Arch Pharm Res Vol 26, No 1, 83-88, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Pharmacokinetics and Bioavailability of Oral Cephalosporins, KR-984055 and its Prodrugs, KR-999001 and KR-999002, in the Rat

Yong Soon Park, Su Kyung Woo, Myung Hee Jung[1], and Kwang-il Kwon

College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea and [1]Korea Research Institute of Chemical Technology, Daejeon 305-343, Korea

(Received November 1, 2002)

KR-984055 is a new oral cephalosporin antibiotic with activity against both gram-positive and gram-negative bacteria. Lipophilic ester-type prodrugs of KR-984055, i.e., KR-999001 and KR-999002, have been synthesized in an attempt to increase the oral bioavailability of this broad-spectrum antibiotic agent. In this study we determined the oral bioavailability of KR-984055 and its prodrugs in the rat, and evaluated the pharmacokinetic model that best describes the plasma concentration behavior following single intravenous (IV) and oral single dose. In addition, concentrations in plasma as well as biliary and urinary recovery of KR-984055 were determined. Also, protein binding of KR-984055 in plasma was examined *in vitro*. The degree of protein binding of KR-984055 was in the range of 92.09~94.77%. KR-984055 exhibited poor oral bioavailability (7.02 ± 1.58%). The observed oral bioavailabilities of KR-984055 from KR-999001 and KR-999002 were 38.77 ± 2.81% and 39.81 ± 5.25%, respectively. These data were calculated from the levels of free KR-984055 in plasma. Oral KR-999001 and KR-999002 were not recovered from plasma, suggesting that it was readily cleaved to free KR-984055. KR-999001 and KR-999002 appear to be an efficient oral prodrug of KR-984055 that deserved further clinical evaluation in human.

Key words: Cephalosporins, Prodrug, KR-984055, KR-999001, KR-999002, Bioavailability, Pharmacokinetics

INTRODUCTION

Cephalosporins usually exhibit poor bioavailabilities when they are given orally. Higher values can be obtained only when cephalosporins are taken up by carrier systems or when the polarity of the carboxylic acid group in the 4 position is reduced by esterification (Stoechel *et al.*, 1999; Campbell *et al.*, 1987). Derivatives produced by esterification of the carboxylic acid group can be absorbed by passive diffusion. Therapeutically useful compounds can, however, be obtained only if the absorbed prodrug ester is readily converted back to the active drug (Stoechel *et al.*, 1999; Ruiz-Carretero *et al.*, 2000).

KR-984055, [2-(aminothiazol-4-yl)-2-hydroxyiminoacetamido -3-[(4-methylthiazol-5yl) vinyl]-3-cephem-4-carboxylic acid, is a new oral cephalosporins antibiotic synthesized by Korea Research Institute of Chemical Technology (KRICT). KR-984055 exhibits potent antibacterial activity against both gram-positive and gram-negative bacteria. Bactericidal effect of KR-984055 is similar or more than that of cefixime, and especially more potent against on *Streptococcus faecium* and *Staphylococcus aureus*. KR-984055 has broad-spectrum bacterioside, but it exhibits poor, variable bioavailability and it is difficult to establish the optimal oral dosage regimen. Since KR-984055 is not well absorbed orally, prodrugs of KR-984055 were used to improve its gastrointestinal absorption. From a large number of newly synthesized lipophilic esters of KR-984055, 2'-cyclopropyloxyethyl ester (KR-999001) and pivaloyloxymethyl ester (KR-999002), respectively, were selected as a potentially useful prodrug of KR-984055.

The aims of the present study were to determine the absolute bioavailability of KR-984055 after oral administration of KR-984055 and its ester prodrugs (KR-999001 and KR-999002), and plasma protein binding of KR-984055. Also, we evaluated the pharmacokinetic model of KR-

Correspondence to: Kwang-il Kwon, Ph.D., College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
E-mail: kwon@cnu.ac.kr

984055 after IV and oral administration that best described the time profile of KR-984055.

MATERIALS AND METHODS

Drugs

KR-984055, KR-999001, and KR-999002 (Fig. 1) were synthesized at Korea Research Institute of Chemical Technology, Daejeon, Korea. KR-984055, KR-999001 and KR-999002 were dissolved in 100% dimethyl sulfoxide (DMSO) and an aliquot of the DMSO solution was diluted 10-fold with distilled water for oral administration; for intravenous injection, all solutions were diluted by 0.9% of sodium chloride solution.

Conversion of the prodrugs to KR-984055 by plasma esterase activity

The prodrugs KR-999001 and KR-999002 were incubated at concentrations of 10, 20 and 50 μg/ml in the presence, or absence of plasma in a water bath for 30 min at 37°C. A suitable intervals, 300 μl aliquots were withdrawn and deproteinated immediately with equal volume of 6% trichloroacetic acid (TCA) (Li *et al.*, 1998). After mixing and centrifugation, 50 μl of the supernatant was injected into chromatograph. The amount of KR-984055 generated during the incubation period was then determined by HPLC, and the extent of conversion observed during incubation was taken as a relative measure of the stability of the prodrug in plasma.

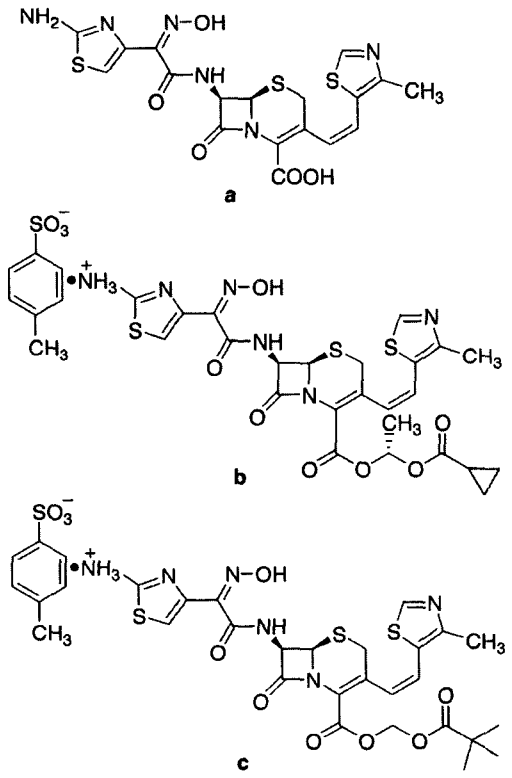

Fig. 1. Structures of KR-984055 (a), KR-999001 (b) and KR-999002 (c)

Plasma protein binding

The plasma protein binding of KR-984055 was determined *in vitro* by an ultrafiltration method (Amicon Division, W.R. Grace & Co., USA). The plasmas spiked with standard solution of 20, 100, and 200 μg/ml KR-984055 to final concentration were incubated for 2 hr in a water bath at 37°C. After incubation, 100 μl of spiked plasma was withdrawn and stored at -20°C. Remained sample was filled the sample reservoir (YMT membrane) and centrifuged with a 35° fixed-angel rotor for 10 min at 2000 g. The concentration of unbound drug in the ultrafiltrate was measured. KR-984055 did not show significant adsorption to the membrane. The unbound fraction (F_U) in plasma was calculated as the concentration in the filtrate divided by the original concentration in plasma. The plasma protein binding rate (%) was calculated as $(1 - F_U) \times 100$ (Wilkins *et al.*, 1997).

Pharmacokinetic study

Male Sprague Dawley rats weighting 250-300 g were used for all the experiments. A random experimental design was used. Animals were randomly assigned to one of the two groups (IV and oral administration).

Anesthesia solution by mixing 800 mg/kg of a urethane and 80 mg/kg of a α-chloralose was administered by an i.p. injection. In order to facilitate blood sampling and IV dosing to rats, a 15-cm long PE 50 tube (bridge-tubing) was connected to the free end of the cannula. The bile duct was exposed and a sterile PE 10 catheter was threaded in the duct and anchored with ligatures. The abdominal incision was closed with stainless steel wound clips. Once normal bile flow was established, each rat was given an i.v. injection of KR-984055 in the femoral artery at a dose of 10 mg/kg. The cannula was immediately rinsed with 0.5 ml of heparinized saline solution. Bile samples were collected continuously at 0 to 1, 1 to 2, 2 to 3, 4 to 5, 5 to 6, 6 to 8 and 8 to 10 hr postdose.

Twenty-four hours before oral administration, under anesthesia, the rats were subjected to femoral artery cannulation with a 30-cm-long fragment of PE 50 tube and the free end of the cannula was subcutaneously conducted to the dorsal base of the neck. The exteriorized end was closed with a polyethylene plug. The cannula was permanently filled with heparinized (20 IU/ml) saline solution. After the surgery and until drug administration, the animals were kept fasted overnight with water free available. All compounds were administered at doses of 10 mg/kg. The animals were placed immediately in metabolic cages, and urine sample was collected from -12 to 0, 0 to 1, 1 to 2, 2 to 3, 4 to 5, 5 to 6, 6 to 8 and 8 to 10 hr after oral administration. The total volume was recorded and the urine samples were

centrifuged to remove food and fecal debris and frozen at -20°C until analysis. The urine samples were analyzed by HPLC as described below.

At predetermined time points up to 360 min after dosing, blood samples (0.3 ml) were collected in heparinized syringes from the cannula at 1, 5, 10, 15, 30, 60, 90, 120, 180, 240 and 360 min for IV administration, and at 5, 15, 30, 60, 90, 120, 180, 240, 300 and 360 min for oral administration. Each sample was immediately deproteinated with an equal volume of 6% TCA and centrifuged for 5 min at 5000 rpm. Then, the supernatant was stored at -20°C until HPLC analysis.

Analytical procedures

The plasma, urine and bile samples were assayed for KR-984055 concentration by high-performance liquid chromatography (HPLC), which provided excellent separation and quantification of the cephalosporins antibiotic.

The plasma mixed with 6% TCA was centrifuged with 5000 rpm for 5 min. After centrifugation, 50 μl of the clear supernatant was injected into the chromatograph. On the other hand, KR-984055, KR-999001 and KR-999002 were extracted from urine and bile samples through SPE (solid phase extractor). SPEs were rinsed with 2 ml of methanol and 2 ml of mixture of acetonitrile and aqueous 0.1 M ammonium acetate (pH 3.5), 18 : 82 (vol./vol.). 500 μl of the urine or bile samples were immediately transferred to the SPE. KR-984055, KR-999001 and KR-999002 were eluted with 4 ml of methanol and elute was collected in a glass tube. The solution obtained was evaporated to dryness under nitrogen gas at 45°C and reconstituted with 500 μl of mobile phase. After centrifugation, 50 μl of the aqueous supernatant was injected into the chromatograph (Ruiz-Carretero *et al.*, 2000).

The mobile phase was a mixture of acetonitrile and aqueous 0.1 M ammonium acetate (pH 3.5), 82 : 18 (vol./vol.). A flow rate of 1.0 ml/min was used. A reversed phase column (μ-Bondapak C18, 300×3.9 mm, I.D. 10 μM) was used. A Shimadzu spectrophotometer, model LC 10, set at 300 nm, was as used to monitor the column effluent.

Calibration curves covering the whole range of KR-984055 concentrations in plasma samples were prepared. The accuracy and precision of the method were established using the concentrations covering the range of the concentrations to be analyzed. The accuracy and precision were evaluated by calculating the relative error and coefficient of variation, respectively, which were always less than 10%. The limit of quantification was 0.1 μg/ml.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated for each individual experiment and averaged. The area under the plasma concentration-versus-time curve (AUC) for the 6 hr following administration of compound (AUC_{0-6}), the terminal-phase half-life ($t_{1/2}$), the maximum concentration of compound in plasma (C_{max}), the time to C_{max} (T_{max}), and the mean residence time (MRT) were calculated by a noncompartmental method with WinNonlin professional version 2.1 (Pharsight, Inc., Mountain, CA). The oral bioavailability (F) of KR-984055 from the prodrugs or from oral administration of the KR-984055 was calculated from the AUC_{0-6} of the oral dose divided by the AUC_{0-6} of the i.v. dose of KR-984055. For example, the oral bioavailability of KR-984055 from orally administered KR-999001 was calculated as follows: $F = [(AUC_{p.o.})/(dose_{p.o.})]/[(AUC_{i.v.})/(dose_{i.v.})]$, where $AUC_{p.o.}$ is the AUC of KR-984055 after oral administration of KR-999001, $dose_{p.o.}$ is the oral dose of KR-999001 in milligram equivalents of KR-984055 per kilogram, $AUC_{i.v.}$ is the AUC of KR-984055 after i.v. administration, and $dose_{i.v.}$ is the i.v. dose of KR-984055. Total clearance (*CL*) from plasma was calculated as dose/$AUC_{i.v.}$. The volume of distribution at steady-state (V_{ss}) of drug administered i.v. was calculated as CL×MRT (Shargel & Yu, 1999).

The equations of the classic compartmental models were fitted to the experimental plasma concentrations of KR-984055 versus time data obtained, using weighted least-squares non-linear regression by means of the WinNonlin program. The weighting factor was $1/C_p^2$ (C_p is the experimental plasma concentration). One and two-compartment open models were used for IV and oral routes. For oral administration, one-compartment open model without lag time was fitted. In the case of the IV route, two-compartment open model was fitted to the experimental data. To select the best model, the Akaike information criteria, AIC was applied (Gabrielsson & Weiner, 1997).

The urinary recovery of KR-984055 was defined as the ratio of the amount of free KR-984055 collected in the urine to the total amount of KR-984055 equivalent administered (Shargel & Yu, 1999).

RESULTS

Prodrugs are readily hydrolyzed to KR-984055 in rat plasma

To test whether plasma esterase activity could convert the prodrugs to the KR-984055 compounds, the prodrugs were incubated with rat plasma at 37°C for 30 min prior to studying. KR-999001 and KR-999002 are rapidly hydrolyzed to the KR-984055, as indicated by the observation that full conversion occurred in less than 1 min at 37°C.

Plasma protein binding

The plasma protein binding of KR-984055 over the concentration of 50, 100 and 200 μg/ml averaged 92.94±0.12, 92.09±0.05 and 94.77±0.07%, respectively.

Pharmacokinetic in rats

To determine the oral bioavailability of KR-984055 and its prodrugs, KR-984055, KR-999001 and KR-999002 were administered to rats by i.v. and oral routes. Concentrations of KR-984055 in plasma were determined by a highly sensitive method using HPLC analysis with UV detection. Following intravenous bolus injection, KR-984055 concentrations rapidly declined in an apparent biexponential manner and fell below the 80% of AUC within 6 hr after injection (Fig. 2). The terminal half-life ($t_{1/2\beta}$) in plasma was 2.05 hr, and the total body clearance was 29.09 ml/hr (Table I). The average pharmacokinetic parameters of KR-984055 were calculated after IV administration of KR-984055 by fitting the two-compartment model to the individual experimental values are summarized in Table I. In this study with a single i.v. dose of 10 mg/kg, KR-984055 was not detected in bile sample over the collection period (0 to 10 hr).

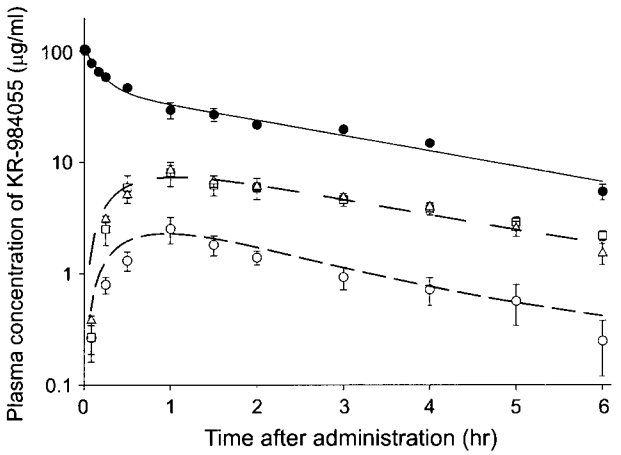

Fig. 2. Plasma concentrations of KR-984055 (Mean ± S.E.M, n = 5) after administration to rats (●, intravenous KR-984055; ○, oral KR-984055; □, oral KR-999001; △, oral KR-999002). All compounds were given at a dose of 10 mg/kg. The lines represent the fitted results to I.V. two-compartment model or to oral one-compartment model.

Table I. Phamacokinetic parameters following intravenous administration of KR-984055 of 10 mg/kg in rats (Mean ± S.E.M., n = 5)

Parameter	KR-984055
$AUC_{0\text{-}6}$ (μg·h/ml)	142.43±13.50
Vss	78.84± 5.14
CL (ml/hr)	29.09± 2.13
$T_{1/2\beta}$ (hr)	2.05± 0.01
MRT (hr)	2.72± 0.04
Kel (hr^{-1})	0.73± 0.05
K_{12} (hr^{-1})	2.44± 0.30
K_{21} (hr^{-1})	2.54± 0.35
α (hr^{-1})	5.37± 0.60
β (hr^{-1})	0.34± 0.01
A (μg/ml)	59.30± 6.63
B (μg/ml)	44.33± 4.66
Vc	40.18± 3.68

The mean concentration vs. time curves for KR-984055 in plasma after oral administration of KR-984055, KR-999001 and KR-999002 are shown in Fig. 2. The oral bioavailabilities of KR-984055, defined as the ratio of the AUC for KR-984055 following oral administration of KR-984055 or its prodrug to the AUC for intravenous KR-984055, were 7.02%, 38.77% and 39.81% for oral KR-984055, for oral KR-999001 and for oral KR-999002, respectively. Table II shows the average pharmacokinetic parameters of KR-984055 were calculated after oral administration of KR-984055, KR-999001 and KR-999002 by fitting the one-compartment model to the individual experimental value. All pharmacokinetic parameters of KR-999001 and KR-999002 were not shown significant differences.

The urinary excretion of KR-984055 was determined from the recovery of KR-984055 in the urine during 10 hr after administration of KR-984055, KR-999001, or KR-

Table II. Pharmacokinetic parameters of K984055 following oral administration of KR-984055, KR-999001 and KR-999002 of 10 mg/kg in rats (Mean ± S.E.M., n = 5)

Parameter	Compound		
	KR-984055	KR-999001	KR-999002
$AUC_{0\text{-}6}$ (μg·h/ml)	9.23±1.96	34.40±3.41	35.15±3.31
C_{max} (μg/ml)	2.29±2.29	7.50±1.79	7.75±0.80
T_{max} (hr)	1.01±0.07	1.31±0.19	1.08±0.09
$t_{1/2}$ (hr)	1.39±0.70	1.87±0.05	2.36±0.17
Ka (hr^{-1})	1.58±0.35	1.44±0.06	1.79±0.13
V/F	926.40±257.75	163.10±16.28	191.25±38.71

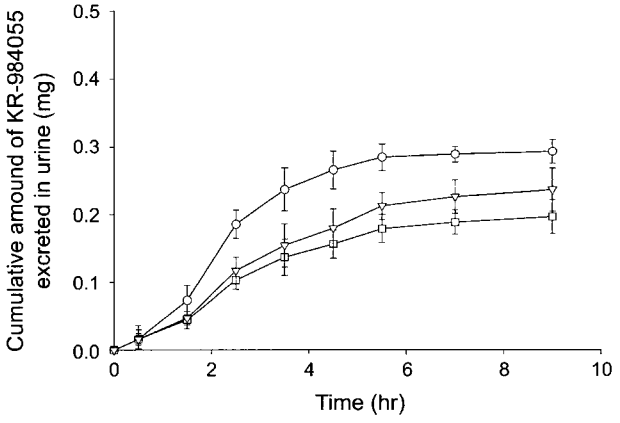

Fig. 3. Urinary excretion of KR-984055 after oral administration of 10 mg/kg of KR-984055 (-○-), KR-999001 (-□-) and KR-999002 (-△-), respectively, in the rat (mean ± S.E.M, n = 5).

999002 (Fig. 3). Following oral administration, KR-984055 was markedly excreted in the urine as an intact form. Only small amounts of KR-984055, however, were recovered in the urine of rat that received oral KR-999001 or oral KR-999002 (6.14 and 7.40%, respectively). The cumulative urinary excretion of KR-999001 and KR-999002 tended to be lower than KR-984055 after oral administration.

DISCUSSION

For long-term treatment and compliance, the compound should be given orally, but for KR-984055, oral bioavailability is rather low (7.02% in rats). Recently, a large number of lipophilic esters of KR-984055 have been synthesized and investigated for their potential as oral prodrugs of KR-984055. KR-999001 and KR-999002 appeared the most promising, with oral bioavailabilities in rats of 38.771% and 39.81%, respectively. Based on a distribution volume of approximately 0.3 L/kg in experimental animals, its distribution was considered to be limited to the extracellular space (Okudaira *et al.*, 2000). Although KR-984055 itself was not orally absorbed well because of its hydrophilicity, increasing the membrane permeability by making the esterification of KR-984055 seemed to improve the oral absorption and, consequently, the oral bioavailability.

KR-984055 exhibits potent activity which is comparable or superior to cefixime against both gram-positive and gram-negative organism *in vitro* antibacterial test. Especially it was about fourteen-fold more active against the two *Streptococcus pyogenes* strain and sixtyfour-fold against the three *Staphylococcus aureus* strains than that of cefixime (our unpublished data).

KR-984055 from its prodrugs, KR-999001 and KR-99902, was found to be rapidly absorbed from the gastrointestinal tract, as indicated by the T_{max}s, which were achieved 1.08 to 1.31 hr after oral administration (Fig. 2).

The two-compartment model for intravenous injection and the one-compartment model for oral administration of KR-984055 gave the best parameters according to the criteria (AIC and correlation of predicted and observed data)

In this study with a single dose of 10 mg/kg, KR-999001 and KR-999002 were not detected in plasma and urine. This is supposed to be hydrolyzed rapidly by nonspecific esterases to KR-984055 in the intestinal mucosa and blood (Umemura *et al.*, 1997). These similar results were found in other pivaloyloxymethyl ester prodrug (Naesens *et al.*, 1996).

The plasma protein binding of KR-984055 ranged from 92 to 95% and was almost constant and independent of the KR-984055 concentration. The blood to plasma concentration ratio for KR-984055 over the concentration range of 10 to 50 μg/ml ranged from 0.64 to 0.68, indicating that these compounds were mainly distributed in the plasma (data not shown). KR-984055 was eliminated in urine primarily as unchanged drug and rapidly eliminated from the body with elimination $t_{1/2}$s of 1.87 to 2.36 hr. KR-984055 was not detected in bile sample over the collection period (0 to 10 hr) after a single i.v. dose of 10 mg/kg. However, only 6 to 7.5% of the administered dose was recovered in urine as KR-984055 up to 10 hr after administration of a single oral KR-999001 and KR-999002, which suggested that unexpected metabolic routes would be possible.

In conclusion, KR-999001 and KR-999002 were well absorbed orally and converted rapidly to the active metabolite, KR-984055, during absorption through the intestinal wall. KR-999001 and KR-999002 are expected to be an effective therapeutic agent in the treatment of various infectious diseases.

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