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Dose-Independent Pharmacokinetics of a New Peroxisome Proliferator-Activated Receptor-γ Agonist, KR-62980, in Sprague-Dawley Rats and ICR Mice

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The pharmacokinetics of a novel peroxisome proliferator-activated receptor-γ agonist, KR-62980, were characterized *in vitro* with respect to liver metabolic stability, cell permeability, and plasma protein binding and *in vivo* using Sprague-Dawley rats and ICR mice. The metabolic half-life of 0.1-10 μM KR-62980 was 11.5-15.2 min in rat liver microsomes and 25.8-28.8 min in human liver microsomes. KR-62980 showed high permeability across MDCK cell monolayers, with apparent permeability coefficients of 20.4×10^{-6} to 30.8×10^{-6} cm/sec. The plasma protein binding rate of KR-62980 was 89.4%, and most was bound to serum albumin. After intravenous administration of KR-62980 (2 mg/kg), the systemic clearance was 2.50 L/h/kg, and the volume of distribution at steady-state was 9.16 L/kg. The bioavailability after oral administration was approximately 60.9%. The dose-normalized AUC values were 0.50 ± 0.09, 0.41 ± 0.20, and 0.62 ± 0.08 h·μg/mL after oral administration of 2, 5, and 10 mg/kg KR-62980, respectively, showing no dose-dependency. The *in vivo* pharmacokinetic parameters in ICR mice were also dose independent. These data suggest that KR-62980 is not significantly dose dependent in rats or mice, although it may disappear rapidly from the systemic circulation via metabolism in the liver.

Key words: KR-62980, Pharmacokinetics, Dose-dependency, Liver microsomal stability, Cell permeability, Plasma protein binding

INTRODUCTION

Peroxisome proliferator-activated receptor (PPAR)-γ is an important nuclear receptor involved in lipid and glucose metabolism (Evans et al., 2004) and is crucial in obesity-related metabolic diseases such as hyperlipidemia, insulin resistance, and coronary artery disease (Adams et al., 1997; Brown et al., 1999; Ye et al., 2001; Calnek et al., 2003; Rangwala et al., 2003; Chui et al., 2005; Odegaard et al., 2007).

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KR-62980, 1-(trans-methylimino-*N*-oxy)-6-(2-morpholineoethoxy)-3-phenyl-1H-indene-2-carboxylic acid ethyl ester (Fig. 1), acts as a selective PPAR-γ agonist and was developed for the treatment of type 2 diabetes. Its half-maximal effective concentration (EC_{50}) as a PPAR-γ agonist in transactivation assays was only 15 nM (Kim et al., 2006a). KR-62980 shows anti-hyperglycemic activity, including *in vivo* glucose lowering activity, with little weight gain (Kim et al., 2006b, 2007). Furthermore, KR-62980 has recently been reported to enhance the interaction between PPAR-γ and nuclear TAZ to suppress adipocyte differentiation (Jung et al., 2009; Won et al., 2010). These data suggest that KR-62980 may be a good candidate drug for the treatment of type 2 diabetes.

To explore the possible use of KR-62980 as a new

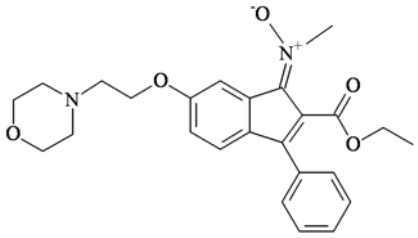

Fig. 1. Structure of the selective PPAR-γ agonist KR-62980, 1-(trans-methylimino-*N*-oxy)-6-(2-morpholineoethoxy)-3-phenyl-1H-indene-2-carboxylic acid ethyl ester

drug for the treatment of type 2 diabetes, this study evaluated the pharmacokinetic profile of KR-62980 after intravenous or oral administration at various doses in Sprague-Dawley rats and ICR mice. In addition, pharmacokinetic properties such as rat liver microsomal stability, MDCK cell permeability, and plasma protein binding rate of KR-62980 were determined *in vitro*.

