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METABOLISM AND DISPOSITION OF NOVEL DES-FLUORO QUINOLONE GARENOXACIN IN EXPERIMENTAL ANIMALS AND AN INTERSPECIES SCALING OF PHARMACOKINETIC PARAMETERS

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ABSTRACT:

Garenoxacin is a novel quinolone that does not have a fluorine substituent at the C-6 position in the quinoline ring. Garenoxacin or ^{14}C-garenoxacin was intravenously or orally administered to rats, dogs, and monkeys. Metabolic profiles and pharmacokinetic parameters were investigated focusing on the species differences and the allometric scaling of pharmacokinetic parameters. Garenoxacin was well absorbed following oral administration then underwent phase II metabolism in all species tested. Major metabolites of garenoxacin were the sulfate of garenoxacin (M1) and glucuronide (M6). Oxidative metabolites were present in very minor concentrations in all species tested. Another minor route of metabolism was the formation of the carbamoyl glucuronide. Garenoxacin is characterized across species by the observation that it circulates systemically, is excreted renally as unchanged drug, and is metabolized to M1 and M6, which are excreted specifically into the bile. The total clearances (CL) were 12.1, 2.43, and 3.39 ml/min/kg for rats, dogs, and monkeys, respectively. The distribution volume values of garenoxacin (Vss) were 0.88, 1.29, and 0.96 l/kg for rats, dogs, and monkeys, respectively. In all animals tested, the extrarenal clearance was larger than the renal clearance, and neither of the clearances was limited by blood flow. Despite these conditions, garenoxacin showed a good correlation for CL and Vss for allometric interspecies scaling.

Fluoroquinolones such as ciprofloxacin and ofloxacin are potent agents with broad spectrum antimicrobial activity and are used as alternatives to β-lactam agents in the treatment of a wide range of infections. The fluorine at the position C-6 of the quinoline ring was proposed to be necessary for expression of the antimicrobial activity of quinolones (Domagala, 1994). Garenoxacin[1] (formerly T-3811 or BMS-284756) is a novel, unique quinolone that does not have a fluorine substituent at the position C-6 but has shown excellent potency particularly against Gram-positive bacteria, including methicillin-resistant staphylococci and penicillin-resistant streptococci, as well as activity against Gram-negative bacteria (Takahata et al., 1999; Fung-Tomc et al., 2000; Hayashi et al., 2002). Quinolones including garenoxacin display a concentration-dependent bactericidal effect on most bacteria (Hyatt et al., 1995). Thus, the maximum plasma concentration (C_{max}) to minimum inhibitory concentration ratio can act as a parameter that correlates to efficacy (Stein, 1996). The area under the curve of a plasma concentration-time profile (AUC) to minimum inhibitory concentration ratio is a more important predictor of efficacy for quinolones in clinical practice (Forrest et al., 1997). Therefore, it is useful to extrapolate pharmacokinetic parameters, especially AUC in the case of quinolones from experimental animal data for predicting efficacy in humans.

Interspecies scaling has been performed to predict drug disposition in humans for some drugs (Mordenti, 1985; Efthymiopoulos et al., 1991; Sanwald-Ducray and Dow, 1997; Grindel et al., 2002). However, interspecies scaling has not yet been widely reported for quinolones, with moxifloxacin a notable exception (Siefert et al., 1999). In the present study, first we examined the disposition and metabolism of garenoxacin in animal species used in the toxicological evaluation of the compound. Second, we performed interspecies scaling of pharmacokinetic parameters.

[1] Abbreviations used are: garenoxacin, 1-cyclopropyl-8-(difluoromethoxy)-7-[(1*R*)-(1-methyl-2,3-dihydro-1*H*-5-isoindolyl)]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid; AUC, the area under the curve of a plasma concentration-time profile; T-3811M1, 1-cyclopropyl-8-(difluoromethoxy)-7-[2-hydroxysulfonyl-(1*R*)-1-methyl-2,3-dihydro-1*H*-5-isoindolyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid; T-3811M4, 1-cyclopropyl-8-(difluoromethoxy)-7-[(1*R*)-1-methyl-3-oxo-2,3-dihydro-1*H*-5-isoindolyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid; T-3811M5, 1-cyclopropyl-8-(difluoromethoxy)-7-(1-methyl-1-hydroxy-3-oxo-2,3-dihydro-5-isoindolyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid; HPLC, high-performance liquid chromatography; LC/MS/MS, liquid chromatography-tandem mass spectrometry; DMSO, dimethyl sulfoxide; LSC, liquid scintillation counter; CL, clearance; MRT, mean resident time; Vss, volume of distribution at steady state; CL_r, renal plasma clearance; CL_{er}, extrarenal plasma clearance; UGT, UDP-glucuronyl transferase; GFR, glomerular filtration rate; CL_{rb}, renal blood clearance; R_b, blood-to-plasma concentration ratio.

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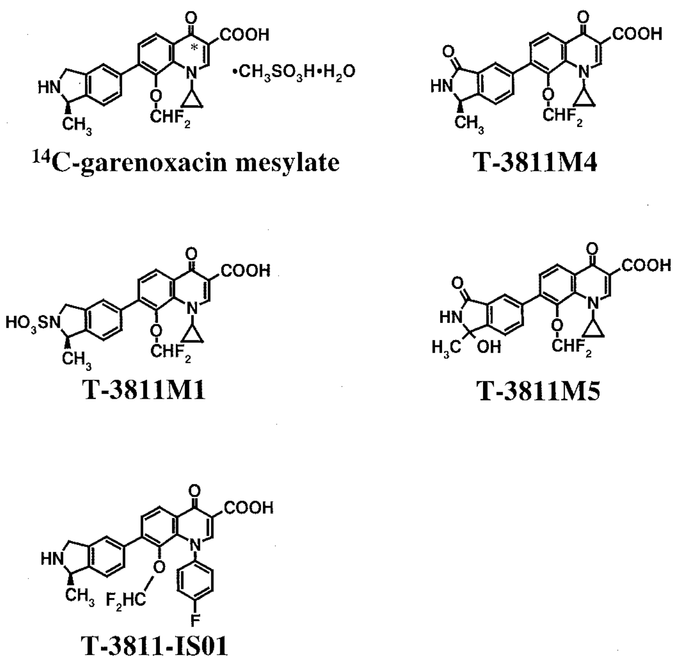

FIG. 1. *Chemical structures of [14]C-garenoxacin (*, labeled position), synthesized authentic metabolites (T-3811M1, T-3811M4, and T-3811M5), and internal standard for garenoxacin determination (T-3811-IS01).*

Garenoxacin was administered to rats, dogs, and monkeys in both single oral and intravenous doses to investigate the rate of absorption, to compare the pharmacokinetics in those species and to perform an interspecies scaling along with the mouse data of our previous report (Takahata et al., 1997). To support the results of interspecies scaling, the profiles of metabolites in plasma and excreta were determined in rats, dogs, and monkeys following oral and intravenous administration of ^{14}C-garenoxacin.

Materials and Methods

Chemicals. ^{14}C-Garenoxacin mesylate was synthesized at Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). The specific activity was 1.62 GBq/mmol, and the radiochemical purity was >96% for rat experiments. For the experiments performed on dogs and monkeys, the specific activity was 335 MBq/mmol, and the radiochemical purity was >98%. The ^{14}C compound was labeled at position C-3 of the quinoline ring (Fig. 1). Cold garenoxacin mesylate was synthesized by Toyama Chemical Co., Ltd. (Toyama, Japan) with chemical purity of >99%. The T-3811M1 disodium salt (sulfate of garenoxacin, HPLC purity >96.0%), T-3811M4 (oxidative product of garenoxacin, HPLC purity 97.7%), and T-3811M5 (oxidative product of garenoxacin, HPLC purity 98.1%) were used as authentic compounds and were also synthesized by Toyama Chemical Co., Ltd. The internal standard for HPLC analysis (T-3811-IS01) was synthesized by Toyama Chemical Co., Ltd. The structures of these compounds are illustrated in Fig. 1. β-Glucuronidase purified from *Escherichia coli* (type IX-A) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were of reagent grade or of the highest purity available commercially.

