Effects of Ritobegron (KUC-7483), a Novel Selective $\beta_3$-Adrenoceptor Agonist, on Bladder Function in Cynomolgus Monkey

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ABSTRACT
We evaluated the pharmacological profile of ritobegron [KUC-7483: (−)-ethyl 2-[4-[2-{{[1S,2R]-2-hydroxy-2-(4-hydroxyphenyl)-1-methyl[4H]-1-imidazol-2-yl}(amino)ethyl]-2,5-dimethylphenoxyl]acetonitrile monohydrochloride] and its effects on the bladder in cynomolgus monkeys by in vitro and in vivo experiments. In vitro, ritobegron decreased the resting tension of the isolated bladder in a concentration-dependent manner ($EC_{50}$ 8.2 ± 2.3 × 10$^{-7}$ M; maximal relaxation 88.7 ± 3.7%). The $\beta_3$-adrenoceptor (AR) antagonist 3-[2-allylphenoxy]-1-[3-(15S)-1,2,3,4-tetrahydronephth-1-ylamino]-2-(5S)-2-propanol hydrochloride (SR58894A) produced a rightward shift of this concentration-response curve without altering the maximal response ($pA_{2}$ value 6.56 ± 0.35). In isolated atria, ritobegron increased the atrial rate only at high concentrations ($EC_{50}$ 6.5 ± 1.2 × 10$^{-8}$ M). Ritobegron had no effect on tracheal contraction at concentrations from 10$^{-9}$ to 10$^{-4}$ M, and even at the highest concentration tested, 10$^{-3}$ M, the maximal relaxation it induced was only 26.7 ± 8.1%. Tests of the selectivity of ritobegron for the bladder gave values of 79.3- and 1200-fold higher versus atria and trachea, respectively. In the in vivo study ritobegron significantly decreased intravesical pressure ($ED_{50}$ 1.44 mg/kg) without affecting either mean blood pressure or heart rate. In conclusion, ritobegron displayed potent and selective $\beta_3$-AR agonistic activity and relaxed the monkey isolated bladder, and in vivo it decreased intravesical pressure without affecting cardiovascular parameters. These results suggest that ritobegron may be a promising potential agent for the treatment of overactive bladder.

Introduction
Overactive bladder (OAB) is a common condition that is characterized by urgency, often accompanied by increased daytime frequency, nocturia, and sometimes urge incontinence (Abrams et al., 2002). Antimuscarinic drugs have been widely used for the treatment of OAB. However, these drugs can have severe side effects (dry mouth, constipation, and blurred vision) and have the potential to cause voiding difficulty in a patient with a poorly contractile bladder. Thus, there is an urgent need for new therapeutic drugs with alternative mechanisms of action.

The urinary bladder is innervated by both the sympathetic and parasympathetic nervous systems, and sympathetic activation contributes to urine storage by relaxing the bladder smooth muscle via the activation of $\beta$-adrenoceptors (ARs) (Andersson, 1999). $\beta$-ARs are currently classified into $\beta_1$-, $\beta_2$-, and $\beta_3$-AR subtypes (Bylund et al., 1994), and there are species differences in the subtypes involved in the relaxation of the mammalian bladder. For example, in cats (Nergardh et al., 1977) and guinea pigs (Li et al., 1992) relaxation of the bladder is mediated mainly via the $\beta_2$-AR; in rats (Anderson and Marks, 1984; Levin et al., 1988; Yamazaki et al., 1998) relaxation of the bladder is mediated mainly via the $\beta_3$-AR; and in ferrets (Takeda et al., 2002) relaxation of the bladder is mediated mainly via both the $\beta_2$-AR and $\beta_3$-AR; and in ferrets (Takeda et al.,...
2000a) and dogs (Yamazaki et al., 1998) relaxation of the bladder is mediated mainly via the β3-AR. Furthermore, in primates such as cynomolgus monkeys (Takeda et al., 2002a) and humans (Igawa et al., 1998, 1999; Yamazaki et al., 1998; Takeda et al., 1999) bladder relaxation is reportedly mediated via the β3-AR, with 97% of the total β-AR mRNA in humans being of the β3-AR subtype (Yamaguchi, 2002; Nomiyama and Yamaguchi, 2003). This evidence indicates that the cynomolgus monkey is an appropriate species for the evaluation of the effects of selective β3-AR agonists on bladder function.

