

# In vivo bladder selectivity of imidafenacin, a novel antimuscarinic agent, assessed by using an effectiveness index for bladder capacity in rats

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**Abstract** Imidafenacin (KRP-197) is a novel antimuscarinic agent for overactive bladder treatment. The inhibitory effect of imidafenacin on detrusor contraction has been adopted for assessing their bladder selectivity, but this is becoming less convincing as an effectiveness index. We, therefore, reevaluated the bladder selectivity of imidafenacin and other antimuscarinics using their effects on the bladder capacity as an effectiveness index. Bladder capacity was measured by intermittent cystometry in urethane-anesthetized rats. In the tissues related to antimuscarinic side effects, the inhibitory actions were measured each on salivary secretion by electrical stimulation of chorda tympani, on rhythmical contractions in colon, and on carbamylcholine-induced bradycardia. Imidafenacin, solifenacin succinate, tolterodine tartrate, and propiverine hydrochloride significantly increased the bladder capacity, with minimum effective doses of 0.003, 1, 0.03, and 3 mg/kg (i.v.), respectively. The antimuscarinics tested, except for propiverine hydrochloride, shared a common property of increasing bladder capacity at a dose which did not affect micturition pressure. The relative bladder selectivity of imidafenacin, solifenacin succinate, and tolterodine tartrate was 15-, 1.7-, and 2.5-fold higher over salivary gland; 150-, 1.9-, and 9.2-fold higher over colon; and 50-, 12-, and 4.6-fold higher over heart, respectively, than that of propiverine hydrochloride. Thus, imidafenacin shows the most highly selective for bladder over the tissues related to major antimuscarinic side effects, compared to the other three well-known antimuscarinics tested in the rat.

**Keywords** Bladder selectivity · Imidafenacin · Antimuscarinic agent · Overactive bladder (OAB) · Bladder capacity · Cystometry

## Abbreviations

OAB	Overactive bladder
ACh	Acetylcholine
CCh	Carbamylcholine
BP	Blood pressure
HR	Heart rate
SNP	Sodium nitroprusside

## Introduction

Overactive bladder (OAB) is a syndrome characterized by urinary urgency, with or without urge incontinence, usually with frequency and nocturia (Abrams et al. 2002). Antimuscarinics are the first-line pharmacotherapy for OAB, and those with a high tissue and/or receptor-subtype selectivity have been intensively developed to attain the aim of reducing side effects, such as dry mouth, constipation, blurred vision, and tachycardia (Abrams and Andersson 2007). Nevertheless, tolerability of patients to these agents has not been improved enough (Hegde 2006).

In urinary bladder, acetylcholine (ACh) released from postganglionic parasympathetic nerve terminals contracts the bladder by activating muscarinic receptors on detrusor muscles at voiding phase (Andersson 1999). Muscarinic receptors are currently divided into five subtypes ( $M_1$ – $M_5$ ), and  $M_3$  receptor plays a predominant role for the detrusor contraction (Hegde 2006). Non-neuronal ACh has been recently revealed to be released from urothelium by the stretching of urinary bladder during storage phase (Yoshida et al. 2010). The

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muscarinic receptors are expressed in urothelium, suburothelium, and afferent nerves (Mansfield et al. 2005), and these receptors stimulated by non-neuronally released ACh are considered to result directly or indirectly (for example, mediated by ATP release) in the activation of afferent nerves (Yoshida et al. 2010). Furthermore, spontaneous myogenic contractions of detrusor muscles have been reported to be involved in the bladder filling sensation and to be strongly regulated by cholinergic mechanism (Gillespie et al. 2009).

Traditionally, the effectiveness of antimuscarinics in relieving OAB symptoms had been linked to their inhibitory effects on involuntary detrusor contraction by blocking muscarinic receptors on the detrusor muscle during storage phase (Andersson 1999). Based on this concept, the inhibitory effect of antimuscarinics on detrusor contraction has been used conventionally as an effectiveness index in assessing their bladder selectivity in various animals (Kobayashi et al. 2007b; McNamara et al. 2009; Ohtake et al. 2004, 2006; Sinha et al. 2010). However, antimuscarinics at their therapeutic doses have been revealed to increase bladder capacity without inhibiting detrusor contractility in OAB patients, suggesting that antimuscarinics primarily act on the bladder afferent pathway during storage phase, thereby increasing bladder capacity and improving OAB symptoms (Andersson and Yoshida 2003; Finney et al. 2006; Yamaguchi 2010). Therefore, the inhibitory effect of antimuscarinics on detrusor contraction seems to be less convincing as an effectiveness index.

Imidafenacin (KRP-197), 4-(2-methyl-1*H*-imidazol-1-yl)-2,2-diphenylbutanamide (Fig. 1), is an antimuscarinic agent with high bladder selectivity and was approved in Japan in 2007 for OAB treatment. Analysis of subtype selectivity using recombinant human muscarinic receptors demonstrated that imidafenacin has higher affinities for  $M_1$  and  $M_3$  than for  $M_2$  subtype, with  $K_i$  values in the low nanomolar range (Kobayashi et al. 2007a). Homma et al. (2009) demonstrated that imidafenacin had an equal efficacy and better tolerability relative to propiverine in OAB patients. In conscious rats, imidafenacin has been reported to be the most highly selective for bladder over salivary gland compared to propiverine, tolterodine, oxybutynin, and darifenacin (Kobayashi et al. 2007b). Furthermore, Yamada et al. (2011) reported that imidafenacin administered orally distributes predominantly to the bladder and exerts more selective and longer-lasting effect on the bladder than other

tissues such as salivary gland, heart, and colon using radioreceptor assay in rats. In this connection, Kobayashi et al. (2007b) had evaluated the bladder selectivity of imidafenacin by using the inhibitory effects on detrusor contraction. We, therefore, reevaluated the bladder selectivity of imidafenacin over salivary gland, gastrointestinal tract, and heart by using an effectiveness index for the bladder capacity and compared it to those of solifenacin succinate, tolterodine tartrate, and propiverine hydrochloride which are widely used in Japan.

