Pharmacokinetics Research Laboratories[1]; Central Research Laboratories[2], Kissei Pharmaceutical Co., Ltd., Nagano, Japan

Absorption, disposition, metabolism, and excretion of ritobegron (KUC-7483), a novel selective β_3-adrenoceptor agonist, in rats

Y. Abe[1], E. Ota[1], T. Endo[1], M. Murakami[1], M. Kobayashi[2]

Received June 5, 2014, accepted July 7, 2014

Yoshikazu Abe, Pharmacokinetics Research Laboratory, Kissei Pharmaceutical Co., 19-48 Yoshino, Matsumoto-City, Nagano-Pref. 399-8710, Japan

Pharmazie 69: 881–888 (2014) *doi: 10.1691/ph.2014.4679*

The pharmacokinetic profile of ritobegron, a novel, selective β_3-adrenoceptor agonist, was investigated in rats. Ritobegron, an ethyl ester prodrug of the active compound KUC-7322, or KUC-7322 itself was orally administered (10 mg/kg). Ethyl esterification resulted in a 10-fold increase in the area under the plasma concentration-time curve ($AUC_{0\text{-}t}$), as compared to KUC-7322. Following intravenous administration of KUC-7322 (1 mg/kg), total blood clearance was 1.36 L/h/kg, suggesting that intrinsic hepatic clearance is the rate-limiting step in KUC-7322 excretion. When ritobegron was orally administered (0.3, 1, 3, and 10 mg/kg), plasma concentrations of KUC-7322 rapidly increased and reached a maximum concentration (C_{max}) at 0.25 to 0.31 h. KUC-7322 levels rapidly decreased, with a half-life ($t_{1/2}$) of 0.42 to 1.37 h thereafter. $AUC_{0\text{-}t}$ did not show a dose-dependent increase. The bioavailability of KUC-7322 was estimated to be 4%. Following oral administration of [^{14}C]ritobegron (3 mg/kg), radioactivity concentrations in tissues rapidly increased and declined in parallel with changes in plasma concentration. In most of tissues, excluding the liver, kidney, urinary bladder, stomach and small intestine, radioactivity concentrations were lower than that in plasma. In plasma, bile, urine, and feces, KUC-7322 and its glucuronide, sulfate, and glutathione conjugates were detected. The glucuronide conjugate of KUC-7322 was the predominant metabolite in bile, plasma, and urine, and KUC-7322 was predominant in feces. Ritobegron was not detected in any of the samples. The cumulative excretion of radioactivity in urine and feces were 28.7% and 68.3% of the dose, respectively, up to 120 h after administration.

1. Introduction

The urinary bladder is innervated by both the sympathetic and parasympathetic nervous system, with sympathetic activation contributing to urine storage through the relaxation of bladder smooth muscle, *via* activation of β-adrenoceptors (ARs) (Andersson 1999). In humans, bladder relaxation is mediated *via* β_3-AR, which comprises 97% of the total β-AR mRNA in the bladder (Igawa et al. 1998, 1999; Yamaguchi et al. 2001; Nomiya et al. 2003).

Abbreviations: AR, adrenoceptor; $AUC_{0\text{-}t}$, area under the plasma concentration-time curve through the final time-point at which the concentration could be determined; AUC_{inf}, area under the plasma concentration-time curve extrapolated to infinity; BA, bioavailability; BDC rats, bile duct-cannulated rats; C_{max}, maximum concentration; CMC-Na, carboxymethyl cellulose sodium; CL_B, total blood clearance; CL_{tot}, total plasma clearance; C_{max}, maximum plasma concentration; f_u, plasma unbound fraction; GSH, glutathione; $NADP^+$, β-nicotineamide adenine dinucleotide phosphate; oxidized form; NADPH, β-nicotineamide adenine dinucleotide phosphate; reduced form; OAB, overactive bladder; PAPS, adenosine 3'-phosphate 5'-phosphosulphate; R_B, ratio of blood to plasma concentration; SPE, solid-phase extraction; $t_{1/2}$: elimination half-life; T_{max}, time to reach maximum plasma concentration; UDPGA, uridine 5'-diphosphoglucuronic acid; V_{ss}, distribution volume at steady state Ritobegron: (-)-ethyl 2-[4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)-2,5-dimethylphenyloxy]acetate monohydrochloride KUC-7322: 2-[4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)-2,5-dimethylphenyloxy]acetic acid.

Ritobegron (KUC-7483, the ethyl ester prodrug of KUC-7322), was synthesized by Kissei Pharmaceutical Co. Ltd. (Nagano, Japan) as a novel, selective β_3-AR agonist for use in the treatment of overactive bladder (OAB). *In vitro* studies using CHO cells expressing human β-ARs revealed that the selectivity of ritobegron for β_3-AR was 301-fold and 32-fold higher than β_1-AR and β_2-AR, respectively (Maruyama et al. 2012a). *In vivo* studies indicated that ritobegron induced a dose-dependent decrease in intravesical pressure without affecting heart rate in rats and cynomolgus monkeys (Maruyama et al. 2012b). Further, in a study using isolated human detrusor muscle, KUC-7322, the active form of ritobegron, relaxed detrusor preparations and decreased carbachol-induced bladder contractions in a concentration-dependent manner (Igawa et al. 2012). Therefore, it is hypothesized that ritobegron may be useful for treatment of OAB. The objective of the present study was to assess the pharmacokinetic profile of ritobegron in rats.

2. Investigations and results

2.1. Comparison of the pharmacokinetic parameters of KUC-7322 after oral administration of ritobegron and KUC-7322

The pharmacokinetic parameters of KUC-7322 after oral administration of ritobegron and KUC-7322 (10 mg/kg), are summarized in Table 1. The $AUC_{0\text{-}t}$ and C_{max} of ritobegron were

Table 1: Pharmacokinetic parameters of KUC-7322 after oral administration of ritobegron and KUC-7322 (10 mg/kg) to rats

Parameters	Ritobegron	KUC-7322
$AUC_{0\text{-}t}$ (h·ng/mL)	682.3 ± 113.9	54.6 ± 32.6
AUC_{inf} (h·ng/mL)	727.9 ± 92.6	113.3 ± 21.9
C_{max} (ng/mL)	835.2 ± 186.2	96.4 ± 31.1
T_{max} (h)	0.50 ± 0.00	0.33 ± 0.14
$t_{1/2}$ (h)	0.42 ± 0.20	0.61 ± 0.37

Each value represents the mean ± standard deviation of three animals.
Dose was indicated as KUC-7322.