Ritobegron [KUC-7483; (−)-ethyl 2-[4-2-([1S,2R)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl][amino][ethyl]-2,5-dimethylphenoxyl]acetate monohydrochloride] is a phenoxy-acetic acid derivative (Tanaka et al., 2001) that was synthesized and developed by Kissei Pharmaceutical Co. Ltd. as a novel selective β3-AR agonist. In this study, we evaluated its pharmacological profile and its effects on the bladder in the cynomolgus monkey by means of in vitro and in vivo experiments.

**Materials and Methods**

**Animals.** This study was conducted according to guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical Co. Ltd. Male and female cynomolgus monkeys (2–5 kg; Toyota Tsusho Corporation, Tokyo, Japan) were used in this study. All monkeys were housed individually at a stable temperature and humidity under a 12-h light/dark cycle, and they were maintained with free access to water and standard laboratory food until the day of the experiment.

**In Vitro Experiments.** Monkeys were anesthetized with ketamine (10 mg/kg i.m.) and sacrificed by rapid exsanguination. The heart, trachea, and urinary bladder were then isolated. After removal of the fat and mucosa, the ventricles were removed from the heart, and the atria were prepared for experimentation. The atria were cut into approximately 10 rings, each 2 mm in length, and they were appropriately prepared. The urinary bladder was opened longitudinally. After removal of the mucosa, a bladder preparation approximately 10 × 3 mm was prepared. Atria were suspended in a 20-ml organ bath, and other preparations were suspended in a 10-ml organ bath, each time containing Krebs’ solution. This bath solution was maintained at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. Each preparation was connected to a force-displacement transducer (SB-1T; Nihon-Kohden, Tokyo, Japan) for continuous recording on a recordograph (Recti-Horiz-8K; NEC Co. Ltd.) for blood pressure and heart rate measurements. Through a midline abdominal incision, the ureter on each side and the proximal urethra were ligated, and a polyethylene catheter (PE-50; Nihon Becton Dickinson) was inserted into the urinary bladder via the top of the bladder dome, then connected through a three-way connector to a pressure transducer and a syringe filled with saline. The initial bladder pressure was adjusted to approximately 5 cmH2O by instillation of warmed saline (37°C) in 5-ml increments. Blood pressure, heart rate, and intravesical pressure were recorded continuously on a rectigraph. At the end of each experiment, isoproterenol (0.1 mg/kg i.v.) was administered to obtain the maximal intravesical pressure response.

**Analysis of Data.** In the in vitro experiments, drug effects on the isolated bladder and trachea were expressed as a percentage of the maximal relaxation response to 10−5 M forskolin, whereas drug effects on atria were expressed as the differences before and after drug treatment. The EC50 value was calculated for each agonist from its concentration-response curve. In the in vivo experiments, drug effects on intravesical pressure were expressed relative to the maximal response to isoproterenol (0.1 mg/kg i.v.) (see legend to Fig. 6). Drug effects on blood pressure and heart rate were assessed as the difference before and after drug administration. All results are expressed as mean ± S.E. Statistical analysis was performed by using one-way analysis of variance followed by Dunnett’s comparison test. A probability less than 0.05 was accepted as significant. The SAS system (version 4.1; SAS Institute, Cary, NC) was used as the resource text for the statistical analysis.