Animals and Preparation of Dosing Solution. All animal studies were performed in accordance with the guideline for the Care and Use of Laboratory Animals published by Toyama Chemical Co., Ltd. Male 8- to 10-week-old Wistar/ST rats (Japan SLC, Inc., Hamamatsu, Japan) with free access to food and water before experiments were used. The body weights of the rats were 245 to 266 g and 214 to 246 g for intravenous and oral dosing, respectively. The rats dosed orally were fasted overnight before dosing. For the collection of excreta, the rats were housed individually in glass metabolism cages (Metabolica type MC-CO_2; Sugiyama Gen Iriki Ltd., Tokyo, Japan), and for other studies they were housed singly in Bollman's cages (Natsume Seisakusho Co., Ltd. Tokyo, Japan). The beagle dogs were purchased from CSK Research Park (Gotenba, Japan) or were obtained from either NARC Co., Ltd. (Matuomati, Japan). The dogs (8.6–12 kg) were fed daily at 4 PM for the studies of pharmacokinetics and metabolic profiles. Dogs that were fed daily in the morning and were fasted overnight before dose administration were used for the isolation of metabolites. The dogs were housed individually in stainless steel metabolism cages with free access to water. Male cynomolgus monkeys from 3 to 4 years old weighing 2.0 to 3.0 kg were purchased from CLEA Japan, Inc. (Tokyo, Japan). Monkeys were kept individually in metabolic cages with free access to water and were fed at 3 and 4 PM daily.

Garenoxacin was dissolved in a 5% (w/v) D-mannitol solution for intravenous administration and suspended in a 0.5% (w/v) methylcellulose aqueous solution for oral administration. The doses and concentrations were represented as the free base of garenoxacin.

Sample Collection for Plasma Kinetics and Urinary Excretion. Blood and urine samples were collected from rats, dogs, and monkeys following garenoxacin administration. Blood samples were collected into heparinized syringes and centrifuged to separate the plasma. All plasma and urine samples were stored at −20°C or lower, pending analysis. Collection of samples were as follows.

Rats. Three or four male rats were intravenously and orally administered garenoxacin at a dose of 10 mg/kg. The femoral artery of each rat was cannulated under light ether anesthesia. After the rats had awakened, garenoxacin was injected into the femoral vein or orally administered by gavage. A series of blood samples (150 μl) were collected from the femoral artery at 2, 10, and 30 min and 1, 2, 4, 6, and 24 h after the intravenous administration and at 5, 10, 15, and 30 min and 1, 2, 4, 6, and 24 h after the oral administration. A volume of blood for transfusion (withdrawn from other rats; blood-heparin 9:1) equal to the volume of blood drawn was injected after collection of each sample. Urine samples were collected over a period of 0 to 24 h after the intravenous dosing.

Dogs. Three male dogs were intravenously administered garenoxacin via the cephalic vein at a dose of 15 mg/kg. Another group of male dogs was orally administered garenoxacin in gelatin capsules at 25 mg/kg. A series of blood samples (0.25 ml) were collected from the cephalic vein of each animal at 5, 15, and 30 min and 1, 4, 8, and 24 h after the intravenous administration and at 15 and 30 min and 1, 2, 4, 6, 8, and 24 h after the oral administration. Urine samples were collected over a period of 0 to 24 h after the dosing.

Monkeys. Three male monkeys were intravenously administered ^{14}C-garenoxacin via the cephalic vein and were orally administered by a rubber catheter tube at a dose of 5 mg/kg (3.78–3.80 MBq/kg). A series of blood samples were collected from the cephalic vein of each animal at 5, 15, and 30 min and 1, 2, 4, 6, 8, and 24 h after the intravenous administration and at 10 and 30 min and 1, 2, 4, 6, 8, 12, and 24 h after the oral administration. Urine samples were collected over a period of 0 to 24 h after the dosing.

Serum Protein Binding. The binding of garenoxacin to serum protein in rats, dogs, and monkeys was determined in vitro using an ultrafiltration device (Microcon; Millipore Corporation, Bedford, MA) as described previously (Hayakawa et al., 2002). Determinations were performed for four concentrations between 0.5 and 100 μg/ml (in the case of monkeys, 0.5 to 50 μg/ml). The concentrations of garenoxacin in the ultrafiltrate were determined by HPLC under the same conditions as for the determination of garenoxacin in rat plasma.

Isolation and Identification of Metabolites. *Sample collection.* From our preliminary study, metabolites of garenoxacin (denoted as M1, M2, M3, M4, M5, and M6) were observed in rat and dog bile following garenoxacin administration. Therefore, the metabolites were isolated and identified using dog bile following garenoxacin administration. Dogs were surgically fitted with bile duct cannulas under pentobarbital anesthesia. A cannula was inserted into the common bile duct and the entrance to the duodenum. The cannulas were connected to a T-shaped stopcock implanted subcutaneously to resume the bile flow into the duodenum. The animals were placed in jackets. After a recovery period of a few days, the bile duct-cannulated dog was constantly infused with garenoxacin (1.6 mg/kg/10 ml/h) after a single intravenous administration (12 mg/kg) to maintain the plasma concentration of garenoxacin at 10 μg/ml. The bile was collected for up to 6 h after initiating the administration.

Isolation and identification of M1. A reverse-phase silica gel column (LRP-2; Whatman, Maidstone, UK) was used to obtain a crude fraction of M1 from dog bile. The crude fraction was extracted with chloroform under acidic conditions (0.2 M hydrochloric acid), and the chloroform layer was evaporated to dryness. The residue was dissolved with water, and M1 was further isolated by HPLC (Hitachi L-6250 pump, Hitachi L-4200 UV detector; Hitachi, Ltd., Tokyo, Japan). The column was Develosil ODS-5 (Nomura Chemical Co., Ltd., Seto, Japan) 20 mm i.d. × 250 mm with a mobile phase of acetonitrile/0.3 M formic acid-ammonium formate (pH 4.0)/water (300:200:500, v/v), and M1 was detected by UV detection at a wavelength of 279 nm. The isolated M1 and its synthetic standard, T-3811M1, were analyzed with LC/MS/MS.

Isolation and identification of metabolite M2. The dog bile was applied to a reverse-phase LRP-2 column to obtain a crude fraction of M2. The crude fraction was extracted with chloroform under acidic conditions (0.2 M hydrochloric acid), then the chloroform layer was evaporated to dryness. The residue was dissolved with a 10% DMSO aqueous solution, and M2 was further isolated by HPLC. HPLC conditions were the same as for the isolation of M1. The eluate was evaporated to dryness under reduced pressure and reconstituted with the 10% DMSO aqueous solution. The fraction including M2 was desalted, evaporated to dryness, and then reconstituted in acetic acid/DMSO for analysis by NMR (JNM-LA500; JEOL, Tokyo, Japan). To characterize the aglycone of M2, the fraction including M2 was treated with an equal volume of 1 M NaOH, incubated at 100°C for 1 h, and then neutralized with 1 M HCl solution. β-Glucuronidase solution (0.1 M phosphate buffer, pH 6.8) was added to the M2 in solution and incubated at 37°C for 1 h. M2 and the samples treated with NaOH or β-glucuronidase were analyzed by HPLC and LC/MS/MS.

