ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1995, p. 2635–2640 Vol. 39, No. 12
0066-4804/95/$04.00+0
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Single- and Multiple-Dose Pharmacokinetics of AM-1155, a New 6-Fluoro-8-Methoxy Quinolone, in Humans

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Received 12 December 1994/Returned for modification 11 May 1995/Accepted 15 September 1995

The pharmacokinetics of AM-1155, a new 6-fluoro-8-methoxy quinolone, was examined in healthy male volunteers after the oral administration of a single dose of 100, 200, 400, or 600 mg and multiple doses of 300 mg twice daily for 6.5 days (13 total doses). Throughout the whole study period, AM-1155 was well tolerated in every subject. In the single-dose study, the concentrations in serum reached a peak between 1 and 2 h, and the peak concentrations were 0.873, 1.71, 3.35, and 5.41 μg/ml at the doses of 100, 200, 400, and 600 mg, respectively. The elimination half-life was 7 to 8 h, independently of the doses. The unchanged drug was excreted mainly in the urine, with 82 to 88% of the doses appearing for 72 h. The fecal recovery of the unchanged drug amounted to 5.7% for 72 h after a single oral administration of a 400-mg dose. Urinary excretion of metabolites was minimal. The serum protein binding was 20%, independently of the concentrations in serum. The concentrations in saliva were approximately 80% of those in serum. The intake of food had no effect on the pharmacokinetic parameters and urinary excretion of AM-1155 except the slight decrease in area under the concentration-time curve. The concurrent administration of probenecid prolonged the elimination half-life, increased the area under the concentration-time curve, and decreased the apparent total body clearance, renal clearance, urinary recovery of unchanged drug, and the excretion ratio (intrinsic renal clearance of AM-1155/creatinine clearance). This indicated that the tubular secretion contributed to the renal excretion of AM-1155. In the multiple-dose study, the concentrations of AM-1155 in serum and urine reached a steady state within 2 to 3 days. The measured concentrations in serum fitted well the simulation curve, which reflected the persistence of linear pharmacokinetics of AM-1155. In conclusion, AM-1155 is expected to be clinically useful because of its potent antibacterial activity and favorable pharmacokinetics.

AM-1155 [(±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate] is a new 6-fluoro-8-methoxy quinolone (Fig. 1) being tested in clinical trials. It has potent and broad antimicrobial activity against gram-positive and gram-negative aerobes, anaerobes, and *mycobacteria* (3, 4, 21, 23). It is more potent than ciprofloxacin and ofloxacin and is comparable to sparfloxacin and tosufloxacin against gram-positive bacteria and anaerobes in vitro; it also has activity comparable to those of ciprofloxacin and sparfloxacin against gram-negative bacteria. Pharmacokinetic studies with laboratory animals demonstrated that this drug was rapidly and completely absorbed from the gastrointestinal tract, well distributed to various tissues except the brain, and principally excreted into the urine (13).

The pharmacokinetics of AM-1155 was examined in healthy humans. The parameters studied included the serum drug concentration-time profiles, urinary excretion of unchanged drug after the administration of single and multiple oral doses, fecal excretion of unchanged drug, urinary excretion of metabolites, excretion in saliva, serum protein binding, the influence of food intake on pharmacokinetics, and the effect of probenecid on renal excretion after the administration of a single oral dose.

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MATERIALS AND METHODS

Chemicals. AM-1155 (lots G955320 and K939931) and its metabolites (Fig. 1) to be analyzed were synthesized and supplied by Kyorin Pharmaceutical Co., Ltd. (Tokyo, Japan). AM-1155 tablets (lots S170030 and S190050), each containing 100 mg of active substance, were prepared by Kyorin Pharmaceutical Co., Ltd. Probenecid tablets (Benecid, 250 mg) were purchased from Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan). Acetonitrile and tetrahydrofuran for high-performance liquid chromatography (HPLC) were from Kanto Chemical Co., Ltd. (Tokyo, Japan). Sodium 1-octane sulfonate and sodium dodecyl sulfate for ion pair liquid chromatography were from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). β-Glucuronidase derived from *Escherichia coli* was purchased from Boehringer-Mannheim Yamanouchi (Tokyo, Japan). All other reagents were of analytical grade and used without further purification.

Subjects. The clinical part of this study was performed at Maruyama Hospital, Hamamatsu, Japan. A total of 30 healthy male Japanese volunteers (Table 1), 20 to 44 years old and weighing 51.3 to 83.2 kg, participated after giving written informed consent. They were determined to be healthy by medical history, physical examination, and laboratory profiles. None of the subjects were taking medications within 1 week before and during the study. The subjects had normal access to water but did not ingest any beverage or food containing alcohol or caffeine. The study protocol was approved by the local ethics committee.

