Urinary excretion contributes to long-lasting blockade of bladder muscarinic receptors by imidafenacin: Effect of bilateral ureteral ligation

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Abbreviations

OAB  overactive bladder

$B_{\text{max}}$  maximal number of binding sites

$K_d$  dissociation constant

$C_{\text{urine}}$  urine concentration

$C_{\text{plasma}}$  plasma concentration
Abstract

Imidafenacin is a potent and selective antagonist of M₁ and M₃ muscarinic receptors that is safe, efficacious, and well tolerated for controlling the symptoms of overactive bladder (OAB). However, the precise mechanisms responsible for the bladder-selective pharmacological effects of this agent remain unclear. The in vivo pharmacologic effects of imidafenacin result from receptor occupancy. Therefore, the present study was performed to characterize in vivo muscarinic receptor binding by tritium-labeled imidafenacin with high specific activity ([³H]imidafenacin) in the bladder and other tissues of mice, and to clarify the mechanisms underlying selective binding of imidafenacin to bladder muscarinic receptors. After intravenous injection of [³H]imidafenacin, its binding to muscarinic receptors in the bladder and other tissues of mice was assessed by a radioligand binding assay. [³H]imidafenacin showed a significantly longer duration of binding to muscarinic receptors in the bladder than in other tissues and muscarinic receptor binding of [³H]imidafenacin was markedly suppressed in the bladder alone after bilateral ligation of the ureters. After intravenous injection, the [³H]imidafenacin concentration was markedly higher in the urine than in the plasma, suggesting that urinary excretion may contribute significantly to the selective and long-lasting binding of imidafenacin to bladder muscarinic receptors. These findings suggest that the intravesicular concentration of an antimuscarinic agent and its active metabolites may have a substantial influence on its pharmacological effect and duration of action in patients with OAB. In addition, factors that modulate urine production may influence the efficacy and safety of antimuscarinic agents.
Introduction

Antimuscarinic agents are an important treatment for overactive bladder (OAB), acting to increase bladder capacity and reduce urgency during the storage phase (Finney et al., 2006), but can cause various adverse effects such as dry mouth, constipation, blurred vision, and cognitive impairment (Colli et al., 2007). Both the therapeutic and adverse effects of these agents are mediated via the blockade of muscarinic receptors in the bladder and in non-target tissues, respectively.

Imidafenacin is a potent antagonist of M₁ and M₃ muscarinic receptors that is used to treat OAB (Homma et al., 2008a and 2009). Clinical studies have shown that imidafenacin is safe, efficacious, and tolerable for controlling the symptoms of OAB, even as long-term therapy (Homma and Yamaguchi, 2008b; Ohno et al., 2008; Zaitsu et al., 2011; Kadekawa et al., 2012). Sakakibara et al. (2014) reported that imidafenacin may be used safely to treat OAB in cognitively vulnerable patients. According to a recent systematic review and meta-analysis performed by Huang et al. (2015), imidafenacin is better tolerated than propiverine or solifenacin based on the incidence of dry mouth and constipation. Pharmacological studies have revealed significant selectivity of imidafenacin for muscarinic receptors in the bladder over those in the salivary gland or brain (Kobayashi et al., 2007a, and 2007b; Yamazaki et al., 2011). We previously demonstrated that imidafenacin binds with a high affinity to muscarinic receptors in human bladder mucosa and detrusor muscle (Seki et al., 2011). Also, after low-dose oral administration of imidafenacin to rats, its binding showed a longer duration to muscarinic receptors in the bladder than to receptors in other tissues such as the salivary gland (Yamada et al., 2011), suggesting preferential binding of bladder muscarinic receptors by this
drug. However, the mechanisms responsible for the longer duration of binding to bladder muscarinic receptors by imidafenacin remain unclear. A previous pharmacokinetic study revealed that imidafenacin reached a higher concentration in the bladder than in the serum or submaxillary gland after oral administration (Yamada et al., 2011). Pharmacologically relevant concentrations of the unchanged drug or active metabolites are excreted in the urine by humans receiving clinical doses of imidafenacin (Masuda et al., 2007) and other antimuscarinic agents (Krauwinkel et al., 2005; Cyong et al., 2006; Ellsworth, 2009; Malhotra et al., 2009). These reports suggested a possible contribution of urinary imidafenacin to the pharmacological effects of this drug on the bladder.