Isolation and identification of M3, M4, and M5. M3, M4, and M5 were isolated from dog bile by HPLC [L-6000 pump (Hitachi, Ltd.), SPD-10A UV detector (Shimadzu, Kyoto, Japan)]. The column was Develosil ODS-HG-5 4.6 mm i.d. × 150 mm with a mobile phase of acetonitrile/0.3 M formic acid-ammonium formate (pH 4.0)/water (300:200:500, v/v), and the metabolites were detected by UV detection at a wavelength of 279 nm. M3, M4, and M5 fractions were evaporated to dryness under reduced pressure. The isolated M3, M4, M5, and synthetic standard (T-3811M4 and T-3811M5) were analyzed with LC/MS/MS.

Isolation and identification of metabolite M6. The dog bile was applied to a reverse-phase LRP-2 column to obtain a crude fraction of M6, then M6 in the crude fraction was finally isolated by HPLC. Conditions were the same as for the isolation of M1, except the mobile-phase acetonitrile/0.3 M formic acid-ammonium formate buffer (pH 4.0)/water (150:200:650, v/v). The desalted fraction was evaporated to dryness and reconstituted in acetic acid/DMSO before analysis by NMR. To characterize the aglycone of M6, the solution of M6 was treated with NaOH as described previously for M2. M6 and the sample treated with NaOH were analyzed by HPLC and LC/MS/MS.

HPLC conditions for identification of M1, M2, M3, M4, M5, and M6. The separation of each metabolite was achieved by HPLC with essentially the same conditions used in the isolative procedure. The column was Develosil ODS-HG-5 4.6 mm i.d. × 150 mm with a mobile-phase flow rate of 1.0 ml/min. Formation of the aglycones of M2 and M6 in bile samples after treatment with NaOH and/or β-glucuronidase was confirmed by the same HPLC condition as used in the isolation of M3, M4, and M5. All metabolites isolated were characterized by the retention time of HPLC used for determining radiometabolite profiles (conditions were described under *Analytical Methods*).

Mass Spectrometry for Identification of M1, M2, M3, M4, M5, and M6. A TSQ 7000 or 7001 triple-stage, quadrupole, tandem mass spectrometer (Thermo Electron Corporation, Waltham, MA) was used for spectra in the electrospray ionization and collision-induced dissociation modes. The electrospray ionization was performed at 4.5 kV with a heated capillary temperature of 225°C, a sheath gas (N_2) pressure of 70 psi, and an auxiliary gas (N_2) flow of 10 units. The product ion mass spectra of garenoxacin and its metabolites were measured with a collision gas (argon) pressure of 2.0 mTorr and a suitable collision offset voltage for each metabolite ranging from −15 to −30 eV for positive ions and between 15 and 30 eV for negative ions. The apparatus was combined with an HPLC system with the column of Develosil ODS-HG-5 (2.0 mm i.d. ×150 mm). The mobile phase was acetonitrile/0.3 M formic acid-ammonium formate buffer (pH 4.0)/water (30:40:130, v/v for M6, and 30:40:50, v/v for other metabolites).

Radiometabolite Profiles. *Sample collection.* Blood and excreta samples for radiometabolite profiles were collected from rats, dogs, and monkeys following ^{14}C-garenoxacin administration. For each species, plasma obtained from blood samples by centrifugation and excreta samples were pooled per time point for the determination of radiometabolite profiles, and stored at −20°C or lower, pending analysis. Total radioactivity of the samples was measured with a liquid scintillation counter (LSC; Tri-Carb 2500TR; PerkinElmer Life Sciences, Boston, MA, or LSC-1000; Aloka Co., Ltd., Tokyo, Japan) with a scintillation cocktail (Hionic-Fluor, PerkinElmer Life Sciences). Before the measurement, the plasma and feces were solubilized by Soluene-350 (PerkinElmer Life Sciences). Quench correction was automatic with the use of the external standard method. Collection of samples were as follows.

Rats. Three male rats were intravenously administered ^{14}C-garenoxacin via the caudal vein or orally administered by gavage at a dose of 5 mg/kg (19 MBq/kg). After the administration of ^{14}C-garenoxacin, blood was collected from the external jugular vein at 15 min and from the abdominal vein at 4 h under ether anesthesia. Urine and feces were collected over a period of 0 to 24 h under freezing conditions. Feces were homogenized with a 5-fold volume (w/v) of ice-cold water. For the collection of bile, the common bile duct was cannulated under a light ether anesthesia. After the rats awoke, ^{14}C-garenoxacin (5 mg/kg) was administered orally or intravenously to the bile duct-cannulated animals, then bile samples were collected under ice-cold conditions for up to 24 h after the administration.

Dogs. Three male dogs were intravenously administered ^{14}C-garenoxacin via the cephalic vein at a dose of 100 mg/kg (370 kBq/kg). A series of blood samples were collected from the jugular vein of each animal at 5 min and 2, 6, and 24 h after the administration. Urine and feces were collected over a period of 0 to 24 h after dosing under dry-ice-cooled and ambient conditions, respectively. The fecal samples were cooled down (−20°C) immediately after the collection. The feces were homogenized with ice-cold water.

Monkeys. The urine and feces samples were collected from the same animals as described under *Sample Collection for Plasma Kinetics and Urinary Excretion*. The feces were collected at 0 to 24 h and 24 to 48 h after the dosing, and those with high concentrations of radioactivity were used for the pooled samples. The fecal sample was homogenized with ice-cold water before measuring radioactivity. For the collection of bile, the common bile duct was cannulated under phenobarbital anesthesia. Another cannula for the intraduodenal administration was inserted into the duodenum through the bile duct. ^{14}C-garenoxacin mesylate was administered intravenously or intraduodenally to one bile duct-cannulated monkey at a dose of 5 mg/kg (3.94–4.06 MBq/kg); then bile was collected over a period of 0 to 6 h under phenobarbital anesthesia. From the same animals, plasma samples were collected from the cephalic vein at 5 min and 4 h after intravenous administration and at 1 and 4 h after intraduodenum administration.

Preparation of samples for HPLC. Radiometabolite profiles were determined by HPLC and quantified using either a radiodetector or fraction collection followed by scintillation counting. Plasma was mixed with acetonitrile/methanol (1:1, v/v), and the supernatant was collected after centrifugation (3000 rpm for 10 min). The precipitate was washed with the same solvent, and the supernatant was collected, combined, and then evaporated to dryness under reduced pressure. The residue was dissolved in HPLC mobile phase and injected into the radio-HPLC system. Bile from all species and urine from the rat and monkey were analyzed directly. The radioactivity in dog urine was extracted using OASIS HLB solid-phase extraction cartridges (Waters, Milford, MA). After the loading of a dog urine sample, the column was washed with distilled water and eluted with acetonitrile/citrate buffer (0.2 M, pH 4.0)/distilled water (80:1:19, v/v). The eluate was evaporated to dryness under reduced pressure; the residue dissolved in a HPLC mobile phase, then injected into the radio-HPLC system. Fecal homogenate samples from all species were extracted using acetonitrile/citrate buffer (0.2 M, pH 4.0)/distilled water (80:1:19, v/v). An aliquot of pooled fecal homogenate was mixed with the extraction solvent (acetonitrile/citrate buffer), centrifuged (3000 rpm for 10 min), and the supernatant collected. The precipitate was washed with the same solvent. The supernatant was combined and evaporated to dryness under reduced pressure. The residue was dissolved in HPLC mobile phase and injected into the radio-HPLC system. An estimate of the recovery of radioactivity was determined by assaying aliquots of the extracts by an LSC. Extraction efficiencies were 85 to 96% for plasma (except dog plasma at 24 h with a value of over 74%), and 88 to 93% for feces. The extraction efficiency was 103% for dog urine.

Analytical Methods. *Determination of garenoxacin in plasma and urine in rats.* Concentrations of garenoxacin in rat plasma and rat urine were determined by HPLC.

