Comparative analysis of the effects of antimuscarinic agents on bladder functions in both non-human primates and rodents

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Number of:
- Text page 14
- Tables 4
- Figures 3
- References 39

Words in the
- Abstract 245
- Introduction 698
- Discussion 1484

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OAB, overactive bladder

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Abstract

Both the physiological role of muscarinic receptors for bladder function and the therapeutic efficacy of antimuscarinic agents for overactive bladder syndrome are well documented. We investigated the effect of antimuscarinic agents with different subtype selectivity on urodynamic parameters in non-human primates and rodents, and compared plasma levels of these agents between species. Anesthetized rhesus monkeys were transurethrally catheterized and the bladder was infused with saline. Urodynamic parameters were measured before and after intravenous drug administration. Tolterodine (non-selective) and oxybutynin (moderately M3 selective) increased bladder capacity at lower doses than those required to decrease micturition pressure. However, higher doses of darifenacin (M3 selective) were needed to increase the bladder capacity than those needed to decrease the micturition pressure. In rats, tolterodine had no effect on the bladder capacity, but decreased the micturition pressure at all doses administered. Oxybutynin also decreased micturition pressure and increased bladder capacity at the highest dose. Plasma levels of these drugs overlap in both species. These results suggest that, in addition to the M3 receptor, other muscarinic receptor subtypes contribute to regulate bladder storage function in non-human primates, since less-subtype selective tolterodine and oxybutynin showed higher specificity to the bladder capacity effect than the effect on micturition pressure, when compared to M3-selective darifenacin. Additionally, the role of muscarinic receptors in bladder storage function varies between primates and rodents. As compared to rodents, muscarinic receptors may play a more active role during the storage phase to regulate the functional bladder capacity in primates.
Introduction

Urinary bladder function is regulated by the sympathetic and parasympathetic nervous system. Acetylcholine released from parasympathetic nerve endings activates muscarinic receptors on the detrusor muscle, and thereafter induces smooth muscle contraction. While all five muscarinic receptor subtypes (M1-M5) are expressed in the bladder, the M3 subtype is predominant in many species including humans (Hegde and Eglen, 1999); however, the M3 receptors play a major role in detrusor contraction (Longhurst et al., 1995; Chess-Williams et al., 2001).

Antimuscarinic agents have been used for the treatment of overactive bladder (OAB) for over three decades (Abrams and Andersson, 2007). It has been generally thought that antimuscarinics inhibit abnormal detrusor overactivity caused in the diseased bladder (de Groat, 1997). However, several lines of evidence support the effect of antimuscarinic agents on not only the bladder-motor system, but also on the sensory system (Andersson and Yoshida, 2003; De Laet et al., 2006; Yoshimura, 2007; Matsumoto et al., 2010). It seems that the sensory effect of antimuscarinics is more likely as a mechanism of action as this class of drugs alleviates bladder symptoms such as the urgency, frequency, and urge urinary incontinence, which occur during the storage phase when parasympathetic efferent activity is normally absent (de Groat et al., 1993).

It is obvious that antagonism of the M3 subtype is crucial for OAB pharmacotherapy because the efficacy of available antimuscarinics is similar, despite differing subtype selectivity against M3. However, there are significant arguments regarding the role of the M2 subtype and additional therapeutic benefits in drugs, which possess M2 antagonism (Abrams and Andersson, 2007; Smith and Wein, 2010). M2 receptors are expressed in the urothelial layer of the bladder, from which non-neuronal mediators are released with bladder distention (Mukerji et al., 2006; Yoshida et al., 2006). Released mediators such as ATP and acetylcholine are believed to activate bladder sensory pathways. Recent studies revealed that selective M2 subtype blockade inhibited non-neuronal ATP release (Yoshida et al., 2010) and also suppressed bladder overactivity (Matsumoto et al., 2010).
In order to measure bladder function, urodynamic studies, which assess bladder storage and voiding function, are commonly used in humans as well as in animals (Abrams, 2005). Bladder capacity is one of the parameters collected in urodynamic studies, and an increase in the bladder capacity is considered to be a preferable effect of OAB therapy, correlating with a decrease in the micturition frequency. Although urodynamic studies are commonly utilized for diagnostic purposes in clinics and rarely to measure pharmacological treatment outcomes, a few papers have reported the urodynamic effect of antimuscarinics in humans. For example, tolterodine and trospium, both antimuscarinics, increased bladder capacity in patients with detrusor overactivity (Stohrer et al., 1991; Jonas et al., 1997). Additionally, in healthy subjects, tolterodine increased the bladder volume evoking the normal desire to void (Stahl et al., 1995). In contrast, many studies in rats, the most frequently used preclinical species, failed to show an increase in bladder capacity with antimuscarinics (Sasaki et al., 1997; Angelico et al., 2005; Nagabukuro et al., 2010) or showed a bladder capacity increase only at the high doses (Ohtake et al., 2007; Hegde et al., 2009). Despite this paradox, these studies successfully demonstrated the inhibitory effects of antimuscarinics on detrusor contractility, by demonstrating a significant decrease in the maximum micturition pressure, and a significant increase in the residual urine volume.

