Journal of Pharmaceutical Investigation
Vol. 41, No. 1, 31-36 (2011)

Determination of Novel Synthetic $5HT_{2C}$ Agonist KOPC20010 by Gas-Chromatography/ Mass Spectrometry and its Bioavailability in Sprague-Dawley Rats

Hye-Yeon Im[1], Ae Nim Pae[2], Ha Yun Yang[2], Woo-Kyu Park[3], Ji Eun Seo[1,4], Md. Mamunul Haque[1,4] and Oh-Seung Kwon[1,4†]

[1]*Doping Control Center*
[2]*Neuro-Medicine Center, Korea Institute of Science and Technology (KIST), Seoul, 136-791, Korea*
[3]*Pharmacology Research Center, Korea Research Institute of Chemical Technology, Daejeon 305-343, Korea*
[4]*Biomolecular Sciences, University of Science and Technology, Daejen 305-333, Korea*

(Received January 26, 2011 · Revised February 19, 2011 · Accepted February 20, 2011)

ABSTRACT – $5HT_{2C}$ receptor among fourteen 5-HT subtypes plays important roles in several disorders such as depression, anxiety, epilepsy, schizophrenia and sleep disorders. The purpose of the study is to investigate pharmacokinetic parameters and bioavailability of a newly synthesized selective agonist of 5-HT_{2C} receptor, KOPC-20010 (KP10) in rats after intravenous and oral administration for the development of therapeutic anti-obesity agents. KP10 was administered orally (40 mg/kg) or intravenously (20 mg/kg), blood was collected via a catheter, and analyzed by GC/MSD. The calibration curve of KP10 in plasma and urine showed high linearity (r^2>0.999). The retention times of KP10 in plasma and urine were 8.7 and 9.7 min, respectively. After oral administration of 40 mg/kg, pharmacokinetic parameters were calculated as follows; C_{max} value was 1242.9±1195.5 ng/mL at 1.1±0.6 hr (T_{max}). $AUC_{0->24hr}$ and $AUC_{0->\infty}$ were 8034.2±960.7 and 10464.1±681.5 ng·hr/mL, respectively. The terminal half-life was 21.9±7.6 hr. $AUC_{0->24hr}$ and $AUC_{0->\infty}$ were 4292.4±523.0 and 6111.2±756.2 ng·hr/mL, respectively, after 20 mg/kg of intravenous administration. The terminal half-life after intravenous administration was 25.1±9.4 hr. Bioavailability of KP10 was determined to 86%. The excretion amount into the urine within 48 hr was approximately 4.7 to 6.7% of the dose administered. These data may be beneficial to the anti-obesity drug development of KP10.

Key words – $5HT_{2C}$ agonist, KOPC20010, Pharmacokinetics, Anti-obesity, Bioavailability

Serotonin (5-Hydroxytriptamine; 5-HT) is produced from the tryptophan. 5-HT receptors are known to be widely distributed in the central and peripheral nervous systems in mammals. The 5-HT plays important roles in regulation and modulation of physiological and behavioral functions. The receptors of serotonin have at least 14 different subtypes that are classified into 7 sub-family from 5-HT_1 to 5-HT_7 (Hoyer et al., 1994, 1997; Halford et al., 2007).

Among these receptors, 5-HT_{2C} receptors have been considered as therapeutic targets for the treatment of various central nervous system disorders such as depression, anxiety, epilepsy, schizophrenia, and sleep disorders (Giovanni et al., 2006; Frank et al., 2002; Issac, 2005; Millan, 2005; Tecott et al., 1995, 2003). 5-HT_{2C} target drugs like fenfluramine and dexfenfluramine have been reported to cause the reduction of body weight and food intake. However, the compounds have showed side effects by mediating the nonspecific action to subtype 5-HT receptors (Halford et al., 2007). Therefore, drug candidates to the 5-HT_{2C} receptor for anti-obesity effects have to possess good selectivity and specificity to the target receptor.

