MECHANISM FOR THE TISSUE DISTRIBUTION OF GREPAFLOXACIN, A FLUOROQUINOLONE ANTIBIOTIC, IN RATS

TAKASHI SUZUKI, YUKIO KATO, HIROYUKI SASABE, MINORU ITOSE, GOHACHIRO MIYAMOTO, AND YUICHI SUGIYAMA

Department of Drug Metabolism, Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima City, Japan (T.S., H.S., M.I., G.M.); and Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan (Y.K., Y.S.)

(Received May 7, 2002; accepted September 4, 2002)

ABSTRACT:

This study was carried out to investigate the most important factor(s) governing the tissue distribution of grepafloxacin (GPFX), a fluoroquinolone antibiotic, in rats. The tissue-to-blood concentration ratio ($K_p$) of GPFX at steady state during constant infusion was highest in the lung, followed by the pancreas, kidney, and spleen. After bolus injection, GPFX was efficiently taken up by most of the organs examined, the uptake clearance other than the lung being almost blood flow-limited. Approximately 10% of the intravenously injected dose was rapidly trapped by the lung, but GPFX distribution rapidly decreased within 30 s due to the washout by the plasma flow. Thus, the higher distribution of GPFX to the lung compared with the other organs cannot be accounted for by a difference in its uptake or efflux. Subcellular fractionation after the infusion indicated that GPFX is primarily distributed to the organelle fractions in most organs, 60% of lung-associated GPFX being recovered in the nucleus and plasma membrane fraction. Such subcellular distribution in the lung was proportional to the phosphatidylserine (PhS) content of each fraction. The steady-state $K_p$ value in each tissue in vivo also correlated with the tissue content of PhS. GPFX preferentially binds to PhS, compared with other phospholipids, and this binding was inhibited by weakly basic drugs, such as quinidine, imipramine, and propranolol, that have also been reported to bind to PhS. The association of GPFX with PhS synthase transformants of Chinese hamster ovary (CHO-K1) cells depends on the PhS content of each cell line, this association being also inhibited by basic drugs. These results suggest that binding of GPFX to PhS is the major determinant of the high distribution of GPFX to the lung.

New quinolone antibacterial agents (NQs), which have been developed since the 1980s, have a high intrinsic antibacterial activity with a wide spectrum of action and have been used in the treatment of a variety of infections. Among them, both grepafloxacin (GPFX) (Akiyama et al., 1995a,b) (Fig. 1) and HSR-903 (Murata et al., 1999) were recently reported to be highly distributed in the tissues compared with the other NQs. The distribution volume of GPFX (7095 ml/kg) and HSR-903 (4900 ml/kg) (Murata et al., 1999) is almost blood flow-limited. Approximately 10% of the intravenously injected dose was rapidly trapped by the lung, but GPFX distribution rapidly decreased within 30 s due to the washout by the plasma flow. Thus, the higher distribution of GPFX to the lung compared with the other organs cannot be accounted for by a difference in its uptake or efflux. Subcellular fractionation after the infusion indicated that GPFX is primarily distributed to the organelle fractions in most organs, 60% of lung-associated GPFX being recovered in the nucleus and plasma membrane fraction. Such subcellular distribution in the lung was proportional to the phosphatidylserine (PhS) content of each fraction. The steady-state $K_p$ value in each tissue in vivo also correlated with the tissue content of PhS. GPFX preferentially binds to PhS, compared with other phospholipids, and this binding was inhibited by weakly basic drugs, such as quinidine, imipramine, and propranolol, that have also been reported to bind to PhS. The association of GPFX with PhS synthase transformants of Chinese hamster ovary (CHO-K1) cells depends on the PhS content of each cell line, this association being also inhibited by basic drugs. These results suggest that binding of GPFX to PhS is the major determinant of the high distribution of GPFX to the lung.