Drug administration. (i) Single dosing. Six volunteers in each group received AM-1155 tablets. The administered doses corresponded to 100, 200, 400, and 600 mg. The study proceeded from the 100-mg dose to successively higher doses as the safety of the preceding dose was established. The tablets were taken with about 150 ml of tap water after an overnight fast; fasting was continued for 4.5 h after drug intake. Two weeks after the first dose, six subjects who had taken the 200-mg oral dose in a fasting state each received the same dose of AM-1155 0.5 h after a light morning meal (two rolls of bread, margarine, cheese, a boiled egg, a glass of milk, and a glass of orange juice). They also received 500-mg oral doses of probenecid (corresponding to two tablets) 1 h prior to and 12 and 24 h after the administration of 200 mg of AM-1155 2 months after the first dose. The volunteers ate the same meals at the same time up to 72 h after dosing.

(ii) Multiple dosing. Six volunteers received 13 total oral doses of 300 mg of AM-1155 twice daily (at 9:00 a.m. and 7:00 p.m.) on seven consecutive days. They

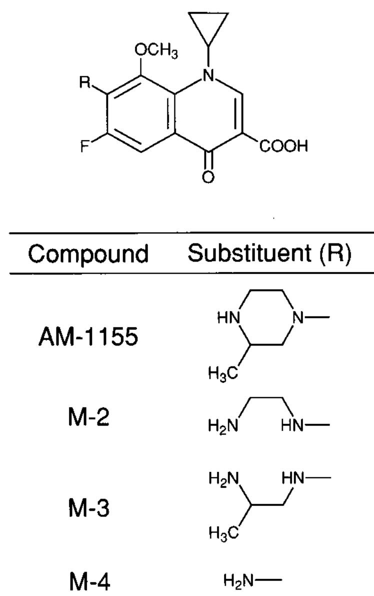

FIG. 1. Chemical structures of AM-1155 and its metabolites.

took only one dose (at 9:00 a.m.) on the last day. The dosing intervals were exactly 10 and 14 h. The drug was taken in a nonfasting state.

Sample collection. (i) Single dosing. In addition to the blank samples taken immediately before drug administration, serial blood samples (7 ml each) were collected by direct venipuncture at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 48 h after drug administration. Blood samples were centrifuged and separated in the usual manner. Serum samples were transferred to the test tubes. Urine samples were collected shortly before and 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 h after the dosing. After complete voiding of the urine at the end of each sampling period, the exact volume of urine was recorded. A 10-ml portion of urine was collected into a test tube. Saliva samples were collected without any stimulation shortly before and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 48 h after the 200- and 400-mg administration. Total fecal samples were collected at 24-h intervals for 72 h after the 400-mg dosing.

(ii) Multiple dosing. Serial blood samples were collected immediately before and at 1, 2, 3, 4, 6, 8, and 10 h after the morning dose on day 1; just before and at 2, 4, 6, 8, and 10 h after the morning dose on day 4, and just before and at 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, and 72 h after the morning dose on day 7. In addition, predose blood samples were taken in the morning on days 2, 3, 5, and 6. Urine was collected just before and 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, and 10 to 24 h after the morning dose on day 1, and the total 24-h urine was collected on day 2 to 9.

Assay procedure. Serum, urine, and other samples were transported in frozen packs to Central Research Laboratories of Kyorin Pharmaceutical Co., Ltd., as soon as possible and then stored at less than −20°C until they were assayed.

(i) Unchanged drug in serum, ultrafiltrate, saliva, urine, and feces. The concentrations of the unchanged drug in serum, ultrafiltrate, and saliva were

TABLE 1. Subjects[a]

Dose (mg)	Age (yr)	Ht (cm)	Wt (kg)
Single			
100	27.7 ± 6.2	169.8 ± 4.0	63.9 ± 12.1
200			
Fasting[b]	27.5 ± 5.6	171.6 ± 3.5	61.9 ± 6.0
No fasting[b]	27.7 ± 5.5		62.6 ± 5.8
Plus probenecid[b]	27.7 ± 5.5		62.5 ± 5.6
400	30.5 ± 5.2	173.7 ± 8.0	69.1 ± 8.7
600	29.7 ± 2.8	172.2 ± 4.3	66.9 ± 7.7
300 b.i.d.[c]	31.5 ± 9.5	173.9 ± 6.5	60.7 ± 6.0

[a] The data are means ± standard deviations for six subjects.
[b] The same subjects participated in these studies with washout intervals of at least 2 weeks.
[c] b.i.d., twice a day.

