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 \( K_p \) 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\(^1\)), 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 ofloxacin (1460 and 1540 ml/kg, respectively) (Okezaki et al., 1998). Okezaki et al. (1998) 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 distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood distribution characteristics to the lung for NQs other than GPFX and HSR-903 because a correlation was observed between the tissue-to-blood 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 \( K_p \) and the plasma unbound fraction \( f_u \) of several NQs, except for these two compounds, whereas the \( K_p \) 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 \( K_p \) 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.

\(^1\) Abbreviations used are: NQ, new quinolone antibacterial agents; GPFX, grepafloxacin; PhS, phosphatidylserine; AUC, area under the curve; HSR-903, (S)-5-amino-7-(7-amino-5-azaspiro[2.4]hept-5-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid methanesulfonate.

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Plasma Protein Binding and Red Blood Cell Distribution. The blood
unbound fraction ($f_{un}$, 0.80) was obtained by $f_{un}$ (0.60) (Akiyama et al., 1995b) divided by the blood-to-plasma concentration ratio ($K_p$, 1.51), which was obtained in the present study.

Subcellular Fractionation. Rat tissues were fractionated according to
the procedures described by Roberti et al. (1962). Briefly, the sampled tissues,
whole or partial, were added to 9 volumes ice-cold 0.32 M sucrose and
homogenized. All subsequent steps were performed at 4°C. To obtain
the nuclear and membrane fractions, lysosomal and mitochondrial fractions and
microsomal fraction, the homogenates were sequentially centrifuged at 1,000g
for 10 min, 13,200g for 20 min, and 100,000g for 60 min, respectively.
The final supernatant was taken as the cytosol fraction. The radioactivity of the
channel homogenates and subcellular fractions was 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 ($f_{un}$) was obtained by extrap-
olating to binding for 100% homogenate 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
was prepared in 0.32 M sucrose. These homogenates were then diluted with
0.32 M sucrose to give 5 and 2% homogenates. The total concentrations of
GPFX in the homogenates of the brain, testis, liver, and lung (0.880, 2.77, 31.6,
and 56.0 µM, respectively) were set so 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 filtrates. Then,
the homogenate was centrifuged (1,800g, 10 min) to give a filtrate containing
the unbound drug. The radioactivities of the tissue homogenates and its filtrates
were measured using a liquid scintillation counter.

Kinetis 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 channel ratio method using an external standard. The following
equation represents the mass balance of GPFX in the tissues:

$$\frac{dX_t}{dt} = CI_{uptake,p}C_p - k_{efflux}X_t$$

where $X_t$ is the amount of unchanged drug in the tissue at time $t$, $CI_{uptake,p}$ is
the tissue uptake clearance, $C_p$ is the plasma concentration of unchanged drug
and $k_{efflux}$ 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:

$$K_p = \frac{CI_{uptake,p}}{AUC}$$

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):

$$C_p = A \cdot e^{-r t} + B \cdot e^{-\beta t}$$

Materials and Methods

Chemicals. GPFX (1.08 MBq/μmol, radiochemical purity 97.1%) was obtained
from Amersham Biosciences UK Ltd. (Little Chalfont, Buckingham-
shire, UK). Inulin (9.0 mCi/g, radiochemical purity >97%) was purchased
from PerkinElmer Life Sciences (Boston, MA). Unlabeled GPFX was synthe-
sized by Otsuka Pharmaceutical Company (Tokyo, Japan). The following
standard phospholipids were obtained from Sigma-Aldrich (St. Louis, MO)
and used without further purification: α-phosphatidyl-L-serine (PS, no.
P-7769), α-phosphatidyl-α-glycerol (PGh, no. P-5650), α-phosphatidyl-
inositol (PI, no. P-0639), α-phosphatidylcholine (PC, no. P-7318), and
α-phosphatidylethanolamine (PE, no. P-6386). Rotenone, quinidine, imip-
}
Using eqs. 2 and 4, the time profile of the data for $X_t$ was fitted to the following equation to estimate $C_L^{\text{uptake,k}}$ and $V_E$:

$$X_t = \frac{C_L^{\text{uptake,k}} A}{\alpha - k_{\text{diss}}} \left(e^{-k_{\text{diss}} t} - e^{-\alpha t}\right) + \frac{C_L^{\text{uptake,b}} B}{B - k_{\text{diss}}} \left(e^{-k_{\text{diss}} t} - e^{-B t}\right) + V_E e^{-\beta t}$$

