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The Forefront for Novel Therapeutic Agents Based on the Pathophysiology of Lower Urinary Tract Dysfunction: Bladder Selectivity Based on In Vivo Drug–Receptor Binding Characteristics of Antimuscarinic Agents for Treatment of Overactive Bladder

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Abstract. We have reviewed the binding of antimuscarinic agents, used to treat urinary dysfunction in patients with overactive bladder, to muscarinic receptors in target and non-target tissues in vivo. Transdermal administration of oxybutynin in rats led to significant binding in the bladder without long-term binding in the submaxillary gland and the abolishment of salivation evoked by oral oxybutynin. Oral solifenacin showed significant and long-lasting binding to muscarinic receptors in mouse tissues expressing the M_3 subtype. Oral tolterodine bound more selectively to muscarinic receptors in the bladder than in the submaxillary gland in mice. The muscarinic receptor binding activity of oral darifenacin in mice was shown to be pronounced and long-lasting in the bladder, submaxillary gland, and lung. In vivo quantitative autoradiography using (+)*N*-[^{11}C] methyl-3-piperidyl benzilate in rats showed significant occupancy of brain muscarinic receptors on intravenous injection of oxybutynin, propiverine, solifenacin, and tolterodine. The estimated in vivo bladder selectivity compared to brain was significantly greater for solifenacin and tolterodine than oxybutynin. Darifenacin occupied few brain muscarinic receptors. Similar findings were also observed with positron emission tomography in conscious rhesus monkeys. The newer generation of antimuscarinic agents may be advantageous in the bladder selectivity after systemic administration.

Keywords: overactive bladder, antimuscarinic, in vivo receptor binding, bladder, other tissue, lower urinary tract

1. Introduction

An overactive bladder (OAB), characterized by an increased frequency of micturition, urgency, and urge incontinence, is very common in the geriatric population, a group that is rapidly increasing in number (1). Antimuscarinic agents (Fig. 1) are widely used as the first-line therapy for OAB because parasympathetic innervation is the predominant stimulus for bladder contraction (2). While anticholinergic agents have proven effective in patients with OAB, they are also associated with anticholinergic side effects, including dry mouth, constipation, somnolence, and blurred vision, because the muscarinic receptor mediates the excitatory and inhibitory actions of acetylcholine in the central and peripheral nervous systems (3). Dry mouth is the most common of these complaints and decreases quality of life. Therefore, numerous studies involving antimuscarinic agents to treat OAB have focused on targeting the urinary bladder over the salivary gland. The incidence of side effects on the central nervous system (CNS) is generally lower than that of dry mouth, but such effects may be of great concern in elderly patients because of an increase of blood-brain barrier (BBB) permeability with aging (4, 5). In this con-

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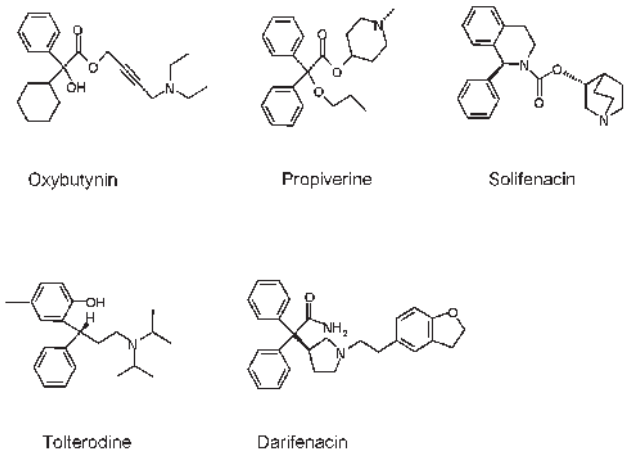

Fig. 1. Chemical structures of oxybutynin, propiverine, solifenacin, tolterodine, and darifenacin.

nection, clinical studies have demonstrated increased cognitive sensitivity to scopolamine (6, 7) and a reduced density of brain muscarinic receptors in the elderly (8). So, it is important to evaluate the bladder selectivity of antimuscarinic agents used to treat OAB for optimal medication.

2. In vivo drug–receptor binding characterization

There are a number of clinically useful drugs targeting receptors for neurotransmitters and hormones. The chain of events from drug administration to a certain pharmacological endpoint is complicated, but can be schematically simplified as illustrated in Fig. 2. It is evident that pharmacological effects of drugs are determined by both pharmacokinetic and pharmacodynamic processes. Pharmacokinetics includes the absorption, distribution, excretion, and metabolism of drugs, while pharmacodynamics includes the affinity of drugs for receptors, signal transduction, and homeostatic mechanisms (9, 10). The drug–receptor interaction results in a measured effect, and the magnitude of the interaction depends on the concentration of the drug in the biophase and on its affinity for the receptors. The biophase concentration depends not only on the amount of drug given, but also on factors such as the pharmacokinetic processes. As in vivo pharmacological specificity may be complicated by several factors including an equilibrium delay in concentrations of drugs between blood and the vicinity of the receptors, the formation of active metabolites, the occurrence of acute tolerance/sensitization, and the involvement of physiological control mechanisms (11), it may be important to examine directly the extracellular concentration, receptor occupancy, and pharmacological responses of drugs in different tissues such as target tissues and non-target

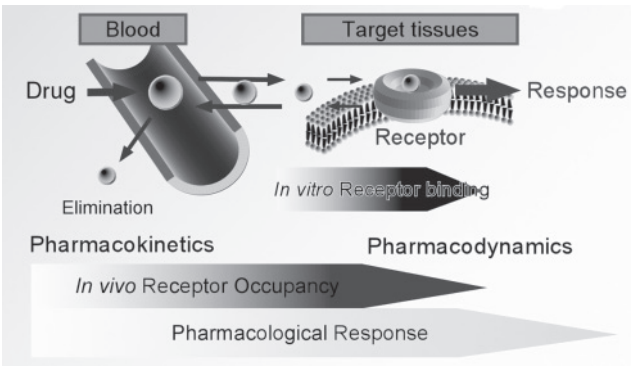

Fig. 2. Schematic representation of in vivo drug–receptor binding in relation to pharmacokinetics and pharmacodynamics (for details, see the text).

tissues under in vivo conditions.