Plasma. An aliquot of plasma (0.5 ml) was added to 1 ml of acetonitrile/methanol solution (1:1, v/v), followed by the addition of internal standard (T-3811-IS01, 50 μl of 25 μg/ml aqueous solution). The mixture was centrifuged, the organic solvent of the supernatant was evaporated under reduced pressure, and then the residual aqueous solution was buffered with 2.5 ml of phosphate buffer (0.5 M, pH 6.0). Solid extraction was carried out with the OASIS HLB 3CC. After loading the sample, the column was washed successively with 3 ml $\times$ 2 of 20% methanol and 3 ml of 10% acetonitrile, and eluted with 3 ml $\times$ 2 of acetonitrile. The eluate was evaporated to dryness under reduced pressure. The residue was reconstituted in 250 μl of HPLC mobile phase and injected into the HPLC system.

Urine. An aliquot of urine (0.1 ml) was added to 3 ml of phosphate buffer (0.02 M, pH 6.0), followed by the addition of the internal standard solution (T-3811-IS01, 50 μl of 25 μg/ml solution). The mixture was applied to a 0.5-ml volume of positive ion exchange resin (CM-Toyopearl 650M column; TOSOH Co., Tokyo, Japan). The column was washed successively with 4 ml of distilled water and 4 ml of 20% acetonitrile, then eluted with 4 ml of 1 M acetic acid/acetonitrile (1:9, v/v). The eluate was evaporated to dryness, then the residue was reconstituted in 250 μl of mobile phase and injected into the HPLC system.

HPLC conditions. HPLC analysis was carried out on a Hitachi L-7100 equipped with a UV detector (L-7400, Hitachi Ltd.). The mobile phase was acetonitrile/ citrate buffer (0.2 M, pH 3.5)/water (280:150:570, v/v), delivered isocratically at a flow rate of 1 ml/min. Samples (100 μl) were injected onto a Develosil ODS-HG-5 column (4.6 mm i.d. $\times$ 150 mm) at 30°C and detected by UV at a wavelength of 280 nm. The assay demonstrated good linearity and reproducibility over the plasma concentration range of 0.03 to 10 μg/ml, and the urine range of 0.5 to 50 μg/ml. The assay accuracy was 1.2 to 17.5% for plasma and 1.8 to 4.9% for urine. The intra- and interday precision, expressed as the coefficient of variance (%CV), was 0.6 to 9.7% for plasma and 1.2 to 4.9% for urine.

Determination of garenoxacin in plasma and urine in dogs. Concentrations of garenoxacin in dog plasma were determined by LC/MS/MS according to a previously validated report (Fukumoto et al., 2003). Concentrations of garenoxacin in dog urine were determined by HPLC by the same method as that used for rat urine. The assay accuracy was 2.6 to 8.3%, and the intra- and interday precision was 1.9 to 9.0%.

Determination of garenoxacin in plasma and urine in monkeys. Total radioactivity in plasma and urine was determined directly by a LSC. To determine concentrations of garenoxacin in plasma, plasma samples were applied to a thin-layer chromatography plate (silica gel $60F_{254}$; Merck Research Labs, West Point, PA) together with cold garenoxacin; then the plate was predeveloped with 2% (w/v) ammonium acetate in methanol for a distance of 10 mm (in height) from the origin. The plate was air-dried at room temperature then developed with chloroform/methanol/formic acid (20:5:1, v/v). The ratio of garenoxacin radioactivity to the total radioactivity in each plasma sample was determined with a Bio-imaging analyzer (Fuji Photo Film Co., Ltd., Tokyo, Japan). The amount of garenoxacin in urine was estimated from the total radioactivity and the metabolic profiles of the urine (see *Radiometabolite Profiles*).

HPLC conditions for radiometabolite profiles. Samples prepared for HPLC (L-6200, Hitachi Ltd.) were injected onto a Develosil ODS-HG-5 column (4.6 mm i.d. $\times$ 150 mm). The column was then eluted with acetonitrile/citrate buffer (0.2 M, pH 4.0)/water (100:150:750, v/v, solvent A), acetonitrile/citrate buffer (0.2 M, pH 4.0)/water (350:150:500, v/v, solvent B), and acetonitrile/citrate buffer (0.2 M, pH 4.0)/water (700:150:150, v/v, solvent C) as the mobile phase, using the following solvent gradient program (expressed as a percentage of solvent): 100% solution A at 0 min; 0 to 60% solution B over 0 to 15 min; 60 to 100% solution B over 15 to 35 min, held at 100% solution B over 35 to 45 min; 0 to 30% solvent C over 45 to 60 min; 100% solution C over 60 to 70 min. A flow rate of 1.0 ml/min was used. The column eluate was monitored continuously using an L-4000 UV detector (Hitachi Ltd.) set at 280 nm and a radiochemical detector (Aloka radio analyzer; Aloka Co., Ltd., or Radiomatic TM525TR; PerkinElmer Life Sciences). For dog and monkey samples that did not have sufficient radioactivity for direct quantification, peak fractions of the eluate were collected and assayed for radioactivity by a LSC.

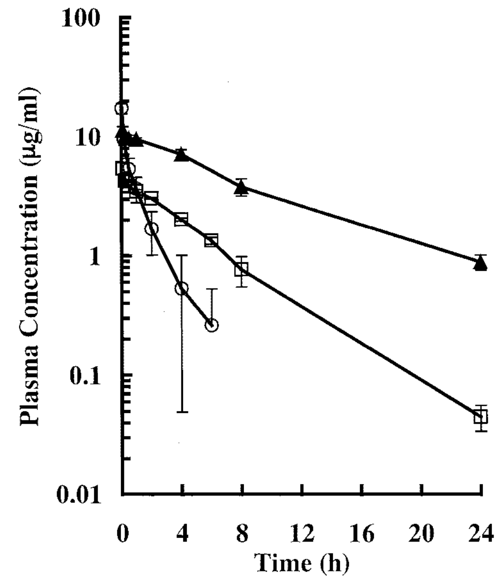

FIG. 2. *Plasma concentration-time profiles of garenoxacin following intravenous administration.*

Garenoxacin concentrations in plasma were determined following intravenous administration of garenoxacin to rats (10 mg/kg; ○), dogs (15 mg/kg; ▲), and monkeys (5 mg/kg; □). Each plot and bar represents the mean ± S.D. of three or four animals.

Pharmacokinetic Analysis. AUC (time 0 to infinity), mean residence time (MRT), and half-life ($t_{1/2}$) were calculated from the garenoxacin concentration in plasma by the noncompartmental analysis method (WinNonlin; Scientific Consulting, Inc., Apex, NC). Systemic plasma clearance (CL) was calculated as the intravenous dose divided by the AUC (time 0 to infinity). The steady-state volume of distribution (Vss) was estimated by the following equation:

$$\mathrm{Vss} = \mathrm{Dose} \times \mathrm{AUMC}/\mathrm{AUC}^2$$

where AUMC is the total area under the first moment of the drug concentration versus time curve from time 0 to infinity. The renal clearance (CL_r) was calculated as the amount of drug excreted in the urine for up to 24 h divided by the AUC_{0-24} (time 0 to 24 h). The extrarenal clearance (CL_{er}) was estimated by subtracting CL_r from CL. Bioavailability (*F*) was estimated with the following equation:

$$F = \mathrm{Dose}_{iv} \times \mathrm{AUC}_{po}/\mathrm{dose}_{po} \times \mathrm{AUC}_{iv}$$

Subscripts denote administration route. The mean absorption rate was estimated by subtracting MRT_{iv} from MRT_{po}. For interspecies scaling, the mean values of MRT, Vss, CL, CL_r, and CL_{er} obtained from each animal and mouse data previously reported were plotted against body weight. Linear least-squares regression analysis was performed to fit the following relationship. The value of pharmacokinetic parameter $= a \times W^b$ where W is body weight, a is the allometric coefficient, and b is the allometric exponent. In a separate analysis, the value of the pharmacokinetic parameter was corrected using a serum free fraction (f). In such a case, the value of the pharmacokinetic parameter was divided by f. Other correction factors were examined such as maximum life span potential, brain weight, UDP-glucuronyl transferase activity (UGT), and bile flow rate. In such cases, the values of the pharmacokinetic parameters were multiplied by the correction factors before allometric scaling. The values of maximum life span potential, brain weight, UGT, and bile flow rate were obtained from a previous report (Ward et al., 1999).