The possible mechanisms involved in the tissue accumulation of drugs are considered likely to be higher tissue uptake and/or tissue binding, or lower efflux from the tissues. Various determining factors have been identified for the tissue distribution of certain types of drugs. For example, binding to the nuclei is involved in the tissue distribution of certain types of drugs. For example, binding to the nuclei is involved in the tissue distribution of doxorubicin (Terasaki et al., 1984). The $K_p$ values for various alkaloids, such as vincristine and vinblastine, correlate with the tissue tubulin concentration (Wierzda et al., 1987, 1988). Okumura et al. (1978, 1989) and Yoshida et al. (1987, 1989) reported specific common binding sites for basic drugs in the lung, the affinity for these sites being dependent on the lipid solubility of the drugs. Yata et al. (1990) and Nishiura et al. (1986, 1987, 1988) reported the involvement of phospholipids in the tissue distribution of basic drugs. Ishizaki et al. (1998a,b) reported that some basic drugs, such as biperiden and trihexiphenidyl, are mainly distributed in the postnuclear fractions containing the acidic organelles (e.g., lysosomes). Concerning the NQs, HSR-903 has been reported to be taken up by active transport into isolated rat lung cells (Murata et al., 1999), although the contribution of this uptake to their lung distribution has not yet been fully characterized.

In the present study, to clarify the mechanism governing the distribution of GPFX to the lung, the uptake, binding, and efflux after intravenous administration of GPFX were evaluated in rats.
Plasma Protein Binding and Red Blood Cell Distribution. The blood unbound fraction \( (f_{u}) \) was calculated using the following equation:

\[
\frac{C_p}{X_t} = \frac{A}{k_{el} X_t} + B
\]

where \( X_t \) is the amount of unchanged drug in the tissue at time \( t \), \( A \) and \( B \) are the intercept and slope, respectively, of the uptake linear regression. The concentration of unchanged drug \( C_u \) was set at 0.01 μg/ml in each tissue, and the distribution ratio in each fraction was calculated by dividing the radioactivity in each fraction by the total radioactivity in each tissue.

Materials and Methods

Chemicals. GPFX (1.08 MBq/μmol, radiochemical purity 97.1%) was obtained from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). Inulin (9.0 mCi/g, radiochemical purity >97%) was purchased from PerkinElmer Life Sciences (Boston, MA). Unlabeled GPFX was synthesized by Otsuka Pharmaceutical Company (Tokyo, Japan). The following standard phospholipids were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification: \( \alpha \)-phosphatidylcholine (PhC, no. P-7769), \( \alpha \)-phosphatidylglycerol (PhG, no. P-5650), \( \alpha \)-phosphatidylinositol (PhI, no. P-0639), \( \alpha \)-phosphatidylethanolamine (PhE, no. P-7318), and \( \alpha \)-phosphatidyl ethanolamine (PhE, no. P-6386). Rotenone, quinidine, imipramine, Tyrode’s, and propranolol were also obtained from Sigma-Aldrich. All other chemicals used were commercially available and of reagent grade.

Tissue Distribution of GPFX during Steady-State Infusion. Under light ether anesthesia, the femoral artery and vein of three different rats (250–300 g male Sprague-Dawley rats; Charles River Japan Inc., Kanazawa, Japan) were cannulated with polyethylene catheters (PE-50) for blood sampling and GPFX injection, respectively. The rats received a constant infusion of GPFX at a dose of 15 μg/min/kg after a bolus i.v. administration of 5 mg/kg. Rats were kept warm by the heat from an electric bulb throughout the experiment. Blood samples (approximately 200 μl) were collected with a heparinized syringe at 70, 90, 110, and 120 min after starting the infusion, and plasma and was prepared by centrifuging a portion of the blood samples (1800 g, 10 min). At 120 min after dosing, the rats were killed and various tissues were excised immediately. The tissues were stored at −20°C until required for assay. The radioactivity in the plasma and blood was measured using a liquid scintillation counter as follows. Fifty microliters of plasma was transferred to a scintillation vial and sonicated with 1 ml of tissue-solubilizing solution (Nakalai Tesque, Inc., Kyoto, Japan) at 50°C for 30 min. The lysate was neutralized with 1 ml of pH adjustment solution (Nakalai Tesque, Inc.) and then a scintillation cocktail (Hionic-Fluor; Packard BioScience B.V., Groningen, The Netherlands) was added. Fifty microliters of blood was transferred to a scintillation vial and sonicated with 1 ml of tissue-solubilizing solution (Nakalai Tesque, Inc., Kyoto, Japan) at 50°C for 30 min. The lysate was neutralized with 1 ml of pH adjustment solution (Nakalai Tesque, Inc.) and then a scintillation cocktail (Hionic-Fluor; Packard BioScience B.V., Groningen, The Netherlands) was added. The radioactivity of the homogenate was measured using a liquid scintillation spectrometer. The distribution ratio in each fraction was calculated by dividing the radioactivity in each fraction by the total radioactivity in each tissue.

