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

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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 (Kp) 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 Kp 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 (NQs1), 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) was higher than that of lomefloxacin and ofloxacin (1460 and 1540 ml/kg, respectively) (Okezaki et al., 1988). Okezaki et al. (1988) proposed common distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood concentration ratio (Kp) and the plasma unbound fraction (fup) of several NQs, except for these two compounds, whereas the Kp of GPFX and HSR-903 in the lung was far removed from this correlation line. This observation suggests the existence of some specific mechanism(s) for the distribution of these two NQs in the lung. The efficacy of an antibiotic is generally thought to depend both on its plasma concentration and minimum inhibitory concentrations for likely pathogens. However, the tissue concentrations at the site of infection may be more relevant for the effects of antibiotics (Baldwin et al., 1990). Consequently, it is important to clarify the distribution mechanism of GPFX in 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 doxorubicin (Terasaki et al., 1984). The Kp values for vinca 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.
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: \( \alpha \)-phosphatidyl\( \delta \)-serine (PhS, no. P-7769), \( \alpha \)-phosphatidyl\( \alpha \)-glycerol (PhG, no. P-5650), \( \alpha \)-phosphatidylinositol (Phl, no. P-0639), \( \alpha \)-phosphatidylcholine (PhC, no. P-7318), and \( \alpha \)-phosphatidylethanolamine (PhE, no. P-6386). Rotenone, quinidine, imipramine, 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 a polyethylene catheter (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 were then collected from the femoral artery and vein of three different rats (250–300 g, 90, 110, and 120 min after starting the infusion, and plasma was prepared from plasma and blood samples. The radioactivities of the tissue homogenates and its filtrates were measured using a liquid scintillation counter. The distribution ratio in each fraction was calculated by dividing the radioactivity in each fraction by the total radioactivity in each tissue.

Intracellular Binding. 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 (\( f_{\text{unb}} \)) was obtained by extrapolating to binding for 100% homogeneate from that of 2, 5, and 10% tissue homogenate, determined by the ultrafiltration method described by Sasabe et al. (1997). Initially, each 10% homogenate of the brain, testis, liver, and lung (0.880, 2.77, 31.6, and 56.0 μM, respectively) were set as to be close to those seen during steady-state infusion. After incubation at 37°C for 5 min, homogenate samples with GPFX were placed in YM-30 tubes, pretreated with blank filtrate. Then, the homogenate was centrifuged (1,800g, 10 min) to give a filtrate containing the unbound drug. The radioactivities of the tissues homogenates and its filtrates were measured using a liquid scintillation counter.

Kinetics for Tissue Uptake of GPFX in Vivo. Under ether anesthesia, GPFX was administered to the rats, weighing approximately 200 g, via the femoral vein at a dose of 5 mg/kg (0.80 MBq/kg). Blood samples were then collected from the femoral artery and the right atrium at designated times over 1, 2, 3, 5, 10, and 30 min with a heparinized syringe. The rats were killed at 1, 2, 3, 5, 10, and 30 min by severing the inferior vena cava and aorta after the final blood sample had been collected and the following tissues were sampled: lung, brain, liver, kidney, thymus, heart, spleen, adrenals, stomach, small intestine, large intestine, and testis. The lung, liver, kidney, brain, stomach, small intestine, and large intestine were homogenized with 2 volumes of saline, and portions of the homogenate and other tissues were weighed and dissolved. The radioactivity of the homogenate was measured using a liquid scintillation counter (LSC-1050; Aloka, Tokyo, Japan). The counting efficiency was corrected by the channels ratio method using an external standard. The following equation represents the mass balance of GPFX in the tissues:

\[
\frac{dX_t}{dt} = \text{CL}_{\text{uptake}}C_p - k_{\text{efflux}}X_t
\]

where \( X_t \) is the amount of unchanged drug in the tissue at time \( t \), \( \text{CL}_{\text{uptake}} \) is the tissue uptake clearance, \( C_p \) is the plasma concentration of unchanged drug and \( k_{\text{efflux}} \) is the efflux rate constant from the tissue. In this case, the plasma sampled at the entrance to the tissues should be measured, i.e., plasma was collected from the right atrium for the lung and from the femoral artery for the other tissues. During this short period, the efflux process can be ignored, and the integration and subsequent normalization by \( C_p \) of the following equation yields:

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

The plot of \( K_p \) versus \( \text{AUC}/C_p \) 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):

\[
C_p = A e^{r_{\text{in}}} + B e^{r_{\text{in}}} P^0
\]
Using eqs. 2 and 4, the time profile of the data for Xr was fitted to the following equation to estimate $CL_{\text{p}}$, $V_{d}$, and $V_{r}$:

$$X_t = \frac{CL_{\text{p}} \cdot A}{\alpha - k_{d}} \left( e^{-\alpha \cdot t} - e^{-\beta \cdot t} \right) + \frac{CL_{\text{p}} \cdot B}{\beta - k_{d}} \left( e^{-\alpha \cdot t} - e^{-\beta \cdot t} \right) + V_{r} e^{-\beta \cdot t}$$

(5)

where $V_{r}$ represents the distribution volume in which the GPFX concentration equilibrates instantaneously with that in plasma. This $CL_{\text{p}}$ is based on the plasma concentration, and the ratio of $CL_{\text{p}}$ to the blood concentration is calculated by dividing the $CL_{\text{p}}$ by $R_{b}$.