\[ ^3H \text{imidafenacin} \] with a high specific activity is a selective radioligand that has been used to label muscarinic receptors in the human bladder and parotid gland (Yoshida et al., 2014), and it shows a high affinity for muscarinic receptors in the M₁ and M₃ dominant tissues of rats (Kuraoka et al., in press). The basis of the in vivo pharmacology of imidafenacin is that its antagonistic effects result from receptor occupancy, which may be determined by an in vivo radioligand-receptor binding assay (Yoshida et al., 2010a), and \[ ^3H \text{imidafenacin} \] may be a useful radioligand for characterizing M₁ and M₃ muscarinic receptor subtypes with such an assay.

The present study was performed to characterize in vivo muscarinic receptor binding of \[ ^3H \text{imidafenacin} \] in the bladder and other tissues of mice, and to elucidate the mechanisms responsible for the bladder-selective pharmacological effects of imidafenacin in OAB patients. It was revealed that \[ ^3H \text{imidafenacin} \] showed a longer duration of binding to muscarinic receptors in the bladder than in other tissues after intravenous injection and that this binding was suppressed by bilateral ligation of the ureters, indicating a significant contribution of the
urinary excretion of imidafenacin to its binding to bladder muscarinic receptors.
Materials and Methods

Materials

[^3H]Imidafenacin (4-(2-methyl-1H-imidazol-1-yl)-2-phenyl-2-(3-tritiophenyl)-butanamide) (851 GBq/mmol) and imidafenacin were donated by Kyorin Pharmaceutical Co., Ltd. (Tokyo). All other chemicals were purchased from commercial sources.

Animals

Male ddY mice aged 11 to 13 weeks (Japan SLC Inc., Shizuoka, Japan) were housed at four to five animals per cage with free access to food and water, and were maintained on a 12 h light dark cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%). Animal care and these experiments were performed in accordance with the guidelines for the care and use of laboratory animals of the University of Shizuoka (registration number:136039).

Measurement of total radioactivity and specific binding of[^3H]imidafenacin

Measurement of the total radioactivity and the in vivo specific binding of[^3H]imidafenacin in mouse tissues after intravenous injection of the radioligand were performed as described previously for[^3H]quinuclidinyl benzilate (QNB) (Maruyama et al., 2008). Briefly,[^3H]imidafenacin (10.2 MBq, 12.0 nmol/kg) was injected into the tail vein and mice were sacrificed under isoflurane anesthesia at 10, 30, 90, and 180 min after injection. A blood sample was collected from the descending aorta and various tissues (bladder, submaxillary gland, heart, colon, lung, and cerebral cortex) were rapidly removed. Each tissue specimen
was homogenized in ice-cold 50 mM Na+/K+ phosphate buffer (pH 7.5) with a Physcotron homogenizer (Nition, Tokyo, Japan). Particulate-bound radioactivity was determined by rapid filtration of 0.5 mL of tissue homogenate through a Whatman CF/C filter, followed by washing with 1 mL of ice-cold buffer. After addition of scintillation fluid, radioactivity in the plasma and the tissue particulate fraction was measured by using a liquid scintillation counter. Then \textit{in vivo} specific binding of $[^3\text{H}]$imidafenacin was estimated from the difference in particulate-bound radioactivity between vehicle-pretreated and atropine (7.28 $\mu$mol/kg i.p.)-pretreated mice, reflecting total and nonspecific binding, respectively.

In order to estimate the \textit{in vivo} affinity constant ($K_d$) and the maximal number of binding sites ($B_{\text{max}}$) in mouse tissues, $[^3\text{H}]$imidafenacin (10.2 MBq, 12.0 nmol/kg) and unlabeled imidafenacin were combined at various ratios over a range between 2.98 and 298 nmol/kg and 150 $\mu$l aliquots were injected into the tail vein to analyze total binding. Nonspecific binding was assessed as described above, and linear regression analysis of nonspecific binding was performed at each dose. Specific binding (Sp) curves were fitted by Graphpad Prism 5 software using non-linear regression analysis to the model as follows: $\text{Sp}=B_{\text{max}}D/(K_d+D)$, where D is the tissue concentration of imidafenacin. Data are presented as Bq/g tissue, which was estimated by converting raw counts to Bq (1 Bq=60 dpm).