Kinetik for Tissue Uptake of GPFX in Vivo. Under ether anesthesia, the rats were killed by severing the inferior vena cava and aorta and the brain, testis, liver, and lung were excised immediately. The tissues were stored at −20°C until required for assay. The unbound fraction of GPFX in tissue \( (f_u) \) was determined using the following equation:

\[
K_p = \frac{C_p}{AUC}\frac{C_p}{C_p}
\]

where \( K_p \) is the distribution ratio between tissue and plasma, \( C_p \) is the plasma concentration of unchanged drug, and \( AUC\) is the area under the curve of tissue concentration against time. The following equation was fitted to the following equation using a pharmacokinetic software package (WinNonlin; Pharsight, Mountain View, CA):

\[
K_p = \frac{AUC}{C_p}
\]

The plot of \( K_p \) versus \( AUC/C_p \) is designated as the integration plot. The time profile for \( C_p \) was fitted to the following equation using a pharmacokinetic software package (WinNonlin; Pharsight, Mountain View, CA):

\[
C_p = A e^{-\alpha t} + B e^{-\beta t}
\]
Using eqs. 2 and 4, the time profile of the data for $X_t$ was fitted to the following equation to estimate $CL_{\text{upake,b}}^{A}$, $CL_{\text{upake,b}}^{B}$, and $V_{cl}$:

$$X_t = \frac{CL_{\text{upake,b}}^{A}}{\alpha - k_{diss}} (e^{-(\alpha - k_{diss})t} - e^{-k_{diss}t}) + \frac{CL_{\text{upake,b}}^{B}}{\beta - k_{diss}} (e^{-(\beta - k_{diss})t} - e^{-k_{diss}t}) + V_{cl} e^{-k_{diss}t}$$

(5)

where $V_{cl}$ represents the distribution volume in which the GPFX concentration equilibrates instantaneously with that in plasma. This $CL_{\text{upake,b}}$ is based on the plasma concentration, and the $CL_{\text{upake,b}}$ for the blood concentration is calculated by dividing the $CL_{\text{upake,b}}$ by $R_B$.

**Single-Pass Lung Uptake Index.** Under ether anesthesia, GPFX and insulin as an extracellular marker were administered to different rats via the femoral vein at a dose of 5 and 1.9 mg/kg/1.6 ml saline, respectively. The rats were killed at designated times over 5 to 120 s and the whole lung was excised immediately. Three to six rats were used at each time point. Lung samples were homogenized with two volumes of saline, and portions of the homogenate were dissolved. The radioactivity of the homogenate was measured using a liquid scintillation counter.

**Phospholipid Binding of GPFX in Vitro.** The binding of GPFX to phospholipids in vitro was performed by partitioning between pH 7.4 buffer (0.25 M sucrose-0.1 M Tris-HCl buffer) and n-hexane (Yata et al., 1990). Briefly, 2 ml of buffer solution containing 1 μM GPFX in the absence or presence of weakly basic drugs such as quinidine, propranolol, and imipramine, which bind to PhS (Nishiura et al., 1986, 1987, 1988; Yata et al., 1990), was shaken with 2 ml of n-hexane solution containing the individual standard phospholipids (8 μg/ml) at 37°C for 2 h. The mixture was then centrifuged at 1,800 g for 10 min and the radioactivity in the separated aqueous and organic phases was measured using a liquid scintillation counter. The concentrations of the weakly basic drugs were set at 10, 100, and 1,000 μM because the dissociation constants of the three compounds to PhS are 0.179 to 4.20 μM (Yata et al., 1990).

