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Pharmacokinetics and Bioavailability of New Synthetic 5-HT_{2C} Agonists, KKHQ80109 and KKHQ80114, in Sprague-Dawley Rats

Hye-Yeon Im[1], Hyun-Ah Choo[2], Ae-Nim Pae[2] and Oh-Seung Kwon[1†]

[1]*Bioanalysis and Biotransformation Research Center*
[2]*Center for Chemoinfomatics Research, Korea Institute of Science and Technology, Seoul, 136-791, Korea*

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ABSTRACT – 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. We chemically synthesized KKHQ80109 (K09) and KKHQ80114 (K14), selective 5-HT_{2C} agonists, with the purpose of developing therapeutic agents for the treatment of obesity. The objective of this work is to investigate pharmacokinetic parameters and bioavailability of K09 and K14 in rats given orally or intravenously. Oral administration of 20 mg/kg K09 results in 4.11 hr of the terminal half-life and 89.16 ng/mL of C_{max} at 5.00 hr (T_{max}). The terminal half-life of K14 was 3.83 hr with 215.81 ng/mL of C_{max} at 3.33 hr (T_{max}) after oral dosing of 20 mg/kg K14, indicating that K14 is more rapidly absorbed than K09. Bioavailability showed 0.17-0.21 for K09 and 0.19-0.23 for K14. Urinary excretion of parent K09 and K14 was less than 1%, indicating that K09 and K14 undergo very extensive hepatic metabolism.

Key words – 5-HT_{2C} agonists, KKHQ80109, KKHQ80114, Pharmacokinetics, Bioavailability, Rats

5-Hydroxytriptamine (5-HT) is widely distributed in the central and peripheral nervous systems in mammals and to play important roles in regulation and modulation of physiological and behavioral functions. At least 14 different 5-HT receptor subtypes exist and these are classified into 7 sub-family from 5-HT_1 to 5-HT_7.[1,2)]

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.[3-8)] 5-HT_{2C} receptor mRNA is found in brain regions of the rat that are involved in feeding behavior, including choroids plexus, the nucleus of the solitary tract, dorsal medial hypothalamic nucleus, paraventricular hypothalamic nucleus, amygdala and other brain regions related with the regulation of appetite.[9-10)]

KKHQ80109 (K09; N-benzyl-N-(2-(2-ethylpiperidin-1-yl) ethyl)-4-propylbenzene sulfonamide hydrochloride) and KKHQ 80114 (K14; N-(benzyl)-N-(1-ethyl-piperidine)-3-trifluomethylbenzenesulfonamide) are new synthetic chemicals with function of selective 5-HT_{2C} agonists with the purpose of developing therapeutic agents for the treatment of obesity (patent submission).[11)] The pharmacokinetic behavior of drug candidates of acting mainly on the central nervous system is considered to be one of significant factors to be pre-clinically evaluated. Bioavailability of drug candidates is specifically a crucial point among several different endpoints to be examined since drug effects are exerted through the circulation into the blood system and is proportional to the concentrations of drugs in the blood. No pharmacokinetic data in the new synthetic 5-HT_{2C} agonist derivatives, K09 and K14, is available yet. The objective of the work is to determine and compare pharmacokinetic parameters and bioavailability of K09 and K14 in rats.

Materials and Methods

Chemicals

KKHQ80114 (K14), KKHQ80109 (K09) and other analogues were chemically synthesized by the KIST chemoinfomatics group.[11)] 1-Methyl-2-pyrrolidine, Cremophore® EL, ethanol, polyethylene glycol 400 and dithiothreitol were obtained from Sigma (St. Louis, MI, USA). Zoletil 50® was purchased from Virbac SA (Carros, France) and Rompun® was from Bayer Korea (Suwon, Korea). Ethyl acetate and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). The other agents used for K14 and K09 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

†본 논문에 관한 문의는 이 저자에게로
Tel : 02)958-5184, E-mail : oskwon@kist.re.kr
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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 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 K09 or K14 to Rats