(5)

where $V_E$ represents the distribution volume in which the GPFX concentration equilibrates instantaneously with that in plasma. This $C_L^{\text{uptake,k}}$ is based on the plasma concentration, and the $C_L^{\text{uptake,b}}$ for the blood concentration is calculated by dividing the $C_L^{\text{uptake,k}}$ by $R_b$.

Single-Pass Lung Uptake Index. Under ether anesthesia, GPFX and inulin as an extracapillary 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 (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 1,800g 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 components to PhS is 0.179 to 4.2 μM (Yata et al., 1990).

Cell Culture. K1/R97K-ppS, 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-ppS transfected with the R97K mutant of PhS synthase II express 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-ppS, CHO-K1 and CDT-1 cells were cultured in Ham’s F-12 medium supplemented with 10% (v/v) newborn calf serum and 44 μg/ml PhS liposomes. K1/R97K-ppS, CHO-K1, CDT-1, and PSA-3 cells were seeded on a 12-well microplate (BD Biosciences, Franklin Lakes, NJ) at approximately 3 × 10⁴ cells/3.8 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 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 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 computer 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 $R_{ki}$ 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 $K_{pi}$ 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 $f_{pv}$ 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 $K_{pi}/f_{pv,pi}$. 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>$K_{pi}$</th>
<th>$k_{pi}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/g</td>
<td>min⁻¹</td>
</tr>
<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 ± 0.20</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>Testis</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>

$^{a}$Tissue-to-blood concentration ratio. $K_{pi}$ was calculated by dividing the tissue concentration (micrograms per gram of tissue) by the plasma concentration (micrograms per milliliter of plasma).

$^{b}$Elimination rate constant in each tissue compartment obtained by dividing blood flow rate by $V_{pi}$.  

\[ \text{Cl}_{\text{uptake,k}} = \frac{C_L^{\text{uptake,k}} A}{\alpha - k_{\text{diss}}} \left(e^{-k_{\text{diss}} t} - e^{-\alpha t}\right) + \frac{C_L^{\text{uptake,b}} B}{B - k_{\text{diss}}} \left(e^{-k_{\text{diss}} t} - e^{-B t}\right) + V_E e^{-\beta t} \]
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 \( \text{CL}_{\text{uptake,b}} \) 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), adrenals (2.38 ml/min/g), and heart (1.86 ml/min/g). The plasma concentrations varied in a concentration-dependent manner (Fig. 6). The solid lines are the fitted curves based on eq. 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-PhS-B (43.3 µl/mg protein), which has the highest PhS content, was approximately 1.5 times as high as that with PSA-3[PhS(−)] (28.4 µl/mg protein), which has the lowest content (Fig. 5B). Quinidine and imipramine reduced the association of GPFX with K1/R97K-PhS-B in a concentration-dependent manner (Fig. 6).

Relationship between Tissue-to-Blood Unbound Concentration Ratio \( K_{p,u} \) and PhS Content in Vivo and PhS Synthase Transformants in Vitro. A correlation was observed between the \( K_{p,u} \) and the PhS content of rat tissues (Fig. 7A). In this plot, the \( K_{p,u} \) 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
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 \( C_{L,uptake,b} \) values, analyzed by integration plot analysis, in the lung, kidney, liver, adrenals, and heart were higher than those in other tissue (Fig. 3). However, this \( C_{L,uptake,b} \) 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 \( \mu \)M. 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-pssB 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-pssB cells). * P < 0.05; ** P < 0.01 (significantly different from control).

(Terasaki et al., 1984), but only a minimal correlation was observed between the $K_{p,u}$ 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 $x$ 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 $k_{el}$ value, because both values are blood flow-dependent parameters. Nevertheless, as discussed above, because both $CL_{uptake,b}$ and $k_{el}$ 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 $K_{p,u}$ 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 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...
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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References