**Cell Culture.** K1/R97K-pssB, CHO-K1, CDT-1, and PSA-3 cells were kindly donated by Drs. M. Nishijima and O. Kuge (National Institute of Infectious Diseases, Tokyo, Japan). PSA-3 cells, a mutant of CHO-K1 cells, lacks the ability to synthesize PhS (Kuge et al., 1986). K1/R97K-pssB transfected with the R97K mutant of PhS synthase II exhibits an approximately 4-fold higher PhS biosynthesis rate than CHO-K1 cells, resulting in a 1.6-fold higher PhS level than that in CHO-K1 cells (Kuge et al., 1999). CDT-1 cells are transformants of PSA-3 cells with a pssA cDNA, which encodes PhS synthase I (Kuge et al., 1991). K1/R97K-pssB, CHO-K1 and CDT-1 cells were cultured in Ham’s F-12 medium supplemented with 10% (v/v) newborn calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, followed by incubation at 37°C under a 5% CO2 atmosphere and 100% humidity. PSA-3 cells were maintained under the same culture conditions except that the medium was supplemented with 44 μg/ml PhS liposomes. K1/R97K-pssB, CHO-K1, CDT-1, and PSA-3 cells were seeded on a 12-well microplate (BD Biosciences, Franklin Lakes, NJ) at approximately 3 × 10^4 cells/3.2 cm². After 2 to 3 days, the medium was replaced with a fresh medium without antibiotics or PhS, whereas the fraction of PSA-3 was maintained with fresh medium containing PhS. After an additional 2 days, these cells were used in the association studies.

**Association to PhS Synthase Transformants.** The culture medium was removed by aspiration, and the monolayers were washed with Hank’s balanced salt solution at 37°C. Association of 10 μM GPFX was initiated by adding 0.05 ml of prewarmed ligand solution to the cell medium after preincubation at 37°C for 3 min. At a designated time, the association was terminated by removing the cell medium immediately. Then, 0.05 ml of medium was transferred to a scintillation vial, the cells were washed twice with 2 ml of ice-cold PBS, and solubilized in 2 N NaOH. After neutralization with 6 N HCl, a scintillation cocktail (ACS-II; Amersham Biosciences UK, Ltd.) was added and the radioactivity was measured. Ligand association was given as the distribution volume, determined as the amount of ligand associated with the cells divided by the medium concentration. Protein concentration in the solubilized cells was determined by the method of Lowry et al. (1951), using a protein assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard. To examine the effect of weakly basic drugs, preincubation was performed with 30 μM rotenone, to exclude any effect of active transport systems, in the absence or presence of inhibitors (quinidine, imipramine, and propranolol) at 37°C for 5 min.

**Quantitation of Phospholipid Content.** Phospholipids were extracted by the method of Folch et al. (1957), with a slight modification. Briefly, the sample obtained from each tissue homogenate (1.2 g) and cell suspension (1.2 ml) was homogenized with 4.5 ml of chloroform/methanol (1, v/v) mixture, and chloroform (1.5 ml) and water (or PBS[−]) (1.5 ml) were added to the homogenates. After centrifugation, the organic layer was transferred to a test tube and evaporated to dryness under nitrogen gas. The phospholipids were separated by two-dimensional thin layer chromatography on 0.5-mm-thick Silica Gel 60 plates (Merck, Darmstadt, Germany) using chloroform/methanol/acetic acid (65:25:10, v/v/v) as the first solvent and chloroform/methanol/formic acid (65:25:10, v/v/v) as the second solvent. After thin layer chromatography, the spots were made visible with iodine vapor. Each spot was scraped off, and the phosphate content was determined chemically by assaying the inorganic phosphorus by the method described by Trudinger (1970).

**Statistical Analysis.** The statistical analyses were performed using Dunnett’s test (SAS software, version 6.12) for the association to PhS synthase transformants and for the phospholipid binding of GPFX in vitro. $P<0.01$ and $P<0.05$ were regarded as significant.

**RESULTS**

**Tissue Distribution of GPFX during Steady-State Infusion.** Plasma concentrations of GPFX at 70, 90, 110, and 120 min during the constant infusion of GPFX at a dose of 15 μg/min/kg were 1.03 ± 0.06, 1.00 ± 0.10, 0.979 ± 0.095, and 0.865 ± 0.088 μg/ml (mean ± S.E.), respectively. The blood concentration at 120 min was 1.30 ± 0.12 μg/ml (mean ± S.E.), and the $R_B$ at 120 min was 1.51 ± 0.03 (mean ± S.E.).