MATERIALS AND METHODS

Chemicals

KR-62980 (> 99.0% purity) was synthesized by the Korea Research Institute of Chemical Technology (KRICT). NADPH regeneration solution was purchased from BD Biosciences. Zoletil 50 was purchased from VIRBAC Laboratories. Imipramine (internal standard), human serum albumin, and other reagents were purchased from Sigma-Aldrich. Acetonitrile and other solvents were HPLC grade or the highest quality available and were purchased from J. T. Baker.

Animals

Male Sprague-Dawley rats and male ICR mice (CD-1) were purchased from NARA-Bio Company. All animals were cared for in an air-conditioned room at a temperature of 22 ± 2°C under specific pathogen-free conditions. Food and water were supplied *ad libitum*. The animals were fasted, except for water, for 12 h before the experiments. All animal procedures were approved by the KRICT Animal Care and Use committee.

Physicochemical properties

The equilibrium solubility of KR-62980 was measured using the shake-flask method. Briefly, KR-62980 was added in excess to water in a flask, and the suspension was shaken at 25°C for 24 h. After equilibration, the solution was filtered, and the concentration of KR-62980 in the filtrate was quantified using ultra-high-performance liquid chromatography (Waters) with detection at a wavelength of 254 nm. To measure the kinetic solubility of KR-62980, test solutions (200 μL) containing various amounts of 10 mM KR-62980 stock solution in DMSO (5% final DMSO concentration) were transferred to microplates, which were placed in a nephelometer (NEPHELOstar Galaxy; BMG Lab Technologies) and incubated for 24 h at 30°C with mild shaking. The nephelometer possessed a 635-nm laser as the radiating source, with a laser beam focus between 1.5 and 3.5 mm. The signal measured by the detector increased linearly with particle concentration. The solubility point was taken as the concentration at which the nephelometric reading deviated from the background, based on a standard deviation algorithm. The pKa constant and lipophilicity profile of KR-62980 were measured by the D-PAS method using a specialized physicochemical instrument (GLpKa; Sirius Analytical Instruments) with a combined electrode. The values were calculated with Sirius pKa LogP software, and the computational predictions were made using ACD/Labs software from Advanced Chemistry Development, Inc.

Liver microsomal stability

The metabolic stability of KR-62980 in human and rat liver microsomes (BD Gentest) was determined. Human or rat liver microsomes (0.5 mg protein/mL) in 100 mM potassium phosphate buffer (pH 7.4) were pre-incubated with 0.1, 1, or 10 μM KR-62980 at 37°C for 5 min, and the reaction was initiated by adding NADPH regenerating solution (BD Biosciences). Samples were collected at 0, 10, 30, and 60 min, and each reaction was terminated by adding three volumes of ice-cold acetonitrile containing an internal standard (imipramine, 80 ng/mL) and mixing on a vortexer. The solution was clarified by centrifugation at 10,000 × g for 3 min at 4°C, and the clear supernatants were collected and transferred to liquid chromatography vials. The samples were analyzed by LC/MS/MS for quantification of KR-62980.

MDCK cell permeability

MDCK cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin in a humidified atmosphere of 5% CO_2 at 37°C. MDCK cells were seeded at a density of 6×10^4 cells/cm^2 in 12-well Transwell plates. The cells were incubated for 3-4 days, with medium changes in both the apical and basolateral compartments on the day after seeding and every other day thereafter. To ensure cell monolayer integrity, the transepithelial electrical resistance (TEER) was measured by voltammetry using an

epithelial volt-ohm meter (EVOM; World Precision Instruments). MDCK cell monolayers with TEER > 700 Ω were used for the transport studies. The cell monolayers were preincubated in transport buffer (HBSS with 10 mM glucose and 25 mM HEPES, adjusted to pH 7.4) for 30 min at 37°C. For apical to basolateral permeability measurements, KR-62980 was added to the apical side, at a final concentration of 10, 50, or 100 μM. Samples (200 μL) were removed from the basolateral side at 30-min intervals for 120 min and added to fresh buffer. The samples were stored at −20°C until LC/MS/MS analysis. At the completion of all experiments, the TEER was measured again to ensure that cell monolayer integrity and viability had not been adversely affected by the experimental conditions. The apparent permeability coefficient (P_{app}, cm/sec) was calculated using following equation:

$$P_{app} = (dQ/dt)/(A \times C_0)$$

where dQ/dt is the rate of permeation across the monolayer, A is the surface area of the monolayer (0.33 cm^2), and C_0 is the initial concentration in the donor compartment.