\textbf{Effects of bilateral ureteral ligation on total radioactivity and specific binding of $[^3\text{H}]$imidafenacin}

Mice were subjected to midline laparotomy under isoflurane anesthesia and both ureters were ligated with silk thread. Then the abdominal incision was sutured and the mice were allowed to recover from anesthesia. The sham group underwent the same surgical procedure without bilateral ureteral ligation. Total radioactivity and specific tissue binding were measured at 30
and 90 min after intravenous injection of \([^3H]\)imidafenacin in mice with bilateral ureteral ligation and sham mice. After tissue specimens were dissected and homogenized in 50 mM Na\(^+/K^+\) phosphate buffer (pH 7.5), the total radioactivity and \emph{in vivo} specific binding of \([^3H]\)imidafenacin were measured in each tissue.

\textit{Determination of \([^3H]\)imidafenacin}

Following injection of \([^3H]\)imidafenacin (10.2 MBq, 12.0 nmol/kg) into mice, the \([^3H]\)imidafenacin concentration in the plasma and urine was analyzed by high performance liquid chromatography (HPLC) after extraction and concentration. Data are presented as the molar concentration (nM) of \([^3H]\)imidafenacin, which was obtained as the raw counts corrected by the specific activity (851 GBq/mmol) of the radioligand.

\textit{Statistical analysis}

Results are reported as the mean ± SEM. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA), followed by Dunnett’s test for multiple comparisons. Significance was accepted at \(p<0.05\).
Results

In vivo identification of specific $[^3]$Himidafenacin binding sites

Particulate-bound radioactivity was measured in mouse tissues at 30 min after the intravenous injection of $[^3]$Himidafenacin. Pretreatment with atropine (7.28 μmol/kg, i.p.) significantly reduced $[^3]$Himidafenacin binding (by 47-70%) in particulate fractions of the bladder, submaxillary gland, heart, and colon, but not in fractions of the lung or cerebral cortex (Fig. 1). Thus, the difference of tissue particulate-bound $[^3]$Himidafenacin radioactivity between vehicle-pretreated and atropine-pretreated mice represents in vivo specific binding of the ligand, as previously reported for $[^3]$HQN (Maruyama et al., 2008a). The in vivo specific binding of $[^3]$Himidafenacin varied among tissues (Fig. 1), and was highest in the submaxillary gland, followed by the bladder, colon, and heart. At 30 min after injection, $[^3]$Himidafenacin binding was negligible in the particulate fractions of the lung and cerebral cortex.

Figure 2A shows total radioactivity in mouse plasma, bladder, submaxillary gland, heart, colon, lung, and cerebral cortex at 10, 30, 90, and 180 min after intravenous injection of $[^3]$Himidafenacin (10.2 MBq, 12.0 nmol/kg). The radioactivity in each tissue, except the cerebral cortex, was markedly higher than the plasma level. Radioactivity peaked at 30-90 min in the bladder, unlike other tissues. Figure 2B displays the time course of specific $[^3]$Himidafenacin binding in the particulate fractions of mouse tissues harvested at 10, 30, 90, and 180 min after the intravenous injection of $[^3]$Himidafenacin. In the bladder particulate fraction, the maximum level of specific $[^3]$Himidafenacin binding was sustained until at least 90 min, with strong binding being noted even at 180 min (Fig. 2B). On the other hand, the maximum level of specific $[^3]$Himidafenacin binding was noted at 10 min in the submaxillary
gland, heart, colon, and lung, after which it decreased significantly over time. Binding to the particulate fraction of lung tissue was only observed at 10 min, and was it negligible in the particulate fraction of the cerebral cortex. In each tissue, the time course of specific $[^3]H$imidafenacin binding appeared to be parallel to that of total radioactivity (Fig. 2A).

**Estimation of $K_d$ and $B_{max}$ for in vivo $[^3]H$imidafenacin binding**

*In vivo* specific $[^3]H$imidafenacin binding in the bladder and submaxillary gland of mice increased with an increase of the radioligand concentrations, and reached a plateau at approximately 30 nM (Fig. 3). As shown in Table 1, the estimated $K_d$ value of $[^3]H$imidafenacin was lowest in the submaxillary gland, followed by the heart, bladder, and colon (Table 1). The $B_{max}$ value was highest in the colon and submaxillary gland, followed by the bladder and heart. The *in vivo* $K_d$ values determined in mice were larger than the *in vitro* $K_d$ values, while *in vivo* $B_{max}$ values were similar to the *in vitro* values (Table 1).