Steady-state tissue distribution of GPFX was examined at 120 min after the start of infusion. The $K_p$ in the lung (15.1 ml/g) was higher than that in other tissues, followed by that in the pancreas, kidney, and spleen (Table 1). The $f_{(p)}$ in the brain, testis, liver, and lung were 0.0523, 0.0571, 0.0334, and 0.0616, respectively, suggesting that most of GPFX exists in bound form in these tissues. The tissue-to-blood unbound concentration ratio ($q$) was calculated as $K_p/\rho f_{(p)} f_{(b)}$. The $q$ in the lung was 2.34, whereas the $q$ in the brain (0.0315) and testis (0.108) was much lower than unity.

**Subcellular Distribution.** The subcellular distribution of GPFX was examined (Table 2). More than 50% of GPFX was localized in the nuclear and membrane fractions in the lung, heart, stomach, muscle, small intestine, large intestine, and brain (Table 2). The

### Table 1

<table>
<thead>
<tr>
<th>Tissues</th>
<th>$K_p$&lt;sup&gt;a&lt;/sup&gt;</th>
<th>$k_{el}$</th>
<th>ml/g</th>
<th>min&lt;sup&gt;−1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>15.1 ± 0.4</td>
<td>8.55</td>
<td>0.0667</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>11.2 ± 0.9</td>
<td>0.421</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>13.6 ± 0.5</td>
<td>0.171</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>11.1 ± 0.8</td>
<td>0.119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymus</td>
<td>6.34 ± 0.17</td>
<td>0.259</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>3.87 ± 0.20</td>
<td>1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>4.52 ± 0.49</td>
<td>0.162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>2.64 ± 0.15</td>
<td>0.0946</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>7.66 ± 0.83</td>
<td>0.489</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>3.67 ± 0.75</td>
<td>0.384</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>0.753 ± 0.091</td>
<td>0.478</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.239 ± 0.003</td>
<td>8.98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Tissue-to-blood concentration ratio $K_p$ was calculated by dividing the tissue concentration (micrograms per gram of tissue) by the plasma concentration (micrograms per milliliter of plasma).

<sup>b</sup>Elimination rate constant in each tissue compartment obtained by dividing blood flow rate by $K_p$. 

---

Downloaded from dmd.aspetjournals.org at ASPET Journals on October 19, 2017
fraction of GPFX distributed to the lung was in the following order: nuclear and membrane fractions (60.3%) > lysosomal and mitochondrial fractions (22.7%) > microsomal fraction (12.9%) > cytosol fraction (4.1%) (Table 2). In contrast, more than 70% of GPFX was recovered in lysosomal and mitochondrial fractions in the spleen and thymus (Table 2). Most of the GPFX (>87%) was distributed to the organelle fractions (nuclear and membrane fractions, lysosomal and mitochondrial fractions, and microsomal fraction) in all tissues examined (Table 2).

Kinetics for Tissue Uptake of GPFX in Vivo. The time profile of GPFX concentrations in the plasma and various tissues was subjected to kinetic analysis after intravenous bolus administration (Fig. 2). The CLuptake,b in the lung (2.86 ml/min/g), kidney (4.25 ml/min/g), and heart (1.86 ml/min/g) was higher than that in other tissues, including the brain (0.0234 ml/min/g). The dose (5 mg/kg) used in this analysis was the same as that used in our previous analysis (Sasabe et al., 1997). The plasma concentrations (1.38–3.18 µg/ml, corresponding to 3.49–8.03 µM) observed at 1 to 10 min postdosing were not far from that for concentrations (1.38 ml/min/g), adrenals (2.38 ml/min/g), and heart (1.86 ml/min/g) were not far from that for concentrations (1.38 ml/min/g), adrenals (2.38 ml/min/g), and heart (1.86 ml/min/g) were not far from that for concentrations (1.38 ml/min/g), adrenals (2.38 ml/min/g), and heart (1.86 ml/min/g) were not far from that for concentrations (1.38 ml/min/g), adrenals (2.38 ml/min/g), and heart (1.86 ml/min/g) were not far from that for concentrations (1.38 ml/min/g), adrenals (2.38 ml/min/g), and heart (1.86 ml/min/g).