**Drugs.** Ritobegron (KUC-7483) is a prodrug, so in the in vitro studies we used the active form [KUC-7322; (−)-ethyl 2-[4-2-([1S,2R)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl][amino][ethyl]-2,5-dimethylphenoxyl]acetate monohydrochloride] but in the in vivo study we used KUC-7483 itself. Ritobegron, its active form (KUC-7322), (R,R)-5-[2-([3-chlorophenyl]-2-hydroxyethyl)-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL316,243), and SR58894A were synthesized in our laboratory. The following drugs were obtained from commercial sources: (−)-isoprenaline (+)-bitartrate, phenolamine hydrochloride (isoproteanol), CCh, hydrcortisone 21-hemisuccinate, and desipramine hydrochloride (Sigma-Aldrich, St. Louis, MO); dimethyl sulfoxide (DMSO). 1 N NaOH, and arabic gum (Nacalai Tesque, Kyoto, Japan); sodium heparin (Aventis Pharma Japan, Tokyo, Japan); forskolin (Wako Pure Chemicals, Osaka, Japan); and CGP-20712A and CGI-118551 (Funakoshi, Tokyo, Japan). The Krebs’ solution was the following composition: 118.1 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 25.0 mM NaHCO3, 1.2 mM KH2PO4, and 11.1 mM glucose, pH 7.4. For the in vitro study, the active form (KUC-7322) was dissolved in distilled water containing two equivalents of NaOH. SR58894A was dissolved in 10% DMSO, whereas forskolin and hydrcortisone 21-hemisuccinate were dissolved in 100% DMSO, and the other drugs were dissolved in distilled water. The reported concentrations are the calculated final concentrations in the bath solution. For the in vivo study ritobegron was dissolved in saline.
Results

Effect of Ritobegron on Monkey Bladder. The chemical structures of ritobegron (KUC-7483) (A), KUC-7322 (B), CL316,243 (C), and isoproterenol (D) are shown in Fig. 1. KUC-7322 is the active form of ritobegron. Ritobegron decreased the resting tension of the isolated bladder in a concentration-dependent manner (Figs. 2 and 3). Isoproterenol (nonselective β-AR agonist) also decreased the resting tension of the isolated bladder in a concentration-dependent manner (Figs. 2 and 3). Isoproterenol increased the resting tension of the isolated bladder in a concentration-dependent manner (Fig. 3).

The EC\textsubscript{50} values obtained for ritobegron, isoproterenol, and CL316,243 were 8.2 ± 2.3 × 10\textsuperscript{-7}, 1.9 ± 0.9 × 10\textsuperscript{-7}, and 5.5 ± 3.1 × 10\textsuperscript{-6} M, respectively (Table 1). The rank order of their relaxing potencies was isoproterenol > ritobegron > CL316,243. The maximal relaxant effects of ritobegron, isoproterenol, and CL316,243 were 88.7 ± 3.7%, 94.2 ± 4.3%, and 73.9 ± 6.7%, respectively (Table 1).

Antagonism by Subtype-Selective β-AR Antagonists of Ritobegron-Induced Relaxation in Isolated Monkey Bladder. In the isolated bladder, addition of the selective \( \beta_1 \)-AR antagonist CGP-20712A (10\textsuperscript{-7} M) plus the selective \( \beta_2 \)-AR antagonist ICI-118551 (10\textsuperscript{-7} M) had no effect on the relaxation induced by ritobegron (Fig. 4A). In the combined presence of CGP-20712A and ICI-118551, the \( \beta_3 \)-AR antagonist SR58894A (3 × 10\textsuperscript{-6} M) produced a rightward shift of the concentration-response curve for ritobegron without altering the maximal response (Fig. 4B). The pK\textsubscript{B} value obtained for SR58894A was 6.56 ± 0.35 (Fig. 4B).

Effects of Ritobegron and Isoproterenol on Isolated Atrial Rate and Isolated Trachea Precontracted with CCh. Both ritobegron and isoproterenol concentration-dependently increased atrial rate (Fig. 5A). The EC\textsubscript{50} values for ritobegron and isoproterenol were 6.5 ± 1.2 × 10\textsuperscript{-5} and 2.2 ± 0.2 × 10\textsuperscript{-5} M, respectively (Table 2). Isoproterenol produced concentration-dependent relaxation of tracheas precontracted with 10\textsuperscript{-7} M CCh (Fig. 5B), the EC\textsubscript{50} value being 1.6 ± 0.4 × 10\textsuperscript{-7} M. In contrast, ritobegron had no such effect between 10\textsuperscript{-9} and 10\textsuperscript{-4} M (Fig. 5B). Even at the highest concentration tested, 10\textsuperscript{-3} M, the maximal tracheal relaxation induced by ritobegron was only 26.7 ± 8.1%, indicating an EC\textsubscript{50} value of 10\textsuperscript{-9} M or more (Table 2). Ritobegron was approximately 30,000 times less potent than isoproterenol in these two tissues (Table 2).

