Physiologically-based Pharmacokinetic Analysis of Grepafloxacin

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Grepafloxacin (GPFX) is a synthetic new quinolone antimicrobial agent that possesses an extensive tissue distribution and exhibits a strong antibacterial activity *in vivo*. In this study, the tissue distribution characteristics of GPFX were examined using tissue concentration–time profiles following intravenous administration to rats. Subsequently, the pharmacokinetics of GPFX were analyzed based on the physiological pharmacokinetic model. The tissue-to-plasma partition coefficients (K_p) of GPFX in rats were high in all tissues except brain. A pharmacokinetic model for rabbits, monkeys and dogs was constructed using the tissue-to-plasma free concentration ratio ($K_{p,f}$) of GPFX in rats to simulate the GPFX concentration–time profile in plasma following intravenous administration of GPFX to each animal. The calculation-derived concentrations correlated well with the experimentally-derived data, suggesting that there are no interspecies differences in the high tissue distribution characteristics of GPFX. The clearance rates of GPFX in humans were predicted from the pharmacokinetic parameters of rats, rabbits, monkeys and dogs by an animal scale-up method and a pharmacokinetic model for humans was constructed. The GPFX concentration–time profiles in plasma, following oral administration of GPFX to humans, were predicted within 0.5–1.0 h of mean absorption time and the calculation-derived results were in good agreement with the experimental data. Thus, it is suggested that the concentration–time profile in plasma and all human organs can be predicted from the pharmacokinetic data of animals.

Key words grepafloxacin; physiological pharmacokinetic model; tissue distribution; animal scale-up

Grepafloxacin (GPFX) is a synthetic new quinolone antimicrobial agent (Fig. 1) that possesses a more potent effect against experimental infectious disease in respiratory organs when compared to other quinolone derivatives.¹⁾ It follows from this that GPFX can be expected to have an extensive tissue distribution, especially to the lung. Several studies have indicated that the peak tissue concentration of GPFX is reached within an hour after oral administration to rats in most organs except testis, and that the maximum concentrations in tissue are much higher than that of the plasma.²⁾ No conspicuous accumulation of GPFX was observed *in vivo* since whole body autoradiography following repeated oral administration to rats was similar to that after single oral administration.³⁾

In general, the new quinolone antimicrobial agents have a good tissue distribution, and the reported tissue-to-plasma free concentration ratios ($K_{p,f}$) of six pyridonecarboxylic acids (PCAs) are high in most tissues.^{4–10)} With regard to the six PCAs, the $K_{p,f}$ values in each tissue are identical and the apparent volume of distribution at steady state ($V_{d,ss}$) is reported to be proportional to the free fraction with plasma proteins (f_p).⁴⁾ However, GPFX would not be expected to obey such a relationship, since the $K_{p,f}$ values of GPFX are several times higher than those of these drugs.

In this study, following intravenous administration to rats, the tissue distribution characteristics of GPFX were examined using tissue concentration–time profiles. Subsequently, the pharmacokinetics of GPFX were analyzed based on the physiological pharmacokinetic model. With regard to the use of antibacterial agents for the treatment of locally infectious diseases, it is important to be able to predict their tissue distribution properties and concentration–time profiles in all organs. Furthermore, this model may become a useful means for predicting human pharmacokinetics from animal data and from the changes in *in vivo* parameters caused by disease. In

this study, we thus examined whether there were interspecies differences in the good tissue distribution characteristics of GPFX, and we further investigated the feasibility of predicting human pharmacokinetics of GPFX from animal data, using an animal scale-up method.