**Single-Pass Lung Uptake Index.** Under anaesthesia, GPFX and inulin 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 was placed in the absence or presence of weakly basic drugs such as quinidine, propranolol, and imipramine, which bind to PhS (Nishihara 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 100, 1,000, and 1,000 μM because the dissociation constants of the three compounds to PhP are 0.179 to 4.20 μM (Yata et al., 1990).

**Cell Culture.** K1/R97K-PhS, 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 PhP (Kuge et al., 1986). K1/R97K-PhS transformants were selected using the R97K mutant of PhS synthase II to exhibit 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 PhP cDNA, which encodes PhS synthase I (Kuge et al., 1991). K1/R97K-PhS, 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 PhP liposomes. K1/R97K-PhS, CHO-K1, CDT-1, and PSA-3 cells were seeded on a 12-well microplate (BD Biosciences, Franklin Lakes, NJ) at approximately $3 \times 10^4$ cells/cm². After 2 to 3 days, the medium was replaced with a fresh medium without antibiotics or PhP, whereas the fraction of PSA-3 was maintained with fresh medium containing PhP. After an additional 2 days, these cells were used in the association studies.

**Association to PhP 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 5 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, 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 3 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 g) was homogenized with 4.5 ml of chloroform/methanol (1:2, v/v) mixture, containing PhS. After an additional 2 days, these cells were used in the association studies.

**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>
<thead>
<tr>
<th>Tissues</th>
<th>$K^b_{\text{tissue}}$</th>
<th>$K^b_{\text{plasma}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>15.1 ± 0.4</td>
<td>8.55</td>
</tr>
<tr>
<td>Liver</td>
<td>8.55 ± 0.17</td>
<td>0.0667</td>
</tr>
<tr>
<td>Kidney</td>
<td>11.2 ± 0.9</td>
<td>0.421</td>
</tr>
<tr>
<td>Pancreas</td>
<td>13.6 ± 0.5</td>
<td>0.171</td>
</tr>
<tr>
<td>Spleen</td>
<td>11.1 ± 0.8</td>
<td>0.119</td>
</tr>
<tr>
<td>Thymus</td>
<td>6.34 ± 0.17</td>
<td>0.259</td>
</tr>
<tr>
<td>Heart</td>
<td>3.87 ± 0.2</td>
<td>1.41</td>
</tr>
<tr>
<td>Stomach</td>
<td>4.52 ± 0.49</td>
<td>0.162</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.64 ± 0.15</td>
<td>0.0946</td>
</tr>
<tr>
<td>Small intestine</td>
<td>7.66 ± 0.83</td>
<td>0.489</td>
</tr>
<tr>
<td>Large intestine</td>
<td>3.67 ± 0.75</td>
<td>0.384</td>
</tr>
<tr>
<td>Tests</td>
<td>0.753 ± 0.091</td>
<td>0.478</td>
</tr>
<tr>
<td>Brain</td>
<td>0.239 ± 0.003</td>
<td>8.98</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.E. of three different rats.*
steady-state infusion. As shown in Fig. 3, the CLuptake,b in most observed at 1 to 10 min postdosing were not far from that for that used in our previous analysis (Sasabe et al., 1997). The plasma ml/min/g). The dose (5 mg/kg) used in this analysis was the same as was higher than that in other tissues, including the brain (0.0234 (1.48 ml/min/g), adrenals (2.38 ml/min/g), and heart (1.86 ml/min/g) 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), liver (1.48 ml/min/g), adrenals (2.38 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 steady-state infusion. As shown in Fig. 3, the CLuptake,b in most tissues except lung was close to the blood flow rate in each tissue. Accordingly, it was concluded that GPFX is efficiently taken up by the different tissues. Because blood flow rate can be affected by the condition of anesthesia, for most organs the information cited herein was obtained in rats under light ether anesthesia. Nevertheless, we cannot rule out the possibility that such anesthesia may differentially affect the blood flow rate in each organ. The fitting in integration plot analysis does not seem to be appropriate, especially for the liver and kidney. This is at least partially due to the rapid decrease after the uptake phase in these organs (Fig. 2). Considering that active transport systems are reported to be involved in basolateral and/or apical membranes of the liver and kidney (Sasabe et al., 1997, 1998b; Matsuo et al., 1998; T. Suzuki, Y. Kato, H. Sasabe, M. Itose, G. Miyamoto, and Y. Sugiyama, unpublished data), nonlinear behavior may occur in GPFX distribution to these organs. Further study is needed to clarify the exact mechanism.