There are only a few reports on urodynamic studies in non-human primates (Shoukry and Ghoniem, 1992; Kimura et al., 1997), but one previous study demonstrated that antimuscarinics, including atropine and oxybutynin, dose-dependently increased bladder capacity at the doses which caused a decrease in micturition pressure (Kimura et al., 1997). In light of these cross-species observations, we hypothesized that there could be a species difference in the physiological role of muscarinic receptors with regard to bladder storage function, and that the effect of antimuscarinics on bladder capacity might vary between species.

In this study, we investigated the effects of three antimuscarinics with different subtype selectivity (tolterodine, nonselective; oxybutynin, relatively M3 selective; darifenacin, highly M3 selective) (Abrams et al., 2006; Hegde, 2006) on urodynamic parameters in both non-human primates and rodents. We also
measured plasma levels of the drugs and directly compared the pharmacokinetic/pharmacodynamic relationship between drugs and species.
Materials and Methods

Subjects. All procedures related to the use of animals were approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories (Rahway, NJ) and conform to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, National Research Council, 1996). The animals were housed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

A total of 12 adult female rhesus monkeys (Macaca mulatta) weighing 5.3-6.2 kg (4-7 year-old) were used. The subjects were either paired or individually housed on a 12-h light/12-h dark cycle (lights on at 7:00 AM). Their diet consisted of 2050 Teklad (Harlan Laboratories, Indianapolis, IN) supplemented with fresh fruit and vegetables. Water was freely available. All animals were observed daily by a veterinary technical staff and caretakers for signs of ill health. Subjects were repeatedly used with ≥13-day resting period.

A total of 54 adult female Sprague-Dawley rats weighing 170-240 g (Charles River, Wilmington, MA) were housed in a temperature- and light- (12-h light/dark cycle) controlled room, and were allowed access to food (Diet 7012, Harlan) and water ad libitum.

Cystometry in rhesus monkeys. Monkeys were anesthetized with an intramuscular injection of either Telazol (3-5 mg/kg) or ketamine (10-20 mg/kg) followed by intravenous constant rate infusion with ketamine (0.2-0.8 mg/kg/min) using a syringe pump (Medfusion 2010, Medex, Carlsbad, CA). Ketamine was selected as this anesthetic minimally affected urodynamic parameters (Ghoniem et al., 1996). Animals were placed in a supine position and a triple lumen balloon transurethral catheter (7.4 Fr, Cook Medical, Bloomington, IN) was aseptically inserted into the bladder and the balloon was inflated with 1 mL of water to secure the tip of catheter at the bladder base. The catheter was connected to an infusion pump (Gemini PC-2TX, ALARIS Medical Systems, San Diego, CA) for bladder filling and to a pressure transducer for intravesical pressure monitoring. Intravesical pressure was continuously recorded using a multiple channel data acquisition system (Power Lab, AD Instruments, Biopac systems, Colorado Springs, CO) at a sampling rate of 20 Hz. After confirming bladder emptiness by ultrasonography (Logiq e vet, GE Medical Systems, Waukesha, WI), saline was intravesically infused at 15 mL/min. When the steep rise in pressure indicative
of the micturition reflex was observed (volume threshold), intravesical infusion was stopped and the bladder was manually drained through the urethral catheter using a syringe. Since the balloon restricts the flow of urine passing through urethra, the bladder was rapidly evacuated within approximately 15-30 seconds immediately after the first peak pressure was identified to avoid bladder overdistension. As a result of the rapid bladder evacuation, micturition via the urethra was not observed in most animals.