Results

Plasma Concentrations after Intravenous Administration. The plasma concentration-time profiles of garenoxacin following intrave-

TABLE 1

Pharmacokinetic parameters of garenoxacin

Parameters were calculated from the plasma concentration-time profiles of garenoxacin and amounts excreted into urine following an administration of garenoxacin. Results were expressed as the mean ± S.D. (n = 3 or 4).

Parameter		Rat	Dog	Monkey
Dose (mg/kg)	i.v.	10	15	5
	p.o.	10	25	5
CL (ml/min/kg)		12.1 ± 6.1	2.43 ± 0.28	3.39 ± 0.30
Vss (l/kg)		0.88 ± 0.26	1.29 ± 0.05	0.96 ± 0.05
MRT (h)	i.v.	1.42 ± 0.61	8.93 ± 0.67	4.7 ± 0.4
	p.o.	2.89 ± 1.43	11.6 ± 3.5	5.9 ± 1.2
$t_{1/2}$ (h)	i.v.	1.42 ± 0.51	7.64 ± 1.99	4.0 ± 0.4
	p.o.	2.38 ± 0.90	7.81 ± 2.85	4.5 ± 0.2
C_{max} (μg/ml)	p.o.	6.25 ± 2.28	7.94 ± 3.12	2.98 ± 0.70
t_{max} (h)		0.25 ± 0.00	3.33 ± 1.16	1.2 ± 0.8
AUC (μg · h/ml)	i.v.	13.6 ± 4.3	104 ± 12	24.7 ± 2.3
	p.o.	10.5 ± 1.4	124 ± 31	18.7 ± 2.7
F		0.772	0.716	0.757
Serum free fraction f		0.131 ± 0.006	0.229 ± 0.007	0.186 ± 0.001
A_e	i.v.	0.145 ± 0.044	0.134 ± 0.029	0.403[a]
CL_r (ml/min/kg)		1.65 ± 0.21	0.368 ± 0.039	1.38[a]
CL_{er} (ml/min/kg)		10.4 ± 6.0	2.06 ± 0.31	2.01[a]

C_{max}, maximum concentration; t_{max}, time to reach the maximum concentration; AUC, area under the curve extrapolated to infinity; F, availability; A_e, fraction excreted unchanged in urine.
[a] The values of A_e, CL_r, and CL_{er} for monkeys were representative mean values (n = 3) because the extent of urinary excretion of garenoxacin was determined in pooled urine of three animals.

nous administration to rats (10 mg/kg), dogs (15 mg/kg), and monkeys (5 mg/kg) are illustrated in Fig. 2. The pharmacokinetic parameters are listed in Table 1. The CL in the animals decreased with increasing body weight. The Vss ranged at comparable levels in the animals tested. The values of Vss were larger than the extracellular space volume, which suggests that the garenoxacin penetrated the cells in all species tested. The MRT values increased with increasing body weight.

Plasma Concentrations after Oral Administration. The plasma concentration-time profiles of garenoxacin following oral administration to rats (10 mg/kg), dogs (25 mg/kg), and monkeys (5 mg/kg) are illustrated in Fig. 3. A comparison of AUC data from oral and intravenous administrations gave information on absorption. Garenoxacin was well absorbed in all animals with high values of F (0.716–0.772). Additionally, absorption was rapid in all animals. The mean absorption rate values for the index of the absorption rate were 1.47, 2.67, and 1.2 h for rats, dogs, and monkeys, respectively.

Urinary Excretion. The fraction excreted unchanged in urine (A_e) and CL_r are listed in Table 1. The CL_r decreased with increasing body weight. The CL_r corrected by the free fraction in serum (CL_r/f) was lower in dogs (1.61 ml/min/kg) than in rats and monkeys (12.6 and 7.42 ml/min/kg, respectively). In each species, the CL_r value was smaller than the CL_{er} value (Table 1).

Serum Protein Binding. Slight species differences were observed; the binding rates were 86.9 to 91.7% for rats at a concentration range of 0.5 to 100 μg/ml, 77.1 to 79.5% for dogs at a concentration range of 0.5 to 100 μg/ml, and 80.6 to 83.7% for monkeys at a concentration range of 0.5 to 50 μg/ml. The binding rates were not affected by increasing the concentration of total drug in serum. The free fractions (f) at 0.5 μg/ml are listed in Table 1 as representative across the concentration range.

Interspecies Scaling. The direct regression of log CL versus log W (body weight) produced an equation (log CL = 0.607 · log W + log 5.84) which showed a good correlation with the coefficient of correlation R^2 = 0.991 (Fig. 4). A good correlation with body weight was also observed for Vss, CL_r, and CL_{er} (Table 2). The predicted CL value in humans (84.1 ml/min for 81 kg) was comparable with the value obtained from the clinical trial following intravenous administration of garenoxacin at 400 mg (86.1 ml/min at a mean body weight of 81 kg) (Gajjar et al., 2001). The predicted Vss value in humans

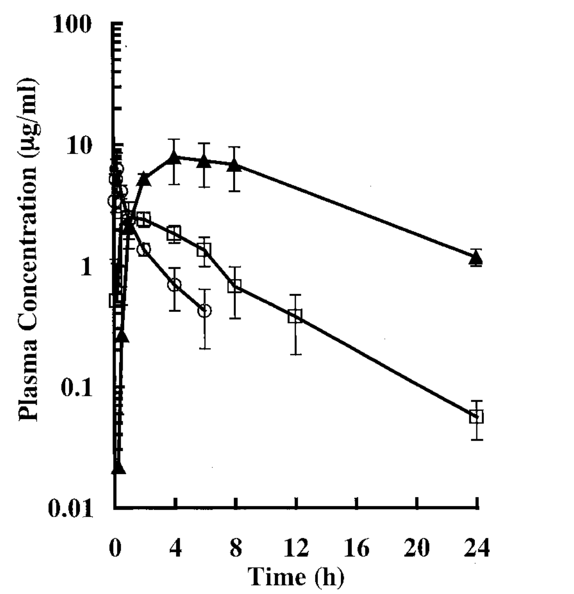

FIG. 3. *Plasma concentration-time profiles of garenoxacin following oral administration.*

Garenoxacin concentrations in plasma were determined following oral administration of garenoxacin to rats (10 mg/kg; ○), dogs (25 mg/kg; ▲), and monkeys (5 mg/kg; □). Each plot and bar represents the mean ± S.D. of three or four animals.

(73.0 liters for 81 kg) was also comparable with the value in the clinical trial (71 liters at a mean body weight of 81 kg) (Gajjar et al., 2001). No improvements were observed in interspecies scaling on using corrections of the free fraction, maximum life span, brain weight, and bile flow rate (Table 2). Corrections based on UGT activity were not appropriate for the interspecies scaling of garenoxacin (Table 2).