MATERIALS AND METHODS

Chemicals and Reagents Grepafloxacin (GPFX, OPC-17116, ($\pm$)-1-cyclopropyl-6-fluoro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-quinoline carboxylic acid hydrochloride) and OPC-17203 (($\pm$)-1-cyclopropyl-5-ethyl-6,8-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-quinoline carboxylic acid), an internal standard for the determination of GPFX, were respectively obtained from the Second Tokushima Factory and Tokushima Research Institute of Otsuka Pharmaceutical Co.,

Fig. 1. Chemical Structures of Grepafloxacin (A) and Internal Standard (B)

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Ltd. (Tokyo, Japan). ^{14}C -Labeled grepafloxacin ($[^{14}\text{C}]\text{GPFx}$) at 2-position of quinolone ring was synthesized by Amersham International plc. (Buckingham, U.K.). The radioactivity and radiochemical purity were 3.29 MBq/mg and higher than 97%, respectively. Other chemicals were of the finest grade or of HPLC grade obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Animals Sprague-Dawley male rats aged 5 weeks weighing 163–192 g were purchased from Charles River Japan Co., Ltd. (Yokohama, Japan) or Shizuoka Laboratory Animal Center (Hamamatsu, Japan) and were given solid food (MR, Oriental Yeast Co., Ltd., Tokyo, Japan). Four male Japan White rabbits aged 10 weeks were purchased from Kitayama Labes Ltd. (Nagano, Japan) and were given solid food (RC-IV, Oriental Yeast). Three male beagle dogs weighing 10.5–13.4 kg at an age of about 16 months and three male cynomolgus monkeys weighing 3–5 kg were purchased from Kasho Co., Ltd. (Tokyo, Japan). Dogs and monkeys were given solid food (CD-5, CREA Japan, Tokyo and PS, Oriental Yeast, respectively). Water was allowed *ad libitum*.

Blood-to-plasma Concentration Ratio (R_B) of GPFx R_B of GPFx in rabbits was determined *in vitro* by comparison of blood and plasma concentrations following incubation of blood and $[^{14}\text{C}]\text{GPFx}$. Incubation of blood was initiated by the addition of 20 μl of 100 or 1000 $\mu\text{g}/\text{ml}$ of GPFx to 2 ml of blood. After incubation for 2, 5, 10, and 15 min at 37 °C, 100- μl aliquot of blood was placed into a glass vial and dissolved with 1 ml of tissue solubilizing solution (Nacalai Tesque Inc., Kyoto, Japan). After dissolving, 1 ml of a solution for pH adjustment (Nacalai Tesque Inc., Kyoto, Japan) and 16 ml of scintillator (Hionic-Fluor, Packard) were added to the mixture for the measurement of radioactivity. Another 250- μl aliquot of blood was placed into a tube to be centrifuged ($9500\times g$ for 2 min) at the same time to obtain the plasma. 100- μl of plasma obtained was placed into a glass vial, and then 1 ml of distilled water and 8 ml of scintillator (ACS-II, Amersham) were added for the measurement of radioactivity. The radioactivity was determined by a liquid scintillation counter (LSC-1050 or 3500, ALOKA Japan, Tokyo). Counting efficiency was corrected by the External Standard Channel Ratio method. R_B was calculated by averaging R_B at the designated time because R_B was kept at a constant through the experiment. *In vivo* R_B of GPFx in rats, dogs, and monkeys were obtained by comparison of blood and plasma concentrations of radioactivity following oral administration of $[^{14}\text{C}]\text{GPFx}$ in previous studies.^{3,11)}

Tissue Distribution Study of $[^{14}\text{C}]\text{GPFx}$ and Unlabeled GPFx in Rats ^{14}C -Labeled or unlabeled GPFx was dissolved in isotonic lactate buffer containing manitol and was intravenously administered at dose of 20 mg/kg (^{14}C -labeled) or 10 mg/kg (unlabeled) to rats through the tail vein. Injected volume was 2.5 ml/kg. Following the administration, blood was withdrawn from the inferior vena cava into a heparinized syringe under anesthesia at designated times in groups of 3 (^{14}C -labeled) or 5 (unlabeled) rats each. The animals were sacrificed by bleeding, and tissue samples were removed. In the $[^{14}\text{C}]\text{GPFx}$, the blood samples (100 μl) were dried in a cone for measurement of radioactivity by the combustion method. The remaining blood was centrifuged at $1800\times g$ for 10 min to obtain the plasma. The plasma (100 μl) was com-