We chemically synthesized KOPC20010 (4-Fluoro-phenyl)-[2-(4-methyl-piperazin-1-yl)-6,7,8,9-tetrahydro-5H-pyrimido[4,5-d]azepin-4-yl]-amine; KP10), a selective 5-HT_{2C} agonist, with the purpose of developing therapeutic agents for anti-obesity. The trace analytical methods of the compound in the biological fluid are required for pharmacokinetic study in rats. No sensitive methods and pharmacokinetic data in the newly synthesized KP10 for 5-HT_{2C} agonist, however, are available yet. The pharmacokinetic behavior and bioavailability of drug candidates are very important to be pre-clinically evaluated.

The purpose of this study is to develop sensitive analytical methods in the plasma and urine of rats by gas chromatography/mass spectrometry for pharmacokinetic studies, and to apply to determine pharmacokinetic parameters and bioavailability of KP10.

†Corresponding Author :
Tel : +82-2-958-5184, E-mail : oskwon@kist.re.kr
DOI : 10.4333/KPS.2011.41.1.031

Materials and Methods

Materials

KOPC20010 (KP10), KOPC20011 (KP11, internal standard), and other analogues were chemically synthesized by the KIST Neuro-Medicine Center. Zoletil 50® was purchased from Virbac SA (Carros, France) and Rompun® was obtained from Bayer Korea (Suwon, Korea). Radioligands, [^{3}H] ketanserin and [^{3}H] imipramine, were purchased from PerkinElmer (PerkinElmer Life and Analytical Sciences, Boston, USA), and [^{3}H] mersulergin was obtained from Amersham Biosciences (Buckinghamshire, UK). Cloned human recombinant serotonin 5-HT_{2A} and 5-HT_{2C} receptors were obtained from Euroscreen (Brussels, Belgium). Ethyl acetate and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). The other agents used for KOPC20010 analysis were of analytical grade.

Animal treatment for blood and urine sampling

Male Sprague-Dawley rats (230±15 g) were purchased from Orient Bio (Chungbuk, Korea). The rats were acclimatized in the KIST animal facility for at least 1 week under the control of constant temperature and humidity. A 12-hr light/12-hr dark cycle was maintained beginning at 6:00 hr. Millipore-filtered tap water and feed (Samyang Co., Seoul, Korea) were provided *ad libitum*. The Internal Animal Care and Use Committee approved animal handling procedure for the experiment. One day before the pharmacokinetic study, the rat were received surgery for catheterization of the carotid artery after anesthesia (0.5 mL/kg) with equal volume of mixture of Zoletin 50 (tiletamine/zolazepam, 25 mg/mL, each; Virbac SA, Carros, France) and Rompun (Xylazine 23 mg/mL, Bayer Korea, Suwon, Korea). Polyethylene tube (PE-50) was inserted to the carotid artery and the catheter was passed subcutaneously to the dorsal nape of the neck, maintaining the catheter with heparin and holding the rat into metabolic cages through the experimental period.

Oral and intravenous administration of KP10 to rats

KP10 (40 mg/mL) was dissolved in saline (0.9%), and further diluted to 20 mg/mL solution. KP10 was either orally administered to Sprague-Dawley rats at 40 mg/kg dose by gavages, or intravenously administered to rats through the cannula of jugular vein by 20 mg/kg dose. The blood (about 400 μL) was withdrawn before administration, at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 34, and 48 hr after oral administration. The blood was collected at 0, 0.167, 0.334, 0.5, 1, 2, 4, 6, 10, 24, 34, and 48 hr after intravenous administration. The plasma was obtained by centrifugation of the blood. Urine was collected at 0-12, 12-24, 24-36, and 36-48 hr periods by using metabolic cages and the samples were stored to a refrigerator (-20°C) until analyzed.