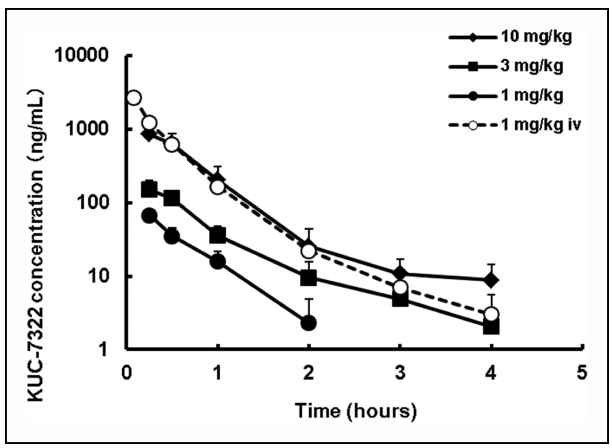

Fig. 1: Plasma KUC-7322 concentration-time profiles after oral administration of ritobegron and intravenous administration of KUC-7322 to rats. Each point represents the mean + standard deviation of four animals.Doses were expressed as free base of ritobegron. Data after oral administration at a dose of 0.3 mg/kg is not plotted because of a few detectable time points.

12 and 9 times higher than those of KUC-7322, respectively, although both the time to reach maximal plasma concentration (T_{max}) and $t_{1/2}$ of ritobegron were similar to those of KUC-7322.

2.2. Plasma concentration of KUC-7322

The plasma concentration-time profile of KUC-7322 is shown in Fig. 1, and the pharmacokinetic parameters of KUC-7322 are summarized in Table 2. Following intravenous administration of KUC-7322 (1 mg/kg), plasma concentrations of KUC-7322 rapidly declined with a $t_{1/2}$ of 0.50 h. Following oral administration of ritobegron at doses of 0.3, 1, 3, and 10 mg/kg, plasma concentrations of KUC-7322 rapidly increased and reached C_{max} at 0.25 to 0.31 h post-administration. Plasma concentrations rapidly declined with $t_{1/2}$ of 0.42 to 1.37 h. The $AUC_{0\text{-}t}$ did not indicate a dose-dependent increase. The area under the plasma concentration-time curve extrapolated to infinity (AUC_{inf}) was 45.3 h ng/mL after oral administration of ritobegron (1 mg/kg) and 1147.2 h ng/mL after intravenous administration of KUC-7322 (1 mg/kg). Based on these results, the bioavailability (BA) of KUC-7322 was estimated to be 4%.

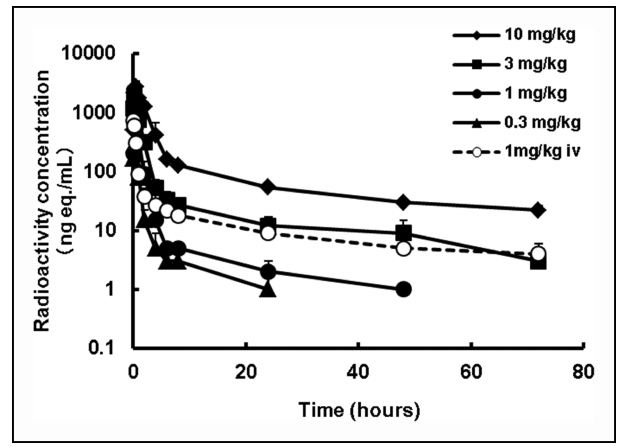

Fig. 2: Plasma radioactivity concentration-time profiles in rats after intravenous administration of [14C]KUC-7322 and oral administration of [14C]ritobegron. Each point represents the mean + standard deviation of three animals. Doses were expressed as free base of ritobegron.

2.3. Plasma concentration of radioactive KUC-7322

The plasma concentration-time profile of radioactive KUC-7322 is shown in Fig. 2, and the pharmacokinetic parameters of radioactive KUC-7322 are summarized in Table 3. Following intravenous administration of [^{14}C]KUC-7322 (1 mg/kg), plasma concentrations of radioactivity declined biphasically, with a $t_{1/2}$ of 0.43 h for the first 2 h after administration, and of 28 h from 8 to 72 h after administration. Following oral administration of [^{14}C]ritobegron to rats at doses of 0.3, 1, 3, and 10 mg/kg, radioactivity was rapidly absorbed and reached C_{max} at 0.25 to 0.5 h post-administration. Plasma concentrations of radioactivity declined biphasically, with a $t_{1/2}$ of 0.66 to 1.4 h, and of 14 to 31 h. $AUC_{0\text{-}t}$ increased in a dose-dependent manner after oral administration of [^{14}C]ritobegron for doses between 0.3 and 10 mg/kg. The amount of radioactivity absorbed was estimated to be 87% of the administered [^{14}C]ritobegron, calcu-

Table 2: Pharmacokinetic parameters of KUC-7322 after intravenous administration of KUC-7322 and oral administration of ritobegron to rats

Parameters	Dose (mg/kg)				
	0.03	1	3	10	1
Route	oral	oral	oral	oral	intravenous
$AUC_{0\text{-}t}$ (h·ng /mL)	–	39.7 ± 12.7	122.7 ± 14.5	643.2 ± 235.6	1144.2 ± 106.1
AUC_{inf} (h·ng /mL)	–	45.3 ± 12.8	–	–	1147.2 ± 107.7
C_{max} (ng/mL)	9.2 ± 2.4	67.5 ± 10.8	157.3 ± 41.3	858.6 ± 180.9	–
T_{max} (h)	0.25 ± 0.00	0.25 ± 0.00	0.31 ± 0.13	0.25 ± 0.00	–
$t_{1/2}$ (h)	–	0.42 ± 0.11	0.68 ± 0.25	1.37 ± 0.35	0.50 ± 0.11
CL_{tot} (mg/h/kg)	–	–	–	–	816.0 ± 75.1
V_{ss} (mL/kg)	–	–	–	–	294 ± 11

Each value represents the mean ± standard deviation of four animals.
Doses were indicated as free base of ritobegron.
–; Not calculated.