bined with 1 ml of distilled water and 8 ml of scintillator (ACS-II, Amersham) for measurement of radioactivity. The wet weight of tissues was measured, and the whole sample or a part was placed in a cone for the combustion. The brain, kidney, stomach, and intestines were homogenized with a 2-fold volume of saline, and part of the homogenates were sampled in a pad. Air-dried samples on a cone were combusted in a sample oxidizer (B-306, Packard Instrument, Meriden, U.S.A.). The resulting $^{14}\text{CO}_2$ was absorbed into Carbo-Sorb (9 ml, Packard) and made up into a premixed scintillation cocktail (12 ml, PermaFluor V, Packard) for measurement of radioactivity. In the unlabeled GPFx, tissue sample removed was homogenized with a 4-fold saline after measurement of the wet weight. Tissue homogenates were centrifuged for 10 min at $1700\times g$, and the supernatant was taken to measure the concentration of GPFx by the HPLC method reported previously by Akiyama.²⁾

Urinary Excretion Studies of GPFx in Rats, Rabbits, Dogs and Monkeys GPFx was dissolved in isotonic lactate buffer containing manitol and was intravenously administered at 10 mg/kg to rats, dogs and monkeys through the tail, forearm and lower extremity vein, respectively. Rabbits were administered 9.3 mg/kg of GPFx through the ear vein. Injected volume was 0.5 ml/kg in dogs and 1 ml/kg in rabbits and monkeys. Animals were placed in a cage after dosing, urine excreted spontaneously was collected during designated periods, and the cage was washed with a small amount of water. The urine samples were measured by the HPLC method.²⁾

Pharmacokinetic Parameters in Animals The total body clearance (CL_{tot}) and the apparent volume of distribution at steady state ($V_{\text{d,ss}}$) were estimated by noncompartmental analysis.¹²⁾ After calculating the renal clearance (CL_r) from the urinary recovery of unchanged GPFx, the hepatic clearance (CL_h) was estimated as the difference between CL_{tot} and CL_r . The blood unbound fraction (f_B) was calculated using the free fraction with plasma proteins (f_p) and the R_B values. The intrinsic clearance in liver ($CL_{\text{int,liv}}$) and the intrinsic clearance in kidney ($CL_{\text{int,kid}}$) were estimated using the values of CL_h , CL_r and blood flow rate in liver (Q_h) and kidney (Q_r), based on the well-stirred model^{13,14)} [Eqs. 1, 2].

$$CL_h = \frac{Q_h \cdot f_B \cdot CL_{\text{int,liv}}}{Q_h + f_B \cdot CL_{\text{int,liv}}} \quad (1)$$

$$CL_r = \frac{Q_r \cdot f_B \cdot CL_{\text{int,kid}}}{Q_r + f_B \cdot CL_{\text{int,kid}}} \quad (2)$$

Estimation of Tissue-to-Plasma Partition Coefficient (K_p) The tissue-to-plasma partition coefficient ($K_{p,\text{app}}$) was estimated by Eq. 3¹⁵⁾ using the concentration ratio after single intravenous administration of GPFx. β is the slope of the β -phase, while Q_T and V_T represent the blood flow rate and the distribution volume of each organ, respectively. Regarding the parameters for organs of excretion, namely liver and kidney, these were calculated by Eq. 5¹⁵⁾ using the value of bioavailability (F) of each tissue estimated from Eq. 4,¹⁶⁾ based on the well-stirred model. The GPFx concentration data, following intravenous administration at 10 mg GPFx per kilogram dose, was used for calculating $K_{p,\text{app}}$ values in lung, brain, heart, kidney, liver, spleen, small intestine, large intestine and stomach. The radioactivity data, following in-

travenous administration at 20 mg [^{14}C]GPFX per kilogram dose, was used for calculation of $K_{p,app}$ values in muscle, bone, skin, fat, thymus and pancreas.

$$K_p = \frac{K_{p,app}}{1 + \beta \cdot V_T \cdot \frac{K_{p,app}}{Q_T} \cdot \frac{1}{R_B}} \quad (3)$$

$$F = \frac{Q}{Q + f_B \cdot CL_{int}} \quad (4)$$

$$K_p = \frac{1}{F} \cdot \frac{K_{p,app}}{1 + \beta \cdot V_T \cdot \frac{K_{p,app}}{Q_T} \cdot \frac{1}{R_B}} \quad (5)$$

Based on the assumption that there are no species differences in $K_{p,f}$ value, K_p values were calculated by multiplying $K_{p,f}$ value obtained by dividing the K_p value of rat by the f_p value of rat by f_p value of each animal or humans.