In vitro radioligand binding assays to 5-HT_{2A} and 5-HT_{2C} receptors

[^{3}H] ketanserin binding to serotonin 5-HT_{2A} receptor-Competition binding assays at serotonin 5-HT_{2A} receptor were performed using 1 nM of [^{3}H] ketanserin, by the protocol provided by the supplier of CHO-K1 membranes (Euroscreen, Brussels, Belgium) with minor modifications. Briefly, receptor membranes (15 μg/well) were incubated at 25°C for 60 min in a final volume of 0.25 ml reaction mixture containing [^{3}H] ketanserin and various concentrations of the drug in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM $CaCl_2$, 0.1% ascorbic acid and 10 μg/mL saponin. Then, the incubations was terminated by rapid filtration using an Innotech cell harvester (Innotech Biosystems, Switzerland) through Whatman GF/C glass fiber filter presoaked in 0.05% Brij. The filter was covered with MeltiLex, sealed in a sample bag followed by drying in a microwave oven, and counted by MicroBeta Plus (Wallac, Finland). Nonspecific binding was determined in the presence of mianserin (0.5 μM). Competition binding studies were carried out with 7-8 varied concentrations of the test compounds run in duplicate tubes, and isotherms from three assays were calculated by computerized nonlinear regression analysis (GraphPad Prism Program, San Diego, USA) to yield IC_{50} values.

[^{3}H] mesulergine binding to serotonin 5-HT_{2C} receptor-Frozen membranes from stable CHO-K1 cell line expressing the human recombinant 5-HT_{2C} receptor were used. For the binding assay, [^{3}H] mesulergine (1 nM), receptor membrane (4 μg/well) and test compounds were added into 50 mM Tris-HCl buffer (pH 7.7) containing 0.1% ascorbic acid and 10 μM pargyline. Nonspecific binding was determined using 0.5 μM mianserin. The incubations were performed for 30 min at 37°C, and these were terminated by rapid filtration through Whatman GF/C glass fiber filters presoaked in 1% BSA.

Gas-chromatography/mass selective detector

The plasma concentrations of KP10 in rat plasma were determined by a gas chromatography/mass selective detector (GC/MSD; HP6890 Series/5972; Hewlett-Packard, CA, USA). The samples were injected to the instrument by an auto-liquid sampler (Agilent 7983 Series), being supported with the GC/MSD ChemStation (Kayak PC/G1701DA, Hewlett Packard, USA). Mass selective detector of electron impact mode and selected ion monitoring mode was used, and ionized energy of

the mode was 70 eV. The KP10 was separated by using the column HP-1 (17 m, length × 0.2 mm, inner diameter × 0.33 μm, film thickness; Agilent Technologies, USA). Initial temperature of the oven was set to 200°C with the holding time of 2 min, and the temperature was increased by a rate of 20°C per min to 260°C where stayed for 1 min, and increased at a rate of 25°C per min to 300°C of the final temperature with holding time of 7 min.

Temperature for urine sample analysis was set initially to 200°C and increased by a rate of 20°C per min to 260°C and 25°C per min to 290°C, at which temperature stayed for 2 and 1 min, respectively, and then increased at a rate of 25°C per min to 300°C of the final temperature where stayed for 7 min. Temperatures of inlet, transfer line, and detector were all set to 300°C. The flow rate of helium as carrier gas was 0.8 mL/min. The characteristic ions selected for GC/MSD/SIM mode were m/z 286, 257, 274 and 356 for KP10, and m/z 298 and 368 for KP 11 as internal standard.

Preparation of calibration curves of KP10

To 0.1 mL of the KP10 and KP11-free plasma and urine, KP10 was added to make final concentrations of 0, 10, 25, 50, 100, 250 and 500 ng/100 μL, each and KP11 (100 μg/mL, 10 μL) as internal standard was added in glass-centrifuged tubes with a stopper. The tubes were vortex-mixed, and 0.1 mL of 0.5 M K_2CO_3 for plasma, or 0.2 mL of 0.5 M K_2CO_3 for urine and 0.5 mL distilled water were added and agitated. After addition of 5 mL ethyl acetate, the tubes were shaken for 30 min on a shaker (100-150 rpm, 7400 Tubingen, Edmund Buchler, Germany) and centrifuged at 2500 rpm (900 g) for 5 min (Varifuge 3.0, Heraeus, Germany). The organic layer was transferred to a new tube after freezing the tube in a freezer. Ethyl acetate was evaporated by a nitrogen evaporator and the tube was placed in a desiccator. The residue was reconstituted with 50 μL of methanol. Two μL of the solution was injected to GC/MSD by an auto liquid sampler.