TABLE 2
Subcellular distribution of GPFX in various tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Nuclear and Membrane Fractions</th>
<th>Lysosomal and Mitochondrial Fractions</th>
<th>Microsomal Fraction</th>
<th>Cytosol Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>60.3 ± 1.4</td>
<td>22.7 ± 0.5</td>
<td>12.9 ± 1.0</td>
<td>4.11 ± 0.50</td>
</tr>
<tr>
<td>Liver</td>
<td>47.1 ± 0.9</td>
<td>28.6 ± 1.7</td>
<td>15.6 ± 0.8</td>
<td>8.67 ± 0.36</td>
</tr>
<tr>
<td>Kidney</td>
<td>38.1 ± 0.5</td>
<td>39.2 ± 1.0</td>
<td>10.5 ± 0.7</td>
<td>12.2 ± 0.7</td>
</tr>
<tr>
<td>Pancreas</td>
<td>25.0 ± 0.5</td>
<td>32.5 ± 2.0</td>
<td>30.7 ± 0.9</td>
<td>11.8 ± 0.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>14.0 ± 2.0</td>
<td>71.2 ± 0.8</td>
<td>11.5 ± 0.7</td>
<td>3.38 ± 0.90</td>
</tr>
<tr>
<td>Thymus</td>
<td>20.7 ± 3.9</td>
<td>71.0 ± 4.4</td>
<td>6.11 ± 0.37</td>
<td>2.25 ± 0.56</td>
</tr>
<tr>
<td>Heart</td>
<td>77.1 ± 2.4</td>
<td>15.6 ± 2.1</td>
<td>4.81 ± 0.73</td>
<td>2.47 ± 0.36</td>
</tr>
<tr>
<td>Stomach</td>
<td>55.8 ± 0.8</td>
<td>21.6 ± 3.0</td>
<td>14.3 ± 1.3</td>
<td>8.25 ± 1.03</td>
</tr>
<tr>
<td>Muscle</td>
<td>87.4 ± 0.8</td>
<td>7.04 ± 0.62</td>
<td>2.60 ± 0.26</td>
<td>2.97 ± 0.11</td>
</tr>
<tr>
<td>Small intestine</td>
<td>66.0 ± 1.7</td>
<td>18.5 ± 1.1</td>
<td>9.94 ± 0.96</td>
<td>5.54 ± 1.42</td>
</tr>
<tr>
<td>Large intestine</td>
<td>70.1 ± 4.5</td>
<td>17.4 ± 3.1</td>
<td>8.91 ± 1.62</td>
<td>3.54 ± 0.52</td>
</tr>
<tr>
<td>Testis</td>
<td>21.0 ± 0.9</td>
<td>47.5 ± 2.0</td>
<td>26.8 ± 1.3</td>
<td>4.67 ± 0.75</td>
</tr>
<tr>
<td>Brain</td>
<td>59.3 ± 1.5</td>
<td>27.9 ± 0.3</td>
<td>8.68 ± 0.44</td>
<td>4.08 ± 2.08</td>
</tr>
</tbody>
</table>

Time profiles of GPFX concentration in plasma and tissues were determined after its bolus injection (5 mg/kg). The solid lines are the fitted curves based on eq. 5. Each plot and bar represents the mean ± S.E. of three rats.

Fig. 2. Integration plot representing the GPFX uptake by each tissue.

Estimation of $k_{41}$ from Tissues. The $k_{41}$ in the tissues was calculated by dividing the blood flow rate by $K_p$ (Table 1), assuming each tissue to be a single compartment. The $k_{41}$ in all tissues was higher than the elimination rate constant calculated from the plasma concentration profile ($\beta$ of 0.00480 min$^{-1}$) obtained by Nakajima et al. (2000).

Phospholipid Binding of GPFX in Vitro. The in vitro binding of GPFX to phospholipids was examined (Fig. 5A). The mean value for the GPFX binding to PhS was 2.67 ml/mg lipid, whereas the binding to other phospholipids was, at most, 0.05 to 0.285 ml/mg lipid, i.e., GPFX preferentially binds to PhS (Fig. 5A). The binding of GPFX to PhS was reduced in the presence of weakly basic drugs, such as quinidine, imipramine, and propranolol (Fig. 6).

