

## Full Paper

**Muscarinic Receptor Binding of the Novel Radioligand, [<sup>3</sup>H]Imidafenacin in the Human Bladder and Parotid Gland**

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**Abstract.** The aim of the current study was to demonstrate highly specific and direct binding activity of tritium ([<sup>3</sup>H])-labeled imidafenacin for labeling muscarinic receptors in human bladder and parotid gland. Specific binding of [<sup>3</sup>H]imidafenacin in human tissues was saturable, reversible, and of high affinity. The  $K_d$  value for specific [<sup>3</sup>H]imidafenacin binding in the human bladder was approximately 3 times higher than that in the parotid gland. Unlabeled imidafenacin as well as the clinically used antimuscarinic agents, oxybutynin, tolterodine, and solifenacin, competed with [<sup>3</sup>H]imidafenacin for binding sites in the human bladder and parotid gland in a concentration-dependent manner, which indicated pharmacological specificity of [<sup>3</sup>H]imidafenacin binding sites. The  $K_i$  for imidafenacin in the human bladder approximately corresponded to pharmacological potency for the competitive blockade of carbachol-induced contractions of bladder, indicating a close correlation between binding affinity of imidafenacin to bladder muscarinic receptors and its pharmacological effects in the bladder. In conclusion, the current study is the first to provide direct evidence to demonstrate that imidafenacin bound muscarinic receptors in the human bladder, supporting its clinical relevance as a therapeutic agent for overactive bladder. [<sup>3</sup>H]Imidafenacin may also be a useful radioligand for labeling the  $M_3$  subtype of muscarinic receptors with higher selectivity than other radioligands.

**Keywords:** overactive bladder, [<sup>3</sup>H]imidafenacin, human bladder, parotid gland, muscarinic receptor

**Introduction**

Overactive bladder (OAB) is characterized by micturition, urgency, and urge incontinence, and it negatively affects many elderly people (1). Pharmacological treatment with antimuscarinic agents has been the main stay of therapy for patients with OAB. The efficacy of these drugs is known to be mediated through the blockade of

muscarinic receptors in the detrusor muscle and urothelium (2). However, antimuscarinic agents have also been associated with systemic adverse effects in patients with OAB, which have been attributed to significant binding to muscarinic receptors in organs other than the bladder. The most frequent adverse effect of antimuscarinic agents, dry mouth, was shown to be mainly due to the blockade of muscarinic receptors in the salivary gland (3).

Imidafenacin [4-(2-methyl-1*H*-imidazol-1-yl)-2,2-diphenylbutanamide, KRP-197/ONO-8025] is a relatively novel antimuscarinic agent used to treat OAB in Japan. A previous study demonstrated that it had higher

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affinity for M<sub>1</sub>- and M<sub>3</sub>-receptor subtypes than the M<sub>2</sub>-receptor subtype (4), which allowed it to regulate acetylcholine release by inhibiting the prejunctional M<sub>1</sub> subtype and inhibit bladder contraction by blocking the post-synaptic M<sub>3</sub> subtype. Further studies revealed that imidafenacin exhibited functional selectivity toward the bladder over the salivary gland and had a negligible pharmacological effect on the CNS even at high doses (5, 6). Imidafenacin is known to be a well-tolerated agent in clinical settings and causes fewer adverse effects (7, 8). Masumori (9), Takeuchi et al. (10), and Maggiore et al. (11) recently reviewed the long-term efficacy, safety, and tolerability of imidafenacin and reported a reduced incidence of severe adverse events such as dry mouth and constipation. In addition, imidafenacin can be used safely for a long period of time even by cognitively vulnerable elderly patients with the symptoms of OAB. Thus, it is highly likely that imidafenacin is a safe, efficacious, and tolerable treatment that can control the symptoms of OAB even in the long term.

Yamada et al. (6) and Seki et al. (12) examined the competitive inhibitory effect of imidafenacin on [*N*-methyl-<sup>3</sup>H]scopolamine methyl chloride (NMS) binding and revealed that it was a potent inhibitor of muscarinic receptor binding sites in rat and human bladders. Thus, investigating the direct binding properties of imidafenacin to bladder muscarinic receptors is of importance. The aim of the current study was to characterize the binding properties of the new tritium (<sup>3</sup>H)-labeled imidafenacin (<sup>3</sup>H]imidafenacin), which exhibits highly specific activity, in the human bladder and parotid gland.