Clean-up and analysis of serum samples

The plasma or urine samples obtained from rats were thawed at room temperature. To tubes, 0.1 mL of the plasma and urine was added to glass-centrifuged tubes with stoppers and the internal standard was added. All the steps are the same as described above. KP10 concentrations in the plasma samples were determined by GC/MSD.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from the time-plasma concentrations of KP10 by non-compartmental analysis by using WinNonlin software (Scientific Consulting Inc., Cary, NC, USA). The area under the curve (AUC) of plasma concentration-time profile was established by a linear trapezoidal rule from the time-plasma concentration curves of KP10. AUCs of time-plasma concentration of KP10 until the last sampling time ($AUC_{0\ to\ last}$) was determined by the equation of $AUC_{0\ to\ inf} = AUC_{0\ to\ last} + C_{last/\beta}$, where β is the slope of the terminal phase of the time-log plasma concentration curve and C_{last} is the concentration at the last sampling time.

Results and Discussion

Method validation of KP10 in rat plasma and urine by GC/MSD

The GC/MSD scan spectra of KP10 were showed in Figure 1. The base peak ions were m/z 286 and m/z 298 of KP10 and internal standard KP11, respectively. Molecular weights of KP10 and KP11 were also clearly observed. The retention times of KP10 in plasma and urine were 8.7 and 9.7 min, respectively (Figure 2). KP11 has a very similar chemical structure to KP10. Retention times of internal standard KP11 in the plasma and urine were showed at 11.2 and 13.5 min, respectively (Figure 2). No interfering peaks were found (Figure 2). The calibration curves of KP10 showed good linearity for plasma (r^2=0.999; y=0.0016 x -0.0064) in the range of 0.1-5 μg/mL, and urine (r^2=0.999; y=4.13 x -1.48) in the range of 10-500 μg/mL, as showed in Figure 3.

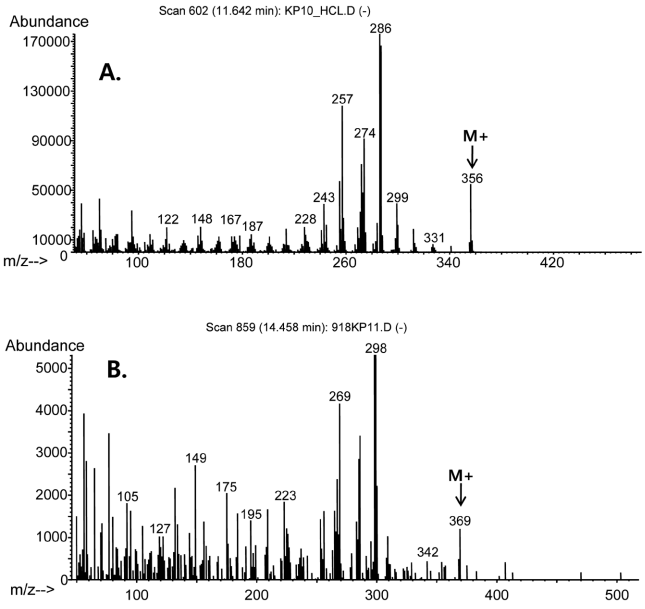

Figure 1. GC/MSD scan spectra of KP10 (A) and internal standard KP11 (B). Base peaks of KP10 and KP11 were m/z 286 and 298, respectively. Molecular weights of the compounds were indicated as M^+ in the spectra of the compounds.