K09 or K14 was dissolved in polyethylene glycol 400/ethanol (95:5, v/v) in 20 mg/kg (dosing volume of 1 mL/kg) for oral administration, 2 and 10 mg/kg (dosing volume of 0.5 mL/kg) for intravenous administration. K09 or K14 of 20 mg/kg dose was orally administered to the rat by a gavage. K09 or K14 (10 and 2 mg/kg, each) was intravenously administered to rats via a catheter of jugular vein. Each group was consisted of 3-4 male rats. The blood (about 450 μL) was withdrawn before administration, at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 hr after oral administration, and at 0.17, 0.33, 0.5, 1, 2, 4, 6, 10, 24, 34, and 48 hr after intravenous administration of K09 or K14. The plasma obtained by centrifugation was stored to a refrigerator (-20°C) until analysis. Urine was collected before administration, 0-12, 12-24, 24-36, and 36-48 hr periods, and the sample was stored to a refrigerator (-20°C) until analysis.

Gas-chromatography/mass Selective Detector

The plasma concentrations of K09 and K14 in rat plasma were determined by a gas chromatography/mass selective detector (GC/MSD; HP 6890 Series/5972; Hewlett-Packard, CA, USA). The samples were injected to the instrument by an autoliquid 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 were used, and ionized energy of the mode was 70 eV. The K09 or K14 was separated by using the column Ultra-2 (17 m length x 0.2 mm inner diameter x 0.33 μm film thickness; Agilent Technologies, USA). Initial temperature of the oven was set to 180°C, at which the temperature was increased by a rate of 30°C per min to 260°C without holding time, and increased at a rate of 10°C per min to 300°C of the final temperature where stayed for 12 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 were m/z 91, 126, 245 and 441 for K14, and m/z 91, 126, 245 and 399 for K09. The ion of m/z 126 was used for the quantitation of K14 and K09, or in reverse way.

Determination of K09 and K14 in Rat Plasma

To 0.1 mL of the K14 and K09-free plasma or 1 mL blank urine, K14 or K09 was added to make final concentrations of 0, 5, 7.5, 10, 25 and 50 ng/mL each and K09 (10 μg/mL, 20 μL) as internal standard to glass-centrifuged tubes with stopper. Alternatively, K14 was used as internal standard for the determination of K09. The tubes were mixed by vortexing, and 0.1 ml of 0.5 N potassium carbonate and 0.5 mL of distilled water were added and agitated. After addition of 5 mL ethyl acetate, the tubes were shaken for 20 min on a shaker (100-150 rpm; 7400 Tubingen, Edmund Buchler, Germany) and centrifuged at 2500 rpm (900 g) for 10 min (Varifuge 3.0, Heraeus, Germany). The organic layer was transferred to a new tube after freezing the tube in a freezer (-30°C, Ecoline RE112, Lauda, Germany). Ethyl acetate was evaporated by a nitrogen evaporator (TurvoVap, Zymark, Hopkinton, MA, USA) and the tube was placed in a desiccator with potassium pentoxide/potassium hydroxide for at least 3 hr. The residue was dissolved in 50 μl of methanol, and 2 μL of the solution was injected to GC/MSD by an auto liquid sampler. The method was validated by measuring intra- and inter-day precision and accuracy in the plasma and urine.

The plasma and urine samples obtained from rats were thawed at room temperature. To the tube, 0.1 mL of the plasma and 1 ml of the urine samples were added to glass-centrifuged tubes with stopper with the internal standard spiked and was prepared as described above. The plasma and urine concentrations of K14 in rats were determined, based on the calibration curve from peak area ratios of K14 to the internal standard.