Single-Pass Lung Uptake Index. To evaluate the single-pass extraction ratio of GPFX in the lung, we measured its association with the lung after intravenous bolus injection over a short time period before the GPFX that enters the circulation again passes through the lung (Fig. 4A). Compared with an extracellular marker, such as inulin, a higher amount (~10% of dose) of GPFX was associated with the lung at 5 s after dosing (Fig. 4A). The lung concentration of GPFX fell over time (Fig. 4A). Thus, GPFX associated with the lung is dissociated by dilution in the plasma flow. To evaluate the saturable distribution of GPFX in the lung, this distribution of GPFX was examined at doses of 0.006 to 15 mg/kg (Fig. 4B). At 5 and 30 s after dosing, the extraction by the lung was approximately 10% and 4%, respectively, showing minimal nonlinear behavior (Fig. 4B).

Estimation of k2 from Tissues. The k2 in the tissues was calculated by dividing the blood flow rate by Kp (Table 1), assuming each tissue to be a single compartment. The k2 in all tissues was higher than the elimination rate constant calculated from the plasma concentration profile (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 GPFX binding to PhS was 2.67 ml/mg lipid, whereas the binding to other phospholipids was, at most, 0.050 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-PhSS (43.3 μM protein), which has the highest PhS content, 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-PhSS in a concentration-dependent manner (Fig. 6).

Relationship between Tissue-to-Blood Unbound Concentration Ratio (Kp,u) and PhS Content in Vivo and PhS Synthase Transformants in Vitro. A correlation was observed between the Kp,u and the PhS content of rat tissues (Fig. 7A). In this plot, the Kp,u in brain and tests were lower than the correlation line, whereas that in pancreas was higher than the correlation line (Fig. 7A). The amount of

### 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>6.67 ± 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>

Each value represents the mean ± S.E. of three different rats.
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 PhS contents were selected and PhS content was determined in the present study. The PhS content of the lung, which shows highest Kp,u, was higher than the other organs (Fig. 7C). In addition, when both the Kp,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, except in the lung, kidney, liver, adrenals, and heart were higher than those in other tissues (Fig. 3). However, this CLuptake,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 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 Kp,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.

**Fig. 3. Comparison of the CLuptake,b and blood flow rate in various tissues.**

The CLuptake,b was estimated by fitting both plasma and tissue concentration profiles to eq. 5. Blood flow rates for all organs except the pancreas, thymus, and testis were determined under light anesthesia (Stanek et al., 1988), and those for the pancreas, thymus, and testis were determined under a conscious condition (Darlington et al., 1995). The solid line has a slope of unity. Lung (a), kidney (b), adrenals (c), heart (d), liver (e), spleen (f), pancreas (g), large intestine (h), small intestine (i), stomach (j), thymus (k), testis (l), and brain (m).

**Fig. 4. Time profile (A) and dose dependence (B) of the single-pass lung uptake index of GPFX after its bolus intravenous administration.**

Both GPFX (●) at 5 mg/kg and inulin (○) were intravenously administered and GPFX associated with the lung was determined (A). The injected dose of GPFX was changed at 0.006 to 15 mg/kg and GPFX association was measured at 5 s (●) and 30 (○) s after injection (B). The data in B represent the intracellular amount of GPFX, which was obtained by subtracting the amount of inulin from that of GPFX. Each plot and vertical bar represents the mean ± S.E. of three to six rats.

**Fig. 5. In vitro phospholipid binding of GPFX (A) and time profile of GPFX association in PhS synthase transformants of CHO-K1 cells (B).**

A, binding of 1 μM GPFX was determined using the n-hexane/buffer partition system at 37°C. Each column represents the mean ± S.E. of three samples. B, association of GPFX was measured by incubating each cell line (K1/R97K-pssB, ●; CHO-K1, ○; CDT-1, ▲; PSA-3[PhS(+)], △; and PSA-3[PhS(-)], ■) with 10 μM GPFX at 37°C. PhS content in K1/R97K-pssB, CHO-K1, CDT-1, PSA-3[PhS(+)], and PSA-3[PhS(-)] was 7.79, 5.38, 5.66, 5.37, and 3.47 μg/mg protein, respectively. The cellular association was normalized in terms of the extracellular concentration. Each plot and vertical bar represent the mean ± S.E. of three samples. PhG, α,α-phosphatidylglycerol; PhC, 1,α-phosphatidylcholine; PhI, 1,α-phosphatidylinositol; PhE, α,α-phosphatidylethanolamine.
The importance of PhS as a determining factor for the tissue distribution of GPFX is evident from the results presented. The association of 1 μM GPFX with PhS was determined after a 2-h incubation, whereas that with K1/R97K-pssB cells was measured after a 20-min incubation. Each GPFX association was normalized in terms of the extracellular concentration. Where a y-intercept (Fig. 7C) is plotted against the PhS content in each tissue, the correlation line was at the lower end of the PhS content (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 factors that are also involved in the association of GPFX with these cell lines.

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 factors 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_s value in all the tissues, including the lung, exceeded the 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_s value, because both values are blood flow-dependent parameters. Nevertheless, as discussed above, because both CL_{uptake,b} and k_s 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_p values 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, Tamai et al. (2000) proposed the involvement of multidrug-resistance protein 1 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...
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.

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