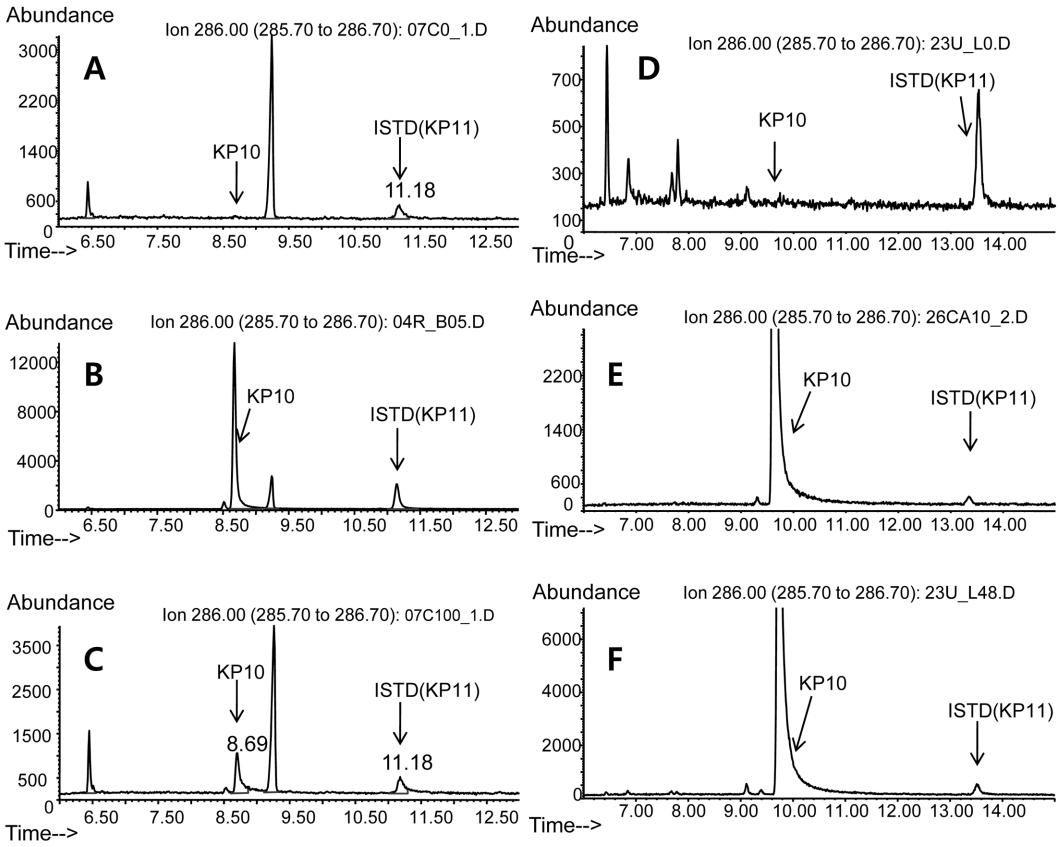

Figure 2. GC/MSD ion chromatograms of KP10. Blank plasma (A), spiked 100 ng KP10 and 1000 ng KP11 in free-plasma (B), the plasma sample 0.5 hr after oral administration of 40 mg/kg KP10 (C), Blank urine (D), spiked 10 ng KP10 and 1000 ng KP11 in free-plasma (E), and the urine sample 48 hr after intravenous administration of 20 mg/kg KP10 (F). Retention times of KP10 and KP11 in plasma were about 8.7 and 11.2 min, and those in urine were about 9.7 and 13.5 min, respectively.

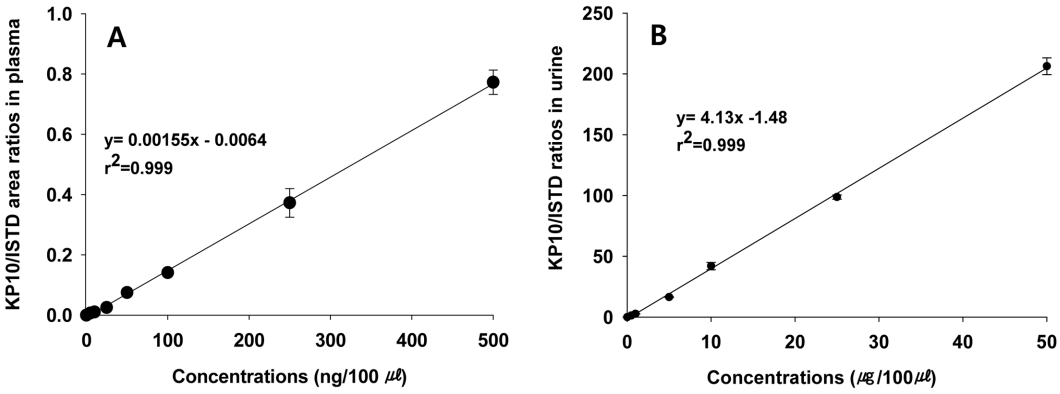

Figure 3. The calibration curves of KP10 in rat plasma (A) and urine (B). The curves showed high linearity (r^2>0.999) at concentrations ranging from 10 to 500 ng/100 μL for plasma, and 10 to 50 μg/100 μL for urine.