Plasma protein binding assay

To evaluate the protein binding rate of KR-62980, either 500 μL of rat plasma or 500 μL of 500 mM human serum albumin were spiked with KR-62980 (final concentration, 2 μg/mL) and incubated for 30 min at 37°C. A 50-μL sample was removed and used to determine the total KR-62980 concentration, and a 350-μL sample was removed to measure protein-bound KR-62980. To separate protein-bound from free KR-62980, the 350-μL sample was filtered through an Amicon® Ultra-0.5 centrifugal filter device (30,000 nominal molecular weight limit; Millipore) by centrifugation at 3,000 × g for 20 min at 37°C. The concentration of KR-62980 was measured in 50-μL aliquots from the upper part of the filter device and in 50-μL aliquots of the filtrate.

In vivo pharmacokinetic studies of KR-62980 in rats

After 1 week of adaptation in the animal facility, male Sprague-Dawley rats (210-240 g each) were cannulated with polyethylene tubing (Intramedic PE50; Becton Dickinson and Co.) in the jugular and femoral veins, under anesthesia induced with an intramuscular injection of a Zoletil-Rompun mixture. The rats were housed individually in metabolic cages and allowed to recover for 1 day prior to being used for experiments. To prevent blood clots, the tubing was flushed with normal saline containing heparin (20 IU/mL). KR-62980 was dissolved in a mixture of DMSO-PEG400-distilled water (0.5:4:5.5) and injected via the femoral vein at a dose of 2 mg/kg. Blood samples were collected prior to administration (blank) and at 2, 10, and 30 min and 1, 2, 4, 8, and 12 h after administration. For oral dosing experiments, the animals were administered 2, 5, or 10 mg/kg KR-62980 dissolved in the same vehicle, and blood samples were collected prior to administration (blank) and at 15 and 30 min and 1, 2, 4, 8, and 12 h after administration. All blood samples were separated by centrifugation (10,000 × g at 4°C), and 200 μL of the plasma were collected and stored at −20°C until analyzed. After the experiments, the pooled urine samples were collected, and the exact volumes were measured; 1 mL aliquots were taken from each sample and kept at −20°C until LC/MS/MS analysis.

In vivo pharmacokinetic studies of KR-62980 in mice

To evaluate the dose-dependency of KR-62980 in ICR mice (22-25 g each), blood samples were taken at 30 min and 4 h after oral administration of KR-62980 at 10, 20, 50, 100, 200, 500, and 1000 mg/kg. After the blood samples were taken, the mice were sacrificed. The plasma was separated by centrifugation of the blood samples (10,000 × g and 4°C) and stored at −20 °C until analyzed for KR-62980.

LC/MS/MS analysis

The concentration of KR-62980 in plasma was determined by LC/MS/MS. According to an analytical method developed in our laboratory (Kim et al., 2011). Briefly, a 50-μL aliquot of each sample was mixed with 20 μL of internal standard (imipraime, 3000 ng/mL in acetonitrile) in a 1.5 mL microfuge tube and extracted with 1 mL of ethyl acetate for 3 min by vortex-mixing. After centrifugation at 10,000 × g for 5 min at 4°C, 1 mL of the supernatant was transferred to a new tube and dried in a centrifugal evaporator (EYELA) at 1,000 rpm and 40°C. The residue was dissolved in 100 μL of the chromatographic mobile phase by mixing on a vortexer for 3 min and transferred to an injection vial. For analysis, 5 μL of the sample were injected into a LC/MS/MS system.