After two baseline cystometry readings, a drug was intravenously administered 3 - 4 times using a rising dose paradigm with a cystometry measurement 10 min after each dose. In the control group, animals were dosed with vehicle in the same manner. Blood samples (0.5 mL) were collected immediately following each cystometric measurement for pharmacokinetic analysis. Heart rate and blood pressure were monitored during the experiment using a lead II ECG and a blood pressure cuff, respectively. The heart rate and blood pressure criteria for discontinuation of compound dosing were defined as >190 bpm and <40 mmHg, respectively. However, none of the compounds tested in this study induced cardiovascular effects that exceeded the criteria.

**Cystometry in rats.** Cystometry was performed as previously described (Nagabukuro et al., 2010). Briefly, animals were anesthetized with urethane (1.1 g/kg, i.p.). A lower abdominal midline incision was made to expose the bladder, and two polyethylene tubes (PE-50) were inserted into the bladder dome, secured with a 4-0 silk suture and connected to both a syringe pump (555920, Harvard Apparatus, Holliston, MA) and a pressure transducer via a 3-way stop cock. Physiological saline was intravesically infused at a rate of 0.05 mL/min. The intravesical pressure signal was recorded using a Power lab unit (16/30, AD Instruments) at a sampling rate of 10 Hz. An intravenous catheter (PE-100) was implanted into a femoral vein for drug administration. The bladder was completely drained off, before beginning intravesical infusion. Bladder infusion proceeded until micturition was observed, and then the bladder was again drained. After two baseline cystometry readings, a drug or vehicle was administered and post-dose cystometry was performed 10-15 min after dosing. Blood samples were collected for pharmacokinetic analysis right after post-dose cystometry was completed.

**Urodynamic parameters.**
In monkeys, bladder capacity, maximum micturition pressure, threshold pressure and bladder compliance were measured as follows: bladder capacity, duration of bladder filling multiplied by intravesical infusion rate (15 mL/min); maximum micturition pressure, the pressure reading of the first peak in intravesical pressure driven by the micturition reflex; threshold pressure, the intravesical pressure at the volume threshold, which was defined as the volume just prior to the initiation of steep rise in pressure indicative of the micturition reflex; bladder compliance, inverse of average slope of intravesical pressure during filling phase.

In rats, bladder capacity, maximum micturition pressure and threshold pressure were measured as follows: bladder capacity, duration of bladder filling multiplied by intravesical infusion rate (0.05 mL/min); maximum micturition pressure, the peak intravesical pressure during the micturition episode; threshold pressure, intravesical pressure at the volume threshold.

**Pharmacokinetic analysis.** Plasma concentrations of tolterodine, 5-hydroxymethyl tolterodine (active metabolite), oxybutynin and darifenacin were determined by liquid chromatography-tandem mass spectrometry following protein precipitation of the incurred plasma samples. An Aria LX-2 (ThermoFisher Scientific, Franklin, MA) was used as the inlet system, coupled to an API 4000 mass spectrometer (Applied Biosystems, Foster City, CA). The lower limits of quantification of tolterodine and 5-hydroxymethyl tolterodine, oxybutynin and darifenacin were 0.92, 1.09, 0.92 and 0.14 ng/mL, respectively.

**Statistical analysis.** Data are presented as the mean ± S.E.M. The mean values were compared with two-way analysis of variance (ANOVA) with Bonferroni post-hoc test or one-way ANOVA with Dunnett’s multiple comparison test. A probability value less than or equal to 0.05 was considered significant.

**Drugs.** Tolterodine, oxybutynin and darifenacin were purchased from Toronto Research Chemicals (North York, Canada), Sigma-Aldrich (St. Louis, MO) and AK Scientific (Union City, CA), respectively. 5-hydroxymethyl tolterodine was synthesized at the Medicinal Chemistry Department, Merck Research Laboratories. For monkey studies, drugs were dissolved in a vehicle of 20% saline-20% ethanol-60% PEG 400 which was sterilized through a 0.22 µm Millipore filter (Fisher Scientific, Pittsburgh, PA), and were
dosed at 0.2 mL/kg. For rat studies, drugs were dissolved in saline at a dosing volume of 0.5 mL/kg. The doses of drugs were determined based on the previous demonstration that these doses affected cystometric parameters via their pharmacological effects (Angelico et al., 2005; Ohtake et al., 2007) and through preliminary pharmacokinetic experiments in rhesus, in order to cover the clinically relevant plasma levels of each drug. Darifenacin was not used in the rat study since the effects of oxybutynin and tolterodine on urodynamic parameters were in accordance with previously reports (Angelico et al., 2005; Ohtake et al., 2007) and similar effects have been reported for darifenacin (Modiri et al., 2002).
Results