Bladder Selectivity of Ritobegron. The selectivity of ritobegron for the bladder was 79.3-fold higher versus the atria and more than 1200-fold higher versus the trachea (Table 2). Ritobegron displayed much higher selectivity for the bladder than isoproterenol (actually, isoproterenol did not display any bladder selectivity) (Table 2).

Effects of Ritobegron in Anesthetized Monkey. The mean values obtained for intravesical pressure before the administration of ritobegron were not significantly different
among the groups. Ritobegron, at a dose of 0.3 mg/kg, had no evident effect on intravesical pressure (Fig. 6A). However, from 1 to 10 mg/kg it significantly decreased intravesical pressure (Fig. 6A). The ED$_{50}$ value obtained for ritobegron at 90 min after its administration was 1.44 mg/kg. Ritobegron had no effect on either mean blood pressure or heart rate (Fig. 6, B and C). At the end of the experiments, the effects of intravenous administration of isoproterenol (0.1 mg/kg) on mean blood pressure and heart rate were assessed in each treated group. Isoproterenol reduced mean blood pressure by 25.92 mm Hg (vehicle group), 35.83 mm Hg (0.3 mg/kg ritobegron group), 37.25 mm Hg (1 mg/kg ritobegron group), 38.87 mm Hg (3 mg/kg ritobegron group), and 33.59 mm Hg (10 mg/kg ritobegron group). Heart rate was increased by isoproterenol by 57.89, 44.12, 44.77, 36.55, and 58.03 beats/min, respectively, in those groups.

**Discussion**

In the present in vitro and in vivo experiments we evaluated the pharmacological profile of the novel selective $\beta_3$-AR agonist ritobegron and its effects on the cynomolgus monkey bladder. It has been reported that in primates, such as cynomolgus monkey (Takeda et al., 2002a) and humans (Igawa et al., 1998, 1999; Yamazaki et al., 1998; Takeda et al., 1999), bladder relaxation is mediated via the $\beta_3$-AR, and that in humans 97% of total $\beta$-AR mRNA is represented by the $\beta_3$-AR subtype (Yamaguchi, 2002; Nomiya and Yamaguchi, 2003). For the above reason, in this study we used the cynomolgus monkey to evaluate the potential usefulness of ritobegron as a drug with beneficial effects on bladder function.

In the first in vitro experiment, we examined the relaxing effects of ritobegron, isoproterenol, and CL316,243. Although all three drugs decreased the resting tension of the isolated bladder, the slopes of their concentration-response curves were different. It has been reported that $\beta_3$-AR agonists [[(R$^*$,R$^*$)-(±)-4-[2-(3-chlorophenyl)-2-hydroxyethylamino]propyll]phenoxyacetic acid (BRL37344) and CL316,243 induce responses with a slower onset than that to isoproterenol (Roberts et al., 1999). Therefore, it seems reasonable that the slopes of the curves differed depending on the drug. The maximal relaxation to ritobegron was equivalent to that induced by isoproterenol, and both agents displayed full agonistic activities. In contrast, CL316,243 had a relatively weak relaxing effect, its EC$_{50}$ value being approximately seven times higher than that of ritobegron. Moreover, CL316,243 displayed evidence of partial agonistic activity in both the monkey and human bladder at the same concentration as the maximal concentration used in this study (1 $\times$ 10$^{-4}$ M) (Igawa et al., 1999, 2001; Takeda et al., 2002a). The above results suggest that in humans ritobegron
might produce sufficient bladder relaxation and be more potent than classic β3-AR agonists.

In the second in vitro experiment we tried, by using subtype-specific β-AR antagonists, to determine which β-AR subtypes might be involved in ritobegron-induced bladder relaxation. The relaxing effect of ritobegron was not antagonized by either CGP-20712A (10⁻⁷ M) or ICI-118551 (10⁻⁷ M), even though at that concentration they occupy virtually all β₁- or β₂-AR, respectively (Takeda et al., 2002a). Thus, neither β₁- nor β₂-AR would seem to be involved in ritobegron-induced bladder relaxation in the cynomolgus monkey. In contrast, in the combined presence of CGP-20712A and ICI-118,551 the selective β₃-AR antagonist SR58894A effectively antagonized the ritobegron-induced bladder relaxation, with the pKᵦ value being 6.56. This pKᵦ value is comparable with the pA₂ value of 6.24 obtained for isoprotenerol-induced relaxation of the human bladder, an effect that is known to be mediated predominantly through the β₂-AR (Igawa et al., 1999). On those grounds, ritobegron-induced cynomolgus monkey bladder relaxation would seem to be mediated via the β₃-AR.