The LC/MS/MS system consisted of an Agilent 1200 series HPLC system, with a CTL PAL autosampler (CTC Analytics), coupled to an API 4000 Qtrap tandem quadrupole mass spectrometer (AB Sciex). The chromatographic separation was performed on a Hypersil Gold C18 column (100 mm × 2.1 mm i.d., 3 μm particle size; Thermo) at a flow rate of 0.2 mL/min in a column oven at 40°C. The HPLC eluent was introduced directly into the mass spectrometer with a nebulizing gas

(GS1) pressure of 50 psi, a heating gas (GS2) pressure of 50 psi, and a collision energy (CE) of 59 V for KR-62980 or 25 V for imipramine. The collision gas was nitrogen. Multiple reaction monitoring mode was used based on the most abundant product ions for KR-62980 (m/z 437.2 → 114.2) and the internal standard (m/z 281.3 → 86.1). The peak areas for all of the components were automatically integrated using Analyst software version 1.4 (AB Sciex). The standard curve was linear within the concentration range of 2 to 8000 ng/mL.

Pharmacokinetic and statistical analysis

The plasma concentration *vs* time profiles were analyzed using a non-compartmental model with WinNolin software version 4.1 (Pharsight). The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule extrapolated to infinity. The terminal elimination half-life ($t_{1/2}$), systemic clearance (CL), mean residence time (MRT), and volume of distribution at steady state (V_{ss}) were determined. The extent of absolute oral bioavailability (F) was estimated by comparing the AUC values after intravenous and oral administration of the same dose of KR-62980. The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) after oral administration were obtained by visual inspection of each rat's plasma concentration-time plot for KR-62980. All data are expressed as means ± S.D. A value of $p < 0.05$ by Student's t-test or ANOVA was considered to indicate statistical significance.

RESULTS

Physicochemical properties of KR-62980

The equilibrium solubility of KR-62980 measured using the shake-flask method was 48.1 ± 3.48 μM in pure water. The kinetic solubility determined by nephelometry was 345 ± 0.92 μM in 5% DMSO in water. The Log P and pKa values were 3.23 ± 0.07 and 6.45 ± 0.03, respectively.

Metabolic stability of KR-62980

The metabolic stability of KR-62980 was investigated *in vitro* using human and rat liver microsomes (Fig. 2). The values for the $t_{1/2}$ of KR-62980 in rat liver microsomes were 15.2 ± 1.1, 11.8 ± 1.8, and 11.5 ± 0.5 min for KR-62980 concentrations of 0.1, 1, and 10 μM, respectively. The $t_{1/2}$ values in human liver microsomes were 27.2 ± 2.2, 28.8 ± 3.6, and 25.8 ± 1.9 min at KR-62980 concentrations of 0.1, 1, and 10 μM, respectively (Table I).

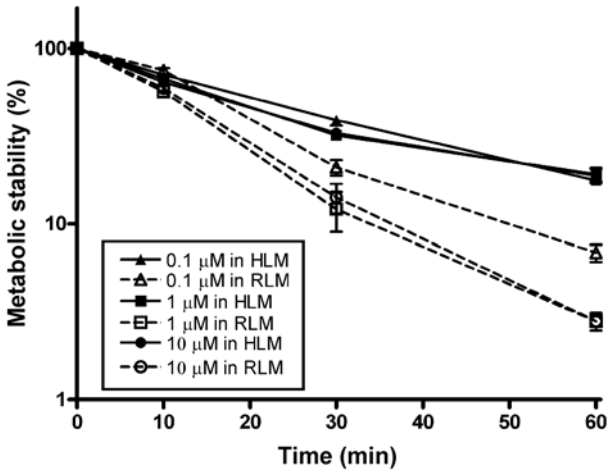

Fig. 2. Metabolic stability of KR-62980 in liver microsomes. The amount of KR-62980 remaining after the incubation of 0.1, 1, or 10 μM KR-62980 with human or rat liver microsomes at 37°C was measured at the different time points (n = 3).

Table I. *In vitro* pharmacokinetic data of KR-62980

In vitro pharmacokinetics	Values
Rat liver microsomal stability	
0.1 μM (half-life, min)	15.2 ± 1.1
1 μM (half-life, min)	11.8 ± 1.8
10 μM (half-life, min)	11.5 ± 0.8
Human liver microsomal stability	
0.1 μM (half-life, min)	27.2 ± 2.2
1 μM (half-life, min)	28.8 ± 3.6
10 μM (half-life, min)	25.8 ± 1.9
MDCK cell permeability	
10 μM (P_{app}, $\times 10^{-6}$ cm/sec)	20.4 ± 0.05
50 μM (P_{app}, $\times 10^{-6}$ cm/sec)	28.0 ± 3.83
100 μM (P_{app}, $\times 10^{-6}$ cm/sec)	30.8 ± 3.33
Plasma protein binding	
2 μg/mL (bound drug %)	89.4 ± 2.0
Human serum albumin binding	
2 μg/mL (bound drug %)	85.4 ± 2.7