## Methods

### Animals

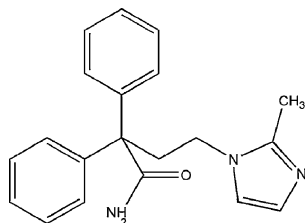
Male Sprague–Dawley rats (Charles River Laboratories Japan, Kanagawa, Japan), weighing 210–300 g, were housed in a room maintained under controlled conditions of  $23\pm 3^\circ\text{C}$ ,  $55\pm 15\%$  RH, and 12:12-h light–dark cycle. The rats were maintained on a solid diet daily with water ad libitum. Different animals were used for each test. The protocols and animal care complied with the Guiding Principles for the Care and Use of Laboratory Animals recommended by The Japanese Pharmacological Society and followed the standard operating procedures for institutional animal care management in our facility.

### Intermittent cystometry

Intermittent cystometry was used to determine bladder capacity in the present study. Takagi-Matsumoto et al. (2004) reported that continuous cystometry under anesthesia was not suitable for evaluating bladder capacity because the residual urine volume increased due to the failure of complete detrusor contraction. For this reason, we removed the residual urine from the bladder after each micturition as described below (intermittent cystometry).

Under urethane anesthesia (1.2 g/kg, s.c.), a saline-filled polyethylene catheter (PE50; Japan Becton Dickinson, Tokyo, Japan) was cannulated into the tip of bladder dome for measurement of the intravesical pressure and another one (PE50) into the femoral vein for injection of test drugs. The bladder catheter was connected via a three-way cock to a pressure transducer (Nihon Kohden, Tokyo, Japan) and a syringe pump (Harvard Apparatus, Holliston, MA, USA) for the infusion of saline. Cystometry was performed after over 30-min recovery period from the surgical procedure. Firstly, saline was infused at a rate of 0.06 mL/min for 15 min in order to confirm the occurrence of micturition reflexes, and then the residual saline was removed from the bladder (priming). Twenty minutes later, saline was infused again at the same infusion rate until micturition was observed. Just after onset of

**Fig. 1** Chemical structure of imidafenacin



micturition, saline infusion was stopped and the residual urine was removed from the bladder. These procedures were repeated at 20-min intervals until two similar consecutive responses were obtained. Five minutes after the last micturition (pre), test drugs were injected, and 15 min later, saline was infused again until micturition was observed (post). No run-down of the micturition reflex was observed and confirmed during experiment. Rectal temperature was maintained at  $37 \pm 1^\circ\text{C}$  with a heat lamp during measurement. The cystometro-gram was recorded by an electric recorder (Nihon Kohden, Tokyo, Japan) to determine the bladder capacity (time to micturition multiplied by infusion rate; milliliters) and micturition pressure (maximum bladder pressure during micturition; millimeters of mercury). The amount of saline removed from bladder for 15 min after the stop of saline infusion was also measured as the residual volume (milliliters). The effects of drugs on bladder capacity, micturition pressure, and residual volume are expressed as a ratio of post- to pre-value.

#### Salivary secretion

Salivary secretion was measured according to a modified method from the previous study (Sugisawa and Takai 1991). Briefly, under urethane anesthesia (1.2 g/kg, s.c.), the chorda tympani was exposed to locate a bipolar stimulus electrode on it, and a Vaseline–paraffin mixture (75:25% (v/v)) was applied to the nerve around the electrode. A polyethylene catheter (PE50) was cannulated into the femoral vein for injection of the vehicle or test drugs. After over 30-min recovery period from the surgical procedure, salivary secretion was confirmed by the 15-s stimulation of the chorda tympani with 3 V, for duration of 5 ms at 40 Hz from the electrode (priming). After removal of saliva in the oral cavity, saliva was collected for 2 min without stimulation (resting salivation). Fifteen minutes later, saliva was collected for 2 min with stimulation under the same condition as described above (pre). Thirty minutes later, test drugs were injected. Then, 15 min later, saliva was collected again for 2 min under the same stimulus condition (post). Rectal temperature was maintained at  $37 \pm 1^\circ\text{C}$  with a heat lamp during measurement. Salivary samples were collected into pre-weighed cotton balls located in the oral cavity. And then, the amount of salivary secretion was calculated by reweighing the cotton balls, where their resting salivation weights were subtracted. Inhibitory effects of drugs on salivary secretion are expressed as percentage inhibition of post- to pre-value.

#### Rhythmical colon contractions

The proximal colon in which the cholinergic nerve is involved in its rhythmical contractions of rats (Li et al. 2002) was used for measurement in the present study. After 23–30 h fasting,

under urethane anesthesia (1.2 g/kg, s.c.), a polyethylene catheter (SP70: O.D. 1.5 mm, Natsume Seisakusho Co., Ltd, Tokyo, Japan) was inserted into the proximal colon approximately 1 cm from the cecum for measurement of internal pressure and another one (PE50) into the femoral vein for injection of the vehicle or test drugs. The SP70 catheter was connected to a pressure transducer (Nihon Kohden, Tokyo, Japan). After intestinal contents were removed as much as possible by infusion of warmed Tyrode's solution of the following composition (millimolars): NaCl 137, KCl 2.7,  $\text{CaCl}_2$  1.8,  $\text{NaH}_2\text{PO}_4$  0.42,  $\text{MgCl}_2$  1.1, and  $\text{NaHCO}_3$  12, glucose 11, containing 10 g/L gelatin, a cul-de-sac (approximately 3 cm in length) was made. In order to induce rhythmical contractions, the intracolonic pressure was raised (5–10 mmHg) by adding, through the colon catheter, Tyrode's solution containing the gelatin. After over 30-min recovery period from the surgical procedure and then confirmation of stabilized rhythmical colon contractions, test drugs were injected. Rectal temperature was maintained at  $37 \pm 1^\circ\text{C}$  with a heat lamp during measurement. The areas under the curve (AUC) of the waveform of rhythmical contractions for 10 min just before the drug injection (pre) and 15 min after drug injection (post) were measured by using a computer-assisted system (PowerLab; AD Instrument Pty Ltd., Castle Hill, Australia). Inhibitory effects of drugs on rhythmical contractions are expressed as percentage inhibition of post- to pre-value.