**Effect of bilateral ureteral ligation on binding to bladder muscarinic receptors**

Figure 4 shows the changes of total radioactivity in the urine over time and specific $[^3]H$imidafenacin binding to bladder tissue 30 and 180 min after intravenous injection of the radioligand (12.0 nmol/kg). There was a significant correlation between these two parameters at 180 min (Fig. 4B), but not at 30 min (Fig. 4A).

Bilateral ligation of the ureters, but not sham surgery, led to marked reduction of total radioactivity (73%) (Fig. 5A) and specific $[^3]H$imidafenacin binding (63%) (Fig. 5B) in the bladder alone at 180 min after intravenous injection of the radioligand. However, there was no significant change of *in vivo* specific $[^3]H$imidafenacin binding to bladder tissue at 90 min after radioligand injection (data not shown).
**Plasma and urine concentrations of [³H]imidafenacin**

In [³H]imidafenacin-treated mice, the urinary concentration of [³H]imidafenacin was markedly higher than the plasma concentration at 30-180 min after injection of the radioligand (Table 2). The C\textsubscript{urine}/C\textsubscript{plasma} ratio was approximately 20-fold larger at 180 min than at 30 min.
Discussion

In vitro studies have demonstrated that [³H]imidafenacin is a selective radioligand that binds to muscarinic receptors in the human bladder and parotid gland (Yoshida et al., 2014) and muscarinic receptors in rat tissues such as the bladder (Kuraoka et al., in press). In the present study, a significant amount of specific [³H]imidafenacin binding was detected in the particulate fractions of the bladder, submaxillary gland, heart, colon, and lungs, but not in the particulate fraction of the cerebral cortex, after intravenous injection of this radioligand in mice (Fig. 1). There was also a significant difference between the bladder and other tissues with regard to the time course of changes in the total radioactivity and specific binding of [³H]imidafenacin after intravenous injection of the radioligand (Fig. 2). In the bladder, peak specific [³H]imidafenacin binding lasted until at least 90 min after injection and only showed a slight decrease at 180 min. On the other hand, specific [³H]imidafenacin binding in the submaxillary gland, heart, colon, and lung was maximal at 10 or 30 min and then declined rapidly. Thus, binding of [³H]imidafenacin persisted for longer in the bladder than in other tissues of mice, which is consistent with the ex vivo findings obtained after oral administration of imidafenacin to rats (Yamada et al., 2011).

Specific [³H]imidafenacin binding was not detected in the cerebral cortex after intravenous injection of the radioligand into mice (Fig. 1), but significant specific cortical [³H]imidafenacin binding was detected in an in vitro binding assay (Kᵢ=0.74 ± 0.03 nM, Bₘₐₓ=120 ± 4 nmol/mg tissue). We previously demonstrated that oral imidafenacin exhibited negligible binding to brain muscarinic receptors in rats (Yamada et al., 2011). Autoradiography and positron emission tomography have previously revealed that there is significant binding to muscarinic receptors in the rat and monkey brain after intravenous injection of oxybutynin, but not imidafenacin.
(Maruyama et al., 2008b; Yoshida et al., 2010b; Yamamoto et al., 2011). Also, oral administration of imidafenacin did not induce cognitive impairment in monkeys. Furthermore, imidafenacin did not affect escape latency in rats performing the Morris water maze task (spatial learning and memory) (Kobayashi et al., 2007b). Taken together, these findings suggest low occupancy of brain muscarinic receptors by imidafenacin, possibly due to low blood-brain barrier permeability. This is also consistent with clinical data showing that imidafenacin is well-tolerated with few adverse effects.

Parameters for in vivo specific [³H]imidafenacin binding were estimated by intravenous administration of various doses of unlabeled imidafenacin and [³H]imidafenacin to mice. The $B_{\text{max}}$ value for in vivo [³H]imidafenacin binding was slightly higher in the submaxillary gland and colon than in the urinary bladder and heart, while the in vivo $K_d$ value was slightly smaller in the submaxillary gland than in other tissues. These findings were similar to those obtained by in vitro binding assays in mice (Table 1) and rats (Kuraoka et al., in press), suggesting a close correlation between in vitro and in vivo binding of [³H]imidafenacin.