Pharmacokinetic Analysis

Pharmacokinetic parameters were determined from the time-plasma concentrations of K09 or K14 by non-compartmental analysis by using WinNonlin software (Scientific Consulting Inc., Cary, NC, USA). The terminal half-life ($t_{1/2}$) was calculated using at least three data points in the terminal phase excluding the C_{max}. The area under the curve (AUC) of plasma

concentration-time profile was established by linear trapezoidal rule from the time-plasma concentration curves of K09 or K14. The area under the curve of time-plasma concentrations of K09 or K14 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 â 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.[12)]

Results and Discussion

The compounds K09 and K14 are synthesized to develop specific 5-HT_{2C} receptor agonists. The binding affinity (Ki) of K09 to 5-HT_{2A} and 5-HT_{2C} receptors were determined to be 909 and 49 nM, and of K14 to be 1232 and 91 nM, respectively, indicating that K09 and K14 possess more potency to 5-HT_{2C} than 5-HT_{2A} receptor by 18.6 and 13.5-fold (patent submitted).[11)]

The plasma disappearance curves of K09 are shown in Figure 1. The pharmacokinetic parameters of K09 after one oral and two different intravenous single doses were determined by non-compartmental methods as shown in Table I. Intravenous administrations of 2 and 10 mg/kg K09 resulted in dose-dependent increases of AUC_{last}, AUC_{inf}, and C_{max}. The terminal half-lives of K09 were about 5.6 to 6.6 hr after intravenous administrations and 4.1 hr after oral administration. Clearance of K09 was about 4382 to 5875 mL/min/kg in 2 and 10 mg/kg doses, and the volume of distribution (V_β) was 32642 and 41522 ml/kg in 2 and 10 mg/kg intravenous doses, respectively, indicating that some variation exists between two different doses. Relative bioavailability of K09 in rats was determined to be 17-21%.

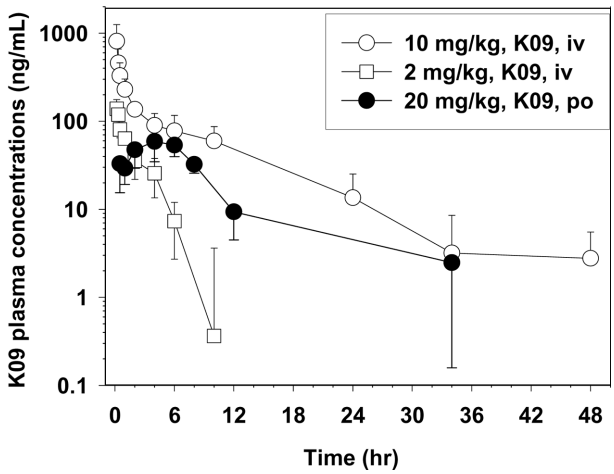

Figure 1–Plasma concentrations–time curves of K09 after intravenous (2 and 10 mg/kg) and oral administrations (20 mg/kg) of K09 to rats (n=3-4 rats /dose group, mean±S.E.). iv, intravenous; po, oral administration.

Table I–*Model-independent pharmacokinetic parameters of K09 after intravenous (2 and 10 mg/kg) and oral (20 mg/kg) administrations of K09 to rats*

	10 mg K09/kg, i.v. (n=4)	2 mg K09/kg, i.v. (n=4)	20 mg K09/kg, p.o. (n=4)
AUC_{inf} (ng·hr/mL)	2037.40±436.59	493.72± 78.13	835.60±169.94
AUC_{last} (ng·hr/mL)	1960.55±435.83	433.69±75.71	713.70±168.78
$AUMC_{inf}$ (ng·hr^2/mL)	20374.71±10194.80	3338.50±1417.56	9777.61±3217.11
$AUMC_{last}$ (ng·hr^2/mL)	17272.80±8919.55	1997.41±1116.68	6535.02±2822.23
CL (mL/hr/kg)	5875.52±1579.33	4382.66±711.53	-
CL/F (mL/hr/kg)	-	-	26681.44±4602.72
C_{last} (ng/mL)	10.08±7.18	8.88±3.81	21.78±4.13
C_{max} (ng/mL)	965.44±470.57	337.89±133.19	89.16±17.74
k (hr^{-1})	0.23±0.12	0.14±0.02	0.21±0.06
MRT_{inf}(hr)	8.13±3.78	5.97±1.73	11.40±2.80
MRT_{last} (hr)	7.01±3.30	3.83±1.51	8.52±2.27
$t_{1/2}$ (hr)	6.63±2.63	5.57±1.26	4.11±0.95
T_{max} (hr)	-	-	5.00±1.29
V_{ss} (mL/kg)	34303.11±13732.69	22858.08±3118.53	-
V_β (mL/kg)	41522.46±12696.67	32642.96±4352.11	-
BA[a]	-	-	0.17-0.21

[a]Bioavailability.

