Effects of ritobegron (KUC-7483), a novel β_3 -adrenoceptor agonist, on both rat bladder function following partial bladder outlet obstruction and on rat salivary secretion: a comparison with the effects of tolterodine

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Received July 24, 2012; Accepted August 28, 2012

Abstract

The objective of this study was to investigate the effects of the β_3 -adrenoceptor (AR) agonist ritobegron on rat bladder function following partial bladder outlet obstruction and on rat salivary secretion. In addition, the effects of ritobegron were compared with those of the anti-muscarinic agent tolterodine. After a 6-week partial bladder outlet obstruction (BOO), drug effects on bladder functions were evaluated using cystometrography. Effects on carbachol (CCh)-induced salivary secretion were evaluated in urethane-anesthetized rats. Ritobegron significantly decreased the frequency of non-voiding contractions (NVC), while both ritobegron and tolterodine each significantly decreased the amplitude of NVC. Ritobegron had no effect on either the micturition pressure (MP) or the residual volume (RV). In contrast, tolterodine dose-dependently decreased MP and increased RV. Ritobegron had no effect on CCh-induced salivary secretion, whereas tolterodine dose-dependently decreased it. Ritobegron decreased both the frequency and amplitude of NVC, which is similar to its effect on the contractions associated with detrusor overactivity (DO) in patients with an overactive bladder (OAB), without affecting MP, RV, or CCh-induced salivary secretion. Although tolterodine reduced the amplitude of NVC, it also markedly increased RV and significantly inhibited CCh-induced salivary secretion. These results suggest that use of ritobegron, a β_3 -AR agonist, is unlikely to lead to the residual urine and dry mouth symptoms that are associated with anti-muscarinic drugs, and that ritobegron may hold promise as a safe and effective agent for OAB treatment.

Key words: β -adrenoceptor, β_3 -agonist, bladder outlet obstruction (BOO), overactive bladder (OAB), ritobegron

Introduction

Overactive bladder (OAB) is a complex of symptoms defined by urgency with or without urge together with urinary incontinence, usually with an associated increase in daytime frequency and nocturia (Abrams, 2003).

Urgency is regarded as a core symptom, and may correspond to the detrusor overactivity (DO) observed during the filling phase in human urodynamic studies (Abrams, 2003; Abrams *et al.*, 2002). It has been reported that DO is seen in half to two-thirds of OAB patients (Wyndaele *et al.*, 2004). A rat model for bladder outlet obstruction (BOO), which was created by partial urethral obstruction, also exhibits DO, as evidenced by non-voiding contractions (NVC) which were observed with cystometry during the filling phase. These NVC are similar to the contractions associated with DO in patients with OAB, and so this animal model is considered to be appropriate for the evaluation of the effect of drugs on DO (O'Connor *et al.*, 1997).

The urinary bladder is innervated by both sympathetic and parasympathetic nerves, activation of which mediate bladder relaxation and contraction, respectively (Andersson, 1999).

Anti-muscarinic drugs have been widely used for the treatment of OAB. However, they can have severe side effects (dry mouth, constipation, blurred vision), and they also have the potential to cause voiding difficulty in patients with a poorly contractile bladder. Thus, there is an urgent need for new therapeutic drugs with alternative mechanisms of action.

In humans, bladder relaxation is reportedly mediated via β_3 -adrenergic receptors (AR), with 97% of the total β -AR mRNA in humans being of the β_3 -AR subtype (Igawa *et al.*, 1998; Igawa *et al.*, 1999; Yamaguchi, 2002; Nomiya *et al.*, 2003), while in rats both β_2 - and β_3 -AR are involved (Yamazaki *et al.*, 1998). To date, the effects of β_3 -AR agonists on bladder function have mainly been studied in rats (Wood *et al.*, 2001; Takeda *et al.*, 2002; Kaidoh *et al.*, 2002). On the basis of such studies, it has been suggested that selective β_3 -AR agonists may be useful for the treatment of OAB (Yamaguchi *et al.*, 2007). Recently, ritobegron (KUC-7483) has been synthesized and developed by Kissei Pharmaceutical Co. Ltd. as a novel selective β_3 -AR agonist (Maruyama *et al.*, 2012a; Maruyama *et al.*, 2012b).