Effect of antimuscarinics in rhesus monkeys. The baseline values for all urodynamic parameters in each group are shown in Table 1. There was no inter-group difference for any parameter (one-way ANOVA). These values are consistent with previous reports (Shoukry and Ghoniem, 1992; Ghoniem et al., 1996). Fig. 1 shows representative cystometrograms from animals dosed with vehicle and tolterodine. While repetitive treatment with vehicle did not alter any urodynamic parameters compared to baseline values, tolterodine, administered with a rising dose paradigm, increased bladder capacity and decreased micturition pressure. Tolterodine, darifenacin and oxybutynin all significantly increased bladder capacity (Fig. 2A, B). The maximum increases were 40 ± 10%, 29 ± 9% and 71 ± 10% of baseline bladder capacity with tolterodine (0.1 mg/kg), darifenacin (0.1 mg/kg) and oxybutynin (1 mg/kg), respectively. These drugs also decreased micturition pressure in a dose-dependent manner (Fig. 2C, D), but only oxybutynin increased threshold pressure at the highest dose (Table 2). None of the drugs affected the bladder compliance at any dose tested.

Effect of antimuscarinics in rats. Table 3 summarizes the effects of tolterodine and oxybutynin on urodynamic parameters. In contrast to the effect in rhesus monkeys, tolterodine had no effect on the bladder capacity of rats, while micturition pressure was significantly decreased at all doses tested. Tolterodine did not affect the threshold pressure. Oxybutynin increased bladder capacity and threshold pressure at only the highest dose (10 mg/kg). At this dose, only a small amount of urine was expelled, indicating that urinary retention was developed due to high doses of oxybutynin. Micturition pressure was decreased by oxybutynin.

Pharmacokinetic/Pharmacodynamic analysis. Results from the pharmacokinetic analysis are summarized in Table 5. Plasma levels of tolterodine and oxybutynin at tested doses overlapped in the two species. Plasma levels of the 5-hydroxymethyl metabolite of tolterodine were very low in rhesus monkeys, which may be due to the acute intravenous dosing paradigm. To compare the potencies of three antimuscarinics in increasing bladder capacity and decreasing micturition pressure, the average plasma levels at minimum effective doses for both parameters were
calculated as follows: (bladder capacity increase : micturition pressure decrease) = (0.111 ± 0.030 μmol/L : 0.346 ± 0.065 μmol/L) for tolterodine; (0.025 ± 0.001 μmol/L : 0.008 ± 0.001 μmol/L) for darifenacin; (0.065 ± 0.006 μmol/L : 0.224 ± 0.015 μmol/L) for oxybutynin. We then calculated the potency ratio of a bladder capacity increase to a micturition pressure decrease (average minimum plasma level to increase bladder capacity / average minimum plasma level to decrease micturition pressure) for each drug: 0.32 (tolterodine), 3.1 (darifenacin), 0.29 (oxybutynin). These ratios suggest that both tolterodine and darifenacin were ~3-fold more potent in increasing bladder capacity than in decreasing micturition pressure, when compared to darifenacin (~3-fold less potent in bladder capacity than micturition pressure).

For tolterodine, the plasma levels that increased bladder capacity in monkeys (~0.1 μM), produced no effect on bladder capacity in rats (Fig. 3A, C), although micturition pressure was decreased at similar range of plasma levels (0.1-0.3 μM) in both species (Fig. 3B, D). Similarly, the plasma levels of oxybutynin that decreased the micturition pressure in monkeys and rats are similar. Although oxybutynin increased the bladder capacity only at very high plasma levels (4 μM) in rats, bladder capacity in monkeys was increased from lower plasma levels of oxybutynin (0.2 μM).
Discussion