The plasma concentration-time curves of K14 and its pharmacokinetic parameters were shown in Figure 2 and Table II, respectively. AUC_{inf} and AUC_{last} were increased dose-dependently in intravenous doses of 2 and 10 mg/kg K14. The ratios of AUC_{last} to AUC_{inf} were 93.8 and 97.5% in 2 and 10 mg/kg K14 treatment, respectively. In rats orally treated with 20 mg/kg K14, the ratio of AUC_{last} to AUC_{inf} was 96.4%. The clearance and volume of distribution of K14 were about 3500 mL/hr/kg and 10106 to 24240 mL/kg. The terminal half-lives ($t_{1/2}$) of K14 were ranged from 2.2 to 4.7 hr, showing shorter half-life than that of K09. Tmax of K14 was 3.33 hr, compared to 5.0 hr of K09. Relative bioavailability of K14 in rats was determined to be 19-23% that was very similar to that of K09.

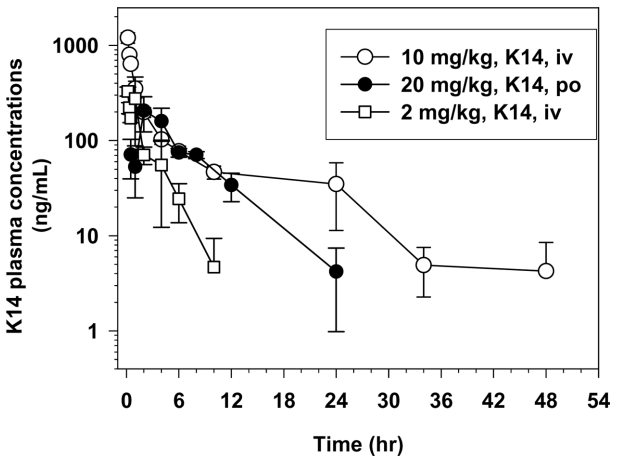

Figure 2–Plasma concentrations–time curves of K14 after intravenous (2 and 10 mg/kg) and oral administrations (20 mg/kg) of K14 to rats (n=4 rats /dose group, mean±S.E.). iv, intravenous; po, oral administration.

Solubility of K09 and K14 was 23 and 43 μM in 0.1% dimethylsulfoxide in 1 mM PBS buffer (pH 7.4), indicating that trifluoromethyl group of K14 has better solubility than the propyl group of K09. The difference in solubility between K09 and K14 may cause the different volume of distribution (V_{β}, 41522 mL/kg for 10 mg/kg K09 *vs.* 24239 mL/kg for 10 mg/kg K14; 32642 mL/kg for 2 mg/kg K09 *vs.* 10105 mL/kg for 2 mg/kg K14) and half-lives ($t_{1/2}$, 5.6 and 6.6 hr for 2 and 10 mg/kg K09 *vs.* 2.2 and 4.7 hr for 2 and 10 mg/kg K14) as shown in Table I and II. Two compounds showed a great difference in absorption when T_{max} and Cmax values were compared after oral administration (Tmax, 5.0±1.3 hr for K09 *vs.* 3.3±1.3 hr for K14; Cmax, 89.2±17.7 ng/mL for K09 *vs.* 215.8±73.2 ng/mL for K14), resulting in a rapid absorption of K14 than K09 from the gastrointestinal tract to the systemic circulation.