In the present study, we have (a) investigated the effects of ritobegron on bladder function in rats with partial bladder outlet obstruction and also on salivary secretion in rats, and (b) compared these effects with those of tolterodine (anti-muscarinic widely used for treating overactive bladder).

Methods

Animals

This study was conducted according to guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical Co. Ltd. Male Sprague-Dawley rats (SLC, Hamamatsu, Japan), weighing 220–270 g, and female Sprague-Dawley rats (SLC, Hamamatsu, Japan) weighing 180–230 g were used in this study. Rats were group-housed with 5 animals per cage at a stable temperature and humidity, with a 12 hr light-dark cycle. They were supplied with free access to water and standard laboratory food until the day of the experiment.

Preparation for partial obstruction of the lower urinary tract

Rats (8 weeks old) were anesthetized with pentobarbital sodium (40 mg/kg, *i.p.*) and the bladder base and proximal urethra exposed through a lower-midline incision. A 4-0 nylon thread (Keisei, Tokyo, Japan) was placed loosely around the proximal urethra. This was then tightened around the urethra and a 1-mm diameter polyethylene tube (No.3; Hibiki, Tokyo, Japan) that had been laid lengthwise along the urethra. After this, the tube was removed, leaving the urethra partially obstructed, and the abdomen wall was closed. A total of 280 rats were operated on for partial obstruction. Sham animals underwent the same preparation, except that the thread placed around the proximal urethra and polyethylene tube was not tightened (*i.e.*, there was no occlusion).

Preparation for catheter implantation and removal of the thread around the lower urinary tract

Five weeks after the above partial obstruction, rats (14 weeks old by then) were anesthetized with pentobarbital sodium (40 mg/kg, *i.p.*) and the bladder exposed through an abdominal midline incision. A polyethylene catheter (PE-50; Nihon Becton Dickinson, Tokyo, Japan) filled with saline was inserted into the urinary bladder via an incision made in the top of the bladder dome. The catheter was tunneled subcutaneously and secured at the back of the animal's neck, then capped off (bladder catheter). Next, through an abdominal midline incision the stomach was exposed and a polyethylene catheter (PE-50) was inserted into it. This catheter, too, was tunneled subcutaneously, secured at the back of the animal's neck, and capped off (gastric catheter). The abdomen-wall incisions were then closed.

Six days later (one day before CMG; see below), the rats were anesthetized with pentobarbital sodium (40 mg/kg, *i.p.*). The bladder was exposed through an abdominal lower-midline incision, the thread was removed from around the lower urinary tract, and the abdominal wall closed. Sham animals underwent the same preparation, but had no occlusion to be reversed (see previous section).

Cystometrogram (CMG)

On the day following the removal of the partial obstruction, conscious, unrestrained rats (14 weeks old by then) were placed in individual plastic metabolic cages (Nalge Nunc International, New York, USA). After its stop-cap had been cut off, the bladder catheter was connected through a three-way connector to a syringe infusion pump (KDS-100; Muromachi Kikai, Tokyo, Japan) for infusion of saline and to a pressure transducer (DT-XX; Nihon Becton Dickinson, Tokyo, Japan) for measurement of bladder pressure. Since variability in bladder capacity among animals is typical of this model, saline was initially infused at 12 ml/hr, then adjusted to 6–18 ml/hr to obtain micturition intervals of around 20 min. Intravesical pressure and micturition volume (MV) were recorded on a rectigraph (Recti-Horiz-8K; NEC San-ei, Tokyo, Japan). Micturition pressure (MP) was determined from the CMG. MV was determined by collecting the voided saline. Immediately after a micturition had ceased, the infusion was stopped and residual volume (RV) measured by allowing the remaining intravesical fluid to flow out through the catheter under gravity. Bladder capacity (BC) was calculated as $MV + RV$. The frequency and amplitude of non-voiding contractions (NVC) were measured during the period 3.5 min–0.5 min before each micturition reflex. NVC were defined as bladder contractions of >2 cm H₂O from baseline pressure.