After oral administration of imidafenacin (0.1 mg) to healthy volunteers, approximately 7.3% of the dose was excreted in the urine as the parent compound by 48 h and the maximum concentration was 293 nM (Masuda et al., 2007). A similar imidafenacin concentration was also found in the urine of rats given a dose of 0.5 mg/kg. We previously showed that the tissue concentration of imidafenacin was markedly higher in the bladder than in the submaxillary gland after oral administration to rats and we also demonstrated significant binding to bladder muscarinic receptors following intravesical instillation of imidafenacin at 200 nM (Yamada et al., 2011). In the present study, it is noteworthy that specific [³H]imidafenacin binding to bladder tissue at 180 min after injection of the radioligand was markedly reduced in mice subjected to bilateral ureteral ligation, but not in sham mice (Fig. 5), and the urine concentration of
[\textsuperscript{3}H]imidafenacin was approximately 4000-fold higher than the plasma level at 180 min (Table 2). These results suggest that a significant amount of imidafenacin is transferred directly from the urine to the bladder tissue by simple diffusion, and that urinary excretion of this drug may promote its selective and long-lasting binding to bladder muscarinic receptors in mice (Fig. 2B). We found a correlation between total urinary radioactivity and specific [\textsuperscript{3}H]imidafenacin binding to bladder tissue at 180 min, but not 30 min, after injection of [\textsuperscript{3}H]imidafenacin in mice (Fig. 4), while bilateral ureteral ligation had a negligible effect on bladder binding of [\textsuperscript{3}H]imidafenacin at 90 min after injection. Based on these results, it seems likely that imidafenacin from the blood mainly binds to muscarinic receptors in the bladder at 30 min, while more than 50\% of the muscarinic receptors are occupied by urinary imidafenacin at 180 min.

**Conclusion**

Imidafenacin shows a longer duration of binding to muscarinic receptors in the bladder than in other tissues, and this bladder selectivity may be largely attributable to urinary excretion of the drug. This may have clinical implications for the efficacy and safety of imidafenacin and other antimuscarinic agents used to treat OAB, because the unchanged form and/or active metabolites of various antimuscarinic agents are excreted in the urine at pharmacologically relevant concentrations (Masuda et al., 2007; Krauwinkel et al., 2005; Cyong et al., 2006; Ellsworth, 2009; Malhotra et al., 2009).

**Authorship Contributions**

*Participated in research design: Ito, Kuraoka, Onoue, and Yamada*

*Conducted experiments: Ito, Kuraoka, Endo, and Takahashi*

*Contributed new reagents or analytic tools: Ito, Kuraoka, and Yamada*
Performed data analysis: Ito, Kuraoka, Endo, and Takahashi

Wrote or contributed to the writing of the manuscript: Ito, Onoue and Yamada
References


Ohno T, Nakade S, Nakayama K, Kitagawa J, Ueda S, Miyabe H, Masuda Y, Miyata...


Legends for figures:

Fig. 1 Effects of pretreatment with atropine on \textit{in vivo} \[^{3}\text{H}]\text{imidafenacin binding to the particulate fraction of mouse tissues. Mice received saline (control, □) or atropine (7.28 \(\mu\text{mol/kg, i.p.}, \text{■}) at 30 min prior to intravenous injection of \[^{3}\text{H}]\text{imidafenacin (10.2 MBq, 12.0 nmol/kg) into the tail vein. Mice were sacrificed at 30 min after injection, and \[^{3}\text{H}]\text{imidafenacin binding to the particulate fraction of each tissue was determined. Each column represents the mean±S.E. for 4 mice. Asterisks show significant differences from control binding (total binding), *P<0.05, **P<0.01, ***P<0.001.}]

Fig. 2 Changes of total radioactivity (A) and \textit{in vivo} specific binding (B) of \[^{3}\text{H}]\text{imidafenacin in mouse tissues (∗: bladder, □: submaxillary gland, ▲: heart, ▼: colon, ◆: lung, ○: cerebral cortex, □: plasma) after intravenous injection of the radioligand. \[^{3}\text{H}]\text{Imidafenacin (10.2 MBq, 12.0 nmol/kg) was injected into the tail vein, and mice were sacrificed after 10, 30, 90, or 180 min. Specific \[^{3}\text{H}]\text{imidafenacin binding was defined as the difference of binding to the particulate fractions of each tissue between saline-pretreated mice (total binding) and atropine (7.28 \(\mu\text{mol/kg, i.p.}) -pretreated mice (nonspecific binding). Each point (A) or column (B) represents the mean±S.E. for 4 to 6 mice. Asterisks show significant differences from the 10 min value, **P<0.01, ***P<0.001 (Dunnett’s test).}