There were two key observations in this study: the differential effects of antimuscarinics with various subtype selectivity on urodynamic parameters in non-human primates and the differential effects of antimuscarinics on the bladder capacity between monkeys and rats. Acute intravenous administration of tolterodine (non-selective), darifenacin (M3 selective) or oxybutynin (moderately M3 selective) significantly increased bladder capacity and decreased micturition pressure in rhesus monkeys. However, tolterodine and oxybutynin were more potent in increasing bladder capacity than in decreasing micturition pressure, when compared to darifenacin. In contrast to rhesus monkeys, but as previously reported (Angelico et al., 2005; Ohtake et al., 2007), tolterodine did not increase bladder capacity in rats and oxybutynin increased bladder capacity only at the highest dose. From the comparison of the plasma levels, there was dissociation in the effect of antimuscarinics on the bladder capacity between the two species, while these drugs significantly decreased micturition pressure at a similar range of plasma levels. Since darifenacin is the most M3 selective antagonist in the three antimuscarinics evaluated, the results suggest that other muscarinic receptor subtypes, in addition to the M3 receptor, contribute to regulate bladder storage function in non-human primates. The results also suggest a species difference in the role of muscarinic receptors in bladder storage function between primates and rodents.

While muscarinic M3 receptors primarily mediate acetylcholine-induced contractile response of the detrusor muscle (Abrams et al., 2006; Hegde, 2006) and are also suggested to play a role in bladder sensory transmission (Andersson and Yoshida, 2003; De Laet et al., 2006; Yoshimura, 2007; Matsumoto et al., 2010), the involvement of M2 receptors in bladder physiology has been proposed. In the detrusor muscle, M2 receptors indirectly mediate smooth muscle tone by inhibiting β-adrenoceptor-mediated relaxation through cAMP signaling (Hegde et al., 1997). Both M2 and M3 receptors are expressed in the urothelial and suburothelial layers (Mukerji et al., 2006), which are a putative site of action for antimuscarinics based on a receptor binding study using human bladder mucosa (Mansfield et al., 2009). In functional studies, a selective blockade of M2 receptors inhibited the release of non-neuronal ATP from the urothelium (Yoshida et al., 2010), and also inhibited bladder overactivity-induced by intravesical administration of a pan-
muscarinic agonist (Matsumoto et al., 2010). In this study, darifenacin, the most M₃ selective antagonist, showed less specificity to the increase in bladder capacity relative to its effect on micturition pressure (decrease in detrusor contraction) than the other two antagonists tolterodine and oxybutynin in monkeys. Since the later two antagonists possess more potent M₂ antagonism than darifenacin (Abrams et al., 2006; Hegde, 2006), the differential effects of the three antimuscarinics on bladder capacity and micturition pressure may be due to the subtype selectivity of these drugs. Although it was reported that selective M₂ antagonists did not increase bladder capacity in normal healthy rats (Kim et al., 2005), this might be due to the species differences in the roles of muscarinic receptors in the bladder, as addressed later.

In addition to M₂ and M₃ receptors, other muscarinic receptor subtypes, such as M₁ and M₄, are suggested to play roles in regulating bladder functions. For example, prejunctional M₁ receptors facilitate and M₄ receptors inhibit acetylcholine release from postganglionic nerve endings (Chapple et al., 2002). Also, central M₁ and M₄ receptors are implicated to modify micturition reflex in rats (Kono et al., 2006). Because both tolterodine and oxybutynin possess substantial activities at these subtypes (Abrams et al., 2006; Hegde, 2006; Ohtake et al., 2007), and also because this study did not examine purely M₂ and M₃ selective antimuscarinics, it is possible that the activities of tolterodine and oxybutynin at multiple muscarinic receptor subtypes were what caused the differential effect on bladder capacity relative to darifenacin in monkeys.