MDCK cell permeability of KR-62980

To assess the cellular transport of KR-62980, its permeability through a MDCK cell monolayer (TEER > 700 Ω) was examined (Fig. 3). The apparent permeability coefficients (P_{app}) at 30-min intervals during the first 90 min after administration were 20.4 ± 0.05, 28.0 ± 3.83, and 30.8 ± 3.33 ($\times 10^{-6}$) cm/sec at KR-62980 concentrations of 10, 50, and 100 μM, respectively. The P_{app} of 50 μM atenolol, as a low-permeability reference compound, was 0.17 ± 0.02 ($\times 10^{-6}$) cm/sec, and the P_{app} of 50 μM metoprolol, as a high-permeability reference compound, was 18.9 ± 0.13 ($\times 10^{-6}$) cm/sec.

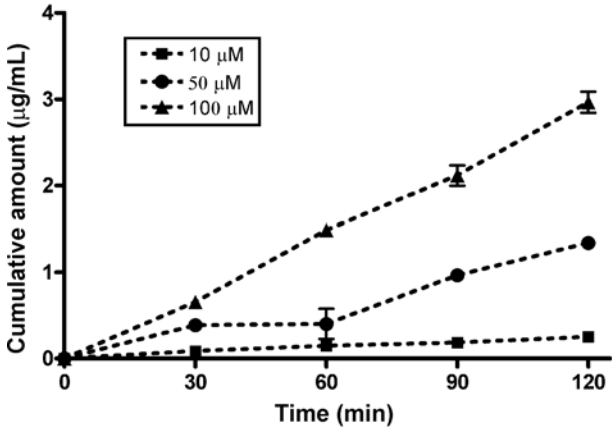

Fig. 3. MDCK cell monolayer permeability of KR-62980. Cell permeability of KR-62980 (10, 50, and 100 μM final concentrations; n = 3) across MDCK cell monolayers was assayed at intervals of 30 min during 2 h.

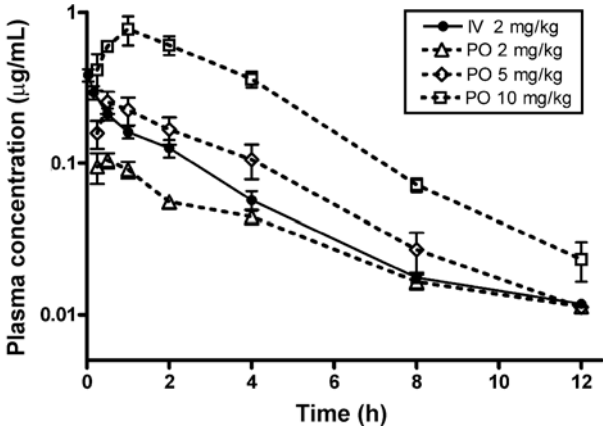

Fig. 4. Plasma concentration-time profiles of KR-62980 after intravenous (2 mg/kg) and oral administration (2, 5, and 10 mg/kg) in Sprague-Dawley rats (n = 5)

Plasma protein binding of KR-62980

The binding rate of KR-62980 (final concentration, 2 μg/mL) to plasma proteins was determined using rat plasma and human serum albumin. The binding rate of KR-62980 to rat plasma proteins was 89.4 ± 2.0%, and the binding rate of KR-62980 to human serum albumin was approximately 85.4 ± 2.7%.