#### Carbamylcholine-induced bradycardia

Heart rate was measured according to a modified method from the previous study (Ikeda et al. 2002). Briefly, under urethane anesthesia (1.2 g/kg, s.c.), a polyethylene catheter (PE50) was inserted into the femoral artery and vein for blood pressure (BP) monitoring and another one (PE50) into the femoral vein for injection of test drugs. The arterial catheter was connected to a pressure transducer (Nihon Kohden, Tokyo, Japan). Heart rate (HR) was counted from the BP pulses. The BP and HR were recorded by the PowerLab. After BP and HR stabilization, rats were injected with 10  $\mu\text{g}/\text{kg}$  of carbamylcholine (CCh) at an interval of 20 min until two similar consecutive responses were obtained. Five minutes after the last CCh injection (pre), test drugs were injected. Then, 15 min later, bradycardia was induced again (post). Rectal temperature was maintained at  $37 \pm 1^\circ\text{C}$  by using a heat lamp during measurement. Inhibitory effects of drugs on CCh-induced bradycardia are expressed as percentage inhibition of post- to pre-value.

#### Baroreflex in conscious rats

As an additional experiment on heart rate, we examined the influence of imidafenacin on baroreflex in conscious rats

because heart rate is indirectly controlled by baroreflex pathway mainly mediated by cholinergic nerve (Olshansky et al. 2008). Baroreflex was measured according to a modified method of the previous study (El-Mas et al. 2002). Briefly, the rats were anesthetized with 3% sevoflurane. Polyethylene catheters (PE50 and PE10; Japan Becton Dickinson), both filled with heparinized saline (100 U/mL), were inserted into the common carotid artery for measurement of BP and into the femoral vein for injection of test drugs, respectively. The catheters were tunneled subcutaneously and exteriorized at the back of the neck between the scapulae. Each rat received an s.c. injection of 20,000 U/body penicillin G potassium and then was housed in a separate cage. Two days after the surgical procedure, the arterial catheter was connected to a pressure transducer (Nihon Kohden, Tokyo, Japan) for measurement of BP and HR. After stabilization of BP and HR, increased i.v. doses of (*R*)-(-)-phenylephrine hydrochloride (phenylephrine) at 1, 3, and 5  $\mu\text{g}/\text{kg}$  or sodium nitroprusside dehydrate (SNP) at 2, 5, and 10  $\mu\text{g}/\text{kg}$  were injected at 4–10 min intervals (pre). This time interval was adequate for BP and HR to regain baseline levels (El-Mas et al. 2002). After stabilization of BP and HR, test drugs were injected. Then, 15 min later, baroreflex was induced by the same method as described above (post). The BP, mean blood pressure (MBP), and HR were recorded by the PowerLab in conscious, freely moving rats. The peak changes in MBP and HR in each dose of phenylephrine or SNP were calculated, and a linear regression analysis was applied to changes in MBP and in HR from individual animals, as described in the previous study (El-Mas et al. 2002). The slope of the regression line was used as an index of baroreflex.

#### Drugs and reagents

Imidafenacin, solifenacin succinate, tolterodine tartrate, and propiverine hydrochloride were synthesized by Kyorin Pharmaceutical Co., Ltd (Tokyo, Japan). CCh, atropine monohydrate sulfate salt (atropine), and phenylephrine were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). SNP was obtained from Calbiochem (San Diego, CA, USA).

Imidafenacin was dissolved in saline with 1 M hydrochloric acid and then neutralized with 1 M sodium hydroxide and serially diluted to desired concentrations. Solifenacin succinate, tolterodine tartrate, propiverine hydrochloride, CCh, atropine, phenylephrine, and SNP were dissolved in saline, and these solutions were serially diluted to desired concentrations. Saline was injected as a vehicle control.

The doses of the drugs used in this study were determined based on the results of preliminary examinations. The test

drugs were injected into separate rats (i.e., one dose of each test drug into a rat).

#### Data analysis

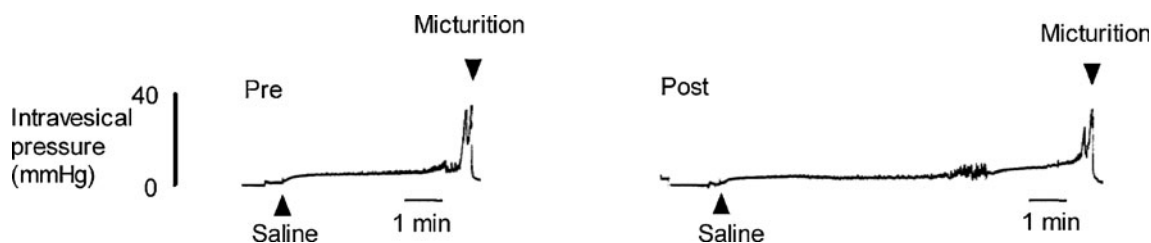
All data are expressed as mean $\pm$ SEM. The statistical differences between the drug-treated and vehicle groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. Differences between pre-injection and post-injection values were tested by paired *t* tests. In all comparisons,  $P < 0.05$  was considered statistically significant.