Intra-day precision and accuracy were determined. Precision % was less than 12.8% and accuracy % was ranged from 81.2 to 109.7% in plasma (Table I). Precision and accuracy % in urine samples were less than 5% and 13.5%, respectively (Table II). Analytical method for determining KP10 in the plasma and urine of rats was validated by using GC/MSD/SIM, and applied to pharmacokinetics of KP10 after oral (40 mg/kg) and intravenous (20 mg/kg) administration.

Binding affinity

Binding affinity to 5-HT_{2A} and 5-HT_{2c} receptors was measured by using the cell membrane of CHO-K1 cell line. IC_{50}s

Table I. *Intra-day precision and accuracy for the determination of KP10 in rat plasma*

Concentrations (ng/mL plasma)	C.V. (%)	Accuracy (%)
100	11.6	109.7
250	8.0	81.2
500	12.2	105.1
1000	6.8	95.0
2500	12.8	97.8
5000	5.2	100.5

Table II. *Intra-day precision and accuracy for the determination of KP10 in rat urine*

Concentrations (μg/mL urine)	C.V. (%)	Accuracy (%)
10	4.68	100.31
50	2.08	86.47
100	7.13	105.12
250	1.78	96.98
500	3.33	100.59

of KP10 for the binding affinity were determined to 215 nM to 5-HT_{2A} receptor and 337 nM to 5-HT_{2c} receptor, indicating that binding affinity to two 5-HT subtypes were very similar and potency of KP10 to the receptors is high as nM ranges of IC_{50}.

Pharmacokinetics of KP10 in rat plasma

The compound KP10 was synthesized to develop specific 5-HT_{2C} receptor agonists for anti-obesity effects. The plasma concentrations-time curves of KP10 are shown in Figure 4. Pharmacokinetic parameters of KP10 were determined by non-compartmental methods as shown in Table III. AUC_{last}, AUC_{inf}, and C_{max} were determined after oral and intravenous administration of 40 and 20 mg/kg KP10, respectively. The terminal half-life of KP10 was about 21.9-25.1 hr after intravenous administration and oral administration. Clearance was about 3.3 L/hr/kg (or, 55 mL/min/kg), and the volume of distribution (V_β) was 117 L/kg after intravenous doses. AUC_{inf} and AUC_{last} were 6111.2 and 4292.4 ng.hr/ml for 20 mg/kg (i.v.) and 10464.1 and 8034.2 ng.hr/ml for 40 mg/kg (p.o.). The ratios of AUC_{last} to AUC_{inf} were 76.8 and 70.2% in 20 and 40 mg/kg KP10 treatment, respectively. C_{max} and T_{max} of KP10 were 1242.93 ng/mL and 1.13 hr after oral administration, indicating a rapid absorption from the gastrointestinal tract to the systemic circulation. The relative bioavailability after oral administration of KP10 to rats was determined to be 86%

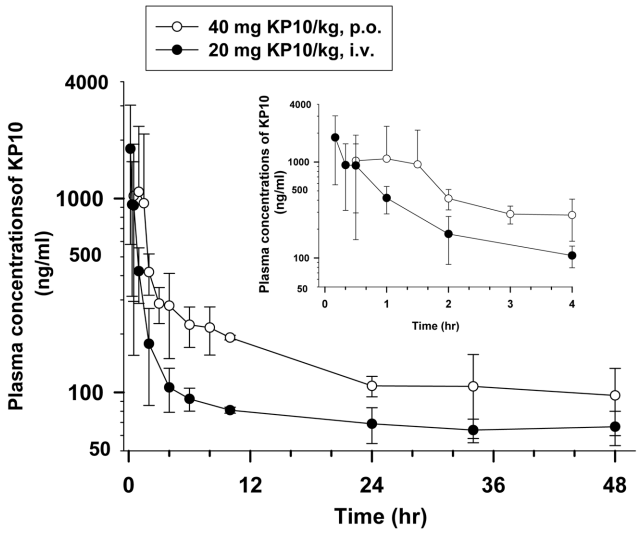

Figure 4. The time-mean plasma KP10 concentration curves after intravenous (20 mg/kg, n=4) and oral (40 mg/kg, n=4) administrations to rats. Inset indicates the plasma KP10 concentrations enlarged between 0 to 4 hr.