In vivo pharmacokinetics of KR-62980 after intravenous and oral administration in rats

KR-62980 was administered intravenously (dose: 2 mg/kg) and orally (doses: 2, 5, and 10 mg/kg) to Sprague-Dawley rats. Fig. 4 presents the mean plasma concentration-time curves, and Table II lists the values for the pharmacokinetic parameters T_{max}, C_{max}, AUC, CL and V_{ss}. After intravenous administration of KR-62980 at a dose of 2 mg/kg, the values of $t_{1/2}$, CL, and V_{ss} were 3.05 ± 0.58 h, 2.50 ± 0.46 L/kg·h, and 9.16 ± 2.46 L/kg, respectively. After oral administration of KR-62980 at doses of 2, 5 and 10 mg/kg, the AUC values were 0.50 ± 0.09, 1.02 ± 0.50, and 3.10 ± 0.39 μg·h/mL, respectively. The values of the dose-normalized AUC (based on 2 mg/kg) were 0.50, 0.408, and 0.62 μg·h/mL for KR-62980 doses of 2, 5, and 10 mg/kg, respectively, and did not differ significantly among the three doses. Similarly, the C_{max} and T_{max} did not differ significantly over the KR-62980 concentration range of 2 to 10 mg/kg (T_{max}, 0.60-0.83 h; C_{max}, 0.12-0.78 μg/mL). The amount of KR-62980 excreted in the urine during 24 h was negligible (0.073 ± 0.019 for an intravenous dose of 2 mg/kg; 0.046 ± 0.026, 0.22 ± 0.096, and 0.74 ± 0.26 for oral doses of 2, 5, and 10 mg/kg, respectively). After intravenous administration, the

Table II. Pharmacokinetic parameters of KR-62980 after intravenous and oral administration at doses of 2, 5, and 10 mg/kg to Sprague-Dawley rats (n = 5). Data represent mean ± S.D.

Parameter	i.v.	p.o.		
	2 mg/kg	2 mg/kg	5 mg/kg	10 mg/kg
Body weight (g)	229.4 ± 14.3	221.6 ± 7.3	217.6 ± 5.0	226.8 ± 12.3
C_{max} (μg/mL)	-	0.12 ± 0.03	0.26 ± 0.10	0.78 ± 0.28
T_{max} (h)	-	0.60 ± 0.38	0.60 ± 0.22	0.83 ± 0.29
$T_{1/2,\lambda}$ (h)	3.05 ± 0.58	3.87 ± 0.99	2.11 ± 0.29	2.09 ± 0.40
$AUC_{0\text{-}12h}$ (h·μg/mL)	0.77 ± 0.15	0.43 ± 0.09	0.98 ± 0.49	3.03 ± 0.38
$AUC_{0\text{-}\infty}$ (h·μg/mL)	0.82 ± 0.15	0.50 ± 0.09	1.02 ± 0.50	3.10 ± 0.39
CL (L/h/kg)	2.50 ± 0.46	-	-	-
CL_r (L/h/kg)	0.0021 ± 0.0006	-	-	-
CL_{nr} (L/h/kg)	2.49 ± 0.46	-	-	-
V_{ss} (L/kg)	9.16 ± 2.46	-	-	-
MRT (h)	3.64 ± 0.34	5.12 ± 1.00	3.22 ± 0.42	3.36 ± 0.38
F (%)		60.9		

Table III. The plasma concentrations at 30 min and $AUC_{0\text{-}4h}$ values of KR-62980 after oral administration at doses of 10, 20, 50, 100, 200, 500, and 1000 mg/kg to ICR mice (n = 3). Data represent mean ± S.D.

Dose (mg/kg)	30 min (μg/mL)	$AUC_{0\text{-}4h}$ in mice (h·μg/mL)
10	0.70 ± 0.164	1.6 ± 0.36
20	0.88 ± 0.282	2.5 ± 0.23
50	3.51 ± 1.171	8.8 ± 2.3
100	7.39 ± 1.312	19.3 ± 3.8
200	12.3 ± 3.68	32.7 ± 6.9
500	28.2 ± 2.28	72.7 ± 7.1
1000	48.1 ± 3.83	159.0 ± 44.7

renal clearance was 0.0021 ± 0.0006 L/kg·h and the non-renal clearance was 2.49 ± 0.46 L/kg·h. These results indicate that KR-62980 is eliminated primarily via a non-renal pathway such as liver metabolism.