Clinical studies have demonstrated that antimuscarinics increased bladder capacity in patients with detrusor overactivity (Stohrer et al., 1991; Jonas et al., 1997), neurogenic bladder dysfunction (Amend et al., 2008) and also in healthy subjects (Stahl et al., 1995). In non-human primates, only a few studies have evaluated antimuscarinics in the urodynamic platform, but Kimura et al. (1997) clearly showed a dose-dependent increase in the bladder capacity after intravenous administration of oxybutynin and atropine in anesthetized rhesus monkeys. Two other studies also showed a bladder capacity increase with atropine in either conscious or anesthetized rhesus monkeys (Craggs and Stephenson, 1985; Shoukry and Ghoniem, 1992). In contrast, many studies using either normal healthy or diseased rodent models did not show any bladder capacity increase with antimuscarinics, or showed an increase only at the high doses (Sasaki et al., 1997;
Angelico et al., 2005; Ohtake et al., 2007; Hegde et al., 2009; Nagabukuro et al., 2010), which were much higher than therapeutic doses. Only in certain disease models, such as cerebral infarction, did antimuscarinics, including tolterodine and solifenacin, increase bladder capacity at reasonable doses from clinical use of these drugs (Suzuki et al., 2005). Our results are consistent with previous observations, but we have tested multiple antimuscarinics with different subtype selectivity and evaluated their pharmacokinetics. These additions further clarify the species differences in the effect of antimuscarinics on bladder capacity.

Pharmacokinetic analysis in this study revealed that the plasma levels of tolterodine and oxybutynin overlapped in the two species, while the effects of these drugs on urodynamic parameters differed. Tolterodine obviously increased bladder capacity in monkeys, but not in rats, whereas oxybutynin increased bladder capacity in both rhesus and rats, but only at the highest doses in rats. Interestingly, the plasma levels of tolterodine and oxybutynin, which decreased the micturition pressure, were similar in two species (0.1-0.3 μM for tolterodine; ~0.2 μM for oxybutynin). However, there are certain caveats when directly comparing plasma drug levels because of the following reasons. Although all three antimuscarinics are known to highly bind to plasma proteins (96-99%) in humans (Abrams et al., 2006; Hegde, 2006), and circulating free drug levels are generally used to interpret target receptor occupancy, the plasma protein binding of each compound may vary between species. Additionally, there may be species differences in the muscarinic receptor antagonistic activity of the drugs, although these differences are expected to be marginal based on previous reports (Sinha et al., 2010; Ohtake et al., 2007). Despite these caveats, as well as existence of active metabolites for tolterodine and oxybutynin in clinical use, if the plasma levels of antimuscarinics in rhesus monkeys are compared to those clinically used to treat OAB, the therapeutic plasma levels of tolterodine (~10 nmol/L), darifenacin (9 nmol/L) and oxybutynin (2 nmol/L) are overall lower than those needed to significantly increase bladder capacity in rhesus monkeys (Abrams et al., 2006; Hegde, 2006). This may suggest a higher sensitivity to muscarinic antagonism in modifying bladder storage function in the OAB diseased state compared to the normal healthy state. In fact, an increase in suburothelial M2 and M3 receptor immunoreactivity has been found in patients with detrusor overactivity, and this increase correlated with urgency scores (Mukerji et al., 2006).
Oxybutynin increased bladder capacity to a greater extent (71% from baseline) at the highest dose (1 mg/kg) compared to tolterodine (40%) and darifenacin (29%) in monkeys, and only oxybutynin increased the threshold pressure at this dose. Also, in rats, oxybutynin significantly increased bladder capacity at the highest dose (10 mg/kg). The results suggest that oxybutynin affected the bladder capacity via other effects than simply through its antimuscarinic activity, which further modify bladder sensory transmission. In fact, it is well documented that oxybutynin acts as a local anesthetic at high concentrations (De Wachter and Wyndaele, 2003).

It is possible that the species differences we observed in this study were due to the technical differences in the two cystometry procedures. Specifically, the definition of micturition pressure was slightly different in the two species due to the distinct intravesical catheterization procedures (transurethral in monkeys and transvesical in rats). This may have resulted in a variable sensitivity to detect a decrease in detrusor contractility with antimuscarinics. In addition, while transurethral catheterization in monkeys does not cause bladder damage, transvesical catheter implantation in rats can cause acute local inflammation. Antimuscarinics are known to reduce detrusor overactivity in a pathogenic model of topical and acute application of proinflammatory prostaglandin E2 to the urethra (Yokoyama et al., 2007), therefore the minor inflammation that may have been induced in rats could have altered the effects of antimuscarinics when compared to the study in monkeys. Additionally, the anesthesia used was different between monkeys (ketamine) and rats (urethane). Since muscarinic receptors in the central nervous system are implicated to modify micturition reflex (Kono et al., 2006), different anesthetics may results in different central effects of antimuscarinics.