After the micturition cycle had stabilized, test drugs or vehicle were administered intra-gastrically and their effects observed for about 1 hr after administration. In sham animals, the test drugs were not administered, but the same parameters were assessed during 2 consecutive micturition cycles. The

means of the values obtained for micturition parameters (NVC frequency, NVC amplitude, MV, MP, and BC) during 2 cycles before drug administration were each taken as 100%. Then, their values during 1 cycle close to 1 hr after drug administration were expressed as a percentage of those “pre” values. RV was expressed as the difference between before and after drug administration. For sham animals, the data reported are the means obtained during of 2 micturition cycles.

CCh-induced salivation secretion

Male rats (8 weeks old) were anesthetized with 25% urethane (1.25 g/kg, *s.c.*). A cannula (No.8; Hibiki, Tokyo, Japan) was inserted into the trachea to support respiration. Through a midline abdominal incision, one cannula (PE-50) was inserted into the duodenum, and another (PE-50) into the femoral vein for intragastric and intravenous administration, respectively, of carbachol (CCh) for salivary stimulation. Test drugs or vehicle were administered intraduodenally 30 min before such CCh administration. At 1 min before CCh administration, five pre-weighed cotton balls were inserted into the oral cavity of each animal, one underneath the tongue and four bilaterally medial to the teeth and oral mucosa. The cotton balls were removed at 5 min after CCh administration and re-weighed. The weight of saliva secreted was calculated from these two weights of the five cotton balls.

Statistical analysis of data

All results are expressed as the mean $\pm$ standard error (S.E.). Body weight, bladder weight, and micturition parameters were compared between the sham and BOO groups using an F-test followed by the Student's *t*-test. Effects of ritobegron and tolterodine on micturition parameters and salivary secretion were evaluated by comparisons with vehicle-treated groups and statistical analysis was performed using a one-way analysis of variance followed by Dunnett's multiple-comparison test. A probability of less than 0.05 ($P < 0.05$) was considered to be significant. The SAS system (version 8.2; SAS Institute Inc., Cary, NC, USA) was used as the resource text for the statistical analysis.

Drugs

Ritobegron ((-)-ethyl 2-[4-(2- {[[(1S,2R)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino} ethyl)-2,5-dimethylphenoxy]acetate monohydrochloride) and tolterodine were synthesized in our laboratory (Kissei Pharmaceutical Co. Ltd., Nagano, Japan). Carbamoylcholine chloride (CCh) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ritobegron and tolterodine were suspended in 0.5% arabic gum in distilled water, and CCh was dissolved in saline.

Results

Effect of BOO on micturition parameters and bladder weight in rats

After the 6-week period of BOO, we evaluated NVC during the filling phase using cystometry (Fig. 1). Both the bladder weight and the frequency of NVC were significantly increased in BOO rats (Table 1), and BC and MV were also significantly increased in BOO rats (Table 1). Although there were no significant differences, both NVC amplitude and RV showed a tendency to be greater in BOO than in sham rats (Table 1).

Fig. 1. Typical cystometrogram tracings. Saline was infused into the bladder at a rate of 6 or 18 ml/hr in a sham rat (A) or BOO rat (B), respectively. NVC were defined as bladder contractions of >2 cmH₂O from baseline pressure. Arrows indicate non-voiding contractions (NVC) during the period 3.5 min - 0.5 min before micturition reflex.