An insignificant amount of parent drugs K09 and K14 administered was excreted into urine (Figure 3). This indicates that K14 and K09 may be extensively metabolized. Bio-

Table II–*Model-independent pharmacokinetic parameters of K14 after intravenous (2 and 10 mg/kg) and oral (20 mg/kg) administrations of K14 to rats.*

	10 mg K14/kg, i.v. (n=4)	2 mg K14/kg, i.v. (n=4)	20 mg K14/kg, p.o. (n=3)
AUC_{inf} (ng·hr/ml)	3041.13±372.35	723.05± 284.20	1386.73±398.95
AUC_{last} (ng·hr/ml)	2966.40±375.72	678.31± 276.08	1336.65±411.64
$AUMC_{inf}$ (ng·hr^2/ml)	31632.79±11045.17	1842.18± 996.74	9369.05±2491.77
$AUMC_{last}$ (ng·hr^2/ml)	28566.28±10986.42	1335.95± 814.88	8105.54±2444.03
CL (ml/hr/kg)	3459.02±474.27	3559.69±1012.84	-
CL/F (ml/hr/kg)	-	-	18188.46±6695.34
C_{last} (ng/ml)	11.00±0.98	13.84± 0.69	8.63±3.38
C_{max} (ng/ml)	1876.98±395.18	491.89± 60.77	215.81±73.22
k (hr^{-1})	0.15±0.02	0.35± 0.09	0.19±0.03
MRT_{inf} (hr)	9.78±2.37	2.27± 0.36	6.87±0.56
MRT_{last} (hr)	8.91±2.49	1.67± 0.38	6.06±0.24
$t_{1/2}$ (hr)	4.70±0.49	2.21± 0.48	3.83±0.70
T_{max} (hr)	0±0	0± 0	3.33±1.33
V_{ss} (ml/kg)	31793.60±5304.45	7363.13±1389.94	-
V_{β} (ml/kg)	24239.66±5585.83	10105.96±2262.09	-
BA[a]	-	-	0.19-0.23

[a]Bioavailability.

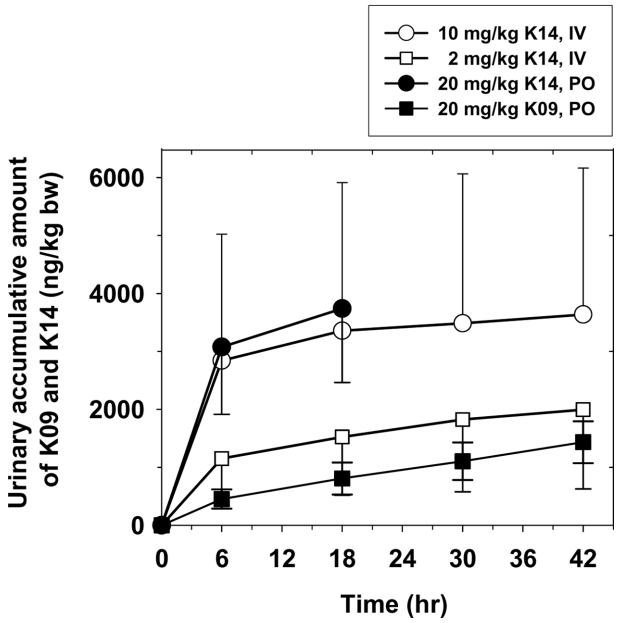

Figure 3–Urinary accumulative amount of K09 and K14 in rats treated with intravenous or oral administration of K09 and K14.

availability was approximately 20% in both K09 and K14 (Table I and II). This low bioavailability may be due to extensive metabolism and low absorption.

In our experiment, urinary excretion of parent compounds of K09 and K14 was much less than 1%, indicating that extensive hepatic metabolism occurs, even though we may consider that urine collection by a metabolic cage has limitation of urinary sampling including the loss of urine volume due to evaporation or spreading to the large surface of glass balls. Further metabolism studies of these compounds are on-going currently.

Among phenyl benzensulfonamide analogues, SB-357134 has been identified as high affinity and selective 5-HT_6 antagonists. Clearance was 840 mL/hr/kg and high bioavailability of 65% was observed. Other analogues showed clearance of 4980 mL/hr/kg and less than 20% of bioavailability in rats,[13] of which the pharmacokinetic parameters were very close to those of K09 and K14.

In summary, pharmacokinetic studies of new synthetic chemicals K09 and K14 were conducted and pharmacokinetic parameters and bioavailability were determined for the first time in rats after oral and intravenous administration of K09 and K14. K14 is absorbed more rapidly than K09. Further metabolism studies are required to elucidate the extensive hepatic metabolism.

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