Table 3: Pharmacokinetic parameters of radioactivity after intravenous administration of [^{14}C]KUC-7322 and oral administration of [^{14}C]ritobegron to rats

Parameters	Dose (mg/kg)				
	0.3	1	3	10	1
Route	oral	oral	oral	oral	intravenous
$AUC_{0\text{-}t}$ (h·ng eq./mL)	317 ± 84	900 ± 223	3030 ± 460	9110 ± 300	1070 ± 370
AUC_{inf} (h·ng eq./mL)	–	973 ± 297	–	–	1230 ± 460
C_{max} (ng eq./mL)	287 ± 42	713 ± 41	1955 ± 517	2781 ± 647	–
T_{max} (h)	0.25 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.50 ± 0.00	–
$t_{1/2}$ −α (h)	0.69 ± 0.25	0.66 ± 0.03	0.75 ± 0.09	1.4 ± 0.2	0.43 ± 0.06
$t_{1/2}$ −β (h)	14[1)]	31 ± 20	24 ± 5	26 ± 3	28 ± 1

Each value represents the mean ± standard deviation of three animals.
Doses were indicated as free base of ritobegron.
–: Not calculated.
1) This value is mean of two animals and standard deviation cannot be calculated

Table 4: Radioactivity concentrations in tissues after oral administration of [^{14}C]ritobegron (3 mg/kg) to rats

Tissues	Radioactivity concentration (μg eq. of KUC-7322/g or mL)				
	0.25 h	2 h	8 h	24 h	120 h
Plasma	2.052	0.494	0.038	0.010	0.002
Blood	1.214	0.280	0.025	0.006	0.002
Cerebrum	0.023	0.007	0.002	ND	ND
Cerebellum	0.023	0.009	0.002	0.001	ND
Pituitary	0.203	0.111	0.035	ND	ND
Thyroid	0.394	0.121	0.024	ND	ND
Thymus	0.184	0.048	0.009	0.002	0.002
Heart	0.281	0.063	0.007	0.003	0.001
Lung	0.636	0.141	0.010	0.004	0.002
Liver	6.683	1.165	0.459	0.099	0.039
Kidney	6.867	1.738	0.053	0.007	0.003
Adrenal gland	0.264	0.093	0.024	0.004	ND
Spleen	0.270	0.070	0.014	0.003	0.002
Pancreas	0.952	0.112	0.014	0.005	0.001
Fat	0.201	0.045	0.003	0.002	0.001
Skeletal muscle	0.145	0.040	0.003	0.002	0.001
Skin	0.591	0.163	0.012	0.006	0.002
Bone marrow	0.330	0.072	0.015	0.004	ND
Aorta	0.984	0.344	0.029	0.005	0.002
Renal artery	0.466	0.576	ND	ND	ND
Testis	0.141	0.057	0.008	0.003	0.002
Prostate gland	0.260	0.084	0.009	0.003	0.001
Urinary bladder	3.703	2.898	0.042	0.009	0.003
Stomach	15.757	29.116	0.041	0.016	0.015
Small intestine	6.238	7.968	0.037	0.018	0.004
Large intestine	1.707	0.666	2.505	0.098	0.011

Each value represents the mean of three animals.
Dose was indicated as free base of ritobegron.
ND: Not detected

lated from AUC_{inf} after oral administration of [^{14}C]ritobegron and intravenous administration of [^{14}C]KUC-7322.

2.4. Tissue distribution of radioactivity in rats

Radioactivity concentrations in the tissues of rats following oral administration of [^{14}C]ritobegron are summarized in Table 4, and representative whole-body autoradiograms are shown in Fig. 3. Following a single oral administration of [^{14}C]ritobegron (3 mg/kg), the radioactivity concentrations in tissues, excluding the gastrointestinal tract, reached a maximum at 0.25 h post-administration, confirming that the radioactivity derived from [^{14}C]ritobegron was rapidly distributed into tissues. At 0.25 h post-administration, radioactivity concentrations in tissue were lower than that in plasma, with the exception of the liver, kidney, urinary bladder, stomach and small intestine, which are involved in administration, absorption, metabolism and excretion of KUC-7322. The radioactivity concentrations in tissue declined rapidly, in parallel with the decline in plasma concentration. Further, 24 h post-administration, radioactivity levels were no more than 4% of the maximum radioactivity concentration or below the detection limit.

2.5. Plasma protein binding in vitro

Plasma protein binding of [^{14}C]KUC-7322 in rats and humans is summarized in Table 5. Plasma protein binding was nearly constant, despite the increase in concentration of [^{14}C]KUC-