The minimum effective dose, defined as the lowest dose required to increase bladder capacity with a statistical significant difference, was used as an index for their potency. ID<sub>50</sub> values, the doses required to induce 50% inhibition of the salivary secretion, rhythmical contraction in colon, and bradycardia, respectively, were determined by linear regression analysis on the basis of the percent inhibition at each log-dose level. The ratio of each ID<sub>50</sub> value in salivary gland, gastrointestinal tract, and heart to the minimum effective dose in bladder (dose ratio) was calculated. Since the experimental conditions of muscarinic stimulations between bladder and each side effect-related tissue are different (for example, micturition reflex induced by raising the intravesical pressure vs. salivary secretion by electrical stimulation of chorda tympani), the potency of each antimuscarinic agent cannot be compared directly among tissues. Thus, the bladder selectivity was expressed relative to that for propiverine hydrochloride.

## Results

#### Increasing effect on bladder capacity in urethane-anesthetized rats

A typical cystometrogram is shown in Fig. 2. The bladder capacity, micturition pressure, and residual volume before drug treatment were  $0.35 \pm 0.01$  mL,  $33.5 \pm 0.7$  mmHg, and  $0.17 \pm 0.01$  mL, respectively. Imidafenacin at doses of 0.003 and 0.01 mg/kg (i.v.), solifenacin succinate at doses of 1 and 3 mg/kg (i.v.), tolterodine tartrate at doses of 0.03 and 0.1 mg/kg (i.v.), and propiverine hydrochloride at doses of 3 and 6 mg/kg (i.v.) all significantly increased bladder capacity compared with vehicle (Fig. 3a). The minimum effective doses of imidafenacin, solifenacin succinate, tolterodine tartrate, and propiverine hydrochloride were 0.003, 1, 0.03, and 3 mg/kg, respectively (Table 1), and the rank order of their potency was, therefore, imidafenacin > tolterodine tartrate > solifenacin succinate > propiverine hydrochloride. Furthermore, all drugs produced a dose-dependent suppression of micturition pressure and also a



**Fig. 2** A typical cystometrogram before (pre) and after (post) imidafenacin (0.003 mg/kg, i.v.) injection in urethane-anesthetized rats. The measurement of the intravesical pressure was started just before the saline infusion

dose-dependent increase in residual volume (Fig. 3b, c). Although propiverine hydrochloride suppressed the micturition pressure and increased the residual volume at the minimum effective dose, imidafenacin, solifenacin succinate, and tolterodine tartrate did not influence these parameters at their minimum effective doses (Fig. 3b, c).

#### Inhibitory effect on salivary secretion in urethane-anesthetized rats

The amount of electrically stimulated salivary secretion of chorda tympani before drug treatment was  $187.5 \pm 4.6$  mg. Imidafenacin, solifenacin succinate, tolterodine tartrate, and propiverine hydrochloride produced a significant dose-dependent decrease in salivary secretion with  $ID_{50}$  values of 0.0078, 0.30, 0.013, and 0.52 mg/kg i.v., respectively (Fig. 6a; Table 1). Vehicle did not affect the salivary secretion ( $1.8 \pm 3.8\%$  inhibition,  $n=5$ ). Bladder selectivity of imidafenacin, solifenacin succinate, and tolterodine tartrate over salivary gland was 15-, 1.7-, and 2.5-fold higher than that of propiverine hydrochloride, respectively, and the rank order was, therefore, imidafenacin > tolterodine tartrate > solifenacin succinate > propiverine hydrochloride (Table 1).

#### Inhibitory effect on rhythmical contractions of colon in urethane-anesthetized rats

A typical trace of rhythmical contractions of colon is shown in Fig. 4. The AUC value before drug treatment was  $1,368 \pm 39$  mmHg 10 min. Imidafenacin, solifenacin succinate, tolterodine tartrate, and propiverine hydrochloride produced a significant dose-dependent decrease in rhythmical contractions induced by raising the intracolonic pressure with  $ID_{50}$  values of 1.8, 7.4, 1.1, and 12 mg/kg i.v., respectively (Fig. 6b; Table 1). Vehicle did not affect the rhythmical contractions ( $1.6 \pm 2.8\%$  inhibition,  $n=6$ ). Bladder selectivity of imidafenacin, solifenacin succinate, and tolterodine tartrate over colon was 150-, 1.9-, and 9.2-fold higher than that of propiverine hydrochloride, respectively, and the rank order was, therefore, imidafenacin > tolterodine tartrate > solifenacin succinate > propiverine hydrochloride (Table 1).