Identification of Metabolites. Authentic compounds were available for the garenoxacin sulfate conjugate (T-3811M1) and garenoxacin oxidative products T-3811M4 and T-3811M5. Assignment of the structures of M1, M4, and M5 was based on molecular weight data and a comparison of the chromatographic retention times and product ion fragmentation patterns of the metabolites with those of the authentic compounds synthesized. The metabolites M1, M4, and M5 were identified as

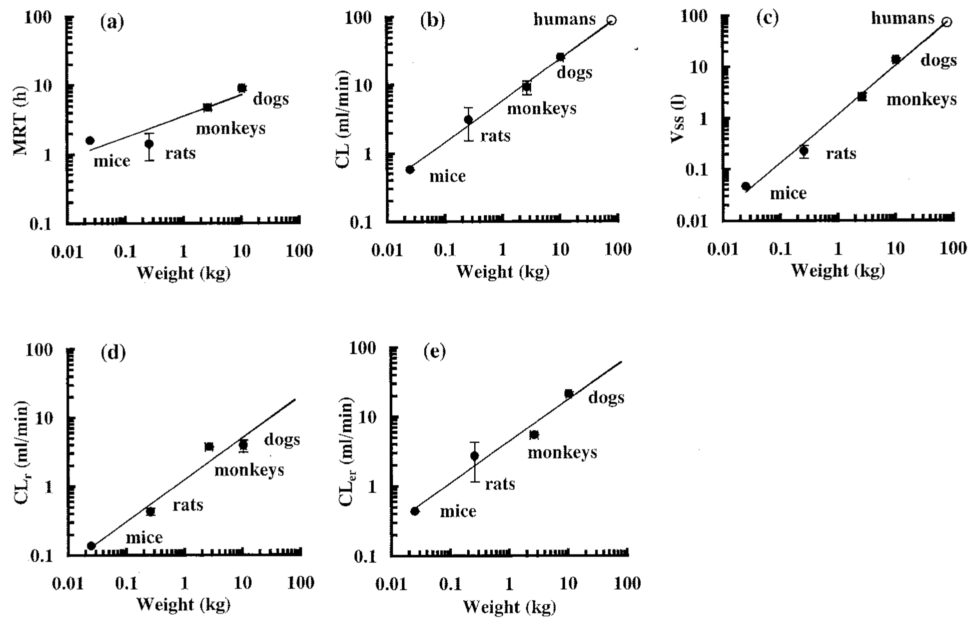

FIG. 4. *Allometric interspecies scaling of the pharmacokinetic parameters of garenoxacin.*

MRT (a), CL (b), Vss (c), CL_r (d), and CL_{er} (e) versus body weight were plotted. Each plot and bar represents the mean ± S.D. of three or four animals. The lines represent the regression of the parameter versus body weight. The mouse data were obtained from our previous report (Takahata et al., 1997); the parameters were 0.571 ml/min for CL, 0.046 liter for Vss, 1.6 h for MRT, 23.7% for urinary recovery, and 25 g for body weight. The human data were obtained from previous reports (Gajjar et al., 2001); the parameters were 86.1 ml/min for CL and 71 liter for Vss after intravenous 400-mg dose.

TABLE 2

Allometric equation coefficients, correlation coefficients, and predicted human pharmacokinetic parameters resulting from interspecies scaling of garenoxacin

Mean data (n = 3 to 4) from mice, rats, dogs, and monkeys were used for interspecies scaling. The mouse data were obtained from our previous report (Takahata et al, 1997). Correction factors tested were free fraction (*f*), maximum life span potential (MLP), brain weight (BW), UDP-glucuronyl transferase activity (UGT), and bile flow rate (Q).

Parameters	Allometric Coefficients	Allometric Exponents	Correlation Coefficients	Human Value	
				Predicted	Observed[a]
MRT (h)	3.56	0.303	0.826	13.5	—[b]
Vss (l)	1.16	0.942	0.986	73.0	71
Vss/*f* (l)	5.82	0.974	0.999	419	284[c]
CL_r (ml/min)	1.24	0.608	0.953	17.9	—[b]
CL_r/f (ml/min)	6.22	0.639	0.931	103	—[b]
CL_{er} (ml/min)	4.40	0.598	0.963	61	—[b]
CL_{er}/f (ml/min)	22.1	0.629	0.904	350	—[b]
CL (ml/min)	5.84	0.607	0.991	84.1	86.1[d]
CL · MLP (ml/min)	59.3	0.985	0.995	48.3	86.1[d]
CL · BW (ml/min)	74.0	1.61	0.981	63.1	86.1[d]
CL · UGT (ml/min)	22.3	0.913	0.979	390	86.1[d]
CL · Q (ml/min)	0.0640	1.06	0.898	77.5	86.1[d]
CL · UGT · Q (ml/min)	0.244	1.37	0.936	359	86.1[d]
CL/*f* (ml/min)	29.3	0.638	0.933	483	344[c]
CL/*f* · MLP (ml/min)	297	1.02	0.978	277	344[c]
CL/*f* · BW (ml/min)	371	1.64	0.978	362	344[c]
CL/*f* · UGT (ml/min)	112	0.944	0.931	2241	344[c]
CL/*f* · Q (ml/min)	0.320	1.09	0.858	445	344[c]
CL/*f* · UGT · Q (ml/min)	1.22	1.40	0.899	2065	344[c]

[a] The human data were obtained from a presentation at the 41st ICCAC (Gajjar et al, 2001).
[b] The results were not obtained.
[c] Estimated from the free fraction in human serum of 0.25 (Bello et al., 2001).
[d] The CL was calculated from the value of the AUC (77.4 μg · h/ml) after intravenous 400-mg dose (Gajjar et al, 2001).

T-3811M1, T-3811M4, and T-3811M5, respectively. MS fragments and molecular ions of garenoxacin and its metabolites are given in Table 3. No authentic compounds were available for a carbamoyl glucuronide (M2), M3, and garenoxacin acyl glucuronide (M6).

Treatment of the dog bile isolate for M2 with β-glucuronidase resulted in the disappearance of the metabolite peak with subsequent formation of garenoxacin (confirmed by retention time and m/z 427). Furthermore, the M2 had a molecular ion (m/z 647) and underwent fragmentations attributable to the loss of the glucuronic acid moiety (molecular weight 176) and the addition of the carbon dioxide (molecular weight 44) to garenoxacin by LC/MS/MS. The ^{1}H NMR spectrum of the M2 was consistent with the presence of a glucuronic acid, and it demonstrated a hindered rotation of the substituent that bound to the nitrogen atom of the isoindolyl group at the C-7 position. The behavior in HPLC (retention time in acidic conditions) and chemical property (extracted by the organic layer under acidic conditions) suggested the absence of a basic moiety in the M2 structure. These results confirmed its structure as a carbamoyl glucuronide (Fig. 5).

TABLE 3

Molecular and product ions observed for garenoxacin and its metabolites by LC/MS/MS

Compound[a]	Retention Time	$(M + H)^+$	Product Ions
	min		*m/z*
Garenoxacin	23.7	427	366, 326,305,298,286
Sulfate conjugate (T-3811M1)	29.1	505[b]	461[b]
Carbamoyl glucuronide (M2)	27.8	647	629, 471, 453
Carbamic acid (M3)	35.4	471	453
Oxidative product (T-3811M4)	38.1	441	423
Oxidative product (T-3811M5)	30.3	457	439, 382
Acyl glucuronide (M6)	11.7	603	586, 427, 366

[a] Authentic compounds synthesized or code numbers of metabolites are indicated in parentheses.
[b] The values were obtained as (M − H).

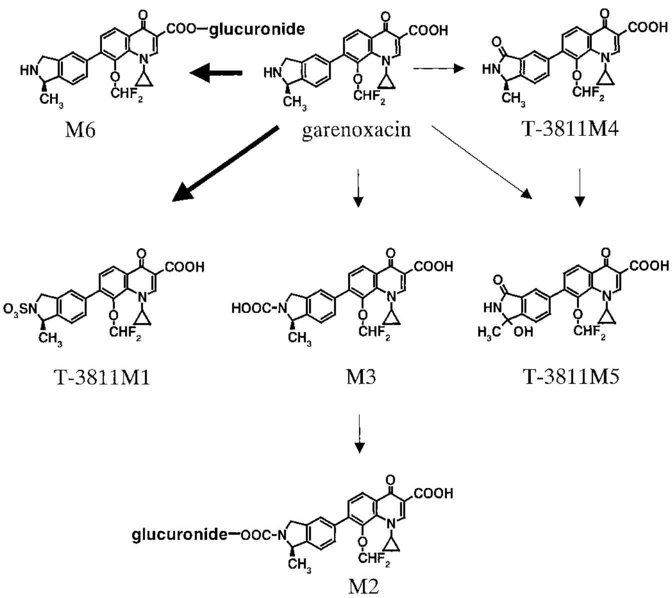

FIG. 5. *Proposed metabolic pathways of garenoxacin in rats, dogs, and monkeys.*

M3 was a very unstable compound after the isolation, although it existed in biological matrixes. It decomposed spontaneously after the isolation, with the concomitant appearance of a peak of garenoxacin. In addition to these results, the molecular ion of M3 (*m/z* 471, consistent with the addition of the carbon dioxide to garenoxacin) and the behavior of M3 in HPLC (loss of basic moiety) supported the speculated structure (Fig. 5). The identity of M3 was based on molecular weight data only; therefore, it is regarded as tentative.