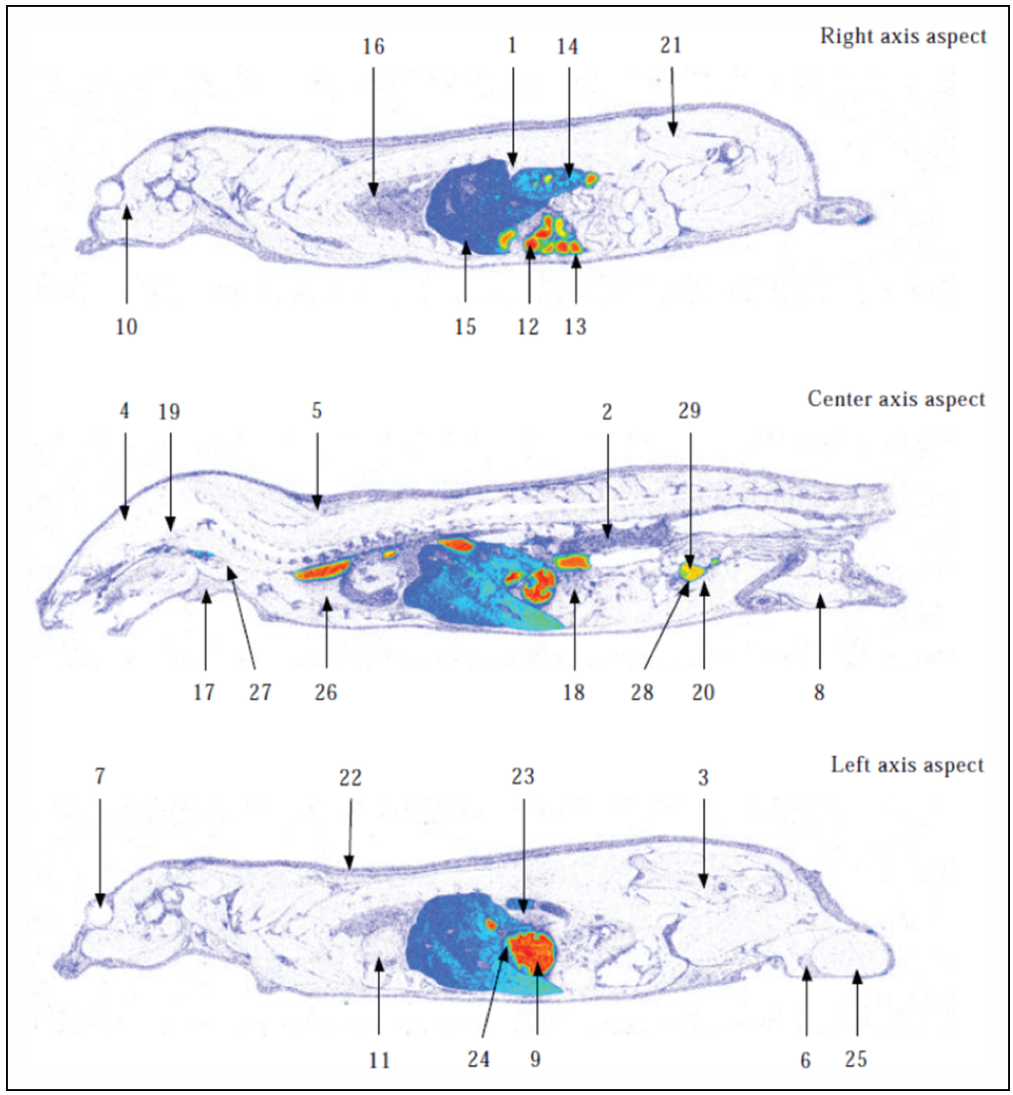

Fig. 3: Representative whole body autoradiograms 15 min after oral administration of [14C]ritobegron at a dose of 3 mg/kg. Dose was expressed as free base of ritobegron. 1. Adrenal gland, 2. Blood, 3. Bone marrow, 4. Brain, 5. Brown fat, 6. Epididymis, 7. Eyeball, 8. Fat, 9. Gastric contents, 10. Harderian gland, 11. Heart, 12. Intestinal contents, 13. Intestine, 14. Kidney, 15. Liver, 16. Lung, 17. Mandibular gland, 18. Pancreas, 19. Pituitary gland, 20. Prostate gland, 21. Skeletal muscle, 22. Skin, 23. Spleen, 24. Stomach, 25. Testis, 26. Thymus, 27. Thyroid gland, 28. Urinary bladder, 29. Urine in bladder.

Table 5: Plasma protein bindings of [^{14}C]KUC-7322 in rats and humans *in vitro*

Plasma concentration of KUC-7322 (ng/mL)	Protein binding (%)	
	Rats	Humans
100	45.7 ± 1.5	71.4 ± 1.5
500	45.3 ± 0.7	70.8 ± 1.9
2000	44.4 ± 0.7	70.3 ± 1.7

Each value represents the mean ± standard deviation of three experiments.

7322 from 100 to 2000 ng/mL in both species. However, plasma protein binding in humans was higher than that in rats, indicating a species difference.

2.6. Distribution of [^{14}C]KUC-7322 in blood cells in vitro

Distribution in blood cells and the blood to plasma concentration ratio (R_B) of [^{14}C]KUC-7322 are summarized in Table 6. The low variation of distribution ratio in the concentration range suggests that [^{14}C]KUC-7322 has a low affinity for blood cells in both species.

2.7. Metabolite analysis

Metabolite profiles in plasma, bile, urine, and feces after oral administration of [^{14}C]ritobegron (3 mg/kg) are summarized in Table 7. The glucuronide conjugate of KUC-7322 was the predominant metabolite in bile, plasma, and urine, comprising 54.7% to 100.0% of the total radioactivity peak area. In addition, KUC-7322 was the predominant metabolite in feces, comprising of 91.7% to 94.2% of the total peak area. The sulfate conjugate of KUC-7322 was present in bile, urine, and feces, and the glutathione conjugate was only present in bile. Ritobegron was not detected in any of the samples.

2.8. Excretion of radioactivity in bile, urine and feces

Cumulative excretion of radioactivity after oral administration of [^{14}C]ritobegron (3 mg/kg) to normal male rats and male bile duct-cannulated (BDC) rats are summarized Table 8 and 9, respectively. The cumulative excretion of radioactivity in urine and feces was 28.7% and 68.3%, respectively, and the total excretion of radioactivity up to 120 h after administration was 97.0%. Following oral administration of [^{14}C]ritobegron to male BDC rats, the cumulative excretion of radioactivity in bile

Table 6: Distribution in blood cells and R_B value of [^{14}C]KUC-7322 in rats and humans *in vitro*

Blood concentration of KUC-7322 (ng/mL)	Distribution ratio (%)		R_B (%)	
	Rats	Humans	Rats	Humans
100	2.1 ± 2.3	2.7 ± 2.5	0.60 ± 0.01	0.57 ± 0.03
500	5.4 ± 1.6	1.4 ± 1.2	0.61 ± 0.01	0.56 ± 0.02
2000	3.1 ± 1.5	2.5 ± 0.7	0.59 ± 0.00	0.57 ± 0.04

Each value represents the mean ± SD of three experiments.
Hematocrit values of rats and humans were 41–43% and 44–45%, respectively.

Table 7: Composition (%) of unchanged drug and metabolites in plasma, bile, urine, and feces after oral administration of [^{14}C]ritobegron (3 mg/kg) to rats

Samples	Composition (%)					
	KUC-7322	Glucuronide conjugate	Sulfate conjugate	Glutathione conjugate	Unchanged (ritobegron)	Others
Plasma 0.25 h	15.7	84.4	0.0	0.0	0.0	0.0
Plasma 2 h	0.0	100.0	0.0	0.0	0.0	0.0
Bile 0-24 h	10.4	54.7	14.7	11.0	0.0	9.3
Urine 0-24 h	10.6	87.4	2.0	0.0	0.0	0.0
Feces 0-24 h	94.2	0.0	5.9	0.0	0.0	0.0
Feces 24-48 h	91.7	0.0	8.4	0.0	0.0	0.0

Each sample was pooled from three animals.
Dose was indicated as free base of ritobegron.