Table III. *Non-compartmental pharmacokinetic parameters of KP10 after 20 mg/kg intravenous and 40 mg/kg oral administrations to rats*

	20 mg/kg, iv (n=4)	40 mg/kg, po (n=4)
AUC_{INF} (ng·hr/mL)	6111.21±756.17	10464.09±681.47
AUC_{last} (ng·hr/mL)	4292.41±522.97	8034.16±960.74
$AUMC_{INF}$ (ng·hr^2/mL)	217991.76±107537.40	320826.88±144996.50
$AUMC_{last}$ (ng·hr^2/mL)	64954.42±28283.87	121804.83±36771.43
CL (mL/hr/kg)	3311.34±417.15	-
CL/F (mL/hr/kg)	-	3834.28±239.34
C_{last} (ng/mL)	67.85±11.23	85.85±36.63
C_{max} (ng/mL)	1802.33±1223.18	1242.93±1195.51
k (hr^{-1})	0.03±0.02	0.04±0.02
MRT_{INF} (hr)	34.78±15.93	30.43±13.16
MRT_{last} (hr)	15.22±6.61	15.60±5.52
$t_{1/2}$ (hr)	25.09±9.40	21.87±7.61
T_{max} (hr)	-	1.13±0.63
V_{ss} (mL/kg)	112001.06±49643.85	-
V_β (mL/kg)	117120.51±41290.18	-
RBA(%)[a]		85.61

(Table III). This high bioavailability may be due to rapid and high absorption. Figure 5 was showed the urinary excretion rate of KP10. In this study, the excretion amount into the urine within 48 hr at oral and intravenous administrations to rats was amount to 1875.8±491.6 and 1332.1±666.2 μg/kg body weight, respectively, and these amount is approximately corresponding

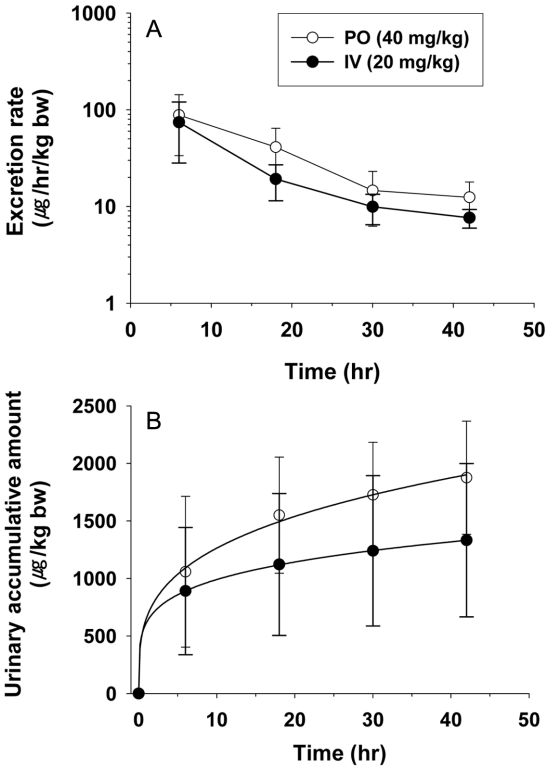

Figure 5. Urinary excretion rates (A) and urinary accumulative amount (B) of KP10 after oral and intravenous administration of a single dose of KP10 to rats.

to 4.7 and 6.7% of the doses administered, respectively.