Table 1. Comparison of body weight, bladder weight, and micturition parameters between sham and BOO rats

	sham	BOO
Body weight (g)	207 $\pm$ 6.6	218 $\pm$ 2.5
Bladder weight (g)	0.25 $\pm$ 0.01	0.85 $\pm$ 0.10*
NVC frequency (times/min)	0.2 $\pm$ 0.1	1.7 $\pm$ 0.1*
NVC amplitude (mmHg)	2.4 $\pm$ 1.0	4.5 $\pm$ 0.2
MP (mmHg)	45.2 $\pm$ 5.6	49.1 $\pm$ 4.4
BC (ml)	2.1 $\pm$ 0.2	8.9 $\pm$ 1.4*
MV (ml)	2.0 $\pm$ 0.2	8.4 $\pm$ 1.3*
RV (ml)	0.08 $\pm$ 0.03	0.49 $\pm$ 0.23

MP, micturition pressure. BC, bladder capacity. MV, micturition volume. RV, residual volume. Data represent means $\pm$ S.E. for 6 animals. *, $P < 0.05$ vs. sham (Student's *t*-test).

Effects of ritobegron and tolterodine on micturition parameters in BOO rats

Ritobegron significantly decreased (vs. vehicle) the frequency of NVC at doses of 1, 3, and 10 mg/kg (Figs. 2 and 3A). Tolterodine only tended (non-significantly) to decrease NVC frequency (Fig. 3A). Ritobegron and tolterodine significantly decreased NVC amplitude at doses of 10 and 3 mg/kg, respectively (Fig. 3B). Ritobegron had no effect on MP or BC (Fig. 3C and 3D), whereas tolterodine displayed a (non-significant) tendency to decrease MP and at a dose of 10 mg/kg significantly decreased BC (Fig. 3, C and D). At a dose of 10 mg/kg, ritobegron significantly increased MV (Fig. 3E), whereas at that dose tolterodine significantly decreased it (Fig. 3E). Ritobegron had no effect on RV (Fig. 3F), and tolterodine tended (non-significantly) to increase it only at a dose of 10 mg/kg (Fig. 3F).

Fig. 2. Typical cystometrogram tracings. Saline was infused into the bladder at a rate of 18 ml/hr. Ritobegron 10 mg/kg was administered intra-gastrically.

Effects of ritobegron and tolterodine on CCh-induced salivary secretion

Ritobegron did not alter carbamoylcholine chloride (CCh)-induced salivary secretion (Fig. 4), whereas tolterodine dose-dependently decreased it (Fig. 4).

Discussion

In the present study, on rats, we investigated the effects of ritobegron on bladder function in animals with partial BOO and on salivary secretion, and compared them with those of tolterodine.

We previously reported that ritobegron is a selective β_3 -AR agonist that displays selective agonistic activity toward the human β_3 -AR and a potent relaxing effect on both the rat isolated bladder and cynomolgus monkey isolated bladder via stimulation of β_3 -AR (Maruyama *et al.*, 2012a; Maruyama *et al.*, 2012b). Furthermore, in our parallel in vivo studies ritobegron decreased intravesical pressure in both rats and cynomolgus monkeys with minimal effects on the cardiovascular system (Maruyama *et al.*, 2012a; Maruyama *et al.*, 2012b).

In the present study of a rat partial BOO model, ritobegron significantly decreased both the frequency and amplitude of NVC, and increased MV, without affecting MP or RV.

CL316,243, another selective β_3 -AR agonist, dose-dependently decreased the frequency of NVC in BOO rats in a previous study (Woods *et al.*, 2001). Furthermore, in bladder strips isolated from BOO rats, the relaxation responses to β -AR agonists were not altered to any major extent (*vs.* non-BOO rats)

Fig. 3. Effects of ritobegron and tolterodine (each at 1, 3, and 10 mg/kg, *i.g.*) on NVC frequency (A), NVC amplitude (B), MP (C), BC (D), MV (E), and RV (F) in rats. Data are expressed as a percentage of the pre-value, except RV. Data represent means $\pm$ S.E. for 6-7 animals. *, $P < 0.05$ vs. vehicle (Dunnett's multiple-comparison test).

(Barendrecht *et al.*, 2009), and the mRNA expressions for the β_2 - and β_3 -AR subtypes were not significantly altered by BOO (Park *et al.*, 2010).