#### Inhibitory effect on CCh-induced bradycardia in urethane-anesthetized rats

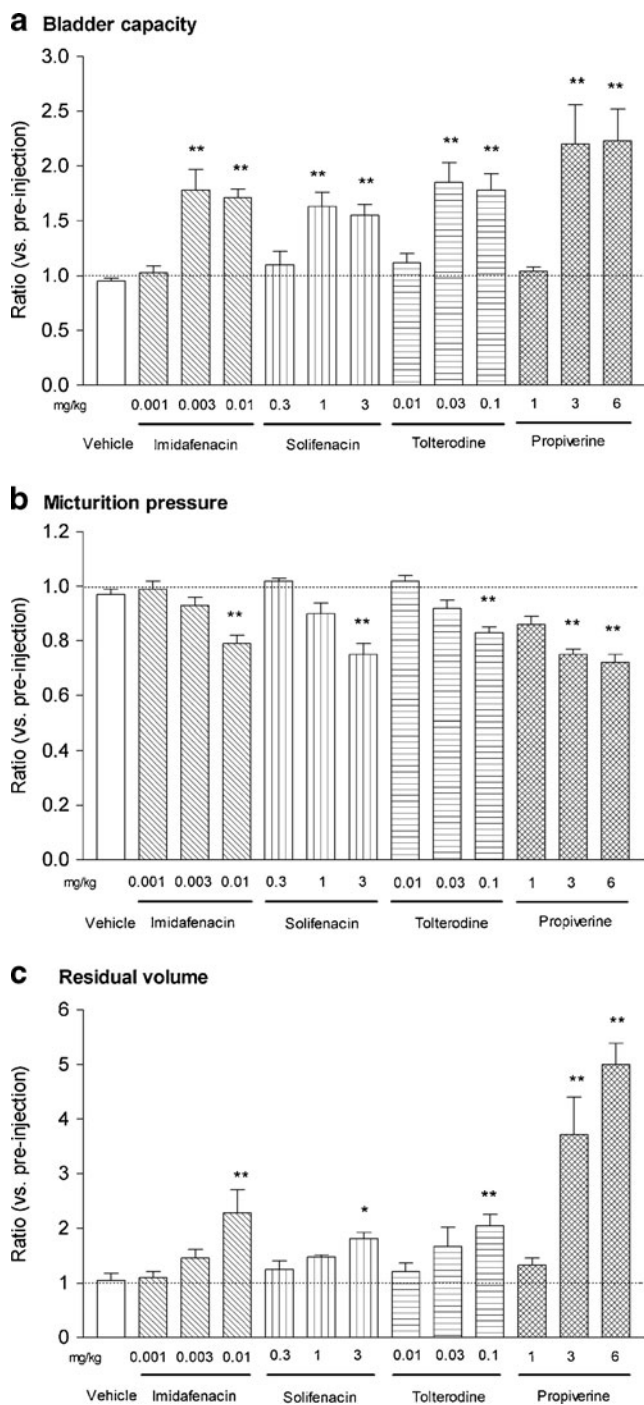
A typical trace of CCh-induced bradycardia is shown in Fig. 5. The mean blood pressure and heart rate before drug treatment were  $87.2 \pm 0.9$  mmHg and  $403.8 \pm 4.6$  beats/min, respectively. Imidafenacin, solifenacin succinate, tolterodine tartrate, and propiverine hydrochloride produced a significant dose-dependent decrease in CCh-induced bradycardia, with  $ID_{50}$  values of 0.0090, 0.71, 0.0082, and 0.18 mg/kg i.v., respectively (Fig. 6c; Table 1). Vehicle did not affect the CCh-induced bradycardia ( $-0.7 \pm 5.8\%$  inhibition,  $n=5$ ). Bladder selectivity of imidafenacin, solifenacin succinate, and tolterodine tartrate over heart was 50-, 12-, and 4.6-fold higher than that of propiverine hydrochloride, respectively, and the rank order was, therefore, imidafenacin > solifenacin succinate > tolterodine tartrate > propiverine hydrochloride (Table 1).

#### Influences on baroreflex in conscious rats

The mean blood pressure and heart rate before drug treatment were  $102.3 \pm 1.9$  mmHg and  $348.9 \pm 5.1$  beats/min, respectively. Atropine monohydrate sulfate salt (0.5 mg/kg i.v.), a positive control, inhibited the phenylephrine-induced baroreflex (slope;  $-2.8 \pm 0.5$  vs.  $-0.7 \pm 0.1$ ,  $P < 0.05$ ,  $n=5$ ). Unlike atropine monohydrate sulfate salt, imidafenacin (0.3 mg/kg i.v., corresponding to the 100 times the minimum effective dose to increase bladder capacity) did not affect the phenylephrine-induced baroreflex (slope;  $-2.6 \pm 0.4$  vs.  $-2.0 \pm 0.5$ ,  $n=5$ ) (Fig. 7a). Similarly, atropine monohydrate sulfate salt (0.5 mg/kg i.v.) inhibited SNP-induced baroreflex (slope;  $-2.6 \pm 0.3$  vs.  $-1.1 \pm 0.3$ ,  $P < 0.01$ ,  $n=5$ ), whereas imidafenacin (0.3 mg/kg i.v.) did not affect it (slope;  $-2.6 \pm 0.3$  vs.  $-2.5 \pm 0.3$ ,  $n=5$ ) (Fig. 7b).

## Discussion

We demonstrated that imidafenacin is the most highly selective for bladder over all of tissues related to major antimuscarinic side effects, using an effectiveness index for



**Fig. 3** Effects of antimuscarinics on bladder capacity (a), micturition pressure (b), and residual volume (c) in urethane-anesthetized rats. Vehicle or antimuscarinics were injected intravenously. Data are expressed as a ratio of post- to pre-value. Each column and bar represents the mean  $\pm$  SEM of five animals. \* $P < 0.05$  and \*\* $P < 0.01$  vs. vehicle group (Dunnett's multiple comparison test). *Solifenacin* solifenacin succinate, *Tolterodine* tolterodine tartrate, *Propiverine* propiverine hydrochloride

bladder capacity, compared with other well-known antimuscarinics. Much evidence showing that antimuscarinics primarily act on afferent pathway rather than detrusor muscle has

been recently accumulated. Based on analysis of published cystometric data in OAB patients, Finney et al. (2006) pointed out that therapeutic doses of antimuscarinics have a clearly significant effect on filling phase such as maximum bladder capacity, but not on voiding phase related to detrusor contraction. Yokoyama et al. (2005) showed that low tolterodine doses significantly increased bladder capacity without inhibiting micturition pressure in cerebral infarcted rats, and this increase is mediated through resiniferatoxin-sensitive (C-fiber) nerves. Kim et al. (2005) also reported a similar result by intravesical instillation of oxybutynin in anesthetized rats. Also in the present study, all antimuscarinics tested, except for propiverine hydrochloride having calcium channel blocking activity (Wada et al. 1995), increased the bladder capacity at lower dose levels than those at which they inhibited micturition pressure, suggesting that this feature is common to antimuscarinics. Not a few studies have already reported on the effects of individual antimuscarinics on the bladder capacity, but little information is still available about the bladder selectivity using bladder capacity as an effectiveness index. To our knowledge, there is only one published study that evaluated the bladder selectivity profiles of tolterodine and oxybutynin by comparing the effects on bladder capacity and visual accommodation in healthy volunteers (Chapple and Nilvebrant 2002). Based on all these recent evidences, we tried to reevaluate the bladder selectivity of antimuscarinics in rats by using an effectiveness index for bladder capacity.