Analysis Using a Physiologically-based Pharmacokinetic Model Through the analysis of the above pharmacokinetic data, a physiologically-based pharmacokinetic model was derived. In the model, the mean of the physiological data for each animal from the literature^{16–20} was used for blood flow rate (Q) and tissue volume (V_d) parameters of each organ. For the small intestine, large intestine, stomach and thymus, the actual weights of the tissue of rats used in our experiments were used for the V_d values. The mass balance equations for the concentration in each organ compartment were solved simultaneously by the Runge–Kutta–Gill method as described in ref. 21, and the concentration in plasma and other tissues after single intravenous administration were calculated.

RESULTS AND DISCUSSION

Pharmacokinetics of GPFX in Rats, Rabbits, Monkeys and Dogs The values of R_B , derived from the plasma and blood samples of each animal (rats, rabbits, monkeys and

dogs), and the f_p values^{3,11} were obtained from previous reports (Table 1). The value of f_p were noted to be almost identical (0.54–0.59) among all four species. A slight variation in R_B values, 1.1–1.4 was noted, however, taking into account the hematocrit (H_c) values for each animal, the erythrocyte free fraction (f_{RBC}) was found to be identical among all four species (Table 1).

Almost all of the GPFX administered was excreted in the feces, whereas most other quinolone derivatives are known to be mainly excreted in urine.^{2,3,11,22} Urinary recoveries of unchanged GPFX were measured in this study after single intravenous administration, and found to be within 10% of dose in all four species (Table 2).

The plasma concentration–time profile data following a single intravenous administration to each animal (rats, rabbits, monkeys or dogs) has been previously reported to exhibit, and showed a biexponential behavior in all animals.² The pharmacokinetic parameters are summarized in Table 2. The V_{dss} value of GPFX for rats was approximately five times and three times larger than those of lomefloxacin and ofloxacin, respectively.⁴

Tissue Distribution of GPFX in Rats The observed concentrations of GPFX or radioactivity in plasma and tissues after intravenous administration of GPFX or [^{14}C]GPFX to rats are listed in Tables 3 and 4, respectively. From this data, the value of $K_{p,f}$ in each tissue was calculated and is summarized in Table 5. The $K_{p,f}$ values of GPFX ranged from 0.6 to 33.1, being greater than unity in almost all major tissues with the exception of brain. In addition to the organs of excretion, namely liver and kidney, the $K_{p,f}$ values were also particularly high in lung and skin, and thus GPFX clearly distributed to such organs efficiently. In contrast, the $K_{p,f}$ values were low in brain and fat and thus, it may be predicted that adverse effects of GPFX in the central nervous system will be low.

The extent of distribution into each tissue can be evaluated from the equation $K_p \cdot V_T / V_{dss} \cdot 100$. GPFX distributed mainly to skin, muscle and liver to the extent of 49%, 28% and 7% of total, respectively. The V_{dss} value represents the sum of $K_p \cdot V_T$ calculated in each tissue.

Okezaki *et al.* (1988) studied the relationship between V_d and f_p of six pyridonecarboxylic acids (PCAs), and recognized a good correlation between the two parameters ($r=0.981$). The V_d value of GPFX predicted from this correlation was about 1.2 l/kg, however, the real value was found to be 5.42 l/kg, thus revealing a great discrepancy between the two values. Therefore the high distribution of GPFX can not be explained purely by the difference in the f_p values. The factors and mechanisms behind the high $K_{p,f}$ values of GPFX remain to be determined. There may be several possibilities such as a specific transporter and/or a specific binding pro-

Table 1. Plasma Free Fraction (f_p), Blood-to-Plasma Concentration Ratio (R_B) and Erythrocyte Free Fraction (f_{RBC}) of GPFX for Rat, Rabbit, Dog, Monkey and Human