## Materials and Methods

### Materials

[<sup>3</sup>H]Imidafenacin [4-(2-methyl-1*H*-imidazol-1-yl)-2-phenyl-2-(3-tritiophenyl)-butanamide] (851 GBq/mmol) and imidafenacin were donated by Kyorin Pharmaceutical Co., Ltd. (Tokyo). Oxybutynin hydrochloride, solifenacin succinate, and tolterodine L-tartrate were donated by Meiji Milk Products Co., Ltd. (Odawara), Astellas Pharma, Inc. (Tokyo), and Pfizer Co., Ltd. (Tokyo), respectively. All other chemicals were purchased from commercial sources.

### Patients and specimens

Human urinary bladder specimens were collected from 12 patients (10 men and 2 women; aged 73.9 ± 5.4, age range 63–80 years) undergoing total cystectomy for bladder carcinoma. All patients were screened by their physician and were confirmed to exhibit no symptoms of OAB or bladder outlet obstruction. Exclusion criteria included previous pelvic radiotherapy, extensive chemo-

therapy, current urinary tract infection, or concomitant carcinoma in situ (CIS). Human normal parotid gland specimens were obtained from 5 patients (3 men and 2 women; age: 75.4 ± 4.7, age range: 69–80 years of age) undergoing the surgical excision of a parotid tumor. None of the patients had diseases or used medications known to interfere with the cholinergic receptor system. Written informed consent was obtained from all patients. This study was conducted according to the Helsinki Declaration, and the protocol of this study was approved by the Ethics Committees of the University of Shizuoka, University of Yamanashi, and Hamamatsu University School of Medicine.

All human bladder specimens were obtained from macroscopically normal tissue in the anterior or lateral wall of the urinary bladder body as described previously (13). They were immediately transported to the laboratory, and the adventitia and connecting fatty tissues were removed. All specimens of the human normal parotid gland were taken from areas macroscopically free of tumors (14). They were then stored at –80°C until used.

### *Tissue preparation and radioligand binding assay of [<sup>3</sup>H]imidafenacin*

Tissue was carefully minced with scissors and homogenized with a Kinematica Polytron homogenizer in 19 volumes of ice-cold 30 mM Na<sup>+</sup>/HEPES buffer (pH 7.5). The homogenate was then centrifuged at 40,000 × *g* for 20 min, and the resulting pellet was resuspended in the same buffer for use in the binding assay.

In the saturation binding experiments with [<sup>3</sup>H]imidafenacin, the tissue homogenates of the human bladder and parotid gland were incubated with various concentrations (0.5–8.0 nM) of [<sup>3</sup>H]imidafenacin in 30 mM Na<sup>+</sup>/HEPES buffer (pH 7.5) at a total assay volume of 1 mL (14). In the competition binding experiments, the tissue homogenate (3.0 mg of original tissue) was incubated with [<sup>3</sup>H]imidafenacin (4.0 nM) in the presence of each anti-muscarinic agent. Incubation was performed for 60 min at 25°C, and the reaction was terminated by rapid filtration (Cell harvester; Brandel Co., Gaithersburg, MD, USA) through Whatman GF/B glass filters. In the case of the kinetics study, [<sup>3</sup>H]imidafenacin binding (4.0 nM) was quantified as a function of time for 60 min from the addition of the ligand, and the dissociation of the [<sup>3</sup>H]imidafenacin-receptor complex was then monitored by the addition of 0.1 μM atropine. The filters were rinsed three times with 3 mL of ice-cold 50 mM Na<sup>+</sup>/K<sup>+</sup> phosphate buffer (pH 7.4). Tissue-bound radioactivity was extracted from the filters overnight in scintillation fluid, and radioactivity was measured with a liquid scintillation counter. Specific [<sup>3</sup>H]imidafenacin binding was determined experimentally from the difference between counts

in the absence and presence of 1  $\mu$ M atropine. Protein concentrations were measured by the BCA method.