The effective dose levels of solifenacin succinate, tolterodine tartrate, and propiverine hydrochloride required to increase bladder capacity in the present study were almost consistent with those in the previous reports using normal rats (Hedlund et al. 2007; Nomura et al. 1989; Ohtake et al. 2007), while the effective dose levels of solifenacin succinate and tolterodine tartrate in the present study were different from those in the previous reports using a rat model of OAB induced by cerebral infarction (Suzuki et al. 2005; Yokoyama et al. 2005). Additionally, the shapes of dose–response curves in terms of bladder capacity increase seem to be relatively steeper in normal rats than in a rat model of OAB in the previous reports (Nomura et al. 1989; Ohtake et al. 2007; Suzuki et al. 2005; Yokoyama et al. 2005). Although the reason for this discrepancy is unclear, the effective dose levels and shapes of dose–response curves of antimuscarinics on increasing bladder capacity may depend on experimental conditions used. Furthermore, when we compared our present data with the available animal data, we found no large differences in the effective dose levels of individual antimuscarinics in terms of CCh-induced salivation and CCh-induced bradycardia (Ikeda et al. 2002; Ohtake et al. 2006; Sinha et al. 2010). Thus, the present experimental system was considered appropriate to evaluate bladder selectivity.

**Table 1** The bladder selectivity of antimuscarinics tested over salivary gland, gastrointestinal tract, and heart in urethane-anesthetized rats

Tissue	Drug	Number	Minimum effective dose or ID <sub>50</sub> (95% confidence interval)	Dose ratio	Bladder selectivity
Bladder	Imidafenacin	5	0.003	–	–
	Solifenacin	5	1	–	–
	Tolterodine	5	0.03	–	–
	Propiverine	5	3	–	–
Salivary gland	Imidafenacin	5	0.0078 (0.0068–0.0088)	2.6	15
	Solifenacin	5	0.30 (0.27–0.33)	0.30	1.7
	Tolterodine	5	0.013 (0.012–0.015)	0.43	2.5
	Propiverine	5	0.52 (0.41–0.62)	0.17	1
Gastrointestinal tract	Imidafenacin	6	1.8 (0.88–6.5)	600	150
	Solifenacin	6	7.4 (4.5–18)	7.4	1.9
	Tolterodine	6	1.1 (0.72–2.1)	37	9.2
	Propiverine	6	12 <sup>a</sup> (6.7–34)	4.0	1
Heart	Imidafenacin	5	0.0090 (0.0071–0.011)	3.0	50
	Solifenacin	5	0.71 (0.57–0.87)	0.71	12
	Tolterodine	5	0.0082 (0.0060–0.011)	0.27	4.6
	Propiverine	5	0.18 (0.11–0.26)	0.060	1

Effects on bladder or other tissues are expressed by the minimum effective dose (milligrams per kilogram) or ID<sub>50</sub> (milligrams per kilogram), respectively. The dose ratio was calculated by dividing the ID<sub>50</sub> value by the minimum effective dose. Since the experimental conditions of muscarinic stimulations are different among tissues as described in “Methods,” the potency of each antimuscarinic agent cannot be compared directly among tissues. Thus, the bladder selectivity is expressed as relative ratios to the dose ratio of propiverine hydrochloride (fold of propiverine hydrochloride)

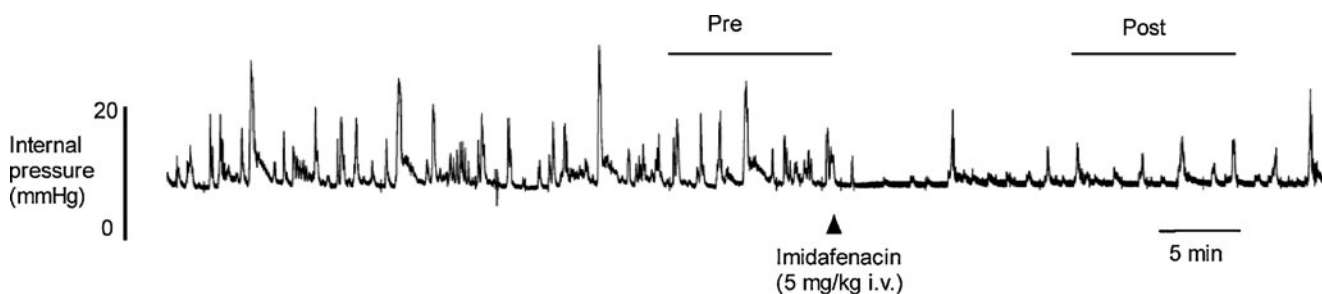
*Solifenacin* solifenacin succinate, *Tolterodine* tolterodine tartrate, *Propiverine* propiverine hydrochloride

<sup>a</sup> Extrapolative value (maximum inhibition was nearly 40%)

In the previous study, the increasing effect of imidafenacin on bladder capacity was assessed by continuous cystometry, but the data were not used for evaluating the bladder selectivity (Kobayashi et al. 2007b). Continuous cystometry is not always suitable for evaluating accurate bladder capacity because the drugs which inhibit detrusor contraction increase the residual urine volume (Takagi-Matsumoto et al. 2004). Thus, in the present study, we applied intermittent cystometry to measurement of bladder capacity.