In view of the above interpretation, and because monkey bladder relaxation has previously been reported to be mediated via β₃-AR (Takeda et al., 2002a), we performed the third in vitro experiment to estimate the β₃-AR-subtype selectivity of ritobegron. To this end, bladder selectivity was evaluated by using the pharmacologically characterized atrium (β₁-AR subtype) and trachea (β₂-AR subtype). The bladder selectivity of ritobegron was 79.3-fold higher and more than 1200-fold higher versus atria and trachea, respectively. Thus, ritobegron is highly selective for the β₃-AR subtype, which is found in the monkey and human bladder. Unfortunately, we do not have data obtained by using recombinant human β-AR; to elucidate the effect on the human bladder would require such experimentation.

Finally, in the in vivo experiment we established that in anesthetized monkeys ritobegron significantly decreased intravesical pressure without affecting either mean blood pressure or heart rate. These results are consistent with previous reports that selective β₃-AR agonists improve bladder functions in rats with minimal effects on the cardiovascular system (Takeda et al., 2000b, 2002b; Kaidoh et al., 2002). Moreover, when we measured the plasma concentration of ritobegron in cynomolgus monkeys that had received intraduodenal administration of 1, 3, or 10 mg/kg the maximum plasma values we obtained were 3.7 × 10⁻⁷, 1.3 × 10⁻⁶, and 4.4 × 10⁻⁶ M, respectively (data not shown). Comparing these plasma concentrations to the concentrations used to obtain the in vitro results suggests that the latter results are well in line with our in vivo results. Actually, the concentration achieved in the plasma after administration of 1 mg/kg ritobegron (3.7 × 10⁻⁷ M) was enough to induce relaxation of the isolated bladder. In addition, 4.4 × 10⁻⁶ M (the plasma concentration achieved by giving 10 mg/kg ritobegron) did not induce a β₁- or β₂-AR-mediated effect.
in the present isolated tissues. Therefore, even at the highest
dose used here, 10 mg/kg ritobegron, the heart rate elevation
and blood pressure reduction attributed to $\beta_1$- and $\beta_2$-AR
(Brode OE, 1988; Ferro et al., 1993) did not occur in our in vivo
study. Thus, we demonstrated that, because of its high selectiv-
ity for $\beta_2$-AR, ritobegron decreased intravesical pressure in
our monkeys without affecting cardiovascular parameters.

There is compelling in vivo evidence to support selective
$\beta_2$-AR agonists improving the urine-storage function in rat
models in which bladder overactivity is induced by bladder-
outlet obstruction (Woods et al., 2001), intravesical infusion
of prostaglandin E$_2$ (Takeda et al., 2002b), or cerebral infarc-
tion (Kaidoh et al., 2002). In a cystometric study in rats,
selective $\beta_2$-AR agonists significantly prolonged micturi-
tion interval and increased bladder capacity without affecting
voiding functions (Takeda et al., 2000b). Collectively, these
pieces of evidence predict a useful role for $\beta_2$-AR agonists in
the treatment of OAB.

In conclusion, our data indicated that ritobegron has po-
tent and selective $\beta_2$-AR agonistic activity and it relaxes the
monkey bladder via the $\beta_2$-AR. Moreover, in vivo
ritobegron decreased intravesical pressure without affecting
vascular parameters. To judge from these results, ritob-

gron shows promise as a potential agent for the treatment of
OAB.

Authorship Contributions
Participated in research design: Maruyama, Tatemichi, Goi, and
Yamazaki.
Conducted experiments: Maruyama, Tatemichi, Goi, and
Yamazaki.
Performed data analysis: Maruyama, Tatemichi, Goi, and
Yamazaki.
Wrote or contributed to the writing of the manuscript: Maruyama,
Tatemichi, Maruyama, Hoyano, Yamazaki, and Kusama.

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