In conclusion, in addition to M3, other muscarinic receptor subtypes may contribute to regulate bladder storage functions in non-human primates. Additionally, the role of muscarinic receptors in bladder storage function varies between primates and rodents. In primates, muscarinic receptors may play a more active role to regulate to the functional bladder capacity during the storage phase than in rodents.
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Authorship contribution

*Participated in research design:* Nagabukuro

*Conducted experiments:* Nagabukuro, Villa, Wickham, Kulick, Gichuru, Donnelly, Voronin, Pereira

*Contributed new reagents or analytic tools:* Villa, Wickham, Kulick, Pereira

*Performed data analysis:* Nagabukuro, Villa, Pereira, Tong

*Wrote or contributed to the writing of the manuscript:* Nagabukuro, Villa, Wickham, Kulick, Gichuru, Pereira, Nichols, Alves, O'Neill, Johnson, Hickey
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Comparison of receptor binding characteristics of commonly used muscarinic antagonists in

Differential roles of M2 and M3 muscarinic receptor subtypes in modulation of bladder afferent


Localization of M2 and M3 muscarinic receptors in human bladder disorders and their clinical

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Footnotes

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Legends for Figures

Fig. 1. Representative cystometrograms in a rhesus monkey treated with vehicle (upper) or tolterodine (lower). Each horizontal and vertical bar represents 2 min and 10 cmH₂O, respectively.

Fig. 2. Effect of tolterodine, darifenacin and oxybutynin on the bladder capacity (A, C) and micturition pressure (B, D) in rhesus monkeys. *P<0.05, **P<0.01, vs. vehicle group, two-way ANOVA with Bonferroni post-hoc test. Each bar represents mean ± S.E.M.

Fig. 3. Pharmacokinetics/pharmacodynamics relationship of antimuscarinics for the effects on bladder capacity (A, C) and micturition pressure (B, D) in rhesus monkeys (A, B) and rats (C, D). *P<0.05, **P<0.01 for urodynamic parameters. Each plot represents mean ± S.E.M.
Table 1.
Baseline urodynamic parameters in treatment groups in rhesus monkeys

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>Bladder capacity (mL)</th>
<th>Maximum micturition pressure (cmH₂O)</th>
<th>Threshold pressure (cmH₂O)</th>
<th>Bladder compliance (mL/cmH₂O)</th>
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</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>136.0 ± 21.8</td>
<td>36.7 ± 1.8</td>
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</tr>
<tr>
<td>Oxybutynin</td>
<td>4</td>
<td>140.4 ± 15.2</td>
<td>35.7 ± 3.4</td>
<td>13.6 ± 1.7</td>
<td>22.1 ± 8.7</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M.
Table 2.

Effect of tolterodine, darifenacin and oxybutynin on threshold pressure and bladder compliance in rhesus monkeys

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Threshold pressure (changes from baseline, cmH₂O)</th>
<th>Bladder compliance (% of baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine</td>
<td>0.01</td>
<td>-0.2 ± 1.2</td>
<td>103 ± 8</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.5 ± 1.5</td>
<td>107 ± 7</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.9 ± 0.8</td>
<td>116 ± 5</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>-0.6 ± 0.9</td>
<td>127 ± 6</td>
</tr>
<tr>
<td>Darifenacin</td>
<td>0.003</td>
<td>-0.4 ± 0.9</td>
<td>114 ± 12</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.3 ± 1.2</td>
<td>105 ± 15</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>-2.3 ± 2.1</td>
<td>101 ± 8</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>-1.8 ± 2.5</td>
<td>100 ± 7</td>
</tr>
<tr>
<td>Oxybutynin</td>
<td>0.1</td>
<td>1.4 ± 1.1</td>
<td>115 ± 7</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>2.4 ± 1.6</td>
<td>105 ± 13</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5.9 ± 2.1**</td>
<td>98 ± 6</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M.