#### Data analyses

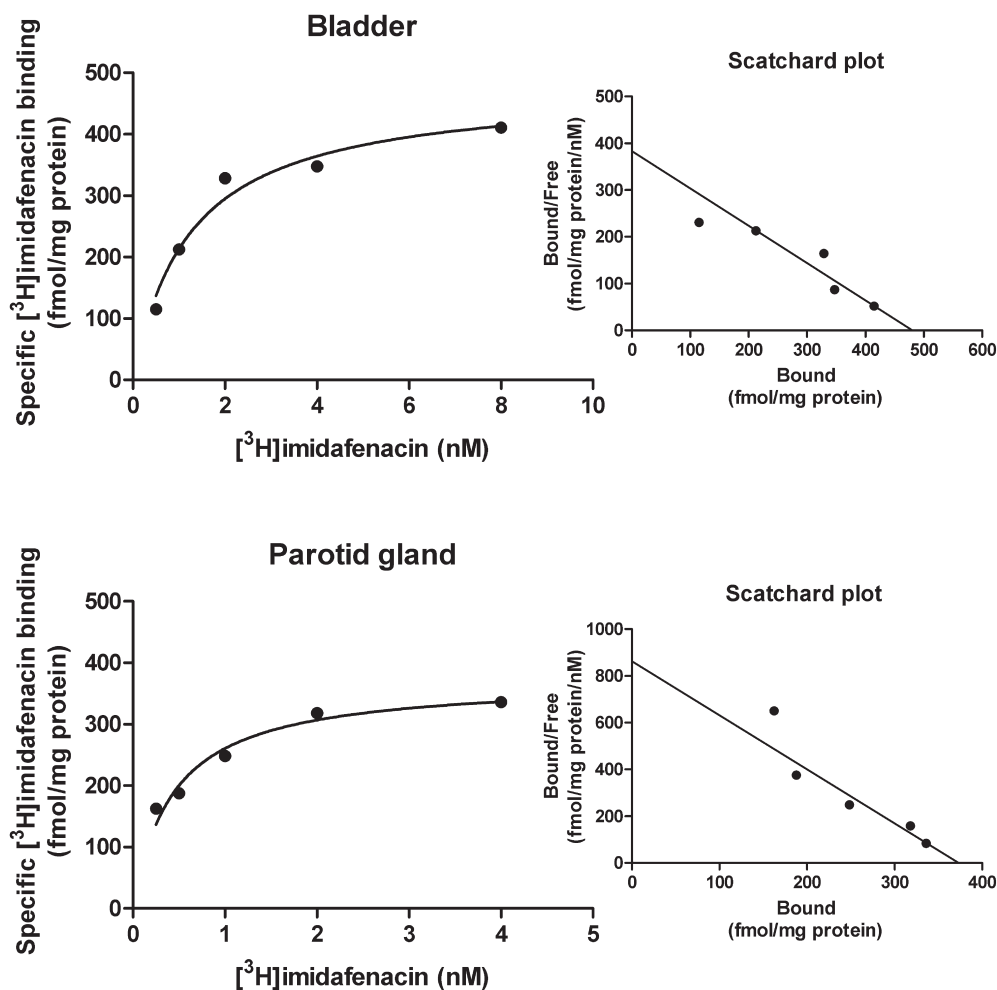
[ $^3$ H]Imidafenacin binding data were subjected to a non-linear regression analysis using Graph Pad PRISM (ver. 4, Graph Pad Software; San Diego, CA, USA) (6, 14). The apparent dissociation constant ( $K_d$ ) and maximal number of binding sites ( $B_{max}$ ) for [ $^3$ H]imidafenacin were estimated. The rate constants of association ( $k_{+1}$ ) and dissociation ( $k_{-1}$ ) from the association and dissociation experiments were also estimated. The ability of nonlabeled agents to inhibit specific [ $^3$ H]imidafenacin binding (4.0 nM) was estimated from the  $IC_{50}$ , which is the molar concentration of antimuscarinic agents necessary to displace 50% of specific [ $^3$ H]imidafenacin binding. The inhibition constant,  $K_i$ , was calculated from the equation  $K_i = IC_{50} / (1 + L/K_d)$ , where  $L$  represents the concentration of [ $^3$ H]imidafenacin, and  $pK_i$  values

were estimated. Statistical analyses of the data were performed with the Spearman test and statistical significance was accepted at  $P < 0.05$ . Data were expressed as the mean  $\pm$  S.E.M.

#### Results

##### Identification and characterization of specific [ $^3$ H]imidafenacin binding in the human bladder and parotid gland

The specific binding of [ $^3$ H]imidafenacin was measured at various concentrations (0.5 – 8.0 nM) in homogenates of the human bladder and parotid gland. The specific binding of [ $^3$ H]imidafenacin was saturable, forming a plateau around 4.0 – 8.0 nM (bladder) and 2.0 – 4.0 nM (parotid gland) of [ $^3$ H]imidafenacin (Fig. 1); however, nonspecific binding increased linearly with an increase in [ $^3$ H]imidafenacin concentrations (data not shown). Nonlinear regression analysis revealed that these data were consistent with a simple one-site model



**Fig. 1.** Specific binding of [ $^3$ H]imidafenacin to homogenates of the human bladder and parotid gland and its Scatchard plot (insert). Each point represents the average from 3 experiments.

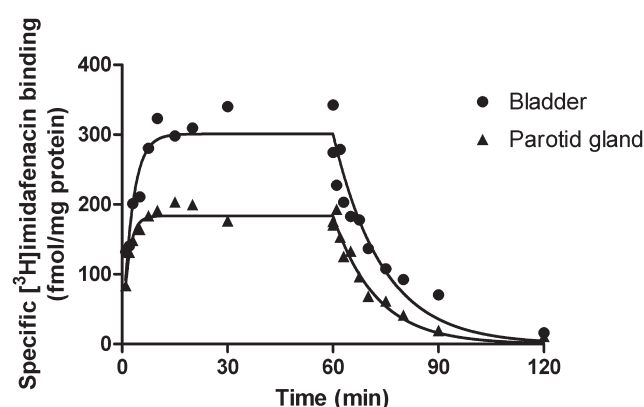
in both tissues. The estimated  $K_d$  values of [<sup>3</sup>H]imidafenacin in the bladder and parotid gland were  $1227 \pm 26$  and  $442 \pm 23$  pM ( $n = 3$ , mean  $\pm$  S.E.M.), respectively, and the corresponding  $B_{max}$  values were  $475 \pm 36$  and  $368 \pm 110$  fmol/mg protein, respectively.

As shown in Fig. 2, [<sup>3</sup>H]imidafenacin (4.0 nM) rapidly associated with its binding sites and reached a steady state by 20 min at 25°C in homogenates of the human bladder and parotid gland. The rate constant for the association ( $k_{+1}$ ) of [<sup>3</sup>H]imidafenacin binding in the bladder and parotid gland was calculated as  $6.99 \pm 1.74 \times 10^7$  and  $1.23 \pm 0.33 \times 10^8$  M<sup>-1</sup>·min<sup>-1</sup> ( $n = 3$ , mean  $\pm$  S.E.M.), respectively. Dissociation after the addition of 0.1  $\mu$ M atropine was also rapid at 25°C, and dissociation rate

constants ( $k_{-1}$ ) were calculated as  $7.09 \pm 1.02 \times 10^{-2}$  min<sup>-1</sup> and  $8.14 \pm 1.34 \times 10^{-2}$  min<sup>-1</sup>, respectively, in the bladder and parotid gland. Thus, the estimated dissociation constants ( $K_d = k_{-1} / k_{+1}$ ) for [<sup>3</sup>H]imidafenacin binding in the human bladder and parotid gland were  $1014 \pm 331$  pM and  $663 \pm 235$  pM, respectively. These kinetic  $K_d$  values were consistent with the  $K_d$  values estimated from saturation studies.

#### *Inhibition of specific [<sup>3</sup>H]imidafenacin binding in the human bladder and parotid gland by antimuscarinic agents*

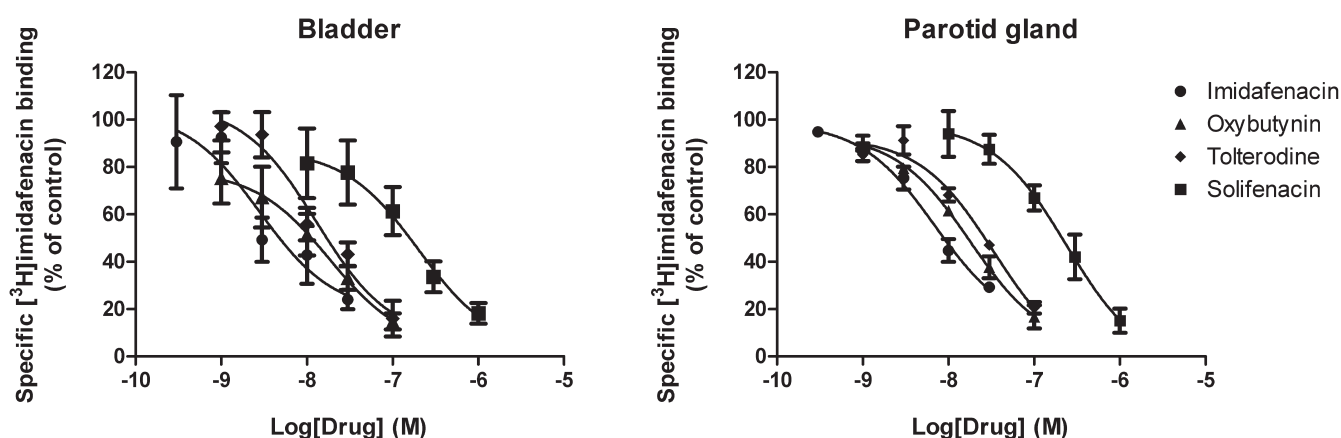
The antimuscarinic agents, imidafenacin, oxybutynin, solifenacin, and tolterodine competed with [<sup>3</sup>H]imidafenacin for binding sites in the human bladder and parotid gland in a concentration-dependent manner (Fig. 3). Their  $pK_i$  values are shown in Table 1, and the rank order of these agents in terms of affinity for binding sites in the bladder was imidafenacin > oxybutynin, tolterodine > solifenacin. The muscarinic binding affinities of imidafenacin in the bladder and parotid gland were 73 and 35 times more potent, respectively, than that of solifenacin. No significant difference was observed in the  $pK_i$  values of antimuscarinic agents between the bladder and parotid gland (Table 1). The Hill coefficients for their inhibition of specific [<sup>3</sup>H]imidafenacin binding in both tissues were close to unity.



**Fig. 2.** Time course of the association and dissociation of specific [<sup>3</sup>H]imidafenacin binding to the human bladder (closed circle) and parotid gland (closed triangle). The specific binding of [<sup>3</sup>H]imidafenacin (4.0 nM) was quantified as a function of time from the addition of the ligand. Dissociation of the [<sup>3</sup>H]imidafenacin–receptor complex was also monitored by the addition of 0.1  $\mu$ M atropine. Each point represents the average from 3 experiments.

#### **Discussion**

Imidafenacin was previously shown to be a well-tolerated therapeutic agent for OAB with fewer adverse effects (7–10). We previously demonstrated that imidafenacin bound muscarinic receptors in the human



**Fig. 3.** Competitive inhibition of specific [<sup>3</sup>H]imidafenacin binding in homogenates of the human bladder and parotid gland by imidafenacin (closed circle), oxybutynin (closed triangle), tolterodine (closed rhombus), and solifenacin (closed square). The abscissa is the logarithmic molar concentration of the antimuscarinic agents. The ordinate is specific [<sup>3</sup>H]imidafenacin binding (% of control). Each point represents the mean  $\pm$  S.E.M. for 3–4 experiments.

**Table 1.** Competitive inhibition by imidafenacin, oxybutynin, tolterodine, and solifenacin of specific [ $^3\text{H}$ ]imidafenacin binding in homogenates of human bladder and parotid gland

Drugs	Bladder		Parotid gland	
	pKi	nH	pKi	nH
Imidafenacin	9.21 $\pm$ 0.43	0.85 $\pm$ 0.15	9.20 $\pm$ 0.15	0.89 $\pm$ 0.06
Oxybutynin	8.40 $\pm$ 0.35	0.78 $\pm$ 0.28	8.78 $\pm$ 0.12	0.96 $\pm$ 0.13
Tolterodine	8.55 $\pm$ 0.22	1.05 $\pm$ 0.20	8.51 $\pm$ 0.15	0.84 $\pm$ 0.12
Solifenacin	7.36 $\pm$ 0.37	0.89 $\pm$ 0.16	7.66 $\pm$ 0.23	1.15 $\pm$ 0.13

Each value represents the mean  $\pm$  S.E.M. of 3 – 4 samples.

bladder mucosa and detrusor muscle and also in the parotid gland with high affinity by characterizing the inhibitory effect of the specific binding of [ $^3\text{H}$ ]NMS, the most commonly used radioligand of muscarinic receptors (12). The current study aimed to demonstrate the direct and highly specific binding activity of the new tritium ( $^3\text{H}$ )-labeled imidafenacin ([ $^3\text{H}$ ]imidafenacin) for labeling muscarinic receptors in the human bladder and parotid gland.

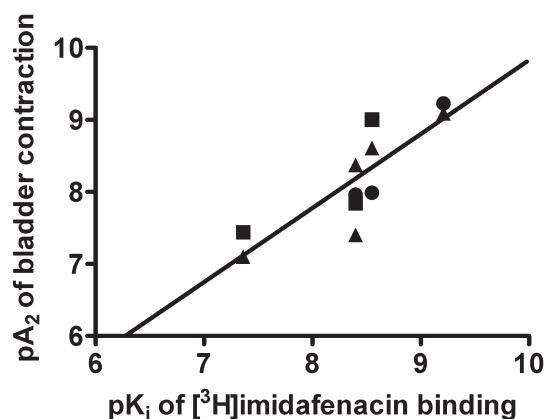
A significant level of specific binding of [ $^3\text{H}$ ]imidafenacin was observed in human tissues; it was saturable, reversible, and of high affinity, as shown by the nanomolar range of  $K_d$  values in the human bladder and parotid gland. Equilibrium binding studies in the human bladder revealed that its  $K_d = 1227$  pM and  $B_{\max} = 475$  fmol/mg protein. The  $K_i$  value for imidafenacin to inhibit bladder [ $^3\text{H}$ ]imidafenacin binding was 0.62 nM ( $pK_i$ : 9.21). These values for imidafenacin in the human bladder approximately corresponded to pharmacological potency for the competitive blockade of carbachol-induced contractions of the human bladder ( $pA_2$ : 9.23) (15) and guinea-pig urinary bladder ( $K_b$ : 0.81 nM) (4), indicating a close correlation between the binding affinity of imidafenacin to bladder muscarinic receptors and its pharmacological effects in the bladder. Previous studies (2, 12, 14) showed that muscarinic receptors were present on not only detrusor but also urothelium, a epithelial lining of the urinary tract. The current study measured specific [ $^3\text{H}$ ]imidafenacin binding in the human detrusor including the urothelium. Thus, this radioligand may bind muscarinic receptors in the urothelium as well as in the detrusor muscle.

Our previous study demonstrated that the affinity of imidafenacin to muscarinic receptors ([ $^3\text{H}$ ]NMS binding sites) was 2.2 times higher affinity in the human parotid gland than in the bladder (12). In the current study, the  $K_d$  value for specific [ $^3\text{H}$ ]imidafenacin binding in the human bladder was approximately 3 times higher than that in the parotid gland, suggesting the higher binding affinity of imidafenacin to muscarinic receptors in the human parotid gland.

The salivary gland predominantly contains  $M_3$  muscarinic receptors (16, 17), whereas the bladder contains both the  $M_2$  and  $M_3$  subtypes, with a predominance of  $M_2$  receptors (18). Furthermore, our previous study with  $M_1 - M_5$ -receptor knockout mice revealed that expression of the  $M_3$  subtype was higher in the salivary gland than in the bladder (19). Maruyama et al. suggested that the human parotid gland predominantly contains the  $M_3$  subtype based on the results of [ $^3\text{H}$ ]NMS binding assays with human tissue and the membranes of CHO-K1 cell lines expressing the human  $M_1 - M_5$ -receptor subtypes (20). Consistent with these findings, the predominance of the  $M_3$  receptor in the human parotid gland was demonstrated directly by a radioligand binding assay using *N*-(2-chloroethyl)-4-piperidinydiphenylacetate (4-DAMP mustard), an irreversible inactivating agent of the  $M_3$  subtype (14). Since imidafenacin exhibited greater selectivity for the  $M_3$  than  $M_2$  subtype in CHO-K1 cells expressing human muscarinic receptors (4), the high affinity of [ $^3\text{H}$ ]imidafenacin for muscarinic receptors in the human parotid gland reflects  $M_3$ -subtype selectivity. In vivo functional and ex vivo muscarinic receptor binding experiments in rats (5, 6) and clinical studies in humans (9, 10) showed that imidafenacin selected the bladder over the exocrine gland. These in vivo results appear to contradict the  $M_3$ -subtype selectivity observed in vitro, but in vivo bladder selectivity has been mainly attributed to the pharmacokinetic property of this agent (6). Orally administered imidafenacin was shown to be distributed at a higher concentration in the bladder of rats than in the salivary gland (6). Our preliminary results showed that imidafenacin might distribute to the bladder tissue from the urine as well as the circulating blood. Such predominant distribution from the urine may contribute to the bladder selectivity over the salivary gland.

Interestingly unlabeled imidafenacin as well as the clinically used antimuscarinic agents, oxybutynin, tolterodine, and solifenacin, competed with [ $^3\text{H}$ ]imidafenacin for binding sites in the human bladder in a concentration-dependent manner, which indicated the pharmacological specificity of [ $^3\text{H}$ ]imidafenacin binding





**Fig. 4.** Correlation between the inhibitory potencies ( $\text{pK}_i$ ) of specific [ $^3\text{H}$ ]imidafenacin binding by antimuscarinic agents (imidafenacin, oxybutynin, tolterodine, and solifenacin) in the human bladder and their pharmacological potencies ( $\text{pA}_2$ ).  $\text{pK}_i$  values were obtained from drug competition experiments (Table 1). Pharmacological data [ $\text{pA}_2$  values for the competitive inhibition of carbachol-induced contractions of isolated bladder tissues from humans (closed circle), guinea pigs (closed triangle), or rats (closed square)] were quoted from the literature (4, 15, 21–23).  $\text{pK}_i$  values for 4 antimuscarinic agents of bladder [ $^3\text{H}$ ]imidafenacin binding were significantly ( $P < 0.01$ , Spearman test) correlated with their pharmacological properties ( $\text{pA}_2$ ). The correlation coefficient for this relationship was  $r = 0.90$ .

sites. Figure 4 shows the strong correlation between the inhibitory potencies ( $\text{pK}_i$ ) of specific [ $^3\text{H}$ ]imidafenacin binding by antimuscarinic agents in the human bladder and their pharmacological potencies ( $\text{pA}_2$  values for the competitive inhibition of carbachol-induced contractions of isolated bladder tissues from humans, guinea pigs, or rats) (4, 15, 21–23). Although some variability in the  $\text{pA}_2$  values of antimuscarinic agents has been reported, the binding affinities of these agents for [ $^3\text{H}$ ]imidafenacin binding sites appears to significantly correlate with their pharmacological potencies. Taken together, the current results indicate that [ $^3\text{H}$ ]imidafenacin selectively binds to pharmacologically relevant muscarinic receptors in the human bladder.

[ $^3\text{H}$ ]Quinuclidinyl benzylate ([ $^3\text{H}$ ]QNB) and [ $N$ -methyl- $^3\text{H}$ ]scopolamine methyl chloride ([ $^3\text{H}$ ]NMS) have been commonly used as a radioligand to measure muscarinic receptor binding in a number of tissues. The advantage of [ $^3\text{H}$ ]imidafenacin over these radioligands may be more selective to the  $\text{M}_3$  subtype of muscarinic receptors.

In conclusion, the current study is the first to provide direct evidence to demonstrate that imidafenacin bound muscarinic receptors in the human bladder, supporting its clinical relevance as a useful therapeutic agent for OAB. [ $^3\text{H}$ ]Imidafenacin may also be a useful radioligand for labeling the  $\text{M}_3$  subtype of muscarinic receptors with higher selectivity than other radioligands.

## Conflicts of Interest

The authors declare that they have no conflicts of interest to disclose.

## References

- Abrams P, Artibani W, Cardozo L, Dmochowski R, van Kerrebroeck P, Sand P. Reviewing the ICS 2002 terminology report: the ongoing debate. *Neurourol Urodyn*. 2009;28:287.
- Finney SM, Andersson KE, Gillespie JJ, Stewart LH. Antimuscarinic drugs in detrusor overactivity and the overactive bladder syndrome: motor or sensory actions? *BJU Int*. 2006;98:503–507.
- Yoshida A, Fujino T, Maruyama S, Ito Y, Taki Y, Yamada S. 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. *J Pharmacol Sci*. 2010;112:142–150.
- Kobayashi F, Yageta Y, Segawa M, Matsuzawa S. Effects of imidafenacin (IMIDAFENACIN-197/ONO-8025), a new anticholinergic agent, on muscarinic acetylcholine receptors. High affinities for  $\text{M}_3$  and  $\text{M}_1$  receptor subtypes and selectivity for urinary bladder over salivary gland. *Arzneimittelforschung*. 2007;57:92–100.
- Kobayashi F, Yageta Y, Yamazaki T, Wakabayashi E, Inoue M, Segawa M, et al. Pharmacological effects of imidafenacin (IMIDAFENACIN-197/ONO-8025), a new bladder selective anti-cholinergic agent, in rats. Comparison of effects on urinary bladder capacity and contraction, salivary secretion and performance in the Morris water maze task. *Arzneimittelforschung*. 2007;57:147–154.
- Yamada S, Seki M, Ogoda M, Fukata A, Nakamura M, Ito Y. Selective binding of bladder muscarinic receptors in relation to the pharmacokinetics of a novel antimuscarinic agent, imidafenacin, to treat overactive bladder. *J Pharmacol Exp Ther*. 2011;336:365–371.
- Homma Y, Yamaguchi O. Long-term safety, tolerability, and efficacy of the novel anti-muscarinic agent imidafenacin in Japanese patients with overactive bladder. *Int J Urol*. 2008;15:986–991.
- Homma Y, Yamaguchi O. A randomized, double-blind, placebo- and propiverine-controlled trial of the novel antimuscarinic agent imidafenacin in Japanese patients with overactive bladder. *Int J Urol*. 2009;16:499–506.
- Masumori N. Long-term safety, efficacy, and tolerability of imidafenacin in the treatment of overactive bladder: a review of the Japanese literature. *Patient Prefer Adherence*. 2013;7:111–120.
- Takeuchi T, Zaitu M, Mikami K. Experience with imidafenacin in the management of overactive bladder disorder. *Ther Adv Urol*. 2013;5:43–58.
- Maggiore UL, Scala C, Venturini PL, Ferrero S. Imidafenacin for the treatment of overactive bladder. *Expert Opin Pharmacother*. 2013;14:1383–1397.
- Seki M, Ogoda M, Kuraoka S, Otsuka A, Ozono S, Takeda M, et al. Muscarinic receptor binding of imidafenacin in the human bladder mucosa and detrusor and parotid gland. *LUTS*. 2011;3:64–68.

- 13 Otsuka A, Shinbo H, Matsumoto R, Kurita Y, Ozono S. Expression and functional role of beta-adrenoceptors in the human urinary bladder urothelium. *Naunyn Schmiedebergs Arch Pharmacol.* 2008;377:473–481.
- 14 Yoshida A, Seki M, Nasrin S, Otsuka A, Ozono S, Takeda M, et al. Characterization of muscarinic receptors in the human bladder mucosa: direct quantification of subtypes using 4-DAMP mustard. *Urology.* 2011;78:721.e7–721.e12.
- 15 Murakami S, Yoshida M, Iwashita H, Otani M, Miyamae K, Masunaga K, et al. Pharmacological effects of IMIDAFENACIN on the human isolated urinary bladder. *Urol Int.* 2003;71: 290–298.
- 16 Giraldo E, Martos F, Gomez, A, Garcia A, Vigano MA, Ladinsky H, et al. Characterization of muscarinic receptor subtypes in human tissues. *Life Sci.* 1988;43:1507–1515.
- 17 Caufield MP. Muscarinic receptors-characterization, coupling and function. *Pharmacol Ther.* 1993;58:319–379.
- 18 Wang P, Luthin GR, Ruggieri MR. Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP binding proteins. *J Pharmacol Exp Ther.* 1995;273:959–966.
- 19 Ito Y, Oyzunzul L, Seki M, Fujino-Oki T, Matsui M, Yamada S. Quantitative analysis of the loss of muscarinic receptors in various peripheral tissues in M<sub>1</sub>-M<sub>5</sub> single subtype knockout mice. *Br J Pharmacol.* 2009;156:1147–1153.
- 20 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.
- 21 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 anti-muscarinic agents. *Biol Pharm Bull.* 2007;30:54–58.
- 22 Schneider T, Fetscher C, Krege S, Michel MC. Signal transduction underlying carbachol-induced contraction of human urinary bladder. *J Pharmacol Exp Ther.* 2004;309:1148–1153.
- 23 Anderson KE. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. *Pharmacol Rev.* 1993;45: 253–308.