Currently, a number of novel drugs exhibiting target organ specificity, receptor subtype selectivity, and long duration of action have been developed to reduce side effects and improve patient compliance (12 – 16). The affinity of compounds for various receptors in the development of novel drugs has been evaluated mainly using in vitro radioligand binding assays in tissue membrane preparations and in intact cells (13, 15, 17 – 20). However, even if a compound displays high affinity towards a certain receptor in vitro, it may not necessarily be able to reach such binding sites under in vivo conditions. In other words, the in vitro receptor binding characteristics may not necessarily assure pharmacological specificity in vivo because various pharmacokinetic and pharmacodynamic factors are not taken into account. Therefore, techniques to measure in vivo receptor binding would be useful in clarifying the pharmacological specificity of drugs in relation to their pharmacokinetics. In this article, we describe in vivo receptor binding characteristics in the bladder and other tissues of antimuscarinic agents (Fig. 1) used to treat OAB and also discuss the rationale for use of in vivo analysis of receptor occupancy in drug development.

3. Muscarinic receptor selectivity in the bladder over the salivary gland

3.1. Transdermal oxybutynin

Oxybutynin was widely used to treat OAB, but its use was often limited by systemic side-effects, such as dry mouth, blurred vision, constipation, and tachycardia, that appear frequently in patients receiving oral oxybutynin (21). It has been shown that the controlled release dosage form of oxybutynin (OROS) causes dry mouth less often than the immediate release form in patients with OAB (22, 23). It is also worth noting that a transdermal thera-

peutic system improved significantly the anticholinergic adverse effects of oral oxybutynin in patients with OAB (24, 25). Although, with the novel dosage form of oxybutynin, a low plasma concentration of its active metabolite, *N*-desethyl-oxybutynin (DEOB), has been suggested to contribute to the low incidence of anticholinergic side effects (24, 25), the underlying pharmacological mechanism for the advantage of transdermal over oral oxybutynin is unknown.

In an ex vivo study, both the oral and transdermal administration of oxybutynin resulted in a significant binding to muscarinic receptors in rat tissues, but a striking difference was seen between the oral and transdermal routes of administration in the effect on muscarinic receptors in the submaxillary gland (26). Namely, although oral oxybutynin significantly decreased the maximal number of [^{3}H]*N*-methylscopolamine (NMS) binding sites (B_{max}) in the rat submaxillary gland in a sustained manner, the transdermal application of oxybutynin produced little reduction in B_{max} values for [^{3}H]NMS binding in rat tissue. Based on a kinetic analysis of binding parameters for radioligand by Yamada et al. (27), it is suggested that oral but not transdermal oxybutynin produced an extremely long-lasting (noncompetitive) blockade of muscarinic receptor sites in the submaxillary gland, and this might be due to slowly dissociating blockade by oral oxybutynin of muscarinic receptors. Furthermore, in dose–response curves of pilocarpine-induced salivation, the antagonism by oral oxybutynin, unlike transdermal oxybutynin, was not simply competitive in that it suppressed the markedly maximal response by pilocarpine. Taken together, it is possible that a significant difference in exocrine muscarinic receptor binding characteristics under in vivo conditions partly underlies the lower incidence of severe dry mouth due to transdermal rather than oral oxybutynin in patients with OAB (24, 25).

It is known that orally administered oxybutynin is rapidly absorbed from the gastrointestinal tract with the major pathway of elimination by hepatic metabolism (28) and that the plasma concentration of DEOB in humans after oral administration is much higher than that of oxybutynin (29). Based on previous reports that muscarinic receptor binding affinity of DEOB was greater in the salivary gland than in the bladder (30, 31), it is considered that DEOB may be responsible for the long-lasting occupation of exocrine muscarinic receptors after oral oxybutynin. In fact, a similar concentration of DEOB to oxybutynin was detected after oral oxybutynin treatment in rats, but little of this metabolite was detected after transdermal oxybutynin treatment. So, it is possible that pharmacokinetic characteristics such as substantially less fluctuation in the plasma oxybutynin level and avoidance of a first-pass effect by transdermal oxybutynin brings about the significant difference in exocrine muscarinic receptor binding characteristics from that by oral oxybutynin, leading to the advantage of transdermal over oral oxybutynin in the treatment of OAB due to a lower incidence of dry mouth. In conclusion, transdermal oxybutynin may lead to a significant degree of binding to bladder muscarinic receptors without causing long-lasting occupation of muscarinic receptors in the submaxillary gland of rats and abolishment of salivation evoked by oral oxybutynin.

3.2. Solifenacin

Solifenacin is a novel muscarinic receptor antagonist intended for the treatment of urinary incontinence and other symptoms of OAB (32, 33). In vitro radioligand studies with human recombinant muscarinic subtypes have revealed that solifenacin exhibits high affinity and specificity for the muscarinic M_3 subtype, mainly mediating contraction of detrusor smooth muscle, relative to the M_1 and M_2 subtypes (32). The inhibitory effect of solifenacin on carbachol-stimulated Ca^{2+} mobilization was as potent as that of oxybutynin in detrusor cells, but 6 – 25 times weaker in submandibular gland cells (32, 33). In vivo studies in anesthetized rats have shown that solifenacin was 4 – 7 times more potent in inhibiting bladder contraction than salivation, whereas oxybutynin had little bladder selectivity (32, 34). Thus, solifenacin may exhibit pharmacological selectivity in the bladder relative to other tissues such as the salivary gland.

After oral administration of solifenacin and oxybutynin, some difference was seen between the two drugs in the time course of the increases in dissociation constant (K_d) for [^{3}H]NMS binding in mouse tissues (35). The increase in K_d with solifenacin in most tissues was greatest at 2 h and was maintained for at least 6 or 12 h, while the increase in K_d in each tissue reached a maximum at 0.5 h after the oral administration of oxybutynin, followed by a rapid decline. This apparent distinction between solifenacin and oxybutynin appears to largely depend on their rate of increase and disappearance in the plasma. Based on the intensity and duration of the increases in K_d values, the muscarinic receptor binding activity of oral solifenacin in mice was suggested to be greatest in the submaxillary gland and lowest in the heart; and it was also suggested to be long-lasting in the bladder, prostate, submaxillary gland, and colon, in contrast to transient binding in the heart and lung. Considering the subtype expression of muscarinic receptors in rat tissues (36, 37), the observed selectivity of oral solifenacin may be interpreted to reflect the muscarinic subtype selectivity shown in the in vitro assay (32). Additionally, the intensity and time course of the inhibitory effects of solifenacin and

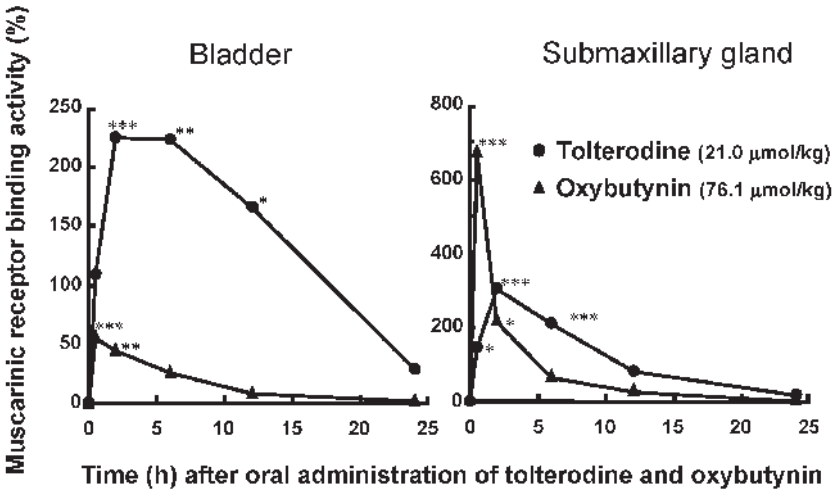

Fig. 3. Time course of muscarinic receptor binding in mouse tissues after the oral administration of tolterodine or oxybutynin. Seven (vehicle) and 5 (drug-treated) mice that received tolterodine (21.0 μmol/kg) or oxybutynin (76.1 μmol/kg), were sacrificed 0.5 – 24 h after administration, and specific binding of [^{3}H]NMS (0.06 – 1.0 nM) in the bladder and submaxillary gland was measured. Muscarinic receptor binding activity (%) was estimated by the fold-increase in K_d values relative to controls. Asterisks show a significant difference from the control values: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

oxybutynin on salivary secretion after oral administration seemed to coincide reasonably well with those for muscarinic receptor binding in the submaxillary gland. In conclusion, oral solifenacin shows significant binding to muscarinic receptors in various tissues of mice, including the bladder, and the receptor binding activity of this agent may be long-lasting in most tissues expressing the M_3 subtype (35, 38).

3.3. Tolterodine

Tolterodine has been developed as a muscarinic receptor antagonist to treat OAB (39), and pharmacological and radioligand binding studies have shown that tolterodine exerts a potent antimuscarinic effect in the isolated detrusor muscle of guinea pigs and humans (40). In addition, tolterodine has been demonstrated to display favorable tissue selectivity for the urinary bladder compared with the salivary glands in cats (40). Tolterodine is extensively metabolized in the liver to form an active metabolite, 5-hydroxymethyl metabolite (5-HM) (41, 42). In in vitro experiments, tolterodine and 5-HM competed concentration-dependently with [^{3}H]NMS for binding sites in the bladder, submaxillary gland, and heart of mice; and the potency of both agents was considerably greater than that of oxybutynin (43). Also, muscarinic receptor binding affinity in the bladder compared with the submaxillary gland was identical for tolterodine and significantly higher for 5-HM, whereas the affinity of oxybutynin was greater in the submaxillary gland than in the bladder.

There seems to be a significant difference between oxybutynin and tolterodine in ex vivo muscarinic receptor binding characteristics in mouse tissues (43). Oral administration of tolterodine compared with oxybutynin resulted in relatively slower and longer-lasting binding in each tissue, as characterized by the increase of K_d values for [^{3}H]NMS, which was greatest at 2 h and lasted for at least 6 or 12 h (Fig. 3). It should be noted that such extremely high receptor binding activity in the submaxillary gland as seen after oral oxybutynin was not observed with oral tolterodine and that the muscarinic receptor binding activity of oral tolterodine was of longer duration in the bladder than in the submaxillary gland. Furthermore, significant receptor binding activity by a lower dose of tolterodine was also observed in the bladder but not in the submaxillary gland. Therefore, oral tolterodine, unlike oral oxybutynin, binds more selectively to muscarinic receptors in the bladder than in the submaxillary gland. At these pharmacological doses, tolterodine was significantly weaker than oxybutynin in inhibiting pilocarpine-induced salivation of mice (43). Although the mechanism by which oral tolterodine causes bladder receptor selectivity in mice is not clear, it has been shown that most of the administered dose in mice receiving oral [^{14}C]tolterodine is preferentially distributed to the eliminating organs, that is, gall bladder, urinary bladder, liver, kidney, and lung (44). Such high concentrations of tolterodine and 5-HM in the bladder may be attributable to the tissue selectivity of this drug. In conclusion, oral tolterodine binds significantly to muscarinic receptors in the mouse bladder and the receptor binding activity of this drug compared with oral oxybutynin is relatively slow and longer lasting.

3.4. Propiverine

Propiverine, a benzilic acid derivative, is one of the antimuscarinic agents most commonly used for the treatment of patients with an OAB (45 – 47). In clinical trials, propiverine has proved to be an effective drug for urinary incontinence due to detrusor overactivity with a moderate incidence of adverse events. In dogs, propiverine increased maximum bladder volume and had inhibitory

effects on acetylcholine-induced periodic contractions of the urinary bladder (48, 49). Furthermore, propiverine exerted competitive anticholinergic and calcium-antagonistic effects in the isolated urinary bladder of rats, dogs, and humans (49 – 51).

It has been shown that propiverine is metabolized in the liver to form active metabolites, 1-methyl-4-piperidyl benzilate hydrochloride (DPr-P-4), 1-methyl-4-piperidyl diphenylpropoxyacetate *N*-oxide [P-4(N→O)], and 1-methyl-4-piperidyl benzilate *N*-oxide [DPr-P-4(N→O)] (52, 53). In in vitro and in vivo studies, these metabolites exerted anticholinergic and calcium antagonistic effects in the urinary bladder of rats and guinea pigs (54 – 56). Therefore, it has been speculated that these metabolites are partially responsible for the spasmolytic activity in the bladder after the oral administration of propiverine.

Propiverine and its *N*-oxide metabolites [P-4(N→O), DPr-P-4(N→O)] competed with [^{3}H]NMS binding sites in the bladder in a concentration-dependent manner in vitro, and the binding activity of DPr-P-4(N→O) was roughly equipotent to that of propiverine, while P-4(N→O) was considerably less active than propiverine (56). Following the oral administration of propiverine, there was relatively selective and longer-lasting binding of muscarinic receptors in the rat bladder compared with the submaxillary gland. Extremely high concentrations of P-4(N→O) and DPr-P-4(N→O) were detected in plasma after the oral administration of propiverine. Notably, following oral treatment with propiverine, the bladder showed the highest concentration of DPr-P-4(N→O), indicating the specific distribution of this metabolite into the target organ. Thus, DPr-P-4(N→O) may contribute greatly to the relatively selective and long-lasting occupation of bladder muscarinic receptors after the oral administration of propiverine at pharmacologically relevant doses.

3.5. Darifenacin

Darifenacin is a muscarinic receptor antagonist intended for the treatment of urinary incontinence and other symptoms of OAB (57, 58). In vitro radioligand studies with human recombinant muscarinic subtypes have revealed that darifenacin exhibits high affinity and specificity for the muscarinic M_3 subtype relative to the other subtypes (59). In in vitro experiments with rats, darifenacin displayed significantly higher affinity for muscarinic receptors in the submaxillary gland than in the bladder and heart. Consistent with this observation, our previous study showed that the muscarinic receptor binding affinity of darifenacin was approximately 10 times greater in the human parotid gland than in the urinary bladder (60). Thus, these data confirm that darifenacin displays a relatively higher affinity for the M_3 subtype than the M_2 subtype.

The muscarinic receptor binding activity of oral darifenacin in mice was shown to be pronounced and long-lasting in the bladder, submaxillary gland, and lung, in contrast to transient or little receptor binding in the heart and colon (61). Because of exclusive expression of the M_3 subtype in the submaxillary gland and moderate expression in the prostate and bladder, the remarkable receptor binding activity of oral darifenacin in the bladder and submaxillary gland is therefore interpreted to largely reflect the M_3-subtype selectivity shown by the in vitro study. The pilocarpine-induced salivary secretion in mice was abolished 0.5 – 6 h after oral darifenacin, followed by the sustained suppression of salivation (61). The time-course of suppression after oral administration of darifenacin coincided well with that for the muscarinic receptor binding in the submaxillary gland. These functional data support further the idea that oral darifenacin may cause a selective and long-lasting blockade of M_3 subtype under in vivo conditions.

4. Muscarinic receptor selectivity in the bladder over the brain

In an ARG study, the intravenous injection of oxybutynin, propiverine, tolterodine, and solifenacin significantly decreased in vivo specific (+)*N*-[^{11}C]methyl-3-piperidyl benzilate ([^{11}C](+)3-MPB, PET ligand of muscarinic receptor) binding in each brain region of rats in a dose-dependent manner (62) (Fig. 4). According to the estimated RO_{50} values, the potency of muscarinic receptor occupancy by each agent in the rat brain appeared to be greatest for oxybutynin, followed by tolterodine, solifenacin, and propiverine. In contrast, darifenacin at pharmacologically effective doses did not significantly reduce in vivo specific [^{11}C](+)3-MPB binding so that the RO_{50} value could not be estimated. RO_{50} values of antimuscarinic agents were similar to intravenous doses inhibiting learning/memory behavior in rats (63). Thus, these values could be regarded as the index of CNS pharmacological effects following the blockade of brain muscarinic receptors. Furthermore, the dose ratios (RO_{50}/ID_{50}) of antimuscarinic agents for the brain receptor occupancy to the inhibitory potency of increases in intravesical pressure were considered to reflect in vivo pharmacological selectivity for the urinary bladder over the brain. This ratio was relatively large for solifenacin (8.1 – 46.7), tolterodine (3.6 – 17.9), and propiverine (2.2 – 8.9), compared with oxybutynin (1.4 – 3.4). Thus, the selectivity for the urinary bladder over the brain was relatively low for oxybutynin, suggesting a high probability of CNS side effects at pharmacological doses to treat OAB. A similar finding was also

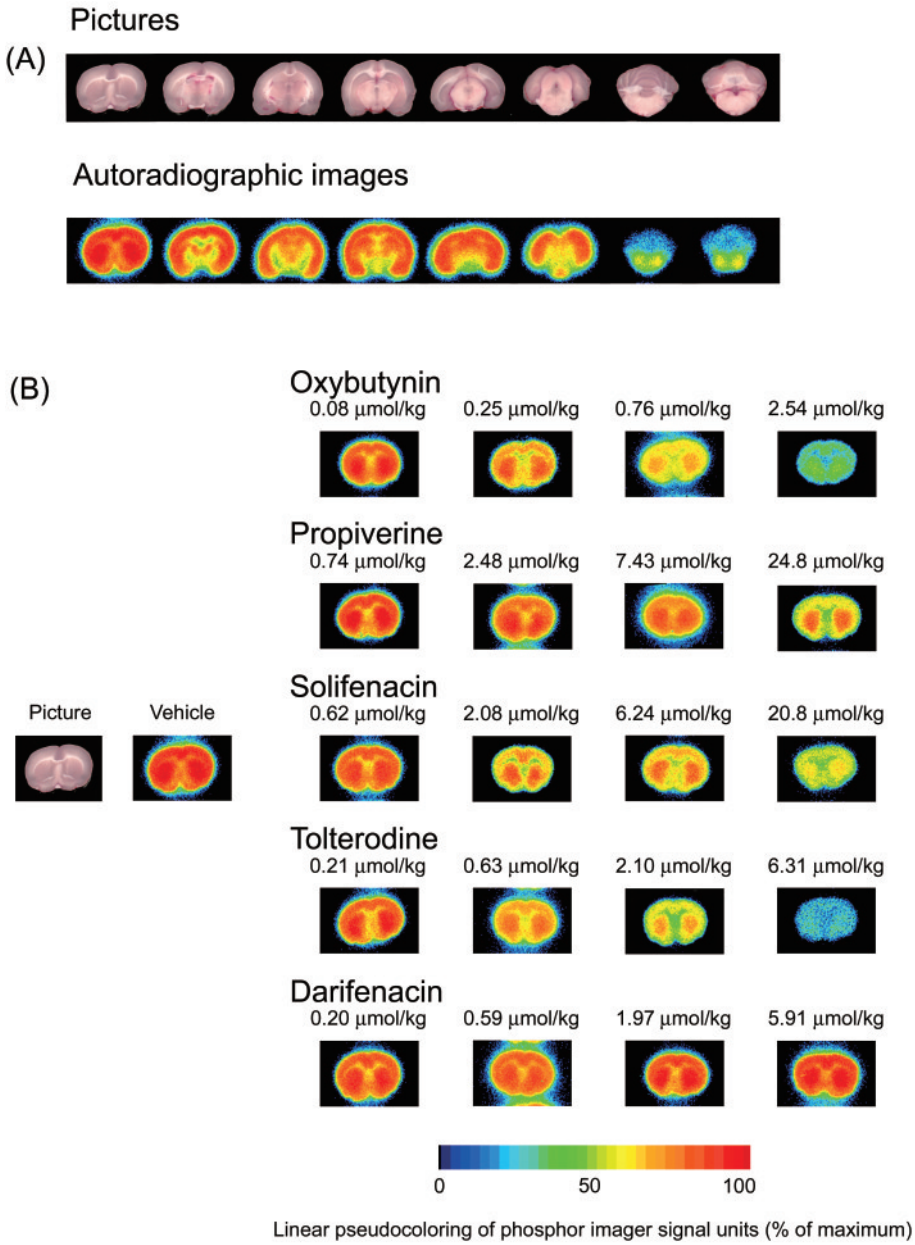

Fig. 4. (A) Representative autoradiographic images (distribution of radioactivity) in the brain of rats given an intravenous (i.v.) injection of [^{11}C](+)3-MPB. At 30 min after the i.v. injection of [^{11}C](+)3-MPB (150 MBq), brain tissue was removed and cut every 2 mm starting from center of the thalamus and analyzed for autoradiography. (B) Effects of i.v. injection of different doses of oxybutynin, propiverine, solifenacin, tolterodine, and darifenacin on autoradiographic images of [^{11}C](+)3-MPB in rat brain. Rats received i.v. injections of different doses of each antimuscarinic agent and 10 min later, given an i.v. injection of [^{11}C](+)3-MPB (150 MBq). After 30 min, brain tissues were removed for autoradiographic determination. This modified figure was reproduced with permission from Ref. 62.

made in a PET study in conscious rhesus monkey that oral administration of oxybutynin at clinically relevant doses exhibited significant (40% – 60%) occupancy of muscarinic receptors in the brain (our unpublished observation). The selectivity for the urinary bladder over the brain of solifenacin and tolterodine was clearly higher than that of oxybutynin in rats. In clinical studies, the incidence of CNS side effects of tolterodine was shown to be lower than that of oxybutynin and comparable to that with placebo (64 – 66). Thus, these data suggest that solifenacin and tolterodine have advantages in the treatment of OAB owing to less CNS effects. Furthermore, it is suggested that darifenacin induces the lowest incidence of CNS effects among antimuscarinic agents examined.

Antimuscarinic agents must first pass the blood-brain barrier (BBB) to occupy central muscarinic receptors. The observed difference among antimuscarinic agents in the potency of brain muscarinic receptor occupancy may be defined by BBB permeability, which is responsible for CNS effects in patients. The passive penetration of drugs through this physiologic barrier generally depends on physicochemical factors such as high lipophilicity, low degree of ionization (neutral charge), and small molecular size (64). The characteristics of chemical properties of oxybutynin relative to tolterodine —lipophilicity (Log $K_{o/w}$: 4.68 vs. 1.83) (67, 68) and neutral polarity (pKa: 6.44 vs. 9.87) (68, 69)— make it the most likely to cross the BBB (70). Additionally, muscarinic receptor subtype selectivity of antimuscarinic agents may be implicated in the appearance of CNS effects. All five muscarinic receptor subtypes are expressed in the brain (71, 72). The M_1 receptor was abundant in the cortex and hippocampus. The striatum contains both M_1 and M_4 receptors. By contrast, the M_2 receptor was predominantly localized to the brainstem and cerebellum. The M_3 receptor displays lower density in the brain compared to the M_1, M_2, and M_4 receptors. The cognitive dysfunction by antimuscarinic agents may be mediated mainly by the M_1 and M_2 receptors in the CNS (73). Oxybutynin shows selectivity for the M_1, M_3, and M_4 receptors, while tolterodine and propiverine are relatively nonselective to muscarinic receptor subtypes (60, 74). Solifenacin and darifenacin show higher selectivity to the M_3 receptor than M_1, M_2, and M_4 receptors. Thus, M_1 selectivity in addition to high BBB permeability of oxybutynin may be more apt to cause CNS side effects. Such muscarinic receptor subtype selectivity may be partly associated with an observed difference among antimuscarinic agents in the in vivo potency of brain receptor occupancy.