Association to PhS Synthase Transformants. The association of GPFX with PhS synthase transformants of CHO-K1 cells, which have different PhS contents, was also examined (Fig. 5B). The mean value for the GPFX association between 10 and 20 min with K1/R97K-PhS (43.3 µM protein) was approximately 1.5 times as high as that with PSA-3[PhS(−)] (28.4 µM protein), which has the lowest content (Fig. 5B). Quinidine and imipramine reduced the association of GPFX with K1/R97K-PhS in a concentration-dependent manner (Fig. 6).

Relationship between Tissue-to-Blood Unbound Concentration Ratio ($K_{p,un}$) and PhS Content in Vivo and PhS Synthase Transformants in Vitro. A correlation was observed between the $K_{p,un}$ and the PhS content of rat tissues (Fig. 7A). In this plot, the $K_{p,un}$ in brain and testis were lower than the correlation line, whereas that in pancreas was higher than the correlation line (Fig. 7A). The amount of
GPFX in each subcellular fraction in the lung also exhibits a linear relationship with the PhS content of each fraction (Fig. 7B).

Because the PhS contents shown in Fig. 7, A and B, were the values reported previously (Nishiura et al., 1988; Yata et al., 1990), the four organs (lung, liver, kidney, and heart) showing the different $K_{p,u}$ were selected and PhS content was determined in the present study. The PhS content of the lung, which shows highest $K_{p,u}$, was higher than the other organs (Fig. 7C). In addition, when both the $K_{p,u}$ in vivo and the association in transformants were expressed as per milligram of protein, all the values similarly depend on PhS contents (Fig. 7C).

Discussion

GPFX is highly distributed to several organs, including the lung (Akiyama et al., 1995a,b; Nakajima et al., 2000) (Table 1), although the determining factors mainly involved in such high tissue distribution remain to be identified. In the present study, we attempted to identify the mechanism(s) involved in GPFX distribution to the lung.

Sasabe et al. (1997) reported that Na$^+$-independent and carrier-mediated active transport system contributes to the hepatic uptake of GPFX. Therefore, tissue uptake system(s) might be present at least in the liver and contribute to the tissue distribution of GPFX. In the present study, the CL$_{\text{uptake,b}}$ values, analyzed by integration plot analysis, in the lung, kidney, liver, adrenals, and heart were higher than those in other tissues (Fig. 3). However, this CL$_{\text{uptake,b}}$ except in the lung, was close to the blood flow rate in each tissue (Fig. 3), suggesting that GPFX is efficiently taken up by tissues in the all organs examined. Thus, higher distribution of GPFX to the lung cannot be explained by the difference in its uptake process. The extraction by the lung in the single-pass lung uptake index was an almost linear behavior at doses of 0.006 to 15 mg/kg (Fig. 4B). In that study, the GPFX concentration in the administered solution was 26 mM at 15 mg/kg. If we assume that the dosing solution is diluted with circulating blood (~80 ml/kg), the GPFX passing through the lung should be at least 520 μM. Thus, the uptake of GPFX in the lung has a low affinity and is not saturated within the micromolar GPFX range.

Other hypotheses accounting for the higher distribution of GPFX to the lung include its specific binding to tissue. In various tissues GPFX was mainly recovered in the nuclear and membrane fractions (Table 2), which contain more phospholipids as membrane components than other fractions. Nishiura et al. (1988) reported that the content of PhS is much higher in the nuclear and membrane fractions than in others in the lung. In addition, compared with the other organs, the content of PhS is higher in the lung (Yata et al., 1990). Therefore, we first examined the relationship between the PhS content and the distribution of GPFX. A linear relationship can be observed between the $K_{p,u}$ of GPFX and the PhS content of each tissue (Fig. 7A) and between the amount of GPFX in subcellular fractions and their PhS content (Fig. 7B). The tissue distribution of doxorubicin is governed by the DNA content of each tissue.
The association of 1 μM GPFX with PhS was determined after a 2-h incubation, whereas that with K1/R97K-psbB cells was measured after a 20-min incubation. Each GPFX association was normalized in terms of the extracellular concentration. Each column represents the mean ± S.E. of three to nine (PhS) or three to 21 samples (K1/R97K-psbB cells). *p < 0.05; **p < 0.01 (significantly different from control).