Table 8: Cumulative excretion of radioactivity after oral administration of [^{14}C]ritobegron (3 mg/kg) to rats

Time (h)	Excretion of radioactivity (% dose)		
	Urine	Feces	Total
0-4	14.3 ± 12.6	–	–
0-8	26.3 ± 4.3	–	–
0-24	27.7 ± 4.1	53.2 ± 9.9	80.8 ± 8.1
0-48	28.5 ± 4.6	66.5 ± 3.0	95.0 ± 2.4
0-72	28.6 ± 4.6	68.1 ± 3.9	96.7 ± 1.1
0-96	28.7 ± 4.7	68.3 ± 4.0	96.9 ± 1.0
0-120	28.7 ± 4.6	68.3 ± 4.0	97.0 ± 0.9

Each value represents the mean ± standard deviation of three animals.
Dose was indicated as free base of ritobegron.
–: Not determined

Table 9: Cumulative excretion of radioactivity after oral administration of [^{14}C]ritobegron (3 mg/kg) to BDC rats

Time (h)	Excretion of radioactivy (% dose)		
	Bile	Urine	Feces
0-1	4.8 ± 6.4	–	–
0-2	10.0 ± 7.2	–	–
0-4	17.3 ± 9.4	15.7 ± 13.3	–
0-8	25.0 ± 5.2	20.9 ± 12.6	–
0-24	28.2 ± 3.6	23.0 ± 11.2	25.3 ± 3.6
0-48	29.4 ± 3.3	23.7 ± 10.7	37.2 ± 8.8

Each value represents the mean ± standard deviation of three animals.
Dose was indicated as free base of ritobegron.
–: Not determined

was 29.4% up to 48 h. The cumulative excretion of radioactivity in urine and feces at 48 h post-administration were 23.7% and 37.2% of the dose.

3. Discussion

As ritobegron is the ethyl ester prodrug of KUC-7322, we first confirmed the effect of ethyl esterification of KUC-7322 on its absorption in rats. Comparing the $AUC_{0\text{-}t}$ and C_{max} after oral administrations of ritobegron and KUC-7322, ethyl esterification enhanced both the $AUC_{0\text{-}t}$ and C_{max} 10-fold. Ester prodrugs, such as enalapril, oseltamivir, famciclovir, adefovir, and pivampicillin, are commonly used to enhance the lipophilicity, and thus the passive membrane permeability, of water soluble drugs by masking charged groups, such as carboxylic acids and phosphates. They are hydrolyzed by ubiquitous esterases including carboxylesterases, acetylcholinesterases, butyrylcholinesterases, paraoxonases, and arylesterases (Rautio et al. 2008). These esterases are likely to be involved in hydrolysis of ritobegron.

As ritobegron was metabolized rapidly to KUC-7322 in rats (discussed below), the BA of ritobegron was estimated using the AUC_{inf} of KUC-7322 after intravenous administration. Following intravenous administration of KUC-7322, KUC-7322 was rapidly eliminated in plasma with a CL_{tot} of 0.82 L/h/kg. The total blood clearance (CL_B), calculated as CL_{tot}/R_B, was 1.36 L/h/kg, was lower than the hepatic flow rate reported in rats (3.5 L/h/kg) (Dedrick et al. 1973). These data suggest that the intrinsic hepatic clearance is the rate-limiting step in KUC-7322 excretion. Following oral administration of ritobegron, the $AUC_{0\text{-}t}$ did not indicate a dose-dependent increase. This is likely attributable to KUC-7322 metabolic saturation, as the $AUC_{0\text{-}t}$ after oral administration of [^{14}C]ritobegron at doses of 0.3 to 10 mg/kg increased in a dose-dependent manner. Following oral administration of [^{14}C]ritobegron, radioactivity was rapidly absorbed. Most of the dose (87%) of [^{14}C]ritobegron was absorbed, likely *via* the gastrointestinal tract. However, the bioavailability of KUC-7322 after oral administration of ritobegron was 4%, which was significantly lower than the absorbed fraction of radioactivity. These findings indicate that the gastrointestinal and hepatic first-pass effect is substantial in rats.

Following oral administration of [^{14}C]ritobegron, radioactivity was distributed rapidly throughout the whole body. However, radioactivity concentrations in tissues were lower than those in plasma, excluding those tissues involved in administration,

absorption, metabolism, and excretion. These results are consistent with the V_{ss} of KUC-7322 being relatively small in rats. The changes in plasma protein binding may sometimes alter the distribution of drugs to target tissues, which is reflected in the V_{ss} or R_B (Øie 1979; Evans et al. 1973). In general, if there was no species difference in blood cell uptake, the R_B value should decrease with corresponding decreases in the plasma unbound fraction (f_u). However, the R_B values of KUC-7322 between rats and humans showed no variation, while f_u in rats was higher than in humans. It is thus presumed these results were attributed to very low blood cell distribution of KUC-7322 in both species.

In the analysis of metabolites, the amount of ritobegron was below the lower limit of detection in plasma, bile, urine, and feces. The primary metabolites of ritobegron were the glucuronide conjugate of KUC-7322 in plasma, bile, and urine and KUC-7322 in feces. These results suggest that, after oral administration to rats, ritobegron is rapidly metabolized to KUC-7322, and then primarily metabolized to the glucuronide conjugate. This metabolite passes through the plasma and is excreted in urine. It can also pass through the bile and be excreted into the intestine. Many compounds are conjugated in the liver to form glucuronides etc., and then excreted into the bile and intestine (Smith 1966). Glucuronides are likely hydrolysed either by enzymes of the intestinal mucosa or by enzymes of any bacteria present in the intestine, releasing the aglycone. Deconjugation of glucuronide by β-glucuronidase in enteric bacteria is primarily mediated by non-sporing anaerobes and lactobacilli, although in the proximal small intestine *Escherichia coli* may make some contribution. The level of β-glucuronidase activity in the small intestine of rats is higher than that of humans (Hawksworth et al. 1971). The glucuronide of SN-38, an active metabolite of irinotecan (CPT-11), is excreted in the intestinal lumen (Ito et al. 2004). It is then extensively deconjugated by bacterial β-glucuronidase, resulting in the regeneration of SN-38, which causes diarrhea (Takasuna et al. 1996). It is also reported that the glucuronide of bisphenol A is deconjugated by β-glucuronidase in the intestine, resulting in the reabsorption of aglycone. The adverse effects of bisphenol A are enhanced by repeated exposure (Sakamoto et al. 2002). Thus, it is likely that the conjugated metabolite of KUC-7322 is also deconjugated in the intestine by enteric bacteria, and then excreted in feces as KUC-7322, as $AUC_{0\text{-}t}$ of KUC-7322 after oral administration of KUC-7322 is less than 10% of that of ritobegron. Excreted fractions of KUC-7322 in urine and feces, estimated using the cumulative excretion of radioactivity and compositions of KUC-7322, were 3.0% and 64.3%, respectively. Assuming that KUC-7322 in feces is the deconjugated and non-absorbed fraction, the excretion ratio of KUC-7322 is estimated to be less than 5% of the dose. Therefore, the main route of clearance of ritobegron is dependent on how the drug is metabolized in rats.