5. Conclusions

The determination of in vivo and ex vivo drug–receptor binding has been shown to be useful in predicting the dose, potency, and duration of pharmacological effects of receptor antagonists. The analysis of muscarinic receptor binding characteristics in the bladder and other tissues after the systemic administration of antimuscarinic agents has revealed that in the treatment of OAB, systemic adverse effects such as dry mouth and cognitive dysfunction could be avoided by the new generation of antimuscarinic agents with high bladder selectivity. Consequently, the in vivo measurement of receptor occupancy by drugs may allow evaluation of pharmacological specificity from the integrated viewpoint of pharmacokinetics and pharmacodynamics.

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References

1 Wein AJ, Rovner ES. Definition and epidemiology of overactive bladder. Urology. 2002;60:7–12.
2 Anderson KE. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol Rev. 1993;45: 253–308.
3 Andersson KE. Antimuscarinics for treatment of overactive bladder. Lancet Neurol. 2004;3:46–53.
4 Ouslander JG. Management of overactive bladder. N Engl J Med. 2004;350:786–799.
5 Pakulski C, Drobnik L, Millo B. Age and sex as factors modifying the function of the blood-cerebrospinal fluid barrier. Med Sci Monit. 2000;6:314–318.
6 Flicker C, Ferris SH, Serby M. Hypersensitivity to scopolamine in the elderly. Psychopharmacology. 1992;107:437–441.
7 Molchan SE, Martinez RA, Hill JL, Weingartner HJ, Thompson K, Vitiello B, et al. Increased cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model. Brain Res Brain Res Rev. 1992;17:215–226.
8 Norbury R, Travis MJ, Erlandsson K, Waddington W, Owens J, Ell PJ, et al. SPET imaging of central muscarinic receptors with (R,R)[^{123}I]-I-QNB: methodological considerations. Nucl Med Biol. 2004;31:583–590.
9 Holford NH, Sheiner LB. Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. Clin Pharmacokinet. 1981;6:429–453.
10 Yamada S, Uchida S, Kimura R. Integration of pharmacokinetics and pharmacodynamics in drug development – *in vivo* receptor occupancy by calcium antagonist and angiotensin II receptor antagonist. Current Topics in Pharmacol (RESEARCH TRENDS). 1998;4:239–250.
11 Dingemanse J, Danhof M, Breimer DD. Pharmacokinetic-pharmacodynamic modeling of CNS drug effects: an overview. Pharmacol Ther. 1988;38:1–52.
12 Ohtsuka M, Yokota M, Kodama I, Yamada K, Shibata S. New generation dihydropyridine calcium entry blockers: in search of greater selectivity for one tissue subtype. Gen Pharmacol. 1989;20:539–556.
13 Yamada S, Tanaka C, Kimura R, Kawabe K. Alpha 1-adrenoceptors in human prostate: characterization and binding characteristics of alpha 1-antagonists. Life Sci. 1994;54:1845–1854.
14 Chapple CR. Selective alpha 1-adrenoceptor antagonists in benign prostatic hyperplasia: rationale and clinical experience. Eur Urol. 1996;29:129–144.
15 Leurs R, Blandina P, Tedford C, Timmerman H. Therapeutic potential of histamine H_3 receptor agonists and antagonists. Trends Pharmacol Sci. 1998;19:177–183.
16 Yamada S, Ohkura T, Kimura R, Kawabe K. *In vivo* receptor binding of novel α_1-adrenoceptor antagonists for treatment of benign prostatic hyperplasia. Life Sci. 1998;62:1585–1589.
17 Palfreyman MG, Dudley MW, Cheng HC, Mir AK, Yamada S, Roeske WR, et al. Lactamimides: a novel chemical class of calcium antagonists with diltiazem-like properties. Biochem Phar-