(Terasaki et al., 1984), but only a minimal correlation was observed between the $K_{pu}$ of GPFX and the DNA content (data not shown). In addition, GPFX preferentially binds to PhS compared with other phospholipids (Fig. 5A). Yata et al. (1990) and Nishiura et al. (1986, 1987, 1988) have also suggested that PhS governs the distribution of weakly basic drugs, such as quinidine, propranolol, and imipramine. In fact, GPFX binding to PhS was inhibited by these three drugs (Fig. 6), although they are basic, whereas GPFX is zwitterionic. Thus, these results suggest the importance of PhS as a determining factor for GPFX distribution.

To further evaluate the contribution of PhS governing the distribution of GPFX, its association was examined by using CHO-K1 mutants that lack the ability to synthesize PhS or are transfected with PhS synthase, resulting in different levels of PhS expression (Kuge et al., 1986, 1991, 1999). The higher PhS content of the cells was tended to result in a higher GPFX association (Fig. 5B). In addition, GPFX association with such a PhS synthase transformant was also inhibited by quinidine, propranolol, and imipramine (Fig. 6). These results suggest that PhS is an important factor governing the association of GPFX with these cell lines. The GPFX association with the PhS synthase transformants in vitro and that to the various tissues in vivo can be shown against the PhS content in a same plot (Fig. 7C). This result is compatible with the hypothesis that PhS is a determining factor for the tissue distribution of GPFX in vivo. However, the linear relationship found in the cell lines may have a y-intercept (Fig. 7C). Furthermore, the weakly basic drugs showed a weaker inhibition of the cellular association in comparison with the GPFX binding to PhS. These results suggest that further studies are needed to clarify the other factor(s) that are also involved in the association of GPFX with these cell lines.

It is unlikely that the difference in the elimination kinetics from the tissues can account for the higher distribution characteristics of GPFX to the lung: The $k_{el}$ value in all the tissues, including the lung, exceeded the $\beta$ value (Table 1), suggesting that the efflux of GPFX from these tissues is not the rate-limiting step in its elimination from tissues. This result is compatible with the finding by Nakajima et al. (2000) that the disappearance curve for the GPFX concentration in most tissues, including the lung, is almost parallel to that in plasma after intravenous bolus injection. In addition, GPFX once trapped by the lung rapidly disappeared in the present study (Fig. 4A), suggesting that the GPFX associated with the lung undergoes ready efflux into the circulation. Note that we cannot discuss differences in the absolute values for intrinsic flux of GPFX among the various tissues based on $CL_{uptake,b}$ or $k_u$ value, because both values are blood flow-dependent parameters. Nevertheless, as discussed above, because both $CL_{uptake,b}$ and $k_u$ are very rapid, the specific distribution of GPFX to the lung cannot be explained either by the uptake or efflux processes. On the other hand, the $q$ values in the brain and testis were much lower than unity and the $K_{pu}$ in these two tissues was at the lower end of the correlation line when plotted against the PhS content (Fig. 7A). However, the method used to determine tissue unbound fraction in the present study is based on the assumption that binding in tissues in vivo is equivalent to that observed in tissue homogenate in vitro. Therefore, the absolute value for such $q$ values should be used carefully in any discussion. However, Tamaia et al. (2000) proposed the involvement of multidrug-resistance protein 1a as an efflux transport system for GPFX in the brain, and these results are compatible with the hypothesis that an active efflux system exists in these tissues.

In conclusion, PhS is the major determining factor for the tissue

![Fig. 6. Effect of weakly basic drugs on GPFX association with PhS (□) and K1/R97K-psbB cells (■).](image)

![Fig. 7. Relationship between PhS content and distribution of GPFX.](image)
distribution of GPFX. This is the first report that the inter-organ variation in the distribution of a zwitterion-type compound can be accounted for, at least in part, by its affinity for PhS and the PhS content of each tissue. However, the data obtained in the present study still provide only indirect evidence for the involvement of PhS in the tissue distribution of GPFX, and further investigation is needed to yield a final conclusion.

Acknowledgments. We thank Drs. Osamu Kuge and Masahiro Nishijima (National Institute of Infectious Diseases, Tokyo, Japan) for donating several cell lines and for valuable discussion. We also thank Dr. Teruo Murakami (Hiroshima University, Hiroshima, Japan) for fruitful discussions and helpful advice about the determination of the PhS content of tissues.

References


