
Regular Article

**Distribution Characteristics of Grepafloxacin, a Fluoroquinolone Antibiotic, in Lung Epithelial Lining Fluid and Alveolar Macrophage**

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**Summary:** The purpose of this study was to investigate the distribution of Grepafloxacin (GPFX), a new quinolone antimicrobial agent, in the lung epithelial lining fluid (ELF) and the alveolar macrophage (AM) in rats, which are potential infection sites in respiratory tract infections. We also aimed to clarify the mechanism governing the transferability of GPFX into the alveolar compartment from a kinetic point of view. The AUC ratios of ELF/plasma and AM/plasma after the oral administration of GPFX were 5.69 ± 1.00 and 352 ± 57, respectively, which were several-fold greater than those of ciprofloxacin (CPFX). Pharmacokinetic analyses of time profiles of GPFX concentrations in ELF and AM revealed that the influx clearance from plasma to ELF across the alveolar barrier is 5-fold greater than the efflux clearance from ELF. In addition, the permeability of GPFX across the cultured AM cell membrane was 7-fold and 11-fold greater than that of levofloxacin (LVFX) and CPFX, respectively. The extent of intracellular binding to AM cells (expressed as a constant (α)) was the greatest for GPFX, followed by CPFX and LVFX. There was a significant correlation between the α value and the partitioning to the immobilized artificial membrane (IAM) column, which consists of phospholipid residues covalently bound to silica. These results suggest that GPFX is highly distributed in ELF and AM, and that the high transferability of GPFX into ELF may be attributable to the existence of asymmetrical transport across the alveolar barrier. In addition, it was suggested that both rapid permeability across the AM cell membrane and avid binding to the membrane phospholipids may be responsible for the high accumulation of GPFX in AM.

**Key words:** alveolar barrier; alveolar macrophage; antimicrobial agent; immobilized artificial membrane column; lung epithelial lining fluid; membrane transport; phospholipid binding

**Introduction**

Grepafloxacin (GPFX) was developed as a new quinolone antibacterial agent, which exerts greater antimicrobial activity against a variety of Gram-positive and Gram-negative bacteria.1) It has been reported that GPFX is highly distributed in tissues except for the testis and the brain.2) The lung has the highest tissue-to-blood distribution ratio (Kp) of all tissues. Recently, Suzuki et al. reported that the binding of GPFX to phospholipid membrane is one of factors that determine the high distribution of GPFX in the lung.3) Such a unique tissue distribution feature of GPFX is consistent with the in vivo efficacy of GPFX against respiratory tract infections.4,5) On the other hand, it is known that the epithelial lining fluid (ELF) which lies in pools on the inside surface of the alveolus, and the alveolar macrophage (AM), which usually reside in the ELF, are potential infection sites in respiratory tract infections, including pneumonia.6) Therefore, the antibiotic concentration at these infection sites would be more relevant than in the rest of the lung for understanding the efficacy of GPFX in respiratory infections. Sampling of ELF and AM cells in the alveolus using the bronchoalveolar lavage (BAL) method made it possible to measure GPFX concentrations both in ELF and AM.7) However, our knowledge about factors governing the transferability and the distribution of GPFX into both ELF and AM is still veiled.

In general, the distribution of antibiotics from plasma to ELF has been considered to be limited by the...
presence of the alveolar barrier, which consists of three layers, including the lung capillary endothelial cells, the connective tissue and the alveolar epithelial cells. In addition, the distribution of antibiotics from ELF to AM would be governed by the permeability across the phospholipid membrane of AM cells and the binding to the constituents in AM cells. Therefore, the distribution of GPFX both in ELF and AM should be evaluated on the basis of the anatomical and physiological nature of the alveolus.

The purpose of this study was to investigate the distribution of GPFX both in ELF and AM in rats, and to clarify factors governing the distribution from a kinetic point of view.

Materials and Methods

Materials: Grepafloxacin hydrochloride (GPFX HCl), ciprofloxacin hydrochloride (CPFX HCl), levofloxacin (LVFX) and OPC-17203 (internal standard for HPLC analysis) were supplied from Otsuka Pharmaceutical Company (Tokyo, Japan) (Fig. 1). Quinidine sulfate and propranolol hydrochloride were purchased from Wako Pure Chemical Co. (Osaka, Japan). 3H-Water (0.037 MBq/μmol) was obtained from PerkinElmer Life Sciences (Boston, MA). Immobilized artificial membrane (IAM) column (4.6 mm × 15 cm) was obtained from Regis Technologies, Inc. (Morton Grove, IL). All other reagents were of analytical grade.

Animals: Adult male Wistar rats weighing 200 g to 250 g were purchased from Japan SLC (Shizuoka, Japan). They were housed in a room with controlled temperature and humidity, and had free access to food and water. They were fasted for 20 h before the experiments. All animal studies were performed according to the Guidelines for the Care and Use of Laboratory Animals that was approved by the Committee of Ethics of Animal Experimentation of Hokkaido College of Pharmacy.

In vivo study: Rats were anesthetized i.p. with pentobarbital sodium at a dose of 50 mg/kg. Either GPFX or CPFX was orally administered to rats at a dose of 24 μmol/kg. At the designated time, blood was collected from the superior vena cava, and then immediately sacrificed by exsanguinations from the descending aorta. The trachea was then cannulated and the lungs were lavaged three times with 5 mL of an ice-cold phosphate buffer saline solution (pH = 7.4) containing 1 mM EDTA. The bronchoalveolar lavage (BAL) fluid was immediately centrifuged at 4°C (650 g for 10 min) to separate AM cells from the ELF. In order to calculate the antibiotic concentrations in ELF, the apparent volume of ELF was estimated by the method using urea, an endogenous marker of ELF dilution. The mean value estimated in the present study was 395 μL/240 g rat. The intracellular volume in AM cells was determined by a velocity-gradient centrifugation technique using 3H-water and was estimated to have a mean value of 4.2 μL/mg protein.