macol. 1989;38:2459–2465.
18 Yamada S, Suzuki M, Kato Y, Kimura R, Mori R, Matsumoto K, et al. Binding characteristics of naftopidil and α_1-adrenoceptor antagonists to prostatic α-adrenoceptors in benign prostatic hypertrophy. Life Sci. 1992;50:127–135.
19 Nozawa Y, Haruno A, Oda N, Yamasaki Y, Matsuura N, Miyake H, et al. Pharmacological profile of TH-142177, a novel orally active AT1-receptor antagonist. Fundam Clin Pharmacol. 1997;11:395–401.
20 Feoktistov I, Polosa R, Holgate ST, Biaggioni I. Adenosine A_{2B} receptors: a novel therapeutic target in asthma? Trends Pharmacol Sci. 1998;19:148–153.
21 Yarker YE, Goa KL, Fitton A. Oxybutynin. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic use in detrusor instability. Drugs Aging. 1995;6:243–262.
22 Anderson RU, Mobley D, Blank B, Saltzstein D, Susset J, Brown JS. Once daily controlled versus immediate release oxybutynin chloride for urge urinary incontinence. OROS Oxybutynin Study Group. J Urol. 1999;161:1809–1812.
23 Sathyan G, Chancellor MB, Gupta SK. Effect of OROS controlled-release delivery on the pharmacokinetics and pharmacodynamics of oxybutynin chloride. Br J Clin Pharmacol. 2001;52:409–417.
24 Davila GW, Daugherty CA, Sanders SW; Transdermal Oxybutynin Study Group. A short-term, multicenter, randomized double-blind dose titration study of the efficacy and anticholinergic side effects of transdermal compared to immediate release oral oxybutynin treatment of patients with urge urinary incontinence. J Urol. 2001;166:140–145.
25 Dmochowski RR, Sand PK, Zinner NR, Gittelman MC, Davila GW, Sanders SW; Transdermal Oxybutynin Study Group. Comparative efficacy and safety of transdermal oxybutynin and oral tolterodine versus placebo in previously treated patients with urge and mixed urinary incontinence. Urology. 2003;62: 237–242.
26 Oki T, Toma-Okura A, Yamada S. Advantages for transdermal over oral oxybutynin to treat overactive bladder: Muscarinic receptor binding, plasma drug concentration, and salivary secretion. J Pharmacol Exp Ther. 2006;316:1137–1145.
27 Yamada S, Isogai M, Kagawa Y, Takayanagi N, Hayashi E, Tsuji K, et al. Brain nicotinic acetylcholine receptors. Biochemical characterization by neosurugatoxin. Mol Pharmacol. 1985;28: 120–127.
28 Douchamps J, Derenne F, Stockis A, Gangji D, Juvent M, Herchuelz A. The pharmacokinetics of oxybutynin in man. Eur J Clin Pharmacol. 1988;35:515–520.
29 Hughes KM, Lang JC, Lazare R, Gordon D, Stanton SL, Malone-Lee J, et al. Measurement of oxybutynin and its N-desethylmetabolite in plasma, and its application to pharmacokinetic studies in young, elderly and frail elderly volunteers. Xenobiotica. 1992;22:859–869.
30 Oki T, Kawashima A, Uchida M, Yamada S. In vivo demonstration of muscarinic receptor binding activity of N-desethyl-oxybutynin, active metabolite of oxybutynin. Life Sci. 2005;76: 2445–2456.
31 Waldeck K, Larsson B, Andersson KE. Comparison of oxybutynin and its active metabolite, N-desethyl-oxybutynin, in the human detrusor and parotid gland. J Urol. 1997;57:1093–1097.
32 Ikeda K, Kobayashi S, Suzuki M, Miyata K, Takeuchi M, Yamada T, et al. M_3 receptor antagonism by the novel antimuscarinic agent solifenacin in the urinary bladder and salivary gland. Naunyn Schmiedebergs Arch Pharmacol. 2002;366:97–103.
33 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 Ca^{2+} mobilization study in monkey cells. Life Sci. 2004;74:843–853.
34 Ohtake A, Ukai M, Hatanaka T, Kobayashi S, Ikeda K, Sato S, et al. In vitro and in vivo tissue selectivity profile of solifenacin succinate (YM905) for urinary bladder over salivary gland in rats. Eur J Pharmacol. 2004;492:243–250.
35 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. 2005;145:219–227.
36 Oki T, Takagi Y, Inagaki S, Taketo MM, Manabe T, Matsui M, et al. Quantitative analysis of binding parameters of [^{3}H]N-methylscopolamine in central nervous system of muscarinic acetylcholine receptor knockout mice. Brain Res Mol Brain Res. 2005;133:6–11.
37 Ito Y, Oyunzul L, Seki M, Fujino Oki T, Matsui M, Yamada S. Quantitative analysis of the loss of muscarinic receptors in various peripheral tissues in M_1-M_5 receptor single knockout mice. Br J Pharmacol. 2009;156:1147–1153.
38 Oki T, Takeuchi C, Yamada S. Comparative evaluation of exocrine muscarinic receptor binding characteristics and inhibition of salivation of solifenacin in mice. Biol Pharm Bull. 2006;29:1397–1400.
39 Guay DR. Tolterodine, a new antimuscarinic drug for treatment of bladder overactivity. Pharmacotherapy. 1999;19:267–280.
40 Nilvebrant L, Andersson KE, Gillberg PG, Stahl M, Sparf B. Tolterodine – a new bladder-selective antimuscarinic agent. Eur J Pharmacol. 1997;327:195–207.
41 Nilvebrant L, Gillberg PG, Sparf B. Antimuscarinic potency and bladder selectivity of PNU-200577, a major metabolite of tolterodine. Pharmacol Toxicol. 1997;81:169–172.
42 Postlind H, Danielson A, Lindgren A, Andersson SH. Tolterodine, a new muscarinic receptor antagonist, is metabolized by cytochromes P450 2D6 and 3A in human liver microsomes. Drug Metab Dispos. 1998;26:289–293.
43 Oki T, Maruyama S, Takagi Y, Yamamura HI, Yamada S. Characterization of muscarinic receptor binding and inhibition of salivation after oral administration of tolterodine in mice. Eur J Pharmacol. 2006;529:157–163.
44 Påhlman I, d'Argy R, Nilvebrant L. Tissue distribution of tolterodine, a muscarinic receptor antagonist, and transfer into fetus and milk in mice. Arzneimittelforschung. 2001;51:125–133.
45 Stöhrer M, Madersbacher H, Richter R, Wehnert J, Dreikorn K. Efficacy and safety of propiverine in SCI-patients suffering from detrusor hyperreflexia – a double-blind, placebo-controlled clinical trial. Spinal Cord. 1999;37:196–200.
46 Madersbacher H, Halaska M, Voigt R, Alloussi S, Höfner K. A placebo-controlled, multicentre study comparing the tolerability and efficacy of propiverine and oxybutynin in patients with urgency and urge incontinence. BJU Int. 1999;84:646–651.
47 Madersbacher H, Mürtz G. Efficacy, tolerability and safety profile of propiverine in the treatment of the overactive bladder (non-neurogenic and neurogenic). World J Urol. 2001;19: 324–335.
48 Kaneko S, Haruno A, Suzuki N, Kitazato K, Yamazaki Y, Nagai M, et al. [Effect of propiverine hydrochloride on the function of