determined by column-switching reversed-phase HPLC with fluorescence detection. Samples (5 μl) were directly injected into the HPLC system (model LC-6A; Shimadzu, Kyoto, Japan) with the automatic injector (model SIL-6B; Shimadzu). First, the sample was loaded on a polyacrylate column, Shimpak SPC-PR2 (inside diameter, 4.6 mm; length, 10 mm; Shimadzu), for deproteinization; then, it was eluted with a mixture of 0.1 M phosphate buffer (pH 2.1) and acetonitrile (95:5, vol/vol; solution A) at a flow rate of 1.0 ml/min. One minute later, the pretreatment column was connected to an analytical column, TSKgel ODS-80TM (inside diameter, 4.6 mm; length, 150 mm; Tosoh, Tokyo, Japan), by valve switching, and it was eluted with a mixture of 0.1 M phosphate buffer (pH 2.1) and acetonitrile (75:25, vol/vol) containing 0.05% sodium 1-octane sulfonate (solution B) for 12 min at a flow rate of 1.0 ml/min. On the other hand, the pretreatment column was separated with the analytical column by valve switching 1.5 min after the sample injection. It was washed with a mixture of 0.2% triethylamine solution and acetonitrile (50:50, vol/vol; solution C) for 5 min at a flow rate of 2.0 ml/min and then equilibrated with 13 ml of solution A for 8 min. The columns were maintained at 40°C with a column oven (model CTO-6A; Shimadzu). The excitation and emission wavelengths of the fluorescence detector (Model RF-535; Shimadzu) were set at 295 and 495 nm, respectively. The data were recorded by a chromato-data processor (model C-R4AX; Shimadzu).

The concentration of the unchanged drug in urine was measured by reversed-phase HPLC with fluorescence detection. The analytical column and conditions of the fluorescence detector were the same as those for the assay of serum. The eluted mobile phase was a mixture of 0.01 M phosphoric acid and acetonitrile (75:25, vol/vol) containing 0.02% sodium 1-octane sulfonate. The flow rate was set at 1.0 ml/min. The column was maintained at 40°C. Urine samples (5 μl) were directly injected into the HPLC system, after dilution with water prior to the assay if necessary.

The feces were homogenized with 3 volumes of 0.1 N hydrochloric acid, and then a portion (10 ml) was extracted four times with the same volume of 0.1 N hydrochloric acid. The combined fraction was neutralized with the same volume of 0.1 N sodium hydroxide. The resulting sample was analyzed in the same way as urine.

Under the HPLC conditions described above, the retention times for AM-1155 in serum and urine were 7 and 8 min, respectively. The chromatograms exhibited no interfering peaks at the retention times of AM-1155. The concentrations of AM-1155 were calculated from the calibration curve constructed by the linear regression of peak area (serum) or peak height (urine) as a function of the spiked concentrations. The standard calibration curves were linear in the concentration ranges of 0.01 to 10 and 0.1 to 250 μg/ml in serum and urine, respectively. The respective minimum quantifiable concentrations were 0.01 and 0.1 μg/ml. The accuracy and precision of the assay value were sufficient for the pharmacokinetic analysis; the within-day and between-day coefficients of variation in the serum assay were less than 2.3 and 9.1%, respectively, in the concentration range of 0.1 to 5 μg/ml; those in the urine assay were 1.5 and 12.5%, respectively, in the concentration range of 1 to 100 μg/ml. AM-1155 was stable in serum and urine at −20°C for at least 1 month.

(ii) Metabolites in urine. The glucuronide conjugate (M-1) of AM-1155 in urine was quantitated by comparison of the concentration of the unchanged drug before and after hydrolysis by incubation of the samples with β-glucuronidase (pH 7.0, 2 h at 37°C with 50,000 U/ml). The optimum hydrolysis condition was examined by using rat bile taken after the dosing of AM-1155. This bile contained a considerable amount of M-1 (13a).

The concentrations of the metabolites (M-2, M-3, and M-4) in urine were measured by reversed-phase HPLC with fluorescence detection. The urine samples (0.5 ml each) were extracted in the same way as described previously with fleroxacin (AM-833) and its metabolites (8). The extracted residue was dissolved with 0.2 ml of a mixture of acetonitrile and 0.01 M phosphoric acid (10:90, vol/vol), and then a portion (5 μl) was injected into the HPLC system. The column was TSKgel Octyl-80TS (4.6 by 150 mm; Tosoh). The eluted mobile phase was a mixture of acetonitrile, tetrahydrofuran, and 0.01 M phosphoric acid (14:2:84, vol/vol) containing 1 mM sodium 1-octanesulfonate. The flow rate was set at 1.5 ml/min. The excitation and emission wavelengths of the fluorescence detector were set at 285 and 490 nm, respectively. The retention times were 7.0, 13.5, 18.7, 16.1, and 25.1 min for the internal standard (pipemidic acid), M-2, M-3, M-4, and unchanged drug, respectively. The chromatograms exhibited no interfering peaks at the retention time of any of the metabolites or internal standard. The concentrations were calculated from the standard calibration curves constructed by linear regression of peak height ratios as a function of the spiked concentrations. The calibration curves were linear in the concentration range of 0.05 to 2 μg/ml. The within-day coefficient of variation was less than 17.5% at 0.05 μg/ml and less than 6% at 0.2 and 2 μg/ml. The minimum quantifiable concentration of these metabolites was 0.05 μg/ml.

(iii) Creatinine in serum and urine. Creatinine was measured by an autoanalyzer (model 7150; Hitachi).

Serum protein binding. The in vivo serum protein binding of AM-1155 was measured by the centrifugal ultrafiltration method using serum samples taken at 1, 3, 6, and 12 h after the administration of a 200-mg dose without probenecid and 3 h after the same dose with probenecid in fasting volunteers. A portion (1 ml each) of serum was loaded onto a membrane (MPS-1; Amicon Corp., Lexington, Mass.) and then centrifuged (1,000 × g for 10 min) at 4°C. The concentrations of AM-1155 in serum and filtrate were determined by HPLC for total

and unbound drug, respectively. The extent of adsorption of AM-1155 to the membrane was negligible (13 ± 6% [n = 3]).

Assessment of safety. In the single-dose study, subjective and objective symptoms and vital signs including blood pressure, pulse rate, and body temperature were checked before administration and periodically until 24 h after administration. A standard 12-lead electrocardiogram was obtained before and 2 and 24 h after administration. Routine laboratory tests including hematology, blood biochemistry, and urinalysis were performed immediately before and 24 h after administration. Light microscopy revealed crystals attributable to the drug in the 2-h urine, collected up to 6 h after the administration of 400- and 600-mg doses. In the multiple-dose study, the same items as described above were checked before, during, and 24 h after the last administration.

Pharmacokinetic analysis. Visual inspection of the log-linear drug concentration-time curves revealed that the serum concentrations of AM-1155 declined biphasically. Thus, the drug concentration-time data for each subject were fitted individually to a two-compartment open model with first-order absorption with or without lag time, corresponding to the equation

$$C_t = Ae^{-\alpha(t-t_0)} + Be^{-\beta(t-t_0)} - Ce^{-ka(t-t_0)}$$

where C_t is the estimated concentration (in micrograms per milliliter) of AM-1155 at time t; A and B are the zero-time intercepts (in micrograms per milliliter) of the distribution (α) and elimination (β) phases, respectively, and α and β represent the slopes (per hour) of the α and β phases, respectively; C is equal to $A + B$; k_a is the absorption rate (per hour); and t_0 is the lag time. Calculations were performed with the nonlinear least-squares computer program PAG-CP, developed by Takebe et al. (19) (Asmedica, Osaka, Japan). A weighting factor was used, if necessary. The goodness of fit of the model with or without lag time was determined by visual inspection of the difference between the observed values and the simulated curve. The terminal elimination half-life ($t_{1/2\beta}$) was calculated as 0.693/β. The peak concentrations in serum (C_{max}) and the time to reach C_{max} (T_{max}) were obtained from the simulated curve. The area under the concentration-time curve (AUC) was determined to the last quantifiable serum concentration by using the trapezoidal rule and extrapolated to infinity by using the terminal-phase rate constant. The apparent total body clearance (CL_T/F [F is the fraction absorbed]) was calculated as dose/$AUC_{0-\infty}$. The renal clearance of total drug (CL_R) was calculated as dose $\cdot f_e/AUC_{0-\infty}$, where f_e is the fraction excreted into urine for 72 h after dosing (f_e = urinary recovery/100). The apparent volume of distribution at the elimination phase (V_β/F) and that at steady state (V_{SS}/F) were calculated as $V_1 \cdot k_{el}/\beta$ and $V_1 + V_2$, respectively, where V_1 and V_2 are the volumes of distribution of the central and peripheral compartments, respectively, and k_{el} is the elimination rate constant.

To clarify the mechanism of renal excretion, creatinine clearance (CL_{CR}), intrinsic renal clearance of AM-1155 ($CL_{R\ int}$), and the $CL_{R\ int}/CL_{CR}$ ratio (excretion ratio) were calculated. $CL_{R\ int}$ was calculated as CL_R/f_u, where f_u is the fraction of unbound drug in serum 3 h after dosing.

A noncompartmental pharmacokinetic analysis was applied to the saliva concentrations from 6 or 8 to 48 h in the single-dose study and serum concentrations from 4 to 10 h on days 1, 4, and 7 in the multiple-dose study.

The simulation curve in the multiple-dose study was obtained by the curve fitting of serum drug concentrations on day 1, based on a linear two-compartment model.

Statistical analysis. The results were expressed as means ± standard deviations. Dose proportionality was evaluated by linear regression analysis. The influences of food intake and probenecid on the pharmacokinetic parameters, such as C_{max}, T_{max}, $AUC_{0-\infty}$, $t_{1/2\beta}$, V_β, V_{SS}, CL_T/F, CL_R, f, $CL_{R\ int}$, CL_{CR}, excretion ratio, and urinary recovery, were examined by the Student paired t test. Differences with $P < 0.05$ (two-tailed) were considered to be significant.

RESULTS

Safety. The applied dose regimens were well tolerated by every volunteer. In the multiple-dose study, one subject showed a transitory elevation of glutamic-pyruvic transaminase (GPT) on the day after the last dosing, but the GPT returned to the normal value 3 days later. Vital signs, hematology, blood chemistry, and urinalysis showed no abnormal changes attributable to the trial medication. No crystalluria was observed after single oral administration of 400- and 600-mg doses or multiple oral administration of a 300-mg dose.

Single-dose pharmacokinetics. Figure 2 shows the serum concentration-time profiles following oral administration of 100, 200, 400, and 600 mg of AM-1155. The best curve fitting for these profiles was achieved with a two-compartment open model. Table 2 shows the resulting pharmacokinetic parameters. The concentrations in serum reached a peak between 1 and 2 h (T_{max}), and the respective C_{max} values were 0.873, 1.71, 3.35, and 5.41 μg/ml. The dose proportionality was calculated by comparison of the C_{max} and $AUC_{0-\infty}$. A good linear correlation was found between the administered doses (x) and the resulting values (y): $y = 0.00901x - 0.0922$ for C_{max} and $y = 0.0933x - 3.46$ for $AUC_{0-\infty}$. The coefficient of correlation was 0.998 with both parameters. The $t_{1/2\beta}$ values were between 7 and 8 h, independently of the doses. The V_β/F and V_{SS}/F values were between 2.0 and 2.3 liters/kg and between 1.7 and 2.0 liters/kg, respectively. The CL_R and CL_T/F values had decreasing trends with the increase of the dose.

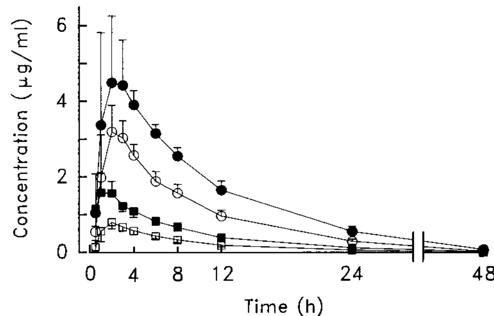

FIG. 2. Mean concentrations (with standard deviations) of AM-1155 in serum following a single oral administration of 100 mg (□), 200 mg (■), 400 mg (○), or 600 mg (●) in humans (n = 6).

The concentrations of the unchanged drug in urine increased with the increase of the doses, and the mean peak concentrations were 221, 240, 675, and 953 μg/ml, which were achieved from 2 to 6 h following oral administration of 100, 200, 400, and 600 mg of AM-1155, respectively. The unchanged drug was excreted mainly in the urine, with 82 to 88% of the doses appearing for 72 h, independently of the doses. The cumulative fecal recovery of the unchanged drug amounted to 5.7% of the dose for 72 h after the administration of a 400-mg dose.

Serum protein binding was 20% ± 5% (n = 24), independently of the concentrations in serum, in the range of 0.29 to 2.00 μg/ml.

Metabolites in urine. M-1 might not be contained in the urine, because there was no difference between the concentrations of unchanged drug before and after hydrolysis with β-glucuronidase. The cumulative urinary recoveries of M-2 and M-3 up to 72 h after the administration of a 400-mg dose were 0.03% ± 0.01% and 0.03% ± 0.01% of the dose (n = 6), respectively. M-4 was not detected in the same urine.

Excretion into saliva. Figure 3 shows the saliva drug concentration-time profiles following the administration of 200- and 400-mg doses. The concentrations in saliva reached a peak between 2 and 3 h (T_{max}) after dosing, and the respective C_{max} values were 1.55 ± 0.51 and 3.05 ± 0.74 μg/ml (n = 6). The $t_{1/2}$ values were 9.28 ± 1.72 and 7.06 ± 0.41 h (n = 6) after the administration of 200- and 400-mg doses, respectively. The concentrations of AM-1155 in saliva correlated well with those in serum, being approximately 80% of the concentrations in serum (Fig. 4).

Effect of food intake. Figure 5 shows the serum drug concentration-time profiles following a single oral administration of a 200-mg dose after a light morning meal. The intake of food had no effect on the pharmacokinetic parameters ($P > 0.05$), such as C_{max}, T_{max}, $t_{1/2\beta}$, V_β/F, and urinary recovery, except $AUC_{0-\infty}$, which slightly decreased from 14.5 to 12.7 μg · h/ml ($P < 0.01$; Table 3).

Influence of probenecid. Figure 6 shows the serum drug concentration-time profiles following a single oral administra-

TABLE 2. Pharmacokinetic parameters of AM-1155 following a single oral administration in fasting healthy volunteers

Parameter	Value for group at a dose (mg) of [a]:			
	100	200	400	600
C_{max} (μg/ml)	0.873 ± 0.187	1.71 ± 0.35	3.35 ± 0.55	5.41 ± 1.13
T_{max} (h)	1.63 ± 0.51	1.41 ± 0.81	1.98 ± 0.65	2.28 ± 1.41
$AUC_{0-\infty}$ (μg · h/ml)	7.00 ± 1.36	14.5 ± 2.6	32.4 ± 4.1	53.5 ± 2.6
$t_{1/2\beta}$ (h)	6.93 ± 0.83	7.10 ± 1.64	8.41 ± 2.23	8.11 ± 1.19
V_β/F (liters/kg)	2.31 ± 0.32	2.29 ± 0.34	2.17 ± 0.41	1.98 ± 0.34
V_{SS}/F (liters/kg)	1.90 ± 0.12	1.96 ± 0.22	1.72 ± 0.21	1.67 ± 0.17
CL_T/F (ml/min)	247 ± 55	236 ± 36	209 ± 26	187 ± 9
CL_R (ml/min)	202 ± 52	197 ± 32	174 ± 23	165 ± 11
Excretion for 72 h (% of dose) in urine	81.6 ± 7.0	83.4 ± 3.3	83.2 ± 4.0	87.9 ± 2.1

[a] The data are means ± standard deviations for six subjects. Excretion for 72 h in feces, 5.7% ± 1.7% of the 400-mg dose.

tion of a 200-mg dose with and without 1.5-g probenecid. With the concurrent administration of probenecid, the $t_{1/2\beta}$ value was prolonged from 7.10 to 10.2 h, and the $AUC_{0-\infty}$ value also increased from 14.5 to 20.6 μg · h/ml. On the other hand, the CL_T/F, CL_R, and $CL_{R\ int}$ values decreased from 235 to 164 ml/min, from 197 to 122 ml/min, and from 242 to 161 ml/min, respectively. In addition, the urinary recovery of AM-1155 decreased from 83.4 to 74.9%. The excretion ratio without probenecid was 2.28, which was significantly reduced to 1.35 with probenecid (Table 3).

Multiple-dose pharmacokinetics. Figure 7 shows the concentrations of AM-1155 in serum and simulation curve following the oral administration of 300 mg twice daily over 7 days. During the multiple oral dosing, AM-1155 was rapidly absorbed: T_{max} was between 1 and 2 h. C_{max} increased from 2.77 ± 0.54 μg/ml on day 1 to 3.45 ± 0.63 μg/ml on day 4 and to 3.36 ± 0.46 μg/ml on day 7. The $t_{1/2}$ values were 5.4 ± 0.6, 7.0 ± 0.9, and 5.7 ± 0.6 h on days 1, 4, and 7, respectively. The concentrations of AM-1155 in serum reached a steady state within 2 to 3 days. The measured concentrations in serum fitted well the simulation curve based on a linear two-compartment model. The theoretical peak and trough concentrations at steady state were 3.7 and 1.0 μg/ml, respectively.

Figure 8 shows the concentrations in urine and cumulative urinary recovery of AM-1155. The concentrations of AM-1155 in urine also reached a steady state within 2 to 3 days. At the steady state, the concentrations in the 24-h urine were between 220 and 290 μg/ml. Within 72 h after the last dosing, 78.8% of the total dose was eliminated renally as unchanged drug.

DISCUSSION

Throughout the entire test period, no abnormalities definitely attributable to trial medication were observed for the subjective and objective symptoms, vital signs, routine laboratory tests, and other test items. These findings indicate that AM-1155 was well tolerated in the healthy subjects.

High concentrations and extensive recovery of the unchanged drug in urine may cause crystalluria, as shown with ciprofloxacin (20). Therefore, we examined the possibility of crystalluria induced by AM-1155. In one subject who had taken a single 600-mg oral dose of AM-1155, a high urinary concentration of more than 1,500 μg/ml was observed. On the other hand, AM-1155 has a high solubility among the newer quinolones. Its solubility in human urine was measured to be 9.6 mg/ml at pH 7.3 and 37°C (13b). At above or below pH 7, the solubility increased markedly, thus excluding the possibility of crystalluria. In fact, no crystals attributable to the drug were observed by light microscopy in this study.

AM-1155 was rapidly absorbed; the peak concentrations in serum were achieved at 1 to 2 h after the single or multiple oral dosing. The extensive urinary recovery of the unchanged drug suggested that this drug was well absorbed from the gastrointestinal tract. The C_{max} of AM-1155 was higher than those of norfloxacin (1), enoxacin (6), tosufloxacin (10), ciprofloxacin (7), and sparfloxacin (14) and was comparable to those of ofloxacin (5), lomefloxacin (12, 18), and fleroxacin (9). The $AUC_{0-\infty}$ was smaller than that of fleroxacin (9) and was comparable to those of ofloxacin (5) and lomefloxacin (12, 18). The $t_{1/2\beta}$ was comparatively long, being similar to those of lomefloxacin (12, 18) and temafloxacin (2, 11). The influence of food intake on the pharmacokinetics of AM-1155 was examined after a light morning meal. No pharmacokinetic change except the slight decrease in $AUC_{0-\infty}$ was observed. This indicated that the effect of food intake was negligible.

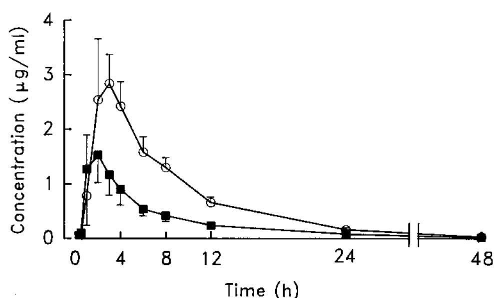

FIG. 3. Mean concentrations (with standard deviations) of AM-1155 in saliva following a single oral administration of 200 mg (■) or 400 mg (○) in humans (*n* = 6).

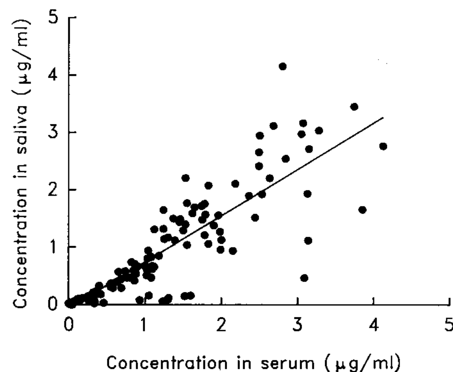

FIG. 4. Correlation between the concentrations of AM-1155 in serum and saliva (coefficient of correlation = 0.857; $y = 0.810x - 0.0668$; $n = 120$).

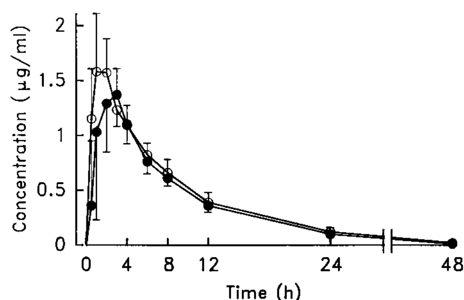

FIG. 5. Mean concentrations (with standard deviations) of AM-1155 in serum following a single oral administration of 200 mg in the fasting (○) or nonfasting (●) state in humans (n = 6).

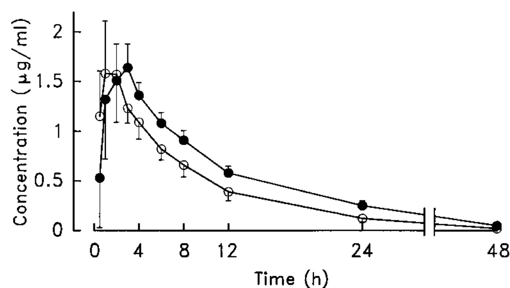

FIG. 6. Mean concentrations (with standard deviations) of AM-1155 in serum following a single oral administration of 200 mg with (●) or without (○) 1.5 g of probenecid in humans (n = 6).

Because the F value of AM-1155 was found to be around unity, V_{β}/F is almost equal to V_{β}. This V_{β} value of AM-1155 is larger than those of ofloxacin, fleroxacin, and pefloxacin and is comparable to those of lomefloxacin, and ciprofloxacin (17). These findings reflect the good tissue penetration of AM-1155.

In general, new quinolones are highly excreted in saliva, and the concentrations in saliva are well correlated with those in serum; the concentrations in saliva were observed to be 30 to 40% of those in serum or plasma with fleroxacin, tosufloxacin, and amifloxacin (22) and 70 to 90% with sparfloxacin, levofloxacin, and temafloxacin (22). In this study, the concentrations of AM-1155 in saliva were also correlated with those in serum, being approximately 80% of those in serum; measurement of the concentrations in saliva may be useful for the therapeutic drug monitoring of these antibacterial agents.

Glucuronide conjugate and three minor metabolites (Fig. 1) of AM-1155 were isolated and identified in rat urine and bile (2a). These four metabolites were, thus, determined by HPLC in the human urine. This study revealed that AM-1155 was hardly metabolized in humans and that its metabolic profile was similar to those of lomefloxacin (12) and temafloxacin (2). However, larger amounts of unchanged drug were recovered in the urine relative to the other agents.

TABLE 3. Effects of food intake and probenecid on the pharmacokinetic parameters of AM-1155 following a 200-mg single oral administration in healthy volunteers

Parameter	Value for group[a]		
	Fasting	No fasting	With 1.5-g probenecid
C_{max} (μg/ml)	1.71 ± 0.35	1.65 ± 0.23	1.79 ± 0.23
T_{max} (h)	1.41 ± 0.81	1.86 ± 0.92	1.86 ± 0.92
$AUC_{0-\infty}$ (μg · h/ml)	14.5 ± 2.6	12.7 ± 2.1[b]	20.6 ± 2.8[b]
$t_{1/2\beta}$ (h)	7.10 ± 1.64	6.52 ± 1.40	10.2 ± 1.9[c]
V_{β}/F (liters/kg)	2.29 ± 0.34	2.37 ± 0.32	2.29 ± 0.38
V_{SS}/F (liters/kg)	1.96 ± 0.22	2.01 ± 0.14	1.92 ± 0.21
CL_T/F (ml/min)	236 ± 36	268 ± 39[b]	164 ± 20[b]
CL_R (ml/min)	197 ± 32	223 ± 40	122 ± 12[b]
f_u	0.815 ± 0.023		0.757 ± 0.023[b]
$CL_{R\ int}$ (ml/min)	242 ± 45		161 ± 14[b]
CL_{CR} (ml/min)	106 ± 6		124 ± 32
Excretion ratio	2.28 ± 0.33		1.35 ± 0.25[b]
Urinary excretion (% of dose, 0–72 h)	83.4 ± 3.3	86.5 ± 2.4	74.9 ± 6.4[c]

[a] The data are means ± standard deviations for six subjects.
[b] Significantly different from the corresponding value for the fasting state (P < 0.01).
[c] Significantly different from the corresponding value for the fasting state (P < 0.05).