Fig. 3 \textit{In vivo} specific binding of \[^{3}\text{H}]\text{imidafenacin to the bladder and submaxillary gland of mice with increasing concentrations of the radioligand. \[^{3}\text{H}]\text{imidafenacin was injected into the tail vein at various doses (2.98 - 298 nmol/kg) and mice were sacrificed after 30 min. Then}
specific $[^3$H]imidafenacin binding to the particulate fraction of bladder and submaxillary gland was measured.

Fig. 4  Relation between urinary radioactivity and specific $[^3$H]imidafenacin binding to bladder tissue at 30 min (A) and 180 min (B) after intravenous injection of the radioligand. $[^3$H]imidafenacin (10.2 MBq, 12.0 nmol/kg) was injected into the tail vein, mice were sacrificed after 30 or 180 min, and urinary radioactivity and specific $[^3$H]imidafenacin binding to the particulate fraction of the bladder were determined. At 30 min and 180 min, Pearson’s correlation coefficient was $r = 0.2114$ and $r = 0.9439$ (P<0.05), respectively.

Fig. 5  Total urinary radioactivity (A) and in vivo specific binding of $[^3$H]imidafenacin in each tissue (B) from sham mice (□) and mice subjected to bilateral ligation of the ureters (■). $[^3$H]imidafenacin (10.2 MBq, 12.0 nmol/kg) was injected into the tail vein, and mice were sacrificed after 180 min. Total urinary radioactivity and specific $[^3$H]imidafenacin binding to the particulate fraction of bladder tissue were measured as described in Figure 2. Each column represents the mean ± S.E. for 5 to 9 mice. Asterisks show significant differences from the control (sham), *P<0.01 (Student’s t-test).
Table 1. Apparent dissociation constant ($K_d$) and maximal number of binding sites ($B_{max}$) for \textit{in vivo} specific $[^3]$Himidafenacin binding to the bladder, submaxillary gland, heart, and colon in mice, and parameters for \textit{in vitro} specific binding of the radioligand to these tissues. Mice were intravenously injected with various doses (2.98-298 nmol/kg i.v.) of $[^3]$Himidafenacin and unlabeled imidafenacin, and were sacrificed after 10 min. $K_d$ and $B_{max}$ were estimated through fitting the specific binding of $[^3]$Himidafenacin at each tissue ligand concentration by non-linear least-squares regression analysis. Values represent the mean ± SEM for 11 to 20 mice.

<table>
<thead>
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<th>Tissue</th>
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<th>In vivo</th>
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<tr>
<td></td>
<td>$K_d$</td>
<td>$B_{max}$</td>
</tr>
<tr>
<td></td>
<td>(nM)</td>
<td>(fmol/mg tissue)</td>
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<tr>
<td>Bladder</td>
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<tr>
<td>Submaxillary gland</td>
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<tr>
<td>Heart</td>
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<tr>
<td>Colon</td>
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Table 2. Plasma and urine concentrations of [3H]imidafenacin and their ratios after intravenous injection of the radioligand in mice. [3H]Imidafenacin (10.2 MBq, 12.0 nmol/kg) was injected into the tail vein, and plasma and urine were collected after 30, 90, 120, or 180 min. Values are the mean ± S.E. for 13 to 15 mice.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>C_{plasma} (nM)</th>
<th>C_{urine} (nM)</th>
<th>C_{urine} / C_{plasma}</th>
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<td>180</td>
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<td>138 ± 29</td>
<td>4313</td>
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</table>
Fig. 1

[3H]Imidafenacine binding (Bq/g tissue)

- Bladder
- S. gallnd
- Heart
- Colon
- Lung
- Cortex

* indicates significant difference from control, ** indicates highly significant difference from control.
Bladder

Submaxillary gland

Specific imidafenacin binding (fmol/g tissue)

Imidafenacin (nM)

Fig. 3