In vivo pharmacokinetic studies of KR-62980 in mice

To evaluate the dose-dependency of KR-62980, the plasma concentration of KR-62980 was measured in ICR mice at 30 min and 4 h after oral administration of KR-62980 at doses of 10, 20, 50, 100, 200, 500, and 1000 mg/kg. The plasma concentration of KR-62980 at 30 min and the $AUC_{0\text{-}4h}$ after oral administration of various doses of KR-62980 to ICR mice are given in Table III.

DISCUSSION

KR-62980, which acts as a PPAR-γ agonist, is under development as a new drug candidate for the treatment of type 2 diabetes, and the pharmacokinetic profile of KR-62980 must be determined before future preclinical and clinical studies can be conducted. Here, the pharmacokinetic profile of KR-62980 was studied *in vitro* and *in vivo*.

The physicochemical properties of KR-62980 are consistent with its use as a drug. Although KR-62980 had poor equilibrium solubility in water (48.1 ± 3.48 μM), this would not preclude its therapeutic application. The Log P value of KR-62980 (3.23 ± 0.07) suggests that its lipophilicity may be involved in its high permeability across MDCK cell monolayers.

The *in vitro* pharmacokinetic parameters (Table I) reveal the rapid disappearance of KR-62980 in human and rat liver microsomes, probably via cytochrome P450 metabolic enzymes. This suggests that KR-62980 may be largely eliminated from the systemic circulation during the hepatic first-pass. Although KR-62980 was unstable in liver microsomes, there was no significant difference in the metabolic $t_{1/2}$ at KR-62980 concentrations ranging from 0.1 to 10 μM, indicating no significant dose-dependency of KR-62980 metabolism in the liver. There was a species difference between human and rat liver microsomal stability of KR-62980. The metabolic $t_{1/2}$ in human liver microsomes (25.8-27.8 min) was approximately twice that in rat liver microsomes (11.5-15.2 min), suggesting greater bioavailability of KR-62980 in humans than in rats. According to a previous study, KR-62980 is metabolized by the liver enzymes CYP1A2, CYP2D6, CYP3A4, and CYP3A5 (Kim et al., 2008). Differences in CYP genes between humans and rats (Nelson et al., 2004) may account for the difference in metabolic stability between the two species. The effect of KR-62980 on the catalytic activities of clinically important human CYPs (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A) is negligible, with IC_{50} values > 50 μM KR-62980 for these enzymes (Kim et al., 2008). Therefore, even though KR-62980 may be metabolized rapidly by CYPs in both human and rat livers, no interactions between the elimination of KR-62980 and other drugs are expected.

KR-62980 showed high permeability across MDCK cell monolayers, with P_{app} values of 20.4 ± 0.05, 28.0 ± 3.83, and 30.8 ± 3.33 ($\times 10^{-6}$ cm/sec) at KR-62980 concentrations of 10, 50, and 100 μM, respectively. The P_{app} values at 50 μM and 100 μM KR-62980 were significantly higher than that at 10 μM KR-62980 ($p = 0.028$ for 50 μM; $p = 0.0058$ for 100 μM). As there was no leakage through the cell monolayers, which had TEER values > 700 Ω, the increased P_{app} at higher KR-62980 concentrations may indicate the cellular transport of KR-62980. Although MDCK cells provide a valid model for estimating drug absorption and disposition (Braun et al., 2000; Chen et al., 2005; Zahner et al., 2010), we may need to perform a Caco-2 cell transport study to further evaluate drug absorption, as Caco-2 cells are one of the best *in vitro* assay tools for estimating drug absorption from the gastrointestinal tract (Artursson and Karlsson 1991; Skolnik et al., 2010). KR-62980 demonstrated middle to high plasma protein binding activity in rat plasma (89.4%) and human serum albumin (85.4%), indicating that most of the KR-62980 binds to serum albumin during movement through the circulatory system.

In *in vivo* pharmacokinetic studies using Sprague-Dawley rats, the CL and V_{ss} after intravenous administration of KR-62980 were 2.50 ± 0.46 L/h/kg and 9.16 ± 2.46 L/kg, respectively, and renal clearance was only 0.0021 ± 0.0006 L/h/kg. Thus, KR-62980 may be eliminated primarily through non-renal pathways such as liver metabolism, which may be the major route

of KR-62980 elimination from the systemic circulation given the short $t_{1/2}$ of KR-62980 in liver microsomes. The $AUC_{0\text{-}12h}$ values were 0.43 ± 0.09, 0.98 ± 0.49, and 3.03 ± 0.38 h·μg/mL after oral administration of KR-62980 at 2, 5, and 10 mg/kg, respectively, and the $AUC_{0\text{-}\infty}$ values at these doses were 0.50 ± 0.09, 1.02 ± 0.50, and 3.10 ± 0.39 h·μg/mL, respectively. At a sampling time (12 h) of more than three times the terminal half-life ($T_{1/2,\lambda}$, 2.09-3.87 h), the $AUC_{0\text{-}12h}$ accounted for 86.9-97.6% of the $AUC_{0\text{-}\infty}$. Thus, the sampling time and AUC during 12 h were sufficient for evaluating the *in vivo* pharmacokinetics of KR-62980 after intravenous and oral administration. The dose-normalized AUCs based on 2 mg/kg showed no significant dose-dependency at KR-62980 doses of 2-10 mg/kg after oral administration (0.50 ± 0.09, 0.41 ± 0.20, and 0.62 ± 0.08 h·μg/mL at 2, 5, and 10 mg/kg). Similarly, the dose-normalized C_{max} (0.12 ± 0.032, 0.11 ± 0.041, and 0.16 ± 0.057 μg/mL) and T_{max} (0.6-0.83 h) showed no significant differences among oral doses of 2, 5, and 10 mg/kg. In a previous pharmacokinetic study (Kim et al., 2006a), the $AUC_{0\text{-}24h}$ of KR-62980 was 2.53 ± 0.47 h·μg/mL after intravenous administration of 10 mg/kg and 8.27 ± 2.81 h·μg/mL after oral administration of 50 mg/kg. Comparing these values of higher doses with both intravenous and oral administration, the pharmacokinetic values in the dose range 2-10 mg/kg showed no significant difference in rats. After oral administration to rats, the bioavailability of KR-62980 was ~60.9%, compared with that after intravenous administration. Even though KR-62980 is unstable in liver microsomes, its probable high permeability across the gastrointestinal tract may contribute to middle to high bioavailability of KR-62980. The *in vivo* data correlated with the metabolic dose-independency demonstrated *in vitro*, despite the instability in liver microsomes. Other compounds have shown no dose-dependency for *in vivo* pharmacokinetics in spite of metabolic issues (Choi et al., 2006; Yang and Lee 2008).

The dose-normalized $AUC_{0\text{-}4h}$ and dose-normalized C_{30min} and C_{4h} showed no significant dose-dependency in ICR mice treated with KR-62980 doses of 10-1000 mg/kg. The $AUC_{0\text{-}4h}$ ranged from 1.6 to 159.0 h·μg/mL, and the dose-normalized $AUC_{0\text{-}4h}$ values based on 10 mg/kg were 1.62 ± 0.36, 1.26 ± 0.12, 1.77 ± 0.46, 1.93 ± 0.38, 1.63 ± 0.34, 1.45 ± 0.14, and 1.59 ± 0.44 h·μg/mL at KR-62980 doses of 10, 20, 50, 100, 200, 500, and 1000 mg/kg, respectively. There was no significant difference in $AUC_{0\text{-}4h}$ between Sprague-Dawley rats and ICR mice after oral administration of 10 mg/kg KR-62980 (1.81 ± 0.27 *vs* 1.62 ± 0.36 h·μg/mL).

In conclusion, KR-62980 showed instability with a short $t_{1/2}$ in rat and human liver microsomes, although there was a difference in metabolic stability between rats and humans. KR-62980 showed high cell permeability across MDCK cell monolayers. In Sprague-Dawley rats, the bioavailability of KR-62980 after oral administration of 10 mg/kg was ~60.9%, compared with that after intravenous administration, and there was no significant dose-dependency after oral administration of KR-62980 at 2-10 mg/kg. In ICR mice, the dose-normalized AUCs and plasma concentrations showed no dose-dependency at higher oral doses of KR-62980 (> 10 mg/kg). These data suggest dose-independency in rats and mice, even though KR-62980 may disappear rapidly from the systemic circulation via liver metabolism.

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