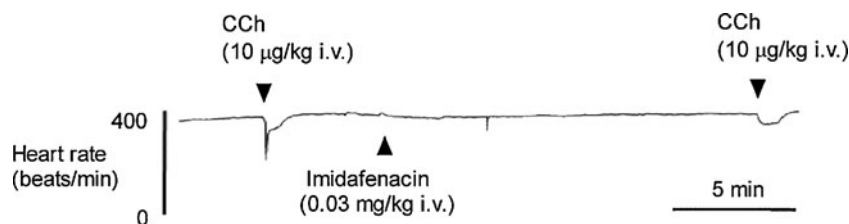
The bladder selectivity rank order of imidafenacin and tolterodine tartrate over salivary gland in the present study agreed with the previous results (Kobayashi et al. 2007b;

Ohtake et al. 2006). On the other hand, the relative bladder selectivity of solifenacin succinate to propiverine hydrochloride in the present study (1.7-fold) was lower than that in the study by Ohtake et al. (2006; 5.9-fold), resulting from the rank order of solifenacin succinate and tolterodine tartrate being reversed between two studies. This discordance is thought to mainly result from the difference in potency of solifenacin between studies; the minimum effective dose to increase bladder capacity in the present study was 1 mg/kg, whereas the ID<sub>30</sub> value to decrease intravesical pressure was 0.023 mg/kg (Ohtake et al. 2006). These suggest that the bladder selectivity of certain

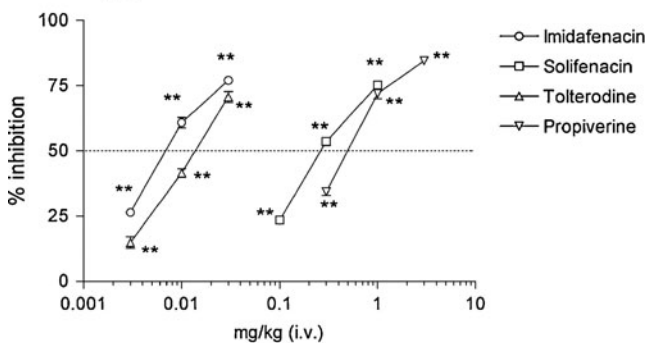


**Fig. 4** A typical trace of rhythmic contractions of colon in urethane-anesthetized rats

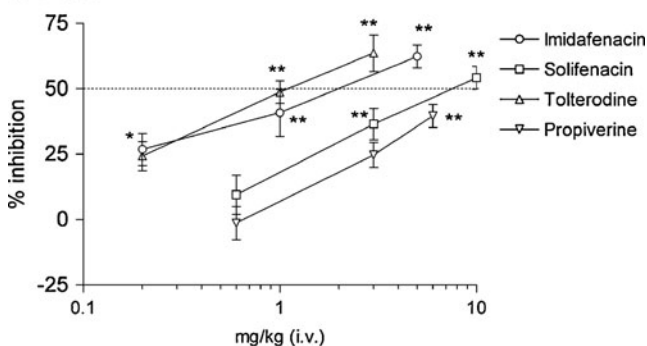
**Fig. 5** A typical trace of CCh-induced bradycardia in urethane-anesthetized rats



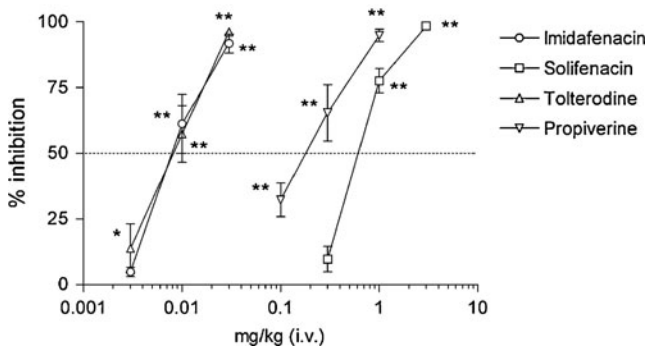
**a Salivary gland**



**b Colon**



**c Heart**

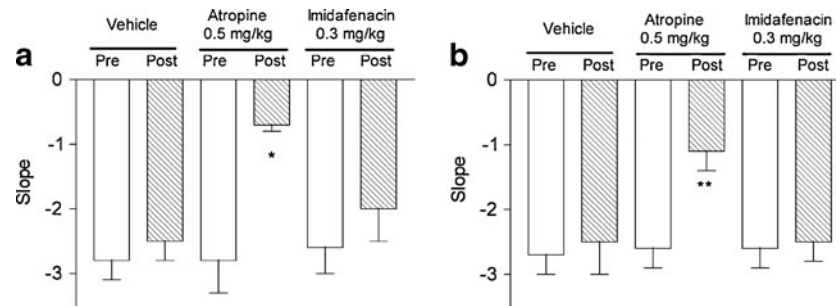


**Fig. 6** Inhibitory dose–response curves of antimuscarinics on salivary secretion (a), rhythmic contractions in colon (b), and CCh-induced bradycardia (c) in urethane-anesthetized rats. Vehicle or antimuscarinics were injected intravenously. Inhibitory effects of drugs on each tissue are expressed as percentage inhibition of post- to pre-value. Each mark and bar represents the mean±SEM of five to six animals. \* $P<0.05$  and \*\* $P<0.01$  vs. vehicle group (Dunnett's multiple comparison test). *Solifenacin* solifenacin succinate, *Tolterodine* tolterodine tartrate, *Propiverine* propiverine hydrochloride

antimuscarinics may depend on the effectiveness index used. Each antimuscarinic may have effects of increasing bladder capacity and inhibiting detrusor contraction, which are separated by mechanisms of action as mentioned above, in a different degree. This speculation, however, is based on limited available data and only solifenacin results. Therefore, further verification is required to ensure whether the bladder selectivity depends on an effectiveness index used.