Many reports that have been published on the renal handling of new quinolones showed different mechanisms, depending on the drugs (15, 16, 24). Because new quinolones were principally excreted into the urine, their concentrations in blood were believed to be closely related to their mechanisms of renal excretion. The decrease of CL_R at the higher doses suggested that carrier-mediated active secretion contributed to some renal excretion of AM-1155 (Table 2). To examine the effect of probenecid on the pharmacokinetics of AM-1155 and to obtain further information on the mechanism of renal excretion, the volunteers each received an oral dose of 200 mg of AM-1155 with or without 1.5 g of probenecid. Probenecid prolonged the $t_{1/2\beta}$, increased the $AUC_{0-\infty}$, and decreased the CL_T/F, CL_R, $CL_{R\ int}$, and urinary recovery of AM-1155. Other pharmacokinetic parameters remained unchanged. This suggested that probenecid has no effect on the gastrointestinal absorption or distribution of AM-1155. The excretion ratio ($CL_{R\ int}/CL_{CR}$) without probenecid was 2.28, which was significantly reduced to 1.35 with probenecid. This effect indicated that the tubular secretion contributed to the renal excretion of AM-1155. The inhibition of secretion with probenecid could explain the pharmacokinetic alterations of AM-1155. An incomplete inhibition of secretion by probenecid was also suggested because the excretion ratio was the value above unity in the presence of probenecid. This assumption is supported by the concentrations of probenecid in serum; its concentrations were insufficient to inhibit tubular secretion immediately before the second dosing and third dosing of probenecid (data not shown).

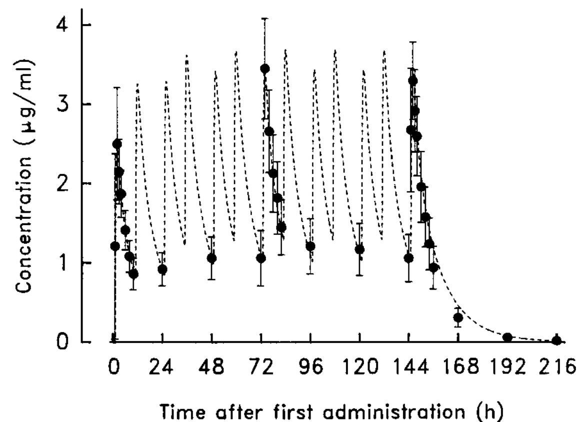

FIG. 7. Mean concentrations (with standard deviations) of AM-1155 in serum during and after multiple oral administration of 300 mg twice daily for 7 consecutive days (n = 6). Dashed line, theoretical simulating curve.

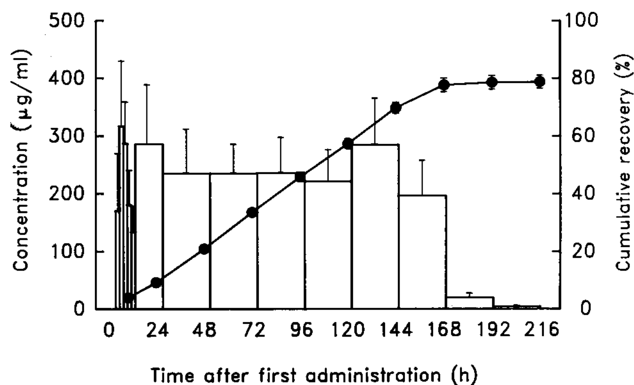

FIG. 8. Mean concentrations of AM-1155 in urine (bars) and its cumulative recovery to total dose (circles) (with standard deviations) during and after multiple oral administration of 300 mg twice daily for 7 consecutive days (n = 6).

During multiple oral administration, the concentrations of AM-1155 in serum and urine reached a steady state within 2 to 3 days. The measured concentrations in serum were close to the simulated value. These findings reflected the persistence of the linear pharmacokinetics of AM-1155.

At the steady state, the trough concentrations in serum were well above the MICs at which 90% of the bacterial strains tested for most AM-1155-susceptible pathogens are inhibited (3, 4, 21, 23) and supported the twice-daily dosage regimen. In Japan, the clinical trials of AM-1155 have been done mainly with the twice-daily dosage regimen, and the once-daily dosage regimen might improve patient compliance, if feasible. In addition, the high concentrations in urine indicated that the drug effect against urinary tract infections might be expected at a dose below 300 mg.

In conclusion, AM-1155 was tolerated well by healthy subjects. This drug is expected to be clinically useful because of its potent antibacterial activity and favorable pharmacokinetics.

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