The *m/z* 603 of M6 isolated from dog bile underwent typical fragmentation attributable to the loss of the glucuronic acid moiety (molecular weight 176) with the formation of parent molecule ions of garenoxacin (*m/z* 427). ^{1}H and ^{13}C NMR spectra of M6 were consistent with the presence of a glucuronic acid. Additionally, incubation of M6 with NaOH resulted in the disappearance of M6, with the concomitant appearance of a peak with the spectral characteristic of garenoxacin. Considering its behavior in HPLC (presence of a basic moiety) and unstable chemical property in alkaline conditions, M6 was identified as an acyl glucuronide of garenoxacin at the carboxylic acid moiety. The postulated metabolic pathways are shown in Fig. 5.

Metabolic Profiles. The metabolic profiles in rats, dogs and monkeys detected by measuring radioactivity are listed in Tables 4, 5, and 6. Garenoxacin was the major component detected in plasma and urine in all species tested. There was no species difference in the metabolic profiles of garenoxacin in plasma and urine. The principal metabolites in bile or feces were conjugate products such as T-3811M1 and M6 in all species tested, although the amount of M6 was larger in rat bile. The results for each animal are described below.

Rat. The radiometabolite patterns observed in the urine were consistent with those seen in the plasma. Almost all radioactivity in urine and plasma was detected as unchanged garenoxacin. The major metabolite of garenoxacin was T-3811M1 in the plasma and urine after both routes of administration. In contrast, most radioactivity in the bile was observed as metabolites. Garenoxacin accounted for only ca. 4.1 and 6.8% of the total radioactivity in the bile after intravenous and oral administration, respectively. The major metabolites in bile were T-3811M1 and M6 after both routes of administration. Several other minor components were detected in the excreta, including the oxidative products of garenoxacin (T-3811M4 and T-3811M5) and conjugation products of garenoxacin (M2, M3), each of which accounted for no more than 1.2% of the total radioactivity in the excreta after both routes of administration.

Dog. Garenoxacin was the major component detected in dog plasma and urine. The principal metabolites detected in the plasma and urine were conjugation products such as T-3811M1 and M6. The principal metabolite in feces was T-3811M1, and the other minor conjugates (M2, M3, and M6) were observed in feces. Each of the minor metabolites accounted for no more than 3.1% of the radioactivity in the feces.

Monkey. As in the case of the rats and dogs, garenoxacin was the major component detected in monkey plasma and urine. The principal metabolites detected in the plasma and urine were conjugates of garenoxacin (T-3811M1, M6). Most radioactivity in the bile was observed as T-3811M1 and other minor conjugates, including M2 and M3. As in the bile, T-3811M1 was observed in feces as the major metabolite.

Discussion

This article reports on the metabolism and disposition of a novel des-fluoro(6)-quinolone garenoxacin in animal species used in the toxicological evaluation of the compound focusing on species differences and the allometric scaling of pharmacokinetic parameters. In all animals tested, garenoxacin was rapidly and well absorbed following oral administration. To clarify the renal handling of garenoxacin, CL_r/f values were compared with the glomerular filtration rate (GFR) values. The GFR values when compared are 4.88 ml/min/kg for rats (our preliminary data), 2.67 ml/min/kg for dogs (Russel et al., 1989), and 2.09 ml/min/kg for monkeys (Schaer et al., 1990). In dogs, the GFR is higher than the CL_r/f (1.61 ml/min/kg). In other species tested, the GFR is lower than the CL_r/f (12.6, and 7.42 ml/min/kg for rats and monkeys, respectively). These results suggest that garenoxacin underwent tubular secretion in the rats and monkeys, whereas in the dogs, the low CL_r was consistent with a dominant tubular reabsorption. The renal handling of garenoxacin differed across species.

TABLE 4

Metabolic profiles of garenoxacin in the plasma and excreta of rats

Metabolic profiles were determined in samples pooled from three animals following oral and intravenous administration of ^{14}C-garenoxacin at 5 mg/kg. Results are expressed as percentage of radioactivity in samples. Amounts excreted into the urine, feces, and bile were expressed as percentage dose (mean ± S.D.).

Sample	Amount (% Dose)	Metabolites (Percentage of Sample Radioactivity)							
		GRX	M1	M2	M3	M4	M5	M6	UN 14 m
		i.v.							
Plasma 15 min	N.A.	94.9	2.6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Plasma 4 h	N.A.	100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Urine 0–24 h	16.1 ± 2.2	90.6	5.6	N.D.	N.D.	N.D.	0.4	N.D.	N.D.
Feces 0–24 h	77.5 ± 6.0	56.2	41.3	N.D.	N.D.	0.6	N.D.	N.D.	N.D.
Bile 0–24 h	65.4 ± 2.6	4.1	52.0	0.6	1.2	N.D.	N.D.	38.2	1.8
		p.o.							
Plasma 15 min	N.A.	96.8	1.3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Plasma 4 h	N.A.	100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Urine 0–24 h	10.3 ± 1.3	94.3	4.9	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Feces 0–24 h	87.6 ± 7.1	75.4	22.3	N.D.	N.D.	0.6	N.D.	N.D.	N.D.
Bile 0–24 h	57.9 ± 5.2	6.8	42.2	0.6	1.1	N.D.	0.6	47.5	1.2

Amount, excretion of radioactivity as percentage of dose; GRX, garenoxacin; M1, T-3811M1; M2, carbamoyl glucuronide; M3, carbamic acid; M4, T-3811M4; M5, T-3811M5; UN 14 m, unknown metabolite at retention time 14 min; N.D., not detected; N.A., not applicable.

TABLE 5

Metabolic profiles of garenoxacin in the plasma and excreta of dogs

Metabolic profiles were determined in samples pooled from three animals following intravenous administration of ^{14}C-garenoxacin at 100 mg/kg. Results are expressed as percentage of radioactivity in samples. Amounts excreted into the urine and feces were expressed as percentage of dose (mean ± S.D.).

Sample	Amount (% Dose)	Metabolites (Percentage of Sample Radioactivity)							
		GRX	M1	M2	M3	M4	M5	M6	UN 2 m
Plasma 5 min	N.A.	100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Plasma 2 h	N.A.	91.8	8.2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Plasma 6 h	N.A.	90.9	9.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Plasma 24 h	N.A.	100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Urine 0–24 h	30.0 ± 2.7	88.3	4.6	0.8	N.D.	N.D.	N.D.	2.1	4.2
Feces 0–24 h	30.7 ± 8.1	70.4	18.5	1.5	3.1	N.D.	N.D.	1.9	4.5

Amount, excretion of radioactivity as percentage of dose; GRX, garenoxacin; M1, T-3811M1; M2, carbamoyl glucuronide; M3, carbamic acid; M4, T-3811M4; M5, T-3811M5; UN 2 m, unknown metabolite at retention time 2 min; N.D., not detected; N.A., not applicable.