Dry mouth and constipation are most common side effects of antimuscarinics and are major causes of their withdrawal (Abrams and Andersson 2007). Imidafenacin exhibited the highest bladder selectivity over the salivary gland and gastrointestinal tract among the antimuscarinics tested. It remains unknown which muscarinic subtypes participate in the activation of bladder afferent pathway. However, at least  $M_3$  receptor seems to have a crucial role in activation of bladder afferent pathway as well as in detrusor contraction. Indeed, it has been reported that known selective  $M_3$  antagonist darifenacin effectively decreases distention-induced A-delta and C afferent nerve activation (Iijima et al. 2007) and that  $M_3$  knockout mice have longer voiding intervals and larger micturition volumes and bladder capacity than wild-type mice (Igawa et al. 2004) and also that darifenacin has comparable efficacies on storage-phase symptoms such as urgency episodes to nonselective antimuscarinics in OAB patients (Zinner et al. 2005). Salivary secretion and gastrointestinal motility are also mediated through  $M_3$  subtype (Abrams et al. 2006). Thus, the high bladder selectivity of imidafenacin cannot be explained only by receptor-subtype selectivity. Previous studies using radioreceptor assay demonstrated that imidafenacin exerts more selective and longer-lasting distribution in the bladder than in salivary gland and colon in rats (Yamada et al. 2011), whereas propiverine and solifenacin distribute in the salivary gland or in other antimuscarinic side effect-related tissue as well as in the bladder (Oki et al. 2001, 2005). Yamada et al. (2011) reported that imidafenacin excreted in urine may contribute to its selective and long-lasting distribution in the bladder because imidafenacin showed a significant binding to bladder muscarinic receptors in rats following its intravesical instillation at a concentration which corresponds to its urine concentration after oral administration in healthy volunteers. Mansfield (2010) suggested that antimuscarinics excreted in the urine may have therapeutic potential;





**Fig. 7** Influences of imidafenacin on phenylephrine (a)- and SNP (b)-induced baroreflex in conscious rats. Vehicle or antimuscarinics were injected intravenously. Data are expressed as a slope (index of baroreflex)

before and 15 min after drug injection. Each column and bar represents the mean±SEM of five animals. \* $P<0.05$  and \*\* $P<0.01$  vs. pre-injection (paired  $t$  test)

the urinary excretion rate (percentage of doses) of imidafenacin in humans (7.8%; Ohno et al. 2008) is relatively high, compared with those of darifenacin, oxybutynin, tolterodine, and trospium (Mansfield 2010). Additionally, Akino et al. (2009) reported that dry mouth and constipation were significantly less severe in patients treated with immediate-release antimuscarinics such as imidafenacin than in those treated with extended-release antimuscarinics such as solifenacin, tolterodine, and propiverine. Similarly, the incidence of dry mouth with imidafenacin was significantly lower than with propiverine in the phase III trial for OAB (Homma et al. 2009). Indeed, the half-life of imidafenacin (3.0 h; Ohno et al. 2008) is markedly shorter than those of other antimuscarinics (Abrams and Andersson 2007). These findings suggest the possibility that pharmacokinetic factors also largely contribute to the high bladder selectivity of imidafenacin.

It has been reported that because elevated heart rate or depressed baroreflex sensitivity increases the mortality and sudden death in patients with cardiovascular disease, drugs with less influence on heart rate are preferable for treating OAB patients concomitant with cardiovascular disease (Andersson and Olshansky 2007; La Rovere et al. 2001). Imidafenacin showed the highest bladder selectivity over heart among the antimuscarinics tested here. As shown in Table 2, imidafenacin is reported to have a high affinity for

$M_3$  (and  $M_1$ ) over  $M_2$  receptor. On the other hand, tolterodine tartrate and propiverine hydrochloride, nonselective muscarinic receptor antagonists, showed lower bladder selectivity than imidafenacin. Muscarinic agonist-induced bradycardia is mediated mainly by  $M_2$  receptor on sinoatrial node (Wess et al. 1988); hence, at least the selectivity for  $M_3$  vs.  $M_2$  receptor probably contributes to the high bladder selectivity over heart. This is further supported by the findings that  $M_3$  selective solifenacin succinate showed high bladder selectivity over heart. Additionally, since imidafenacin distributes less in heart than in bladder but propiverine distributes similarly in heart and in bladder (Oki et al. 2001; Yamada et al. 2011); such pharmacokinetic factors may also serve favorably to its bladder selectivity over heart. Indeed, a randomized, controlled trial proved that imidafenacin has less influence on heart rate than propiverine (Homma et al. 2009). We also examined whether imidafenacin interferes with baroreflex that is associated with cholinergic nerves (Olshansky et al. 2008) and found that imidafenacin had no influence on heart rates mediated through baroreflex even at 0.3 mg/kg (i.v.), which corresponded to 100 times the minimum effective dose to increase bladder capacity.

In the present study, all antimuscarinics tested, except for propiverine hydrochloride, increased the bladder capacity at lower dose levels than those at which they inhibited micturition pressure. Similarly, Yokoyama et al. (2005, 2011) showed that low-dose tolterodine or imidafenacin increased the bladder capacity without inhibiting micturition pressure in cerebral infarcted rats, and this increase is mediated through resiniferatoxin-sensitive (C-fiber) nerves. Although it remains unclear whether the bladder afferent nerve is involved in increasing effects of antimuscarinics on bladder capacity observed in the present study, all these results seem to agree with the recent concept that antimuscarinics primarily act on the bladder afferent pathway during storage phase (Andersson and Yoshida 2003; Finney et al. 2006; Yamaguchi 2010). Thus, it would be reasonable to adopt an effectiveness index for bladder capacity for assessing bladder selectivity.

**Table 2** Reported inhibitory constants ( $K_i$ ) of antimuscarinics for human recombinant muscarinic acetylcholine receptor subtypes

Drug	$K_i$ (nM)				
	$M_1$	$M_2$	$M_3$	$M_4$	$M_5$
Imidafenacin <sup>a</sup>	7.6	23	1.4	8.9	2.6
Solifenacin <sup>b</sup>	26	170	12	110	31
Tolterodine <sup>a</sup>	7.9	7.4	7.9	18	4.2
Propiverine <sup>b</sup>	490	1400	350	900	490

<sup>a</sup> Kobayashi et al. 2007a

<sup>b</sup> Ohtake et al. 2007

In conclusion, when an effectiveness index for the bladder capacity was used in assessing the bladder selectivity, imidafenacin showed the highest bladder selectivity over salivary gland, gastrointestinal tract, and heart among the tested antimuscarinics in the rat.

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