Oral Administration of Anticholinergic Agents

At 0.5 and 2 hours after oral administration of oxybutynin (76.1 μmol/kg) there was a significant increase in K_d values for specific [^{3}H]NMS binding in mouse bladder compared with the corresponding control value (54.7% and 40.6%, respectively, table 2). Similarly at 0.5 or 2 to 6 hours after oral administration of tolterodine (6.31 and 21.0 μmol/kg) there were significant and dose related increases in K_d values for specific [^{3}H]NMS binding in the bladder. The increases at 2 and 6 hours were 21.4% and 37.0% (6.31 μmol/kg), and the increases at 0.5, 2 and 6 hours were 99.5%, 309% and 308% (21.0 μmol/kg), respectively. The maximal enhancement in K_d values by oxybutynin was seen

TABLE 1. K_i *for* in vitro *inhibition by oxybutynin, DEOB, tolterodine, 5-HM and darifenacin of specific [^{3}H]NMS binding in mouse bladder and cerebral cortex*

	Mean ± SE K_i (nM)			p Value	
Drugs	Bladder	p Value vs Oxybutynin	Cerebral Cortex	Vs Bladder	Vs Oxybutynin
Oxybutynin	14.3 ± 1.9		5.55 ± 0.57	<0.01	
DEOB	5.20 ± 1.08	<0.05	3.25 ± 0.21		
Tolterodine	1.22 ± 0.14	<0.01	2.60 ± 0.25	<0.01	<0.05
5-HM	0.81 ± 0.12	<0.01	1.61 ± 0.13	<0.01	<0.01
Darifenacin	21.6 ± 4.9		28.4 ± 1.5		<0.01

Values represent 3 to 5 mice.