In conclusion, gastrointestinal and hepatic first-pass effects, the hydrolysis of ritobegron to KUC-7322, and the conjugation metabolism of KUC-7322 significantly affect the pharmacokinetics of ritobegron in rats.

4. Experimental

4.1. Materials

Ritobegron (KUC-7483), KUC-7322, and UL092 were synthesized by Kissei Pharmaceutical Co. Ltd. Their chemical structures are shown in Fig. 4. [^{14}C]ritobegron was synthesized by Amersham Pharmacia Biotech UK Ltd. (Buckinghamshire, UK). [^{14}C]KUC-7322 was prepared by the addition of 100 mM NaOH to [^{14}C]ritobegron, followed by incubation for 1 h at room temperature before use. Prepared [^{14}C]KUC-7322 was neutralized with 100 mM HCl. The specific activities of [^{14}C]Ritobegron and [^{14}C]KUC-7322 were all 2.15 GBq/mmol, and both radiochemical purities were greater than 95% as determined by HPLC. Solubilizer Soluene-350, the scintillation cocktail for HPLC Uitima Flo-M, and the scintillation cocktail Hionic-Fluor were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). All other chemicals used in this study were of analytical grade and purchased commercially.

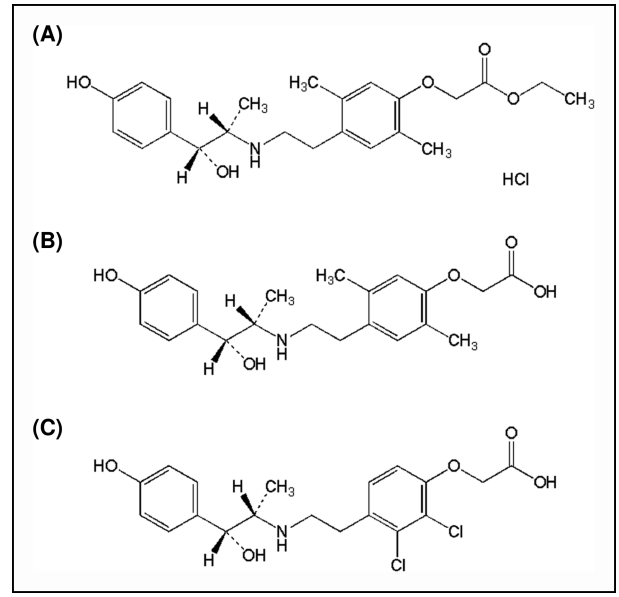

Fig. 4: Chemical structures of ritobegron (A), KUC-7322 (B), and UL092 (C). *14C labeled position of labeled compound.

4.2. Animals

Male Sprague-Dawley rats (7 to 9 weeks old: Japan SLC Inc., Shizuoka, Japan or Charles River Laboratories Japan, Inc., Kanagawa, Japan) were maintained on a 12-h light/12-h dark cycle in a room with controlled temperature (20 to 25 °C), relative humidity (45 to 65%) and acclimatized for 7 days before drug administration. The animals were fed a commercial pellet diet (CE-2; CLEA Japan, Inc., Tokyo, Japan), and were given free access to food and water. For determination of excretion of radioactivity in bile, the animals were anesthetized with ether, and their common bile ducts were cannulated with polyethylene tubes (BDC rats).

This study was conducted according to guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical Co. Ltd.

4.3. Administration of ritobegron and KUC-7322

Rats were fasted overnight and weighed prior to administration of ritobegron or KUC-7322. [^{14}C]Ritobegron or ritobegron dissolved in 0.5% arabic gum solution was orally administered to rats at doses of 0.3, 1, 3, or 10 mg/kg (expressed as free base of ritobegron; 0.327, 1.09, 3.27, and 10.9 mg/kg of ritobegron, respectively, n = 3-4/group). [^{14}C]KUC-7322 or KUC-7322 dissolved in phosphate buffered saline (pH 7.4) was intravenously administered at a dose of 1 mg/kg (expressed as the dose of ritobegron free base; 0.93 mg/kg of KUC-7322, n = 3-4/group). However, for the comparison studies of the concentration profile of KUC-7322, ritobegron and KUC-7322 were administered to rats at a dose of 10 mg/kg KUC-7322 in 0.5% methylcellulose solution. The doses of ritobegron and KUC7322 were 11.67 and 10 mg/kg, respectively.

4.4. Sample collection

Following intravenous or oral administration, blood (0.25 or 0.5 mL) was collected from the jugular or the caudal vein using a heparinized syringe. Plasma was separated by centrifugation (15,900 × g, 1 min, room temperature) and stored at −20 °C until analysis.

Following oral administration of [^{14}C]ritobegron at a dose of 3 mg/kg, rats were anesthetized with diethyl ether and sacrificed at 0.25, 2, 8, 24, and 120 h by exsanguination from the abdominal aorta. Tissues of interest were removed immediately after exsanguinations.

Rats were kept in metabolism cages after oral administration of [^{14}C]ritobegron, and urine and feces were collected up to 120 h post-administration. Bile was collected after oral administration of [^{14}C]ritobegron to BDC rats up to 48 h post-administration. For the determination of radioactivity and metabolite analysis, fecal samples were homogenized in distilled water. These samples were stored at −80 °C until analysis.

4.5. Whole-body autoradiography

Following oral administration of [^{14}C]ritobegron at a dose of 3 mg/kg, rats were euthanized with diethyl ether overdose at 0.25, 2, and 24 h post-administration. Hair was rapidly clipped, and the nasal cavity and anus were filled with 5% carboxymethyl cellulose sodium (CMC-Na). The carcass was frozen in a dry ice-acetone mixture. After the forelimbs, hindlimbs and tail were surgically removed, the frozen carcass was embedded in 5% CMC-Na on a microtome stage, frozen again in a dry ice-acetone mixture, and held in a Cryomicrotome (CryoMacrocut, Leica Microsystems, Wetzlar, Germany). Approximately 35 μm thick sections were cut and collected on an adhesive tape (No. 810, Sumitomo 3 M, Tokyo, Japan) at –25 °C and freeze-dried. The dried sections were covered with protective membrane (4 μm, Dia Foil, Mitsubishi Plastic, Tokyo, Japan) and placed in contact with imaging plates (BAS SR2040, Fujifilm, Tokyo, Japan). The plates were exposed for a fixed period in lead shield boxes. After exposure, the radioactive images on the imaging plates were read (conditions: resolution 50 μm, gradation 256, sensitivity 30,000, latitude 5) using a bio-image analyzer (BAS2500, Fujifilm) to prepare radioluminograms.