**p<0.01 vs. vehicle, two-way ANOVA with Bonferroni post-hoc test.
Table 3.
Effect of tolterodine and oxybutynin on urodynamic parameters in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Bladder capacity</th>
<th>Maximum micturition pressure</th>
<th>Threshold pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% of baseline (baseline, mL)</td>
<td>changes from baseline, cmH\textsubscript{2}O (baseline)</td>
<td>changes from baseline, cmH\textsubscript{2}O (baseline)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>5</td>
<td>101 ± 10 (0.13 ± 0.03)</td>
<td>2.5 ± 2.2 (21.6 ± 4.2)</td>
<td>0.36 ± 0.39 (3.5 ± 0.5)</td>
</tr>
<tr>
<td>Tolterodine</td>
<td>0.1</td>
<td>6</td>
<td>99 ± 20 (0.16 ± 0.06)</td>
<td>-3.0 ± 0.9 (16.9 ± 2.7)</td>
<td>-0.59 ± 0.33 (2.6 ± 0.6)</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>7</td>
<td>90 ± 15 (0.17 ± 0.04)</td>
<td>-8.9 ± 1.3** (27.8 ± 2.4)</td>
<td>-0.73 ± 1.11 (5.1 ± 0.4)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>82 ± 16 (0.15 ± 0.04)</td>
<td>-8.1 ± 1.6** (22.1 ± 3.4)</td>
<td>0.35 ± 1.23 (4.1 ± 1.1)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>6</td>
<td>125 ± 20 (0.11 ± 0.03)</td>
<td>0.0 ± 1.3 (24.3 ± 2.3)</td>
<td>0.44 ± 0.58 (6.0 ± 0.7)</td>
</tr>
<tr>
<td>Oxybutynin</td>
<td>0.1</td>
<td>5</td>
<td>86 ± 12 (0.10 ± 0.01)</td>
<td>-3.2 ± 2.0 (21.3 ± 2.7)</td>
<td>-0.21 ± 0.96 (4.8 ± 1.1)</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>5</td>
<td>77 ± 14 (0.17 ± 0.02)</td>
<td>-7.9 ± 2.8** (24.6 ± 4.0)</td>
<td>-1.73 ± 2.41 (6.6 ± 1.7)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>107 ± 8 (0.18 ± 0.03)</td>
<td>-6.6 ± 1.1** (30.7 ± 2.0)</td>
<td>0.28 ± 1.05 (7.0 ± 1.1)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>230 ± 33** (0.15 ± 0.03)</td>
<td>-4.6 ± 0.7 (22.1 ± 3.5)</td>
<td>4.59 ± 0.24* (4.7 ± 1.0)</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M.

*p<0.05, **p<0.01 vs. vehicle, Dunnett’s multiple comparison test.
Table 4.

Plasma levels of tolterodine, darifenacin and oxybutynin in rhesus monkeys and rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Plasma concentration (nmol/L)</th>
<th>Rhesus monkey</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolterodine</td>
<td>0.01</td>
<td>5.5 ± 0.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>34.8 ± 7.8</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>111.0 ± 30.2</td>
<td>29.2 ± 4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 (5-hydromethyl metabolite: 7 ± 2)</td>
<td>346.0 ± 65.4</td>
<td>84.8 ± 10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>-</td>
<td>278.2 ± 30.9</td>
<td></td>
</tr>
<tr>
<td>Darifenacin</td>
<td>0.003</td>
<td>1 ± 0.1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>2.5 ± 0.2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>8.3 ± 0.8</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>25.1 ± 1.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin</td>
<td>0.1</td>
<td>64.7 ± 6.2</td>
<td>43.9 ± 4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>223.8 ± 15.4</td>
<td>188.3 ± 13.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>697.3 ± 64.1</td>
<td>702.6 ± 57.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-</td>
<td>3850.0 ± 205.9</td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M.
Fig. 1
Fig. 2

A

B

C

D

Changes from baseline (cmH2O)

Changes from baseline (cmH2O)

Tolterodine

Darifenacin

Oxybutynin

Tolterodine

Darifenacin

Oxybutynin

0

5

10

15

20

25

30

35

% of baseline

% of baseline

0

50

100

150

200

Veh

Veh

Veh

0.01

0.03

0.1

0.3

Veh

Veh

Veh

0.003

0.01

0.03

0.1

0.01

0.03

0.1

0.3

1

(mg/kg)

(mg/kg)

AB

CD

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Fig. 3

A

B

C

D

Figures A, B, C, and D show the relationship between plasma levels and % of baseline for different drugs. The graphs illustrate the effects of Tolterodine, Darifenacin, and Oxybutynin on plasma levels at various baseline conditions.