WAY-163909 was developed as a 5-HT_{2C} agonist. This compound was administered either intravenously as 2 mg/kg or orally as 5 mg/kg. Its clearance was 0.76 L/hr/kg and high bioavailability of 71% was observed in rats (Dunlop et al., 2006). Recently, lorcaserin in Arena Pharmaceuticals Inc. was also reported to have anti-obesity effects as potent 5-HT_{2C} agonists (Thomsen et al., 2008). We have reported 5-HT_{2C} agonist KKHQ80119 and 80114 in rats, and its bioavailability showed very low values less than 0.23. And urinary excretion amount was found to be less than 1% (Im et al., 2009). Compared to previous reports, KP10 shows excellent property in bioavailability. The pharmacokinetic behavior of WAY-163909 or lorcaserin was very similar to those of KP10 in terms of having high bioavailability.

In summary, pharmacokinetic studies and analytical method of new synthetic chemical KP10 were conducted. Pharmacokinetic parameters and bioavailability were determined for the first time in rats after oral and intravenous administration of KP10. The information will be beneficial to further study of KP10 for anti-obesity drug development.

References

DiPiro, J.T., Spruill, W.J., Wade, W.E., Blouin, R.A., Pruemer, J.M., 2005. Half-life, elimination rate and AUC: In Concept in Clinical Pharmacokinetics (4th Ed.), American Society of Health-System Pharmacists, pp. 29-44.

Dunlop, J., Marquis, K.L., Lim, H.K., Leung, L., Kao, J., Cheesman, C., Rosenzweig-Lipson S., 2006. Pharmacological Profile of the 5-HT_{2C} Receptor Agonist WAY-163909; Therapeutic Potential in Multiple Indications. CNS Drug Reviews, 12(3-4), 167-177.

Frank, M.G., Stryker, M.P., Tecott, L.H., 2002. Sleep and sleep homeostasis in mice lacking the 5-HT_{2c} receptor. Neuropharmacology. 27, 869-873.

Giovanni, G. Di, Matteo, V., Pierucci, M., Benigno, A., Espoito, E., 2006. Central serotoinin 2C receptor: from physiology to pathology. Curr. Top. Med. Chem. 6, 1909-1925.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharrane E.J., Saxena P.R., Humphrey P.P.A., 1994. Internation Union of Pharmacology classification of receptors for hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157-204.

Hoyer, D., Martin, G.R., 1997. 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. Neuropharmacology. 36, 419-428.

Im, H.Y., Choo, H.A., Pae, A.N., Kwon, O.S., 2009. Pharmacokinetics and bioavailability of new synthetic 5-HT_{2C} agonists, KKHQ80109 and KKHQ80114, in Sprague-Dawley rats. J. Kor. Pharm. Sci., 39(5), 327-331.

Issac, M., 2005. Serotonergic 5-HT_{2C} receptors as a potential therapeutic target for the design of antiepileptic drugs. Curr. Top Med. Chem. 5, 59-67.

Halford, J.C.G., Harrold, J.A., Boyland, EJ., Lawton, C.L., Blundell, J.E., 2007. Serotonergic drugs effects on appetite expression and use for the treatment of obesity. Adis Data Information BV. 67, 27-55.

Millan, M.J., 2005. Serotonin 5-HT_{2c} receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. Therapie. 60, 441-460.

Tecott, L.H., Sun, L.M., Akana, S.F., Strack, A.M., Lowenstein, D.H., Dallman, M.F., Julius, D., 1995. Eating disorders and epilepsy in mice lacking 5-HT_{2c} serotonin receptors. Nature, 374 (6522), 542-546.

Tecott, L.H., Abdallah, L., 2003. Mouse genetic approaches to feeding regulation: serotonin 5-HT_{2C} receptor mutant mice. CNS Spectr. 8, 584-588.

Thomsen, W.J., Grottick, A.J., Menzaghi, F., Reyes-Saldana, H., Espitia, S., Yuskin, D., Whelan, K., Martin, M., Morgan, M., Chen, W., Al-Shamma, H., Smith, B., Chalmers, D., Behan, D., 2008. Lorcaserin, a novel selective human 5-Hydroxytryptamine$_{2C}$ Agonist: in vitro and *in vivo* pharmacological characterization. The Journal of Pharmacology and Experimental Ttherapeutics. 325, 577-587.