Species	f_p	R_B	f_{RBC}^c
Rat	0.593 ± 0.035^a	1.34 ± 0.37	0.32
Rabbit	0.575 ± 0.023^a	1.1 ± 0.07	0.44–0.48
Dog	0.59 ± 0.021^b	1.13 ± 0.25	0.26–0.30
Monkey	0.544 ± 0.020^b	1.42 ± 0.21	0.45–0.47
Human	0.495 ± 0.027^a	1.1^d	—

a) The values reported previously.³ b) The values reported previously.¹¹ c) The values of f_{RBC} calculated according to the equation: $f_{RBC} = f_p \cdot H_c / (R_B - (1 - H_c))$. d) The R_B value calculated from the mean f_{RBC} value of other four animals according to the equation: $R_B = f_p / f_{RBC} \cdot H_c + (1 - H_c)$.

Table 2. Pharmacokinetic Parameters of GPFX Following Intravenous Administration to Rats, Rabbits, Monkeys and Dogs

Species	Dose (mg/kg)	n	AUC ^a ($\mu\text{g} \cdot \text{h/ml}$)	MRT (h)	CL_{tot} (ml/min/kg)	V_{dss} (l/kg)	Urinary excretion (% of dose)	CL_r (ml/min/kg)	CL_h (ml/min/kg)	$CL_{int,kd}$ (ml/min/kg)	$CL_{int,liv}$ (ml/min/kg)
Rat	10	5	6.29 ± 0.16	3.76 ± 0.20	24.1 ± 1.53	5.42 ± 0.61	14.4 ± 0.5	3.47	20.6	8.48	71.5
Rabbit	9.3	4	7.42 ± 0.22	2.21 ± 0.22	19.0 ± 0.76	2.51 ± 0.22	9.23 ± 1.10	1.75	17.2	3.63	44.1
Monkey	10	3	28 ± 0.59	10.19 ± 0.59	5.41 ± 0.29	3.31 ± 0.46	11.6 ± 2.6	0.629	4.78	1.68	14.2
Dog	10	3	25.1 ± 1.23	10.30 ± 1.32	6.04 ± 0.48	3.73 ± 0.71	7.04 ± 0.49	0.425	5.61	0.830	13.6

a) AUC value calculated from the data reported previously.²

Table 3. Tissue Concentration of GPFX Following an Intravenous Administration of GPFX (10 mg/kg dose) in Rats

Tissue	Concentration ($\mu\text{g/ml}$)					
	0.25 h	0.5 h	1 h	2 h	4 h	8 h
Plasma	2.25 $\pm$ 0.06	1.65 $\pm$ 0.06	1.13 $\pm$ 0.05	0.53 $\pm$ 0.03	0.31 $\pm$ 0.03	0.07 $\pm$ 0.01
Lung	24.5 $\pm$ 2.0	21.9 $\pm$ 1.6	16.1 $\pm$ 0.7	9.51 $\pm$ 0.68	4.58 $\pm$ 0.31	1.33 $\pm$ 0.12
Brain	0.26 $\pm$ 0.01	0.25 $\pm$ 0.01	0.24 $\pm$ 0.00	0.17 $\pm$ 0.01	0.11 $\pm$ 0.00	0.04 $\pm$ 0.00
Heart	7.71 $\pm$ 0.30	5.44 $\pm$ 0.24	3.99 $\pm$ 0.22	2.22 $\pm$ 0.15	1.18 $\pm$ 0.08	0.37 $\pm$ 0.02
Kidney	26.0 $\pm$ 0.6	21.2 $\pm$ 1.4	15.6 $\pm$ 0.6	9.29 $\pm$ 0.29	5.16 $\pm$ 0.21	1.52 $\pm$ 0.10
Liver	11.4 $\pm$ 1.1	8.75 $\pm$ 0.57	7.84 $\pm$ 1.00	4.21 $\pm$ 0.13	2.31 $\pm$ 0.14	0.95 $\pm$ 0.11
Spleen	13.1 $\pm$ 0.8	10.5 $\pm$ 0.8	8.27 $\pm$ 0.43	4.60 $\pm$ 0.08	2.35 $\pm$ 0.15	0.76 $\pm$ 0.08
Small intestine	7.21 $\pm$ 0.40	8.97 $\pm$ 0.40	8.23 $\pm$ 0.63	8.03 $\pm$ 1.34	5.48 $\pm$ 0.97	1.41 $\pm$ 0.14
Large intestine	5.78 $\pm$ 0.21	5.02 $\pm$ 0.44	4.75 $\pm$ 0.57	2.61 $\pm$ 0.15	1.53 $\pm$ 0.11	1.16 $\pm$ 0.36
Stomach	7.25 $\pm$ 0.37	7.96 $\pm$ 0.30	5.23 $\pm$ 0.43	2.63 $\pm$ 0.15	1.68 $\pm$ 0.14	0.51 $\pm$ 0.04

Table 4. Tissue Concentration of Radioactivity Following an Intravenous Administration of ^{14}C -GPFX (20 mg/kg Dose) in Rats

Tissue	Concentration ($\mu\text{g eq/ml or g}$)					
	0.083 h	0.25 h	1 h	4 h	8 h	24 h
Plasma	6.51 $\pm$ 0.05	3.99 $\pm$ 0.18	2.19 $\pm$ 0.17	0.31 $\pm$ 0.04	0.11 $\pm$ 0.01	n.d.
Lung	83.6 $\pm$ 1.9	70.1 $\pm$ 2.7	39.4 $\pm$ 4.6	6.58 $\pm$ 0.62	1.76 $\pm$ 0.12	0.14 $\pm$ 0.01
Brain	1.31 $\pm$ 0.03	1.44 $\pm$ 0.23	0.92 $\pm$ 0.10	0.38 $\pm$ 0.05	0.04 $\pm$ 0.02	n.d.
Heart	31.2 $\pm$ 0.9	21.3 $\pm$ 1.0	9.76 $\pm$ 0.72	1.51 $\pm$ 0.17	0.42 $\pm$ 0.02	n.d.
Muscle	24.2 $\pm$ 0.4	19.8 $\pm$ 1.14	10.0 $\pm$ 0.6	1.57 $\pm$ 0.09	0.42 $\pm$ 0.15	n.d.
Kidney	71.4 $\pm$ 7.8	56.1 $\pm$ 2.6	20.4 $\pm$ 2.2	5.38 $\pm$ 0.50	1.63 $\pm$ 0.10	0.29 $\pm$ 0.03
Liver	49.1 $\pm$ 1.6	34.4 $\pm$ 2.6	17.9 $\pm$ 1.4	3.47 $\pm$ 0.21	1.52 $\pm$ 0.10	0.29 $\pm$ 0.00
Spleen	40.6 $\pm$ 2.0	43.7 $\pm$ 2.5	22.7 $\pm$ 3.1	3.43 $\pm$ 0.37	0.94 $\pm$ 0.08	0.11 $\pm$ 0.01
Small intestine	17.9 $\pm$ 1.3	17.7 $\pm$ 1.3	18.1 $\pm$ 0.5	7.01 $\pm$ 1.03	1.24 $\pm$ 0.21	0.11 $\pm$ 0.04
Large intestine	13.9 $\pm$ 0.7	15.5 $\pm$ 1.4	10.4 $\pm$ 1.3	3.96 $\pm$ 0.30	5.78 $\pm$ 2.27	0.24 $\pm$ 0.06
Thighbone	7.31 $\pm$ 0.34	7.63 $\pm$ 0.78	3.70 $\pm$ 0.55	0.68 $\pm$ 0.11	0.20 $\pm$ 0.04	n.d.
Skin	11.9 $\pm$ 0.9	11.5 $\pm$ 1.4	12.0 $\pm$ 2.0	8.82 $\pm$ 0.99	5.55 $\pm$ 1.27	2.22 $\pm$ 0.82
Fat	3.95 $\pm$ 0.74	2.10 $\pm$ 0.16	3.28 $\pm$ 1.64	0.24 $\pm$ 0.07	0.20 $\pm$ 0.13	n.d.
Thymus	11.8 $\pm$ 0.9	15.5 $\pm$ 0.3	17.2 $\pm$ 1.7	4.18 $\pm$ 0.27	0.76 $\pm$ 0.06	0.05 $\pm$ 0.00
Stomach	24.1 $\pm$ 2.3	15.1 $\pm$ 0.7	8.28 $\pm$ 0.56	2.09 $\pm$ 0.17	0.66 $\pm$ 0.07	0.06 $\pm$ 0.01
Pancreas	64.2 $\pm$ 2.9	56.3 $\pm$ 6.2	29.9 $\pm$ 3.3	4.13 $\pm$ 0.41	1.06 $\pm$ 0.05	0.12 $\pm$ 0.02

Table 5. Tissue-to-Plasma Free Concentration Ratio ($K_{p,f}$) of GPFX and 6 PCAs Reported Previously for Various Organs of Rat

Organ	$K_{p,f}$	
	GPFX	Mean value ^{a)}
Lung	27.6 $\pm$ 1.0	1.97 $\pm$ 0.30
Brain	0.569 $\pm$ 0.090	0.39 $\pm$ 0.05
Heart	6.73 $\pm$ 0.33	2.14 $\pm$ 0.26
Muscle	6.70 $\pm$ 0.29	2.25 $\pm$ 0.21
Kidney	31.0 $\pm$ 1.4	6.34 $\pm$ 1.00
Liver	19.8 $\pm$ 0.6	4.82 $\pm$ 0.64
Spleen	13.3 $\pm$ 0.9	2.46 $\pm$ 0.33
Small intestine	27.4 $\pm$ 0.5	1.89 $\pm$ 0.18
Bone	3.15 $\pm$ 0.23	2.36 $\pm$ 0.40
Skin	33.1 $\pm$ 0.7	1.63 $\pm$ 0.24
Large intestine	8.29 $\pm$ 0.18	—
Thymus	15.7 $\pm$ 0.4	—
Stomach	8.7 $\pm$ 0.18	—
Pancreas	22.6 $\pm$ 1.3	—
Fat & other	2.23 $\pm$ 0.32	0.30 $\pm$ 0.04

a) The mean $K_{p,f}$ values reported previously.⁴⁾

tein.

Predicted Time Course of GPFX Concentration in Plasma and Tissues in Animals The GPFX concentration–time profiles in plasma and tissues, following intravenous administration of GPFX to rats at a dose of 10 mg/kg, were es-

timated using the physiologically-based pharmacokinetic model, incorporating the characteristically high $K_{p,f}$ values of GPFX. The calculated (solid lines) and the observed values²⁾ are shown in Fig. 2. The simulation results were in fairly good agreement with the experimental data in plasma and all tissues, with the exception of the early phase in brain and intestine. For intestinal tissue, the simulated concentration reached a maximum immediately after intravenous administration and declined promptly thereafter. In contrast, the observed peak was slowly reached half an hour after administration, and the high concentration was maintained for a few hours. Previous reports on the oral administration of [^{14}C]GPFX to rats have recognized that the enterohepatic circulation may lead to the apparent slow uptake and elimination of GPFX. For brain, the observed concentrations were at lower levels than the simulated values for up to about 1 hour after administration. The factors controlling drug concentration in brain are likely to be more complex than those in other tissues, for example, GPFX permeability including transport systems through the blood-brain barrier and/or brain-cerebrospinal fluid barrier, and the cerebrospinal fluid flow. Therefore, the following assumptions proposed in this model may not apply to brain, namely (1) each tissue acts as a well-stirred compartment, (2) intercompartmental transport occurs by blood flow.

An adequate pharmacokinetic model for each animal was

Fig. 2. Plasma and Tissue Concentration Profiles of GPFX Following Single Intravenous Administration to Rats

The solid lines are calculation curves using the physiologically-based pharmacokinetic model. The closed circles and bars are the observed values and standard errors, respectively. (A) venous plasma, (B) lung, (C) liver, (D) kidney, (E) heart, (F) stomach, (G) brain, (H) spleen, (I) small intestine, (J) large intestine.

constructed using the physiological parameters of rabbits, monkeys or dogs and the GPFX concentration–time profiles in plasma and tissues were simulated following intravenous administration of GPFX to rabbits (9.3 mg/kg), monkeys (10 mg/kg) and dogs (10 mg/kg), respectively. The calculation-derived results corresponded comparatively well with the experimental data, suggesting that there are no inter-species differences in the tissue distribution characteristics (Fig. 3).