the bladder in dogs.] Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi). 1989;93:55–60. (text in Japanese with English abstract)
49 Kaneko S, Nakano D, Ohara M, Nishimori T. [Effects of propiverine hydrochloride (propiverine) on isolated rat and dog urinary bladder.] Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi). 1999;113:145–156. (text in Japanese with English abstract)
50 Riotte J, Mutschler E. The spasmolytic activity of propiverine and some of its structural analogs. Arzneimittelforschung. 1987;37:300–302.
51 Wada Y, Yoshida M, Kitani K, Kikukawa H, Ichinose A, Takahashi W, et al. Comparison of the effects of various anticholinergic drugs on human isolated urinary bladder. Arch Int Pharmacodyn Ther. 1995;330:76–89.
52 Haustein KO, Hüller G. On the pharmacokinetics and metabolism of propiverine in man. Eur J Drug Metab Pharmacokinet. 1988;13:81–90.
53 Wengler A, Schneider T, Zschiesche M, Siegmund W. Studies on the metabolism of the bladder spasmolytic, propiverin (mictonorm) in humans. Pharmazie. 1988;43:652–653.
54 Haruno A, Yamasaki Y, Miyoshi K, Miyake H, Tsuchiya K, Kosaka M, et al. [Effects of propiverine hydrochloride and its metabolites on isolated guinea pig urinary bladder.] Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi). 1989;94:145–150. (text in Japanese with English abstract)
55 Nomura N, Kaneko S, Hamakawa T, Nagai M, Iriki M. [Effects of propiverine hydrochloride (P-4) and its metabolites on urinary bladder function in anesthetized rats.] Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi). 1989;94:173–180. (text in Japanese with English abstract)
56 Uchida S, Kurosawa S, Fujino Oki T, Kato Y, Nanri M, Yoshida K, et al. Binding activities by propiverine and its N-oxide metabolites of L-type calcium channel antagonist receptors in the rat bladder and brain. Life Sci. 2007;80:2454–2460.
57 Wallis RM, Burges RA, Cross PE, MacKenzie AR, Newgreen DT, Quinn P. Darifenacin, a selective muscarinic M3 antagonist. Pharmacol Res, 1995;31S:54.
58 Quinn P, McIntyre P, Miner WD, Wallis RM. In vivo profile of darifenacin, a selective muscarinic M3 receptor antagonist. Br J Pharmacol. 1996;119:198P.
59 Napier C, Gupta P. Darifenacin is selective for the human recombinant M3 receptor subtype. Proceedings of the 32nd Annual Meeting of the International Continence Society (Heidelberg, Germany), 2002; abstract 445.
60 Maruyama S, Oki T, Otsuka A, Shinbo H, Ozono S, Kageyama S, et al. Human muscarinic receptor binding characteristics of antimuscarinic agents to treat overactive bladder. J Urol. 2006;175: 365–369.
61 Yamada S, Maruyama S, Takagi Y, Uchida S, Oki T. In vivo demonstration of M_3 muscarinic receptor subtype selectivity of darifenacin in mice. Life Sci. 2006;80:127–132.
62 Maruyama S, Tsukada H, Nishiyama S, Kakiuchi T, Fukumoto D, Oku N, et al. In vivo quantitative autoradiographic analysis of brain muscarinic receptor occupancy by antimuscarinic agents for overactive bladder treatment. J Pharmacol Exp Ther. 2008;325:774–781.
63 Suzuki M, Noguchi Y, Okutsu H, Ohtake A, Sasamata M. Effect of antimuscarinic drugs used for overactive bladder on learning in a rat passive avoidance response test. Eur J Pharmacol. 2007;557:154–158.
64 Scheife R, Takeda M. Central nervous system safety of anticholinergic drugs for the treatment of overactive bladder in the elderly. Clin Ther. 2005;27:144–153.
65 Chapple CR. Muscarinic receptor antagonists in the treatment of overactive bladder. Urology. 2000;55:33–46.
66 Clemett D, Jarvis B. Tolterodine: a review of its use in the treatment of overactive bladder. Drugs Aging. 2001;18:277–304.
67 Watanabe T. The present conditions and the prospects of a skin application drug. Drug Delivery System. 2007;22:450–457.
68 Abrams P. Evidence for the efficacy and safety of tolterodine in the treatment of overactive bladder. Expert Opin Pharmacother. 2001;2:1685–1701.
69 Yokoyama O, Ishiura Y, Nakamura Y, Ohkawa M. Urodynamic effects of intravesical oxybutynin chloride in conscious rats. J Urol. 1996;155:768–771.
70 Kay GG, Granville LJ. Antimuscarinic agents: implications and concerns in the management of overactive bladder in the elderly. Clin Ther. 2005;27:127–138.
71 Flynn DD, Reever CM, Ferrari-DiLeo G. Pharmacological strategies to selectivity label and localize muscarinic receptor subtypes. Drug Dev Rev. 1997;40:104–116.
72 Oki T, Takagi Y, Inagaki S, Taketo MM, Manabe T, Matsui M, et al. Quantitative analysis of binding parameters of [^{3}H]N-methylscopolamine in central nervous system of muscarinic acetylcholine receptor knockout mice. Brain Res Mol Brain Res. 2005;133:6–11.
73 Kay GG, Abou-Donia MB, Messer WS Jr, Murphy DG, Tsao JW, Ouslander JG. Antimuscarinic drugs for overactive bladder and their potential effects on cognitive function in older patients. J Am Geriatr Soc. 2005;53:2195–2201.
74 Ohtake A, Saitoh C, Yuyama H, Ukai M, Okutsu H, Noguchi Y, et al. Pharmacological characterization of a new antimuscarinic agent, solifenacin succinate, in comparison with other antimuscarinic agents. Biol Pharm Bull. 2007;30:54–58.