When taken together, the above evidence suggests that the observed effects of ritobegron on NVC frequency and amplitude and on MV in the present BOO model were mediated by direct relaxation of

Fig. 4. Effects of ritobegron and tolterodine (each at 1, 3, and 10 mg/kg, *i.d.*) on CCh-induced salivary secretion in rats. Data represent means $\pm$ S.E. for 6 animals. *, $P < 0.05$ vs. vehicle (Dunnett's multiple-comparison test).

bladder smooth muscle via the β_3 -AR present in that muscle.

Recently, it was reported that β_1 -, β_2 -, and β_3 -AR are present in the urothelium of the human bladder (Otsuka *et al.*, 2008; Tyagi *et al.*, 2009), and that in rat isolated urothelium cells, β -AR stimulation induces NO release (Birder *et al.*, 2002). Furthermore, intravenous administration of β_3 -AR agonists, as well as that of an NO donor, has been found to suppress bladder afferent nerve activity (Aizawa *et al.*, 2011; Aizawa *et al.*, 2010). Those results are consistent with β_3 -AR agonists having inhibitory effects on afferent nerve activities in the bladder urothelium, in which β_3 -AR agonists may induce NO release (Yamaguchi *et al.*, 2007). Collectively, the above evidence suggests that not only bladder smooth muscle relaxation, but also suppression of bladder afferent nerve activity might be involved in the effects of ritobegron observed in the present rat BOO model.

Compared to ritobegron, tolterodine had weak effects in the present rat BOO model. Indeed, only at a dose of 3 mg/kg did it decrease the amplitude of NVC. In previous studies, tolterodine significantly decreased both the frequency and amplitude of NVC (Jin *et al.*, 2011). The explanation for this discrepancy is unclear, but it may be related to the experimental conditions (*i.e.*, drug administration route and/or duration of partial BOO). Actually, the bladder weight in our study was about 3 times that in the previous study (Jin *et al.*, 2011), and the muscarinic receptor-subtype mediating bladder contraction reportedly changes with bladder hypertrophy in a rat BOO model (Braverman *et al.*, 2003). So, the effects induced by tolterodine may depend on the severity of the BOO model.

Clinically, anti-muscarinic drugs have the potential to cause voiding difficulty in a patient with BOO accompanied by a poorly contractile bladder. Ouslander (2004) proposed that when anti-muscarinic drugs are used for the treatment of OAB with BOO, physicians should carefully monitor the development of urinary retention. In the present study, ritobegron did not induce a significant change in MP or in RV. In contrast, tolterodine displayed a tendency to decrease MP and markedly increases RV at a dose of 10 mg/kg. These results are consistent with the clinical concern mentioned above, and hence we consider the effect of tolterodine on RV to be attributable to an inhibition of bladder contractility. If it

also applies in humans, the above evidence suggests that ritobegron may be the safer drug since there appears to be little or no danger of it inducing urinary retention.

Finally, dry mouth is one of the commonest side effects of anti-muscarinic drugs, and is major cause of their withdrawal (Abrams *et al.*, 2007). We therefore compared effects on salivary secretion between ritobegron and tolterodine. Ritobegron had no effect on salivary secretion, whereas tolterodine significantly decreased it, at the same dose as that at which it increased RV. These results suggest that a β_3 -AR agonist such as ritobegron may prove to be clinically useful, if it does not induce dry mouth in humans.

In conclusion, in a rat partial BOO model ritobegron decreased both the frequency and amplitude of NVC (which are similar to the contractions associated with DO in patients with OAB), without affecting MP or RV. Moreover, ritobegron did not alter salivary secretion. In contrast, tolterodine markedly increased RV and significantly inhibited salivary secretion. If these results are transferable to humans, ritobegron (β_3 -AR agonist), unlike anti-muscarinic drugs, holds promise to be a safe and effective agent for the treatment of OAB that may not increase the residual urine or cause dry mouth.

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