Preparation of the cultured AM cells: The AM
cells were isolated from rats by the BAL method as described above. The cells were plated at 5 × 10⁵ cells per 2 cm² well in a RPMI-1640 medium (Gibco BRL, Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum (Gibco BRL), 50 μM 2-mercaptoethanol and 10 μg/mL gentamicin. The plate was incubated for 18 h at 37°C in 95% air + 5% CO₂. The non-adherent cells were removed by washing three times with the RPMI-1640 medium. The viability of cells was routinely checked by the trypan blue (0.1% (w/v)) exclusion test.

**In vitro uptake and efflux study:** The AM cells were washed twice with a SFM medium (Gibco BRL), supplemented with 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), and then incubated at 37°C with the SFM medium containing either GPFX, CPFX, LVFX, quinidine or propranolol to give a final concentration in the medium of 500 μM in each case. At the time designated (0 to 120 min), the medium was removed by aspiration and washed three times with ice-cold PBS. The cells were solubilized for HPLC analysis by the addition of 0.5 ml 1 N NaOH. Protein concentration in the solubilized cells was measured with Coomassie Protein Assay Reagent (Pierce Chemical Company, Rockford, IL). The stability of quinolone antibiotics in 1 N NaOH solution was checked. No appreciable degradation was observed.

Efflux of quinolone antibiotics from AM cells was examined after the cells were incubated for 30 min at 37°C in a SFM medium containing either GPFX, CPFX or LVFX (each 500 μM). The cells were washed twice with SFM medium and then incubated in the drug-free medium at 37°C. The amount of a drug remaining in the cells was quantified.

**IAM chromatographic conditions:** The extent of binding of GPFX, CPFX, LVFX, quinidine and propranolol to the phospholipid membrane was evaluated using an HPLC system with an IAM column (IAM.PC.MG, 15 cm × 4.6 mm I.D.) in which the phosphatidylcholine (PhC) is covalently bound to silica via the propylamine group. An aliquot of the drugs, dissolved in PBS (each 100 μM), was injected onto the IAM column and the effluent was monitored at the UV wavelength of 215 nm. The mobile phase was composed of 0 to 30% acetoniitrile in a 0.01 M phosphate buffer saline solution (PBS, pH = 7.4). The capacity factor (KIAM) was calculated as KIAM = tR − t₀) / t₀, where t₀ and tR are the retention times for the test compounds, and a solvent which is not retained by the column to indicate the column dead time, respectively. The KIAM value was extrapolated to that of a 100% aqueous eluent.

**Analytical Method**

The antibiotic concentrations in the plasma, ELF and AM were determined by HPLC. For GPFX, 0.2 mL of biological samples was mixed well with 0.1 mL of 1 μM OPC-17203 (an internal standard) and 1 mL of 1 M PBS (pH = 6.86), and then extracted with 5 mL chloroform by shaking vigorously for 10 min. After centrifugation at 3,000 rpm for 10 min, 4 mL of organic layer was removed and evaporated to dryness at 37°C under reduced pressure. The residue was reconstituted in 1 mL of mobile phase (see below) and an aliquot was subject- ed to HPLC. In case of CPFX and other compounds, biological samples were mixed well with an equal volume of methanol for deprotenization and then, the supernatant was subjected to HPLC. The HPLC system consisted of LC-10AD pump (Shimadzu, Kyoto, Japan), RF-10AXL fluorescence detector (Shimadzu), C-TO-6A column oven (Shimadzu), C-R6A data processing integrator (Shimadzu) and a STR ODS-II column (4.0 mm × 250 mm, Shinwa Chemical, Kyoto, Japan). The mobile phase, composed of 0.01% phosphoric acid and 20 mM sodium sulfate: acetoniitrile (3:1), was used for GPFX, CPFX and LVFX. The separation was carried out at a flow rate of 0.8 mL/min at 40°C and the eluate from the column was monitored by fluorescence detection (excitation wavelength (λE,) of 325 nm and emission wavelength (λEm,) of 448 nm). An alternative mobile phase of 0.05 M PBS (pH = 3.0): acetoniitrile: methanol (55:20:25) was used for quinidine and propranolol. The optimal fluorescence condition was as follows: λE, 327 nm and λEm, 382 nm for quinidine; λE, 290 nm and λEm, 340 nm for propranolol.

**Pharmacokinetic Model**

We employed the hybrid pharmacokinetic model of Fig. 2 to describe the transferability of GPFX into the alveolar compartment, across the alveolar barrier. The alveolar compartment consists of ELF and AM. The ELF compartment serves as a bridge connecting the plasma compartment with the AM compartment, since AM cells usually reside in ELF. The alveolar barrier between plasma and ELF was assumed to be a homogenous single membrane. For this model, it was assumed that lung blood flow was much greater than transport rates of GPFX across the alveolar barrier, and that the distribution in the alveolar compartment does not affect the plasma concentration. Therefore, the mass exchange between the plasma compartment and the alveolar compartment was not considered for the analysis of the plasma concentrations of GPFX.

**Intestine**

\[
\frac{dX_r}{dt} = -k_a X_r
\]  

(1)

**Central compartment**

\[
V_i \frac{dC_p}{dt} = k_{10} X_r + