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Pharmacokinetics of LB20304, a New Fluoroquinolone, in Rats and Dogs

Mi-Kyeong Seo, Sun-Hwa Lee, Yun-Jeong Choi, Yi-Na Jeong, Sung-Hack Lee, In-Chull Kim and Yong-Hee Lee

LG Chem Biotech Research Institute, Moonji-Dong 104-1, Yu Song, Taejon 305-380, South Korea

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The pharmacokinetics of LB20304 was investigated following intravenous (IV) and oral administration to rats and dogs. Additionally, *in vitro* metabolism and serum protein binding studies were also conducted. The total body clearance, apparent volume of distribution, terminal half-life, and extent of bioavailability were 21.8 ml/min/kg, 2265 ml/kg, 93.6 min, and 30.8% for rats; and 7.95 ml/min/kg, 4144 ml/kg, 363 min, and 81.1% for dogs, respectively. LB20304 was stable in the liver microsome containing NADPH generating system and its serum protein binding was 58.5-65.8% for rats, 19.1-26.6% for dogs, and 56.9-59.6% for humans. Its tissue concentration levels in liver, stomach, small intestine, and kidney were 9.5 to 26.1 times greater than plasma level, but the concentration in testis was quite low and that in brain was negligible in rats. The 48 hr urinary recovery of the dose was 44% for IV dosing and 14% for oral dosing, whereas the 48 hr biliary recovery of the dose was 6.4% for IV dosing and 4.5% for oral dosing in rats. In summary, the pharmacokinetic properties of LB20304 were characterized by its good oral absorption, long plasma half-life, and good tissue distribution.

Key words : Pharmacokinetics, LB20304, Fluoroquinolone, Rats, Dogs

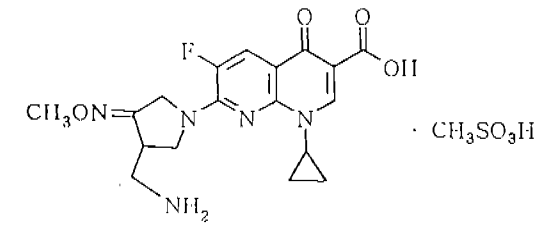
INTRODUCTION

Quinolones are a class of antibiotics structurally related to nalidixic acid. They exhibit bactericidal activity primarily by inhibiting bacterial DNA gyrase which controls the shape and the function of bacterial DNA through its unique supercoiling and relaxing activities (Sugino *et al.*, 1977). A number of new fluoroquinolone derivatives such as norfloxacin, ofloxacin, enoxacin, ciprofloxacin, lomefloxacin, and fleroxacin have recently been developed and introduced into the market. These new fluorinated quinolones exhibited high potency, low incidence of bacterial resistance, high oral bioavailability, extensive tissue penetration, and long elimination half-life. However, these fluoroquinolones possessed only moderate activities against many gram-positive cocci, including *Staphylococci* and *Streptococci* which are major pathogenic strains of respiratory tract infections (Raviglione *et al.*, 1990; Thys *et al.*, 1989). Since it has been suggested that increasing resistance to the new fluoroquinolones is due to the moderate activity of these drugs against gram-positive bacteria (Blumberg *et al.*, 1991; Jones, 1992; Kaatz *et al.*, 1991), recent efforts have been directed toward the synthesis of novel quinolone that can provide improved activities against gram-positive organisms while retaining the broad spectrum activity of ciprofloxacin (Fuchs *et al.*, 1991; Piddock, 1994; Sato *et al.*, 1992).

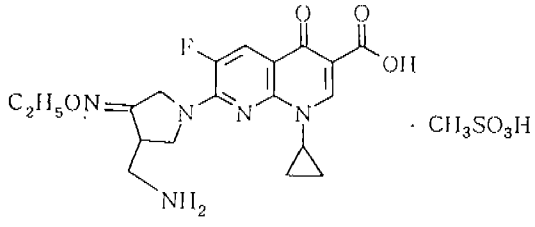
LB20304a (mesylate salt of LB20304, 7-(3-aminomethyl-4-methyloxyiminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthylidine-3-carboxylic acid methane sulfonic acid) is a new quinolone antibacterial agent (Fig. 1). It showed a broad spectrum activity, particularly potent *in vitro* antibacterial activities against gram-positive bacteria (Kim *et al.*, 1996). Hence the improved activity against gram-positive pathogens, particularly *Streptococci* and *Staphylococci*, suggests a significant therapeutic potential for this compound. In view of its activity against not only gram-positive strains but also the family of *Enterobacteriaceae*, LB20304 could be used to treat a broad spectrum of human infections, including those of respiratory tract, urinary tract, skin and skin structures, bone, and gastrointestinal tract.

The peak concentration (C_{max}) and the area under the plasma concentration-time curve (AUC) in relation to the minimal inhibitory concentration (MIC) of the pathogen are of primary importance for the successful antibiotic treatment. Pharmacokinetics of LB

Correspondence to: Yong-Hee Lee, LG Chem Biotech Research Institute, Moonji-Dong 104-1, Yu Song, Taejon 305-380, South Korea



LB20304a



LB20326a

Fig. 1. Chemical structures of LB20304a and LB20326a (the internal standard of HPLC assay)

20304 was studied in the present rats and dogs. The resultant pharmacokinetic information will be used to address efficacy, safety, and regulatory aspects associated with LB20304.

MATERIALS AND METHODS

Chemicals

LB20304a (Lot No.: Q002, purity: 98%) was synthesized at LG Chem. (Taejon, Korea). The dosing solution (LB20304a) was prepared in water and expressed as a mesylate salt whereas the drug concentration (LB20304) in biological samples was expressed as a free form. Nicotinamide-adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G 6P), glucose-6-phosphate dehydrogenase (G6PD), $MgCl_2$, and β-glucuronidase were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and heparin was a product of Green Cross Co. (Yongin, Korea). The other chemicals were of analytical grade.

HPLC assay

All biological samples were stored at -20°C until analysis and analyzed within 1 week by HPLC methods reported from our laboratory (unpublished data). Stock solution (1 mg/ml) and diluted working solutions (0.2, 0.5, 1, 5, 10, 20, 50, and 100 μg/ml) were prepared in methanol in a 14-ml polystyrene (PS) tube (Becton Dickinson Labware, Franklin Lakes, NJ, USA) to construct calibration curve from 0.2 to 100 μg/ml of LB20304 in biological samples including plasma, tissue homogenate, urine, and bile. Throughout the experiment, the storage and handling of samples were conducted with the prevention of light because LB20304 was light labile.

For the analysis of LB20304 in plasma or tissue homogenate samples, a deproteinization method (unpublished data) was applied using methanol and 10% zinc sulfate. A 100-μl of diluted working solution was mixed with 50 μl of 10% $ZnSO_4$ and 100 μl of blank sample for calibrator, whereas 100 μl of sample was mixed with 50 μl of 10% $ZnSO_4$ and 100 μl of methanol for study sample in a 1.5-ml polypropylene (PP) tube (Becton Dickinson Labware). After centrifugation of sample at 14,000 g for 20 min to obtain a clear supernatant, 100 μl of the supernatant was injected directly onto the HPLC column. The procedures for sample handling and processing were carried out at 4°C.

For the analysis of LB20304 in urine or bile samples, a liquid-liquid extraction method (unpublished data) was applied using chloroform (J.T. Baker Inc., Phillipsburg, NJ, USA), 50 mM phosphate buffer of pH 7.0, 10 mM phosphate buffer of pH 2.0, and an internal standard (LB20326a, 50 μg/ml in methanol, LG Chem.). A 200-μl of diluted working solution was evaporated to dryness, then 200 μl of blank sample was spiked and mixed for calibrator, whereas 200 μl of sample was spiked for study sample in a 15-ml PP tube. Afterwards, 10 μl of the internal standard, 200 μl of 50 mM phosphate buffer of pH 7.0, and 5 ml of $CHCl_3$ were added, then vortexed for 10 min and centrifuged at 1,500 g for 20 min. The $CHCl_3$ layer was transferred into a 15-ml PP tube containing 200 μl of 10 mM phosphate buffer of pH 2.0, then vortexed for 10 min and centrifuged at 1,500 g for 20 min. Thereafter, 100 μl of aqueous phase transferred into HPLC injection vial containing 100 μl of methanol for HPLC injection.

The calibration curves were linear in the range of 0.2 and 100 μg/ml of LB20304 in all biological samples with the absolute recoveries of 61-71% for plasma and liver tissue homogenate samples and 90-95% for urine and bile samples. The absolute recoveries of LB20304 in the other tissue homogenates at 10 μg/ml were 76% for brain, 51% for submaxillary gland, 35% for thymus, 70% for heart, 59% for lung, 45% for spleen, 56% for stomach, 70% for small intestine, 55% for large intestine, 63% for cecum, 67% for kidney, 70% for testis, 99% for fat, 81% for muscle, and 62% for lymph node samples.

In vitro study

Metabolism in liver microsome (n=3) : A portion of liver of rats, dogs, and humans was excised, chilled, and blotted dry with paper tissue, then weighed and homogenized with Tempest tissue homogenizer (The Virtis Company, Gardiner, NY, USA) with 2 volumes of 0.1 M Tris-acetate buffer (pH 7.4) con-

taining 0.1 M KCl and 1 mM EDTA. Afterthen, the homogenate was centrifuged at 9,000 g for 20 min twice using Beckman Model J2-M1 (Palo Alto, CA, USA). The supernatant was collected and centrifuged at 105,000 g for 1 hr using Beckman Ultracentrifuge LA-80, then the pellet was resuspended in 0.1 M tetrasodium pyrophosphate containing 1 mM EDTA, and then centrifuged again at 105,000 g for 1 hr. Afterwards, a liver microsome was obtained from the pellet by resuspending with 2 volumes of 50 mM Tris-acetate buffer (pH 7.4) containing 20% glycerol and 1 mM EDTA. All processing procedures were conducted at 4°C. Protein content of liver microsome was measured by the Bradford method (1976), and then liver microsome was diluted to make 1.5 mg/ml protein content. The metabolism of LB20304 was determined at 37°C and 150 opm in a 3 ml incubation mixture containing 0.1 M Tris buffer (pH 7.4), 16 μl/ml substrate, 0.5 mg/ml microsomal protein, 1 mM NADP, 10 mM G6P, 2 units G6PD, and 5 mM $MgCl_2$. The reaction was initiated by the addition of substrate and stopped by the addition of 50 μl 10% $ZnSO_4$ and 100 μl methanol into 100 μl sample at different time intervals for 1 hr. Additionally, metabolism study was conducted at enzyme induced liver microsome which was prepared at 5 day following intraperitoneal administration of polychlorinated biphenyl mixture, aroclor 1254 (500 mg/kg) into rats (Czygan *et al.*, 1973). Aroclor 1254 was used in the induction of liver enzyme because its exposure showed the striking dose- and time-dependant increases in hepatic levels of CYP 1A1 and CYP1A2, and limited induction of epoxide hydrolase, quinone oxidoreductase, and aldehyde dehydrogenase in rats (Dragnev *et al.*, 1994).

Serum protein binding (n=3): Binding of LB20304 to rat, dog, and human serum protein was determined by an ultrafiltration method. After incubation of 2 and 10 μg/ml serum samples for 10 min at 37°C, 0.5 ml of the serum was immediately transferred to Ultrafree-MC Millipore (5000 NMWL, Millipore Cor., Bedford, MA, USA), vortex mixed for 1 min, and the tube was then centrifuged at 3,000 g for 15 min at 37°C. Afterwards, two-fold of methanol was added into the filtrate for HPLC analysis. For the estimation of total drug concentration in the serum, 50 μl of serum was sampled from the tube before centrifugation. The bound fraction of drug was calculated by the following equation, bound fraction (%)=100× (1- drug concentration in the ultrafiltrate/total drug concentration in the serum). Preliminary experiments indicated that the adsorption of LB20304 to the device or to the membrane was negligible (less than 7%).

In vivo study in rats

Male Sprague-Dawley rats (200-300 g) were housed in standard laboratory rat cages, with food and water *ad libitum,* in a light-controlled room at a temperature of 22±1°C and a humidity of 55±10%. Each rat was housed individually in a Ballmann cage (Dae Jong Instrument Company, Seoul, Korea) for plasma disposition and biliary excretion experiments or in a metabolic cage for tissue distribution and urinary excretion experiments. Rats were fed *ad libitum* for IV studies, whereas rats were fasted 12 hr before/6 hr after dosing for oral studies.

Plasma disposition study (n=3-10): The femoral artery and the femoral vein (infusion study only) of rats were cannulated with polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ, USA) under light ether anesthesia. After complete recovery from anesthesia, 5-200 mg/4 ml/kg of LB20304a was infused for 1 min via the femoral vein for intravenous (IV) infusion, but 20-500 mg/4 ml/kg was administered via gavage for oral dosing. Afterwards, approximately 250 μl alliquots of blood were withdrawn into heparinized tube (25 U/ml) from the femoral artery at 1, 5, 10, 20, 40, 60, 120, 240, and 360 min for IV infusion or at 5, 15, 30, 60, 90, 120, 240, 360, and 480 min for oral dosing. Plasma samples were separated by centrifuging the blood samples at 14,000 g for 1 min.

Tissue distribution study (n=3): Rats were exsanguinated by blood collection from the posterior vena-cava at 30, 60, and 240 min following oral administration of 20 mg/4 ml/kg of LB20304a via gavage. Approximately 1 g of liver, lung, heart, spleen, and kidney were quickly removed, rinsed, weighed, minced, and homogenized with 4 volumes of isotonic phosphate buffer (pH 7.4) using a Tempest tissue homogenizer to obtain aliquots of tissue homogenate. Also, an aliquot of plasma was obtained by centrifuging the blood sample at 14,000 g for 1 min. Additionally, tissue distributions including 18 tissues, such as plasma, blood, brain, submaxillary gland, thymus, heart, lung, liver, spleen, stomach, small intestine, large intestine, cecum, kidney, testis, fat, muscle, and lymph node, were studied at 30 min following oral administration of 20 mg/kg of LB20304a to rats.

Urinary excretion study (n=4): Urine samples were collected at 0-12, 12-24, and 24-48 hr post-dosing of 20 mg/4 ml/kg of LB20304a in rats. Dosing solution was administered via the tail vein for IV bolus dosing or via gavage for oral dosing. Finally, urine sample was aliquoted after recording its volume. For the assay of glucuronide conjugation, 0.2 ml of urine sample was added to 400 μl of β-glucuronidase (10,000 U/ml in water), then incubated for 24 hr at 37°C and 150 opm to hydrolyze the conjugate.

Biliary excretion study (n=4): The bile duct of rats was cannulated with polyethylene tubing (PE-10) under light ether anesthesia. After complete recovery

from anesthesia, 20 mg/4 ml/kg of LB20304a was administered via the tail vein for IV bolus dosing or via gavage for oral dosing. Afterwards, bile samples were collected at 0-2, 2-4, 4-6, 6-12, 12-24, and 24-48 hr. Finally, bile sample was aliquoted after recording its volume. For the assay of glucuronide conjugation, 0.2 ml of bile sample was added to 400 μl of β-glucuronidase (10,000 U/ml in water), then incubated for 24 hr at 37°C and 150 opm to hydrolyze the conjugate.

In vivo study in dogs (n=5-6)

Male beagle dogs (8-12 kg, Hazleton Research Product Inc., Calamazoo, MI, USA) were housed in standard laboratory dog cages (Dae Jong Instrument Company), with food and water *ad libitum*, in a light-controlled room at a temperature of 22±1°C and a humidity of 55±10%. Each dog was housed individually in a metabolic cage for plasma disposition study and fasted 12 hr before/6 hr after dosing for oral studies. 10 mg/0.2 ml/kg of LB20304a was IV infused for 0.5 min via the cephalic vein of left leg or 10 mg/2 ml/kg of LB20304a was orally administered via gavage. Afterwards, approximately 300 μl alliquots of blood were withdrawn via the cephalic vein of right leg at 0. 5, 5, 10, 20, 40, 60, 90, 120, 180, 240, 300, 360, 480, 600, and 720 min for IV infusion or at 5, 10, 20, 40, 60, 90, 120, 180, 240, 300, 360, 480, 600, and 720 min for oral dosing to obtain 100 μl of plasma.

Pharmacokinetic analysis

The data after IV administration of LB20304 were plotted as plasma concentration vs. time and fitted to a two-exponential decay model using the non-linear regression program SIPHAR (Version 4.0, Simed, Creteil Cedex, France). The following pharmacokinetic parameters, such as the half-life at terminal phase ($t_{1/2\beta}$), area under the plasma concentration-time curve from time zero to time infinity (AUC), time-averaged total body clearance (CL), and apparent volume of distribution at steady-state (Vd_{ss}) were estimated. The data after oral administration of LB20304 were plotted as plasma concentration vs. time and the model independent parameters, such as peak concentration (C_{max}), peak time (t_{max}), AUC, and extent of bioavailability (BA) were estimated from the observed data. AUC from time zero to time infinity was calculated by trapezoidal rule-extrapolation method: This method employed the logarithmic trapezoidal rule recommended by Chiou (1978) for the calculation of area during the declining plasma level phase and linear trapezoidal rule for the rising plasma level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant. Standard method by Gibaldi and Perrier (1982) was used to calculate CL and Vd_{ss}. BA was calculated by $(AUC_{Oral} \times Dose_{IV})/(AUC_{IV} \times Dose_{Oral})$. The data were expressed as mean±standard deviation (SD).

Statistical data analysis

Statistical significance of difference was first tested with one-way analysis of variance (ANOVA). Where there was a statistical difference, multiple comparisons were conducted with the use of Fisher's Protected Least Significance Difference (PLSD) test. a P value of less than 0.05 was chosen as the level of statistical difference.

RESULTS

The concentration-time profiles of LB20304 after incubation with liver microsomes of rats, dogs, and humans are shown in Fig. 2. In the normal liver microsomes of all species, the concentration of LB20304 did not decrease with time after incubation. However, the concentration of LB20304 decreased to 57±1% in the rat microsome treated by aroclor.

The serum protein bindings of LB20304 in rats, dogs, and humans are listed in Table I. LB20304 was not highly bound to rat, dog, and human serum, and the bound fraction for this compound was 58.5-65. 8%, 19.1-26.6%, and 56.9-59.6%, respectively. The serum protein bindings of LB20304 were not significantly different between low (2 μg/ml) and high (10 μg/ml) concentrations in each species, but they were significantly different among species.

The plasma concentration-time profiles of LB20304 following IV infusion and oral administration of various doses in rats are shown in Figs. 3 and 4, respectively; and the relevant pharmacokinetic parameters

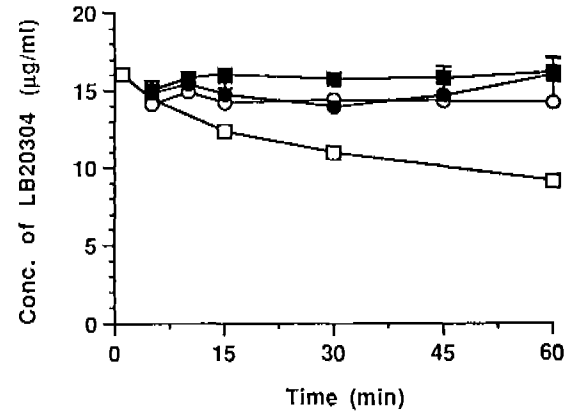


Fig. 2. The concentration profiles of LB20304 after incubation in the liver microsomes of rats, dogs, and humans at 16 μg/ml concentration. Each point represents the mean ±SD (n=3). Key: ●, Rat; ○, Dog; ■, Human; □, Rat (aroclor treated)

are listed in Table II. After IV infusion for 1 min, the plasma concentrations seemed to decrease parallel each other and biexponentially at the dose ranges studied over 5-200 mg/kg. There was no significant difference in the CL values. However, the $t_{1/2\beta}$ and Vd_{ss} at 5 mg/kg dose were significantly different from those of others, which might come from the incomplete determination of terminal phase due to the non-detection at 360 min. The $t_{1/2\beta}$, Vd_{ss}, and CL were in the range of 71.6-128 min, 1144-3025 ml/kg, and 14.6-21.8 ml/min/kg, respectively. After oral administration of LB20304 to rats, the elimination profiles of plasma concentration seemed to change linearly each other over 20-200 mg/kg doses studied. The BA was 30.8, 27.3, 41.6, and 36.0%, respectively. However, the elimination profile of plasma concentration at 500 mg/kg dose seemed to change differently from those at

Table I. Serum protein binding (%) of LB20304 in rats, dogs, and humans (mean±SD, n=3)

Conc. [μg/ml]	Rat	Dog	Human	P[2]
2	65.8±1.94	19.1±4.28[3]	59.6±3.72	0.0001
10	58.5±7.00	26.6±11.2[3]	56.9±4.24	0.0045
P[1]	0.1549	0.3352	0.4515	

[1]Statistical significance of difference at P<0.05 by one-way analysis of variance (ANOVA) between concentrations
[2]Statistical significance of difference at P<0.05 by one-way analysis of variance (ANOVA) among species
[3]Significantly different from other species at P<0.05 by Fisher's Protected Least Significance Difference (PLSD) test

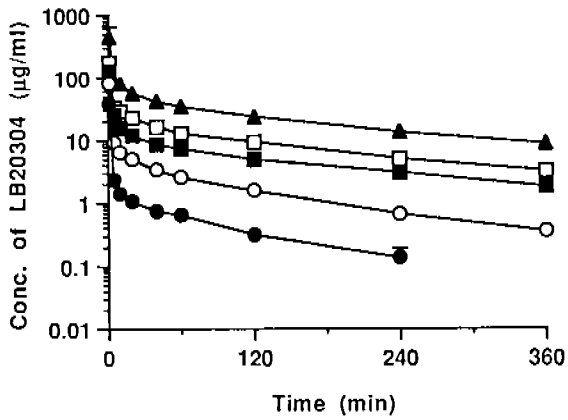


Fig. 3. The plasma concentration-time profiles of LB20304 following IV infusion for 1 min of various doses to rats. Each point represents the mean±SD (n=3-10). Key: ●, 5 mg/kg; ○, 20 mg/kg; ■, 50 mg/kg; □, 100 mg/kg; ▲, 200 mg/kg

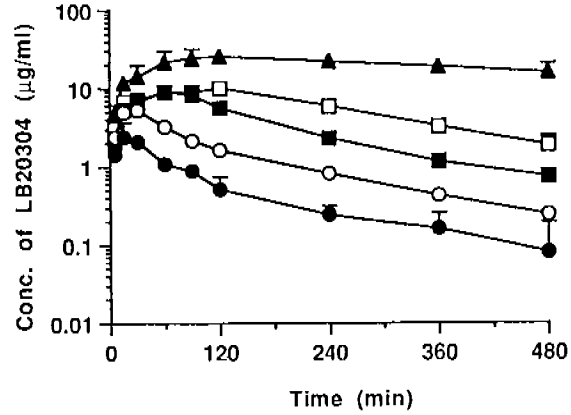


Fig. 4. The plasma concentration-time profiles of LB20304 following oral administration of various doses to rats. Each point represents the mean±SD (n=3-10). Key: ●, 20 mg/kg; ○, 50 mg/kg; ■, 100 mg/kg; □, 200 mg/kg; ▲, 500 mg/kg

Table II. Pharmacokinetic parameters of LB20304 in the plasma following IV infusion for 1 min and oral administration of various doses in rats (mean±SD, n=3~10)

Parameters	5 mg/kg	20 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	500 mg/kg	P[1]
IV							
$t_{1/2\beta}$ [min]	71.6±16.8[2]	93.6±11.6	128±2.51	117±7.04	124±4.13		0.0001
AUC [μg · min/ml]	224.5±26.00	778.4±196.8	2485±206.4	4189±729.6	11004±591.7		
CL [ml/min/kg]	18.0±2.25	21.8±6.57	16.4±1.23	19.5±3.04	14.6±0.777		0.1245
Vd_{ss} [ml/kg]	1144±663.3[3]	2265±959.5	2625±280.8	3025±308.6	2415±253.0		0.0055
oral							
AUC [μg · min/ml]		239.6±48.24	678.4±70.32	1741±176.0	3957±381.6	16120±2082	
t_{max} [min]		18.3±11.7[4]	26.3±7.50[5]	70.0±17.3[6]	120±0.00	130±96.4	0.0001
C_{max} [μg/ml]		2.23±0.640	5.42±0.856	9.02±0.616	12.5±1.50	27.7±6.42	
BA [%]		30.8	27.3	41.6	36.0	58.6[7]	

[1]Statistical significance of difference at P<0.05 by one-way analysis of variance (ANOVA) among doses
[2]Significantly different from other doses at P<0.05 by Fisher's Protected Least Significance Difference (PLSD) test
[3]Significantly different from 20, 50, and 100 mg/kg doses at P<0.05 by Fisher's Protected Least Significance Difference (PLSD) test
[4]Significantly different from 100, 200, and 500 mg/kg doses at P<0.05 by Fisher's Protected Least Significance Difference (PLSD) test
[5]Significantly different from 200 and 500 mg/kg doses at P<0.05 by Fisher's Protected Least Significance Difference (PLSD) test
[6]Significantly different from 500 mg/kg doses at P<0.05 by Fisher's Protected Least Significance Difference (PLSD) test
[7]Relative to 200 mg/kg IV dose

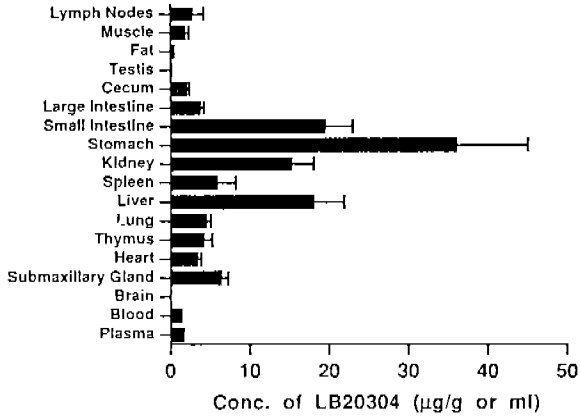


Fig. 5. The tissue concentration of LB20304 in the various tissues at 30 min following oral administration of 20 mg/kg to rats. Each point represents the mean±SD (n=3)

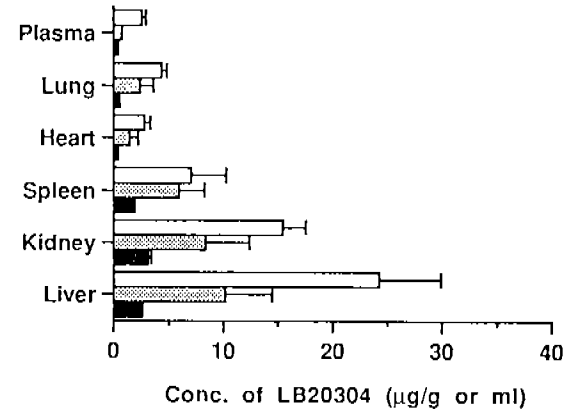


Fig. 6. The tissue concentration-time profiles of LB20304 following oral administration of 20 mg/kg to rats. Each point represents the mean±SD (n=3). Key: □, 30 min; ▩, 60 min; ■, 240 min

20-200 mg/kg doses. By the increase of dose 2.5 times from 200 to 500 mg/kg, the AUC increased 4.1 times and the BA increased from 36.0% to 58.6%. By contrast, the peak concentrations seemed to change non-linearly and the peak times increased significantly over 20-500 mg/kg oral doses ($P<0.05$ by ANOVA). The increase of C_{max} was 2.4, 4.0, 5.6, and 12.4 times by the increase of dose 2.5, 5, 10, and 25 times from 20 to 500 mg/kg.

After single oral administration of 20 mg/kg to rats, the various tissue concentrations of LB20304 at 30 min are shown in Fig. 5; and the major tissue concentrations of LB20304 at 30, 60, 240 min are shown in Fig. 6. The tissue concentration levels at 30 min in liver, stomach, small intestine, and kidney were 9.5 to 26.1 times, and those in spleen, submaxillary gland, heart, thymus, lung, large intestine, cecum, muscle, and lymph node were 1.3 to 4.4 times greater than plasma level, whereas blood concentration was similar to plasma level. The concentration in

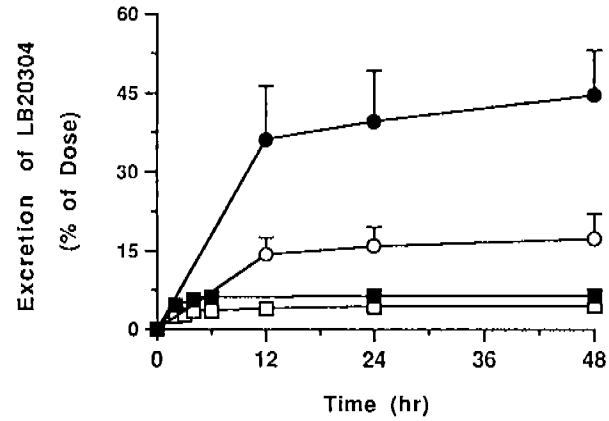


Fig. 7. The urinary and biliary cumulative excretion-time profiles of LB20304 following IV bolus and oral administration of 20 mg/kg to rats. Each point represents the mean±SD (n=4). Key: ●, Urine in IV; ○, Urine in oral; ■, Bile in IV; □, Bile in oral

testis was quite low and that in brain was negligible. The tissue concentrations decreased time-dependently with the the highest concentration at the first sampling time point (30 min).

After single IV bolus and oral administration of 20 mg/kg to rats, the cumulative excretion-time profiles of LB20304 (% of dose) into the urine and bile are shown in Fig. 7. The excretion profiles showed that LB20304 was mainly excreted within 12 hr. The 48 hr urinary recovery of the dose was 44±9.0% for IV dosing and 14±1.4% for oral dosing, whereas the 48 hr biliary recovery of the dose was 6.4±1.5% for IV dosing and 4.5±1.6% for oral dosing. After β-glucuronidase treatment, the recovery of the dose was 17±3.5% in urine and 19±4.7% in bile for oral dosing. Therefore, the urinary excretion of LB20304 was higher than its biliary excretion, whereas the biliary excretion of LB20304 glucuronide conjugate was higher than its urinary excretion.

The plasma concentration-time profiles of LB20304 following IV and oral administration of 20 mg/kg to rats and 10 mg/kg to dogs are shown in Figs. 3, 4, and 8; and the relevant pharmacokinetic parameters are listed in Table III. The CL, Vd_{ss}, $t_{1/2\beta}$, and BA were 21.8 ml/min/kg, 2265 ml/kg, 93.6 min, and 30.8% in rats; and 7.95 ml/min/kg, 4144 ml/kg, 363 min, and 81.1% in dogs, respectively. Therefore in dogs, the CL decreased whereas the $t_{1/2\beta}$ and BA increased significantly compared to those of rats ($P<0.05$ by ANOVA). However, there was no difference in the volume of distribution (Vd_{ss}) between rats and dogs.

DISCUSSION

After IV administration of 20 mg/kg to rats and 10 mg/kg to dogs, the CL, Vd_{ss}, and $t_{1/2\beta}$ of LB20304 were

Table III. Pharmacokinetic parameters of LB20304 in the plasma following IV and oral administration of 20 mg/kg to rats and 10 mg/kg to dogs (mean±SD, n=5~10)

Parameters	Rat	Dog	P[1)]
IV			
$t_{1/2\beta}$ [min]	93.6±11.6	363±64.4	0.0001
AUC [μg · min/ml]	778.4±196.8	1090±322.4	
CL [ml/min/kg]	21.8±6.57	7.95±2.67	0.0034
Vd_{ss} [ml/kg]	2265±959.5	4144±1834	0.1082
Oral			
AUC [μg · min/ml]	239.6±48.24	884.0±162.4	
t_{max} [min]	18.3±11.7	53.3±32.0	0.0059
C_{max} [μg/ml]	2.23±0.640	1.72±0.225	
BA [%]	30.8	81.1	

[1)]Statistical significance of difference at P<0.05 by one-way analysis of variance (ANOVA) between rats and dogs

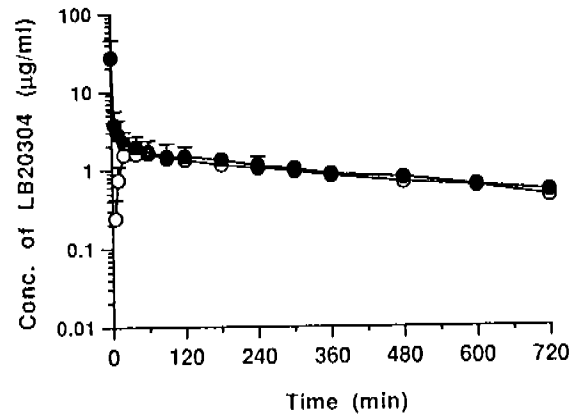


Fig. 8. The plasma concentration-time profiles of LB20304 following IV infusion for 0.5 min and oral administration of 10 mg/kg to dogs. Each point represents the mean±SD (n=5-6). Key: ●, IV; ○, oral

21.8 ml/min/kg, 2265 ml/kg, and 93.6 min in rats; and 7.95 ml/min/kg, 4144 ml/kg, and 363 min in dogs, respectively. The CL, Vd_{ss}, and $t_{1/2\beta}$ of ciprofloxacin (CFLX) were 57.3 ml/min/kg, 3780 ml/kg, and 45.6 min in rats (unpublished data); 10.6 ml/min/kg, 3330 ml/kg, and 223 min in dogs (unpublished data); and 7.9 ml/min/kg, 1700 ml/kg, and 156 min in dogs, respectively (Nakamura *et al.*, 1990). Therefore, LB20304 seemed to have longer half-life than CFLX and similar tissue distribution to CFLX in rats and dogs. By contrast, after oral administration of 20 mg/kg to rats and 10 mg/kg to dogs, the C_{max}, AUC, and BA of LB20304 were 2.23 μg/ml, 239.6 μg.min/ml, and 30.8% in rats; and 1.72 μg/ml, 884.0 μg · min/ml, and 81.1% in dogs, respectively. The C_{max}, AUC, and BA of CFLX were 1.5 μg/ml, 154 μg · min/ml, and 42% in rats (unpublished data); 3.1 μg/ml, 570 μg · min/ml, and 75% in dogs (unpublished data); and 1.1 μg/ml, 458 μg · min/ml, and 44% in dogs, respectively (Nakamura *et al.*, 1990). Therefore, LB 20304 seemed to have similar oral absorption characteristics to CFLX in rats and dogs. When LB20304 was orally administered at the dose of 20 mg/kg to rats and 10 mg/kg to dogs (Figs. 4 and 8), the plasma concentrations of LB20304 were maintained at least for 7.4 hr in rats and 26.2 hr in dogs above its *in vitro* MICs for the most pathogenic organisms (0.1 μg/ml, Kim *et al.*, 1996).

The phase I metabolism of LB20304 was considered to be negligible in rats, dogs, and humans, because of no metabolism in the *in vitro* liver microsomes where phase I metabolisms generally take place. By contrast, LB20304 was considered to receive phase I metabolism by the enzyme induction, because LB20304 concentration decreased in the rat microsome treated with aroclor (Fig. 2). It was reported that aroclor 1254 showed the striking dose- and time-dependant increases in hepatic levels of CYP1A1 and CYP1A2, and limited induction of epoxide hydrolase, quinone oxidoreductase, and aldehyde dehydrogenase in rats (Dragnev *et al.*, 1994).

After IV infusion of LB20304 in rats, its plasma concentrations decreased linearly, which indicated that its distribution, metabolism, and excretion were not changed over 5-200 mg/kg doses studied. After oral administration of LB20304 to rats, the BA was in the range of 27.3-41.6%, which indicated that its first-pass metabolism and/or absorption fraction were not changed over 20-200 mg/kg doses. However, the BA was 58.6% at 500 mg/kg oral dose, which indicated that its first-pass metabolism decreased at higher dose. By contrast, the absorption of LB20304 seemed to be governed by the saturation process at absorption site over 20-500 mg/kg, because the peak concentrations changed non-linearly and the peak times increased by the increase of dose.

The concentration levels of LB20304 in tissues were generally higher than plasma level in rats. Such good tissue uptake of LB20304 was consistent with its relatively large Vd_{ss} (2265 ml/kg in rats) and explained by its lipophilic property as well as its moderate serum protein binding (Table I). In the various quinolones, the tissue concentration levels were also higher than plasma level in rats (Nakamura *et al.*, 1990; Siefert *et al.*, 1986a) and the serum protein bindings were low to moderate (Okezaki *et al.*, Gilfillan *et al.*, 1984; Montay *et al.*, 1984; Siefert *et al.*, 1986b; Fujii *et al.*, 1984; Okazaki *et al.*, 1984; Kasajima *et al.*, 1986). However, irrespective of the good tissue penetration of LB20304, its brain concentration level was negligible, which may indicate its poor penetration into the central nervous system and/or increased pumping out from the cerebrospinal fluid

(CSF) to plasma via the presence of an active transport system. Ooie *et al.* (1996) reported that the low central nervous system (CNS) to plasma concentration ratio of quinolones was characterized by the presence of an active transport system for the efflux of quinolones across the choroid plexus.

The recovery of LB20304 into urine and bile was incomplete in rats, because the 48 hr urinary/biliary recovery of the dose was 50.4% for IV dosing and 18.5% for oral dosing. Based on the *in vitro* metabolism and deconjugation studies, the unrecovered fraction (49.6%) of LB20304 for IV dosing was partly explained by the glucuronide conjugation, because there was a considerable excretion of LB20304 glucuronide into the bile and urine of rats. The 18.5% recovery of LB20304 for oral dosing seemed due to extensive first-pass metabolism or incomplete absorption, which was supported by low oral bioavailability (30.8%).

In the pharmacokinetics of LB20304 for dogs, the CL decreased significantly, and the $t_{1/2\beta}$ and BA increased significantly, whereas the Vd_{ss} did not change compared to those of rats. Compared to those of rats, the increase of $t_{1/2\beta}$ by 3.9-fold in dogs seemed to the decrease of LB20304 elimination (metabolism and excretion); and the increase of BA by 2.6-fold in dogs seemed to the decrease of first-pass metabolism and/or the increase of absorption.

In summary, the pharmacokinetic properties of LB 20304 were characterized by its good oral absorption, long plasma half-life, and good tissue penetration. In the infectious models in mice (Oh *et al.*, 1996), LB 20304 was effective against systemic pulmonary and urinary tract infections caused by a variety of organisms, and its efficacies were generally better than those of ciprofloxacin and ofloxacin due to its good *in vitro* MIC efficacy (Kim *et al.*, 1996) as well as *in vivo* pharmacokinetic characteristics.

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