To understand the drug disposition, it is important to know whether the drug is eliminated in a blood flow rate-limiting manner or not. The renal clearance based on the blood concentration (CL_{rb}) was calculated as CL_r divided by the blood to plasma concentration ratio of garenoxacin (R_b). To compare the CL_{rb} with the renal blood flow rate, CL_{rb} was calculated using our unpublished data of R_b (0.89, 0.81, and 0.71 for the rats, dogs, and monkeys, respectively). The CL_{rb} values calculated (1.85, 0.45, and 1.94 ml/min/kg for rats, dogs, and monkeys, respectively) were not more than 7% of the renal blood flow in each species (36.8, 21.6, and 27.6 ml/min/kg in the rat, dog, and monkey, respectively) (Davies and Morris, 1993). Assuming that extrahepatic metabolism was not involved in the metabolism of garenoxacin, extrarenal blood clearance (calculated as CL_{er} divided by R_b) could compare with the hepatic blood flow. The extrarenal blood clearance values (11.7, 2.54, and 2.83 ml/min/kg for rats, dogs, and monkeys, respectively) were not more than 21% of the hepatic blood flow in each species (55.2, 30.9, and 43.6 ml/min/kg in the rat, dog, and monkey, respectively) (Davies and Morris, 1993). These results indicate that garenoxacin was not subject to extensive renal extraction and the renal blood flow was not a rate-limiting factor of garenoxacin clearance in any species tested. Additionally, the hepatic blood flow was not a rate-limiting factor of garenoxacin clearance if the liver was the only organ for the metabolism of garenoxacin.

The major metabolic routes for garenoxacin were phase II metabolism. The principal metabolite commonly observed in rats, dogs, and monkeys was the sulfate of garenoxacin (T-3811M1). Another common metabolic pathway in all animals tested was the conjugation of the carboxylic acid substituent (formation of M6), which is similar to previous reports (Hayakawa et al., 1995; Dalvie et al., 1996; Ramji et al., 2001). In contrast to conjugation products, oxidative metabolites (T-3811M4 and T-3811M5) were very minor in all species. The results were consistent with our preliminary study using human hepatocytes. Only T-3811M1 and M6 were formed from ^{14}C-garenoxacin after incubation with human hepatocytes (data not shown). In a previous study, garenoxacin did not undergo metabolism in human microsomes and did not inhibit the activities of several cytochrome P450 enzymes in human microsomes (Furuhata et al., 2000). Garenoxacin is probably metabolized to only a limited extent by cytochrome P450 in all species including humans and undergoes mainly phase II metabolism. Of the other minor routes of metabolism observed for garenoxacin in all species tested, the formation of the carbamoyl glucuronide (M2) is a novel route for the basic moiety at position C-7 of the quinoline ring. It is speculated that M2 was formed from subsequent glucuronide conjugation of carbamic acid (M3). The formation of a similar metabolite has previously been reported for rimantadine (Brown et al., 1990), carvedilol (Schaefer, 1992), and mofegiline (Dow et al., 1994).

Through all species tested, the major substance that existed in the plasma was unchanged garenoxacin. In the urine, as well as the plasma, unchanged garenoxacin was the major substance in all species

TABLE 6

Metabolic profiles of garenoxacin in the plasma and excreta of monkeys

Metabolic profiles were determined in samples pooled from three animals following oral, intraduodenal, and intravenous administration of ^{14}C-garenoxacin at 5 mg/kg (plasma and bile samples were determined from one animal). Results are expressed as percentage of radioactivity in samples. Amounts excreted into the urine, feces, and bile were expressed as percentage of dose (mean ± S.D.).

Sample	Amount (% Dose)	Metabolites (Percentage of Sample Radioactivity)								
		GRX	M1	M2	M3	M4	M5	M6	UN 2 m	UN 32 m
		i.v.								
Plasma 5 min[a]	N.A.	98.6	1.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Plasma 4 h[a]	N.A.	92.3	7.7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Urine 0–24 h	44.3 ± 9.4	91.0	5.6	0.9	N.D.	0.4	N.D.	1.6	N.D.	N.D.
Feces 0–24 h	35.6 ± 3.8	24.1	73.5	N.D.	N.D.	0.6	N.D.	N.D.	0.2	N.D.
Bile 0–6 h[a]	20.5	4.5	80.5	2.7	4.0	N.D.	N.D.	0.6	2.3	4.5
		p.o.								
Urine 0–24 h	35.9 ± 10.7	88.5	8.5	0.9	N.D.	0.4	N.D.	1.6	N.D.	N.D.
Feces 0–24 h[b]	43.3 ± 10.6	25.4	72.4	N.D.	N.D.	0.4	N.D.	N.D.	0.3	N.D.
		i.d.								
Plasma 1 h[a]	N.A.	75.6	24.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Plasma 4 h[a]	N.A.	74.6	25.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bile 0–6 h[a]	12.3	2.9	81.1	2.9	3.7	N.D.	N.D.	0.3	2.7	4.4

Amount, excretion of radioactivity as percentage of dose; GRX, garenoxacin; M1, T-3811M1; M2, carbamoyl glucuronide; M3, carbamic acid; M4, T-3811M4; M5, T-3811M5; UN 2 m, unknown metabolite at retention time 2 min; UN 32 m, unknown metabolite at retention time 32 min; N.D., not detected; N.A., not applicable.

[a] Results obtained are from one animal.

[b] The feces of one animal was obtained at 24 to 48 h because radioactivity was not recovered from 0- to 24-h feces.

tested. On the other hand, conjugates of garenoxacin such as T-3811M1 and M6 were excreted in the bile of rats and monkeys (Tables 4 and 6). The results are consistent with the observation that acyl glucuronide of grepafloxacin was transported from the liver to the bile by some transporters in rats (Sasabe et al., 1998). From these results, garenoxacin is characterized by virtue of the observation that it circulates systemically as an unchanged form and is excreted via the renal route; it is also partially metabolized to glucuronide or sulfate as major metabolites, which are excreted specifically into the bile.

Interspecies scaling of pharmacokinetic parameters obtained from preclinical studies has been performed for some drugs (Mordenti, 1985; Efthymiopoulos et al., 1991; Lave et al., 1997; Feng et al., 1998). However, poor information has been reported about the interspecies scaling for quinolones. We tried allometric interspecies scaling of CL, CL_r, CL_{er}, Vss, and MRT using the parameters in the present investigations and in a previous report for mice (Takahata et al., 1997). Generally, serum protein binding is taken into consideration in allometric interspecies scaling if a species difference in protein binding is observed. The serum binding rate of garenoxacin in each animal tested (77.1–91.7%) was comparable with that of mice (70.1–71.9%) (our unpublished data) and humans (75%) (Bello et al., 2001). Because of these slight species differences in protein binding, the correction of pharmacokinetic parameters using the free fraction gave no improvement in the correlation of each pharmacokinetic parameter versus body weight (Table 2). For metabolized compounds characterized by a low or moderate hepatic excretion ratio, physiological correction factors were used to improve the interspecies scaling, such as maximum life span potential (Boxenbaum, 1984), brain weight (Mahmood and Balian, 1996a), and other factors (Lave et al., 1996; Ward et al., 1999). In the case of garenoxacin, a good correlation between pharmacokinetic parameters and body weight was observed without any correction despite the relatively large contribution of metabolic clearance to total clearance (Fig. 4, Table 2). The result was consistent with a previous report (Mahmood and Balian, 1996b). The validity of this scaling strategy was confirmed by the good agreement between the observed and predicted value in humans (Table 2).

In summary, characteristics of the metabolism and disposition of garenoxacin were common across species. Garenoxacin underwent phase II metabolism and the metabolites were excreted into the bile, whereas unchanged garenoxacin was excreted into the urine. Allometric interspecies scaling was applicable to garenoxacin for CL and Vss in humans, although the extrarenal clearance was larger than the renal clearance and neither of the clearances was limited by blood flow.

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