4.6. Determination of radioactivity in plasma, urine bile, and feces

Aliquots of plasma (100 μL) and fecal homogenate (200 μL) were dissolved with 2 mL of Soluene-350. These solutions were then mixed with 10 mL of Hionic-Fluor. In addition, aliquots of urine (0.1–1 mL) and bile (0.2 mL) were mixed with 10 mL of Hionic-Fluor without dissolution in Soluene-350. Radioactivity was measured for 2 min using a liquid scintillation counter (LSC, Tri-Carb 1900CA and 2900TR, PerkinElmer Life and Analytical Sciences). Counting efficiency was corrected by an external standard radiation source. Detection limit of radioactivity was defined as 2-fold of background value. Radioactivity concentration in the plasma was expressed as equivalents of KUC-7322 (ng eq./mL).

4.7. Determination of radioactivity in tissue

An aliquot of blood (100 μL) was dissolved in 2 mL of Soluene-350 and decolorized with 0.4 mL of benzene saturated with benzoyl peroxide. Cerebrum, liver, and kidney were cut into small pieces, and then homogenized in approximately 2-fold volume of saline. Aliquots of homogenates (0.5 mL) were solubilized in 2 mL of Soluene-350 with heating. Stomach, large intestine, and the small intestine were solubilized in 10, 10, and 20 mL of 0.5 M sodium hydroxide solution with heating, and diluted with distilled water to 20, 20 and 40 mL, respectively. Aliquots of diluted stomach, large intestine, and small intestine (1 or 2 mL) were collected in vials for measurement of radioactivity. Small intestine was collected from the duodenum to the ileum, and large intestine was collected from the cecum to the colon. The contents were removed and the tracts were washed with saline. Other tissues (entire tissue or 0.2 g) were solubilized in 2 mL of Soluene-350 with heating.
After preparation, each sample was mixed with 10 mL of Hionic-Fluor, and allowed to stand at room temperature. The radioactivity was measured using the LSC and the concentrations and distribution of radioactivity in the tissues were determined.

4.8. Determination of KUC-7322 in plasma by HPLC

To an aliquot of rat plasma (200 μL), 10 μL of UL092 (10 μg/mL: internal standard), and 500 μL of 100 mM potassium phosphate (pH 3.0) was added. The solution was then applied to an Oasis MCX solid-phase extraction (SPE) cartridge (30 mg/1 cc, Waters) that had been equilibrated with 1 mL of acetonitrile/25% ammonia solution (95/5, v/v) and 1 mL of 100 mM potassium phosphate (pH 3.0). The SPE cartridge was then washed with 1 mL of distilled water, 1 mL of acetonitrile/100 mM hydrochloric acid (9/1, v/v), and 1 mL of acetonitrile and eluted with 1 mL of acetonitrile/25% ammonia solution (95/5, v/v). The solvent in the eluate was then dried under a stream of nitrogen gas, and the residue was reconstituted in 100 μL of 20 mM potassium phosphate (pH 3.0)/acetonitrile (8/2, v/v). The product (30 μL) was injected into the HPLC system (L-7000, Hitachi, Ibaraki, Japan). Separation of KUC-7322 and its internal standard was achieved with a Luna C8 (2) (5 μm, 150 mm × 4.6 mm i.d., Phenomenex, Torrance, CA). The mobile phase was a mixture of 20 mM potassium phosphate (pH 3.0) (A) and 20 mM potassium phosphate (pH 3.0)/acetonitrile (50:50, v/v) (B). The mobile phase was passed through the column at 1 mL/min in the following linear gradient mode: starting with 30% B composition, increasing to 40% for 0 to 4 min, maintaining 40% for 4 to 7 min, increasing 100% for 7 to 8 min, maintaining 100% for 8–13 min, decreasing 30% for 13 to 14 min, and finally maintaining 30% for 14 to 24 min. The column was maintained at 40 °C. KUC-7322 was detected with a fluorescence detector (L-7480, Hitachi) with an excitation at 225 nm and emission at 302 nm. The D-7000 HPLC System Manager, version 3.1.1, (Hitachi) was used to determine the plasma concentrations of KUC-7322.

4.9. Plasma protein binding in vitro

Plasma protein binding of KUC-7322 in rats and humans were determined by ultrafiltration (Wolfer et al. 1987). [^{14}C]KUC-7322 saline solution was added to aliquots of rat and human plasma (3.5 mL), until final concentration of 100, 500, or 2,000 ng/mL was obtained. The volume of [^{14}C]KUC-7322 solution was no more than 1% of the plasma volume. After incubation for 5 min at 37 °C, an aliquot of plasma sample (1 mL) was transferred to a Centrifree (Millipore, Billerica, MA) and centrifuged (1,000 × g, 4 °C, 30 min). For the calculation of the unbound [^{14}C]KUC-7322 concentration in plasma (C_u), an aliquot of filtrate (100 μL) was dissolved in 2 mL of Soluene-350, and radioactivity was measured for 2 min using the LSC after addition of 10 mL of Hionic-Fluor. Radioactivity in plasma (100 μL) was measured in the same manner, and total [^{14}C]KUC-7322 concentration in plasma (C_t) was calculated. Plasma protein binding was calculated using the following equation:

$$\%\text{bound} = (1 - C_u/C_t) \times 100$$

4.10. Distribution of [^{14}C]KUC-7322 in blood cells in vitro

[^{14}C]KUC-7322 saline solution was diluted in an aliquot of rat or human blood (3.5 mL), to a final concentration of 100, 500, or 2,000 ng/mL. The volume of [^{14}C]KUC-7322 solution was no more than 1% of the blood volume. After incubation for 5 min at 37 °C, an aliquot of blood (100 μL) was dissolved in 2 mL of Soluene-350 and decolorized with 0.4 mL of benzene saturated with benzoyl peroxide. The remaining blood was centrifuged (8,000 × g, 4 °C, 5 min) to separate plasma. An aliquot of plasma (100 μL) was dissolved in 2 mL of Soluene-350. The solubilized blood and plasma were mixed with 10 mL of Hionic-Fluor, and radioactivity was measured for 2 min using the LSC to determine [^{14}C]KUC-7322 concentration in plasma (C_P) and blood (C_B).
Next, blood was collected in a capillary tube for hematocrit and the hematocrit value (Ht) was determined using a centrifuge for hematocrit. Distribution ratio of [^{14}C]KUC-7322 in blood cells (%) and R_B value were calculated using the following equations:

$$\text{Distribution ratio in blood cells (\%)} = (1 - C_P/C_B \times [100 - H_t]/100 \times 100)$$

$$R_B = C_B/C_P$$

4.11. Metabolite analysis in plasma, bile, urine, and feces

To aliquots of plasma, bile, urine, and feces after oral administration of [^{14}C]ritobegron to rats, a threefold amount of methanol was added and mixed for approximately 30 min on ice. The solution was centrifuged (15,900 × g, 2 min, room temperature), and the supernatant was evaporated to dryness at room temperature under nitrogen stream. The residue was reconstituted in 200 μL of 10 mM ammonium acetate solution/methanol (80/20, v/v). After centrifugation (15,900 × g, 2 min, room temperature), the supernatant (20 μL) was injected into the HPLC system (HP1100, Agilent Technologies, Santa Clara, CA). Separation of KUC-7322 and its metabolites was achieved with an reverse phase analytical column, Mightysil RP-18 GP (3 μm, 150 mm × 3.0 mm i.d., Kanto Kagaku, Tokyo, Japan), and a mobile phase was mixture of 10 mM ammonium acetate (A) and methanol (B). The mobile phase was passed over the column at 0.5 mL/min in the flowing linear gradient mode: starting with 20% B composition, increasing to 30% for 0 to 10 min, increasing to 100% for 10 to 20 min, maintaining 100% for 20 to 25 min, decreasing to 20% for 25 to 25.1 min, and finally maintaining 20% for 25.1 to 35 min. The column was maintained at 30 °C. The column eluate was added to a flow scintillation analyzer (FSA, Radiomatic 525TR, PerkinElmer Life and Analytical Sciences). The elution pattern of metabolites was determined using the FSA with 6 s integration. Ultima Flo-M (PerkinElmer Life and Analytical Sciences) was delivered to HPLC eluate at a 3-fold flow rate of mobile phase. Peak integration of the UV and radioactivity were performed using FLO-ONE for Windows Analysis, version 3.6.0, (PerkinElmer Life and Analytical Sciences).

4.12. Calculation of pharmacokinetic parameters

The following pharmacokinetic parameters were calculated from individual animal data by non-compartmental methods using WinNonlin Professional, Version 3.1 (Pharsight, Mountain View, CA): C_{max}, T_{max}, $AUC_{0\text{-}t}$, AUC_{inf}, CL_{tot}, and V_{ss} of radioactivity and KUC-7322 concentration in plasma. The terminal elimination rate constant (λ) was estimated by linear regression of the terminal phase of the log plasma concentration-time profile, and the $t_{1/2}$ was calculated as ln2/λ. The AUC extrapolated to infinity (AUC_{inf}) was determined by the trapezoidal rule up to the last time point ($AUC_{0\text{-}t}$) and thereafter extrapolated to infinity on the basis of λ. BA of KUC-7322 and the amount of radioactivity absorbed were calculated from percentage of

AUC_{inf} after oral administration to intravenous administration at a dose of 1 mg/kg.

References

Andersson KE (1999) Advances in the pharmacological control of the bladder. Exp Physiol 84: 195–213.

Dedrick RL, Zaharko DS, Lutz RJ (1973) Transport and binding of methotrexate *in vivo*. J Pharm Sci 62: 882–890.

Evans GH, Nies AS, Shand DG (1973) The disposition of propranolol. III. Decrease half-life and volume of distribution as a result of plasma binding in man, monkey, dog, and rat. J Pharmacol Exp Ther 186: 114–122.

Hawksworth G, Drasar BS, Hill MJ (1971) Intestinal bacteria and the hydrolysis of glycosidic bonds. Med Microbiol 4: 451–459.

Igawa Y, Yamazaki Y, Takeda H, Akahane M, Ajisawa Y, Yoneyama T, Nishizawa O (1998) Possible β3-adrenoceptor-mediated relaxation of the human detrusor. Acta Physiol Scand 164: 117–118.

Igawa Y, Yamazaki Y, Takeda H, Hayakawa K, Akahane M, Ajisawa Y, Yoneyama T, Nishizawa O, Andersson KE (1999) Functional and molecular biological evidence for a possible β3-adrenoceptor in the human detrusor muscle. Br J Pharmacol 26: 819–825.

Igawa Y, Schneider T, Yamazaki Y, Tatemichi S, Homma Y, Nishizawa O, Michel MC (2012) Functional investigation of β-adrenoceptors in human isolated detrusor focusing on the novel selective β3-adrenoceptor agonist KUC-7322. Naunyn Schmiedebergs Arch Pharmacol 385: 759–767.

Itoh T, Takemoto I, Itagaki S, Sasaki K, Hirano T, Iseki K (2004) Biliary excretion of irinotecan and its metabolites. J Pharm Pharm Sci 7: 13–18.

Maruyama I, Goi Y, Tatemichi S, Maruyama K, Hoyano Y, Yamazaki Y, Kusama H (2012a) Bladder selectivity of the novel β3-agonist ritobegron (KUC-7483) explored by *in vitro* and *in vivo* studies in the rat. Naunyn Schmiedebergs Arch Pharmacol 385: 845–852.

Maruyama I, Tatemichi S, Goi Y, Maruyama K, Hoyano Y, Yamazaki Y, Kusama H (2012b) Effect of ritobegron (KUC-7483), novel selective β3-adrenoceptor agonist, on the bladder function in cynomolgus monkey. J Pharmacol Exp Ther 342: 163–168.

Nomiya M, Yamaguchi O (2003) A quantitative analysis of mRNA expression of α1 and β-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. J Urol 170: 649–653.

Øie S (1979) Effect of altered plasma protein binding on apparent volume of distribution. J Pharm Sci 68: 1203–1205.

Rautio J, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Järvinen T, Savolainen J (2008) Prodrugs: design and clinical applications. Nat Rev Drug Discov 7: 255–270.

Smith RL (1966) The biliary excretion and enterohepatic circulation of drugs and other organic compounds. In: Jucker E (ed.) Progress in Drug Research, Vol. 9, Basel, p. 299–360.

Takasuna K, Hagiwara T, Hirohashi M, Kato M, Nomura M, Nagai E, Yokoi T, Kamataki T (1996) Involvement of β-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. Cancer Res 56: 3752–3757.

Wolfer GK, Rippon WB (1987) Protocols for use of ultrafiltration in determination of free ligand concentration and complexity of ligand/protrein interactions. Clin Chem 33: 115–117.

Yamaguchi O (2002) β3-adrenoceptors in human detrusor muscle. Urology 59: 25–29.