Prediction of Plasma Profiles after Intravenous or Oral Administration of GPFX to Humans As shown Fig. 4, an allometric relationship²³⁾ was observed between $CL_{int,lv}$ or $CL_{int,kd}$ and body weight (W), respectively. The regression coefficients for both were 0.94 and statistically significant ($p > 0.1$). The $CL_{int,lv}$ and $CL_{int,kd}$ values in humans were predicted from the following Eqs. 6 and 7.

$$CL_{int,lv} = 37.8 \times BW^{0.604} \quad (6)$$

$$CL_{int,kd} = 3.81 \times BW^{0.483} \quad (7)$$

The $CL_{int,lv}$ and $CL_{int,kd}$ values of the subjects, following oral administration of GPFX at a dose of 100 mg/body, in the phase I study were calculated as 7.46 and 0.458 ml/min/kg, respectively. The R_B value of humans estimated using the mean f_{RBC} value was 1.1, because the f_{RBC} values were found to be identical among all four species taking into account the H_c values for each animal (Table 1). The CL_h and CL_r values were 2.93 and 0.203 ml/min/kg, respectively, giving a CL_{tot} value of 3.13 ml/min/kg in humans. The ratio of area under the concentration–time curve (AUC) to minimum inhibition concentration (MIC) is a general predictor of antibacterial ef-

Fig. 3. Plasma Concentration Profiles of GPFX Following Single Intravenous Administration to Rabbit (A), Dog (B) and Monkey (C)

The solid lines are calculation curves using the physiologically-based pharmacokinetic model. The closed circles and bars are the observed values and standard errors, respectively.

Fig. 4. Allometric Relationship between Body Weight (W) and Either (A) $CL_{int,lv}$ or (B) $CL_{int,kd}$

Each point represents a calculated value derived from intravenous data in rats, rabbits, monkeys and dogs based on the well-stirred model. The solid lines were calculated by the least squares method using unweighted logarithmically transformed data.

Fig. 5. Plasma Concentration Profiles of GPFx Following Single Oral Administration to Healthy Volunteers

The lines are calculation curves using the physiologically-based pharmacokinetic model. Panel (B) represents an enlargement of panel (A). The closed circles and bars are the observed values and standard errors, respectively.

ficacy for quinolones, and thus it is important to be able to predict the value of CL_{tot} in humans. The bioavailability in the liver (F_H) of humans, calculated based on the well-stirred model, was 0.87. Using F_H as the value of the bioavailability (F), given that the main disposing organ is the liver, a physiologically-based pharmacokinetic model for humans was constructed. The GPFx concentrations in human plasma were calculated based on the model in the case when the value of mean absorption time (MAT) is set at (a) 0.5 h, (b) 0.7 h and (c) 1.0 h (namely, at an absorption rate constant (k_a) at (a) 2.0/h, (b) 1.4/h and (c) 1.0/h). Such concentrations are shown in Fig. 5, and are compared with the observed data.²⁴⁾ From the results, it is suggested that the MAT value of GPFx was 0.5–1.0 h in humans. Considering that it takes about one hour to absorb such tablets from stomach to intestine in healthy subjects, it would appear that GPFx is quickly absorbed in the intestine.

In conclusion, we have successfully analyzed the tissue distribution characteristics of GPFx based on the physiological pharmacokinetic model. The $K_{p,f}$ values of GPFx for rats were found to be several times higher than the mean $K_{p,f}$ values of six PCAs reported by E. Okezaki *et al.* The GPFx concentration–time profiles in plasma, estimated using the physiologically-based pharmacokinetic model, incorporating the characteristically high $K_{p,f}$ values of GPFx, corresponded well to the experimental data regardless of species, thus good tissue distribution characteristics were maintained in all ani-

mals. Furthermore, the pharmacokinetic data in animals allowed us to predict clearances in humans, and thus it may be possible to predict concentration–time profiles in all human organs.

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