Physiologically-based Pharmacokinetic Analysis of Grepafloxacin

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Grepafloxacin (GFX) 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 GFX were examined using tissue concentration–time profiles following intravenous administration to rats. Subsequently, the pharmacokinetics of GFX were analyzed based on the physiological pharmacokinetic model. The tissue-to-plasma partition coefficients (Kp,i) of GFX 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 (Kp,i) of GFX in rats to simulate the GFX concentration–time profile in plasma following intravenous administration of GFX to each animal. The calculation-derived concentrations correlated well with the experimentally-derived data, suggesting that there are interspecies differences in the high tissue distribution characteristics of GFX. The clearance rates of GFX 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 GFX concentration–time profiles in plasma, following oral administration of GFX 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 (GFX) 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 GFX can be expected to have an extensive tissue distribution, especially to the lung. Several studies have indicated that the peak tissue concentration of GFX 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 GFX 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 (Kp,i) of six pyridonecarboxylic acids (PCAs) are high in most tissues.4–10 With regard to the six PCAs, the Kp,i values in each tissue are identical and the apparent volume of distribution at steady state (Vd,app) is reported to be proportional to the free fraction with plasma proteins (fpl).5 However, GFX would not be expected to obey such a relationship, since the Kp,i values of GFX are several times higher than those of these drugs.

In this study, following intravenous administration to rats, the tissue distribution characteristics of GFX were examined using tissue concentration–time profiles. Subsequently, the pharmacokinetics of GFX 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 GFX, and further investigated the feasibility of predicting human pharmacokinetics of GFX from animal data, using an animal scale-up method.

MATERIALS AND METHODS

Chemicals and Reagents Grepafloxacin (GFX, OPC-17116, (±)-1-cycloprenyl-6-fluoro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-quinoline carboxylic acid hydrochloride) and OPC-17203 ((±)-1-cycloprenyl-5-ethyl-6,8-fluoro-7-(4-methyl-1-piperazinyl)-4-oxo-quinoline carboxylic acid), an internal standard for the determination of GFX, 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 ([$^{14}$C]GPFX) at 2-position of quinolone ring was synthesized by Amer-
sham International plc. (Buckingham, U.K.). The radioac-
tivity 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 Ki-
tayama 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
canine monguls monkeys weighing 3—5 kg were pur-
blished from Kasho Co., Ltd. (Tokyo, Japan). Dogs and mon-
keys were given solid food (CD-5, CREA Japan, Tokyo
and PS, Oriental Yeast, respectively). Water was allowed ad lihi-
num.

Blood-to-plasma Concentration Ratio ($R_b$) of GPFX
$R_b$ of GPFX in rabbits was determined in vitro by compar-
sion of blood and plasma concentrations following incubation
of blood and [${^{14}}$C]GPFX. Incubation of blood was initiated
by the addition of 20 μl of 100 or 1000 μg/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 solution of tissue solubilizing
solution (Nacalai Tesque Inc., Kyoto, Japan). After dissolving, 1 ml of
a solution of 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×g for 2 min) at the same time to obtain the
plasma. 100-μl plasma of plasma obtained was placed into
a glass vial, and then 1 ml of distilled water and 8 ml of scintil-
lator (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 aver-
inging $R_b$ 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 [${^{14}}$C]GPFX in previous studies.11

Tissue Distribution Study of [${^{14}}$C]GPFX and Unlabeled
GPFX in Rats 14C-Labeled or unlabeled GPFX was dis-
solved 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, the 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 [${^{14}}$C]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×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 con-
busted in a sample oxidizer (B-306, Packard Instrument,
Meriden, U.S.A.). The resulting $^{14}$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×g, and the supernatant
was taken to measure the concentration of GPFX by the HPLC
method reported previously by Akiyama.21

Urinary Excretion Studies of GPFX in Rats, Rabbits,
Dogs and Monkeys GPFX was dissolved in isotonic lactic
buffer containing manitol and was intravenously admin-
istered 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 design-
ated periods, and the cage was washed with a small amount
of water. The urine samples were measured by the HPLC
method.3

Pharmacokinetic Parameters in Animals The total
body clearance ($CL_{\text{tot}}$) and the apparent volume of distribu-
tion at steady state ($V_{\text{dss}}$) were estimated by noncompartmen-
tal analysis.12 After calculating the renal clearance ($CL_r$)
from the urinary recovery of unchanged GPFX, the hepatic
clearance ($CL_{\text{hep}}$) was estimated as the difference between
$CL_{\text{tot}}$ and $CL_r$. The blood unbound fraction ($f_U$) was calcu-
lated using the free fraction with plasma proteins ($f_P$) and the
$R_b$ values. The intrinsic clearance in liver ($CL_{\text{liver}}$) and
the intrinsic clearance in kidney ($CL_{\text{kidney}}$) were estimated using the values of $CL_{\text{tot}}$, $CL_r$, and blood flow rate in liver ($Q_L$) and kid-
ney ($Q_K$), based on the well-stirred model13,14 [Eqs. 1, 2].

$$CL_{\text{hep}} = \frac{Q_L \cdot f_U \cdot CL_{\text{tot}}}{Q_L + f_P \cdot CL_{\text{tot}}}$$

$$CL_r = \frac{Q_K \cdot f_U \cdot CL_{\text{tot}}}{Q_K + f_P \cdot CL_{\text{tot}}}$$

Estimation of Tissue-to-Plasma Partition Coefficient ($K_{\text{pp}}$) The tissue-to-plasma partition coefficient ($K_{\text{pp}}$) was estimated by Eq. 315 using the concentration ratio after single intravenous administration of GPFX. $\beta$ is the slope of the $\beta$-phase, while $Q_\beta$ and $V_\beta$ represent the blood flow rate and the distribution volume of each organ, respectively. Regard-
ing 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 kilograms dose, was used for calculating $K_{\text{pp}}$ values in
lung, brain, heart, kidney, liver, spleen, small intestine, large
intestine and stomach. The radioactivity data, following in-
travenous administration at 20 mg [14C]GPFX per kilogram dose, was used for calculation of $K_{p,app}$ values in muscle, bone, skin, fat, thymus and pancreas.

$$K_p = \frac{K_{p,app}}{1 + \beta V_1 \frac{\Delta R}{\Delta t}} \cdot \frac{1}{Q_h}$$  \hspace{1cm} (3)$$

$$F = \frac{Q}{Q + f_p CL_{int}}$$  \hspace{1cm} (4)$$

$$K_p = \frac{Q}{1 + \beta V_1 \frac{\Delta R}{\Delta t}} \cdot \frac{1}{Q_h}$$  \hspace{1cm} (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 was used for blood flow rate ($Q$) and tissue volume ($V_j$) parameters of each organ. For the small intestine, large intestine, stomach and thymus, the actual weights of the tissues of rat were used for 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 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 ($H_j$) 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. 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. The pharmacokinetic parameters are summarized in Table 2. The $V_{ss}$ value of GPFX for rats was approximately five times and three times larger than those of lomefloxacin and ofloxacin, respectively.

**Tissue Distribution of GPFX in Rats**

The observed concentrations of GPFX or radioactivity in plasma and tissues after intravenous administration of GPFX or [14C]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 other tissues, 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 $K_p \cdot V_j/V_{ss} < 100$. GPFX distributed mainly to skin, muscle and liver to the extent of 49%, 28% and 7% of total, respectively. The $V_{ss}$ value represents the sum of $K_p \cdot V_j$ calculated in each tissue.

Okezaki et al. (1988) studied the relationship between $V_j$ and $f_j$ of six pyridonelactam antibiotics (PCAs), and recognized a good correlation between the two parameters ($r=0.981$). The $V_j$ 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 $f_j$ 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-

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**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

<table>
<thead>
<tr>
<th>Species</th>
<th>$f_p$</th>
<th>$R_b$</th>
<th>$f_{RBC}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.593 ± 0.035</td>
<td>1.34 ± 0.37</td>
<td>0.32</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.575 ± 0.023</td>
<td>1.1 ± 0.07</td>
<td>0.44 - 0.48</td>
</tr>
<tr>
<td>Dog</td>
<td>0.59 ± 0.021</td>
<td>1.13 ± 0.25</td>
<td>0.26 - 0.30</td>
</tr>
<tr>
<td>Monkey</td>
<td>0.544 ± 0.020</td>
<td>1.42 ± 0.21</td>
<td>0.45 - 0.47</td>
</tr>
<tr>
<td>Human</td>
<td>0.495 ± 0.027</td>
<td>1.14 ± 0.25</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>$n$</th>
<th>$AUC^{[a]}$ (µg·h/ml)</th>
<th>MRT (h)</th>
<th>$CL_{int}$ (ml/min/kg)</th>
<th>$V_{ss}$ (l/kg)</th>
<th>Urinary excretion (% of dose)</th>
<th>$CL_L$ (ml/min/kg)</th>
<th>$CL_S$ (ml/min/kg)</th>
<th>$CL_{total}$ (ml/min/kg)</th>
<th>$CL_{extra}$ (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>10</td>
<td>5</td>
<td>6.29 ± 0.16</td>
<td>3.76 ± 0.20</td>
<td>24.1 ± 1.53</td>
<td>5.42 ± 0.61</td>
<td>14.4 ± 0.5</td>
<td>3.47</td>
<td>20.6</td>
<td>8.48</td>
<td>71.5</td>
</tr>
<tr>
<td>Rabbit</td>
<td>9.3</td>
<td>4</td>
<td>7.42 ± 0.22</td>
<td>2.21 ± 0.22</td>
<td>19.0 ± 0.76</td>
<td>5.21 ± 0.22</td>
<td>9.23 ± 1.10</td>
<td>1.75</td>
<td>17.2</td>
<td>3.63</td>
<td>44.1</td>
</tr>
<tr>
<td>Monkey</td>
<td>10</td>
<td>3</td>
<td>28 ± 0.59</td>
<td>10.19 ± 0.59</td>
<td>5.41 ± 0.29</td>
<td>3.31 ± 0.46</td>
<td>11.6 ± 2.6</td>
<td>0.629</td>
<td>4.78</td>
<td>1.68</td>
<td>14.2</td>
</tr>
<tr>
<td>Dog</td>
<td>10</td>
<td>3</td>
<td>25.1 ± 1.23</td>
<td>10.30 ± 1.32</td>
<td>6.04 ± 0.48</td>
<td>3.73 ± 0.71</td>
<td>7.04 ± 0.49</td>
<td>0.425</td>
<td>5.61</td>
<td>0.830</td>
<td>13.6</td>
</tr>
</tbody>
</table>

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*a* $AUC$ value calculated from the data reported previously.
Table 3. Tissue Concentration of GPFX Following an Intravenous Administration of GPFX (10 mg/kg dose) in Rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (µg/ml)</th>
<th>0.25 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td>2.25±0.06</td>
<td>1.65±0.06</td>
<td>1.12±0.05</td>
<td>0.53±0.03</td>
<td>0.31±0.03</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>24.5±2.0</td>
<td>21.9±1.6</td>
<td>16.1±0.7</td>
<td>9.51±0.08</td>
<td>4.58±0.31</td>
<td>1.33±0.12</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>0.26±0.01</td>
<td>0.25±0.00</td>
<td>0.24±0.00</td>
<td>0.17±0.01</td>
<td>0.11±0.00</td>
<td>0.04±0.00</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>7.71±0.30</td>
<td>5.44±0.24</td>
<td>3.99±0.22</td>
<td>2.22±0.15</td>
<td>1.18±0.08</td>
<td>0.37±0.02</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>26.0±0.6</td>
<td>21.2±1.4</td>
<td>15.6±0.6</td>
<td>9.29±0.29</td>
<td>5.16±0.21</td>
<td>1.52±0.10</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>11.4±1.1</td>
<td>8.75±0.57</td>
<td>7.84±1.00</td>
<td>4.21±0.13</td>
<td>2.31±0.14</td>
<td>0.95±0.11</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>13.1±0.8</td>
<td>10.5±0.8</td>
<td>8.27±0.43</td>
<td>4.60±0.08</td>
<td>2.35±0.15</td>
<td>0.76±0.08</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td>7.21±0.40</td>
<td>8.97±0.40</td>
<td>8.23±0.63</td>
<td>8.03±1.34</td>
<td>5.48±0.97</td>
<td>1.41±0.14</td>
</tr>
<tr>
<td>Large intestine</td>
<td></td>
<td>5.78±0.21</td>
<td>5.02±0.44</td>
<td>4.75±0.57</td>
<td>2.61±0.15</td>
<td>1.53±0.11</td>
<td>1.16±0.36</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td>7.25±0.37</td>
<td>7.96±0.30</td>
<td>5.23±0.43</td>
<td>2.63±0.15</td>
<td>1.68±0.14</td>
<td>0.51±0.04</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (µg eq/ml or g)</th>
<th>0.083 h</th>
<th>0.25 h</th>
<th>1 h</th>
<th>4 h</th>
<th>8 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td>6.51±0.05</td>
<td>3.99±0.18</td>
<td>2.19±0.17</td>
<td>0.31±0.04</td>
<td>0.11±0.01</td>
<td>n.d.</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>83.6±1.9</td>
<td>70.1±2.7</td>
<td>39.4±4.6</td>
<td>6.58±0.62</td>
<td>1.76±0.12</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>1.31±0.03</td>
<td>1.44±0.23</td>
<td>0.92±0.10</td>
<td>0.38±0.05</td>
<td>0.04±0.02</td>
<td>n.d.</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>3.12±0.9</td>
<td>21.3±1.0</td>
<td>9.76±0.72</td>
<td>1.51±0.17</td>
<td>0.42±0.02</td>
<td>n.d.</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>24.2±0.4</td>
<td>19.8±1.4</td>
<td>10.0±0.6</td>
<td>1.57±0.09</td>
<td>0.42±0.15</td>
<td>n.d.</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>71.4±7.8</td>
<td>56.1±2.6</td>
<td>20.4±2.2</td>
<td>5.38±0.50</td>
<td>1.63±0.10</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>49.1±1.6</td>
<td>34.4±2.6</td>
<td>17.9±1.4</td>
<td>3.47±0.21</td>
<td>1.52±0.10</td>
<td>0.29±0.00</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>40.6±2.0</td>
<td>43.7±2.5</td>
<td>22.7±3.1</td>
<td>3.43±0.37</td>
<td>0.94±0.08</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td>17.9±1.3</td>
<td>17.7±1.3</td>
<td>18.1±0.5</td>
<td>7.01±0.3</td>
<td>1.24±0.21</td>
<td>0.11±0.04</td>
</tr>
<tr>
<td>Large intestine</td>
<td></td>
<td>13.9±0.7</td>
<td>15.5±1.4</td>
<td>10.4±1.3</td>
<td>3.96±0.30</td>
<td>5.78±2.27</td>
<td>0.24±0.06</td>
</tr>
<tr>
<td>Thighbone</td>
<td></td>
<td>7.31±0.34</td>
<td>7.63±0.78</td>
<td>3.70±0.55</td>
<td>0.68±0.11</td>
<td>0.20±0.04</td>
<td>n.d.</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>11.9±0.9</td>
<td>11.5±1.4</td>
<td>12.0±2.0</td>
<td>8.82±0.99</td>
<td>5.55±1.27</td>
<td>2.22±0.82</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td>3.95±0.74</td>
<td>2.10±0.16</td>
<td>3.28±1.64</td>
<td>0.24±0.07</td>
<td>0.20±0.13</td>
<td>n.d.</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td>11.8±0.9</td>
<td>15.5±0.3</td>
<td>17.2±1.7</td>
<td>4.18±0.27</td>
<td>0.76±0.06</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td>24.1±2.3</td>
<td>15.1±0.7</td>
<td>8.28±0.56</td>
<td>2.97±0.17</td>
<td>0.66±0.07</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td>64.2±2.9</td>
<td>56.3±6.2</td>
<td>29.9±3.3</td>
<td>4.13±0.41</td>
<td>1.06±0.05</td>
<td>0.12±0.02</td>
</tr>
</tbody>
</table>

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

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

^{a) The mean K_{p,i} values reported previously.}

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 estimated using the physiologically-based pharmacokinetic model, incorporating the characteristically high K_{p,i} values of GPFX. The calculated (solid lines) and the observed values 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 [14C]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
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 interspecies differences in the tissue distribution characteristics (Fig. 3).

**Prediction of Plasma Profiles after Intravenous or Oral Administration of GPFX to Humans**

As shown in Fig. 4, an allometric relationship was observed between \( CL_{int,iv} \) or \( CL_{int,kd} \) and body weight (\( W \)), respectively. The regression coefficients for both were 0.94 and statistically significant \((p>0.1)\). The \( CL_{int,iv} \) and \( CL_{int,kd} \) values in humans were predicted from the following Eqs. 6 and 7.

\[
CL_{int,iv} = 37.8 \times BW^{0.994} \tag{6}
\]

\[
CL_{int,kd} = 3.81 \times BW^{0.483} \tag{7}
\]

The \( CL_{int,iv} \) 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 \( R_0 \) 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 \( H_v \) values for each animal (Table 1). The \( CL_{iv} \) and \( CL_{iv} \) values were 2.93 and 0.203 ml/min/kg, respectively, giving a \( CL_{iv} \) 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. 2. Plasma and Tissue Concentration Profiles of GPFX Following Single Intravenous Administration to Rats](image)

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.

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

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,ld}

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_{int} in humans. The bioavailability in the liver (F_l) of humans, calculated based on the well-stirred model, was 0.87. Using F_l 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. 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_{x,y} values of GPFX for rats were found to be several times higher than the mean K_{x,y} 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_{x,y} values of GPFX, corresponded well to the experimental data regardless of species, thus good tissue distribution characteristics were maintained in all animals. 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.

Acknowledgments We thank Dr. Y. Sugiyama (The University of Tokyo, Japan) for helpful advice for this study. This work is supported in part by funds from the Private School Promotion Foundation, Clinical Pharmacology Foundation, and Public Welfare Grant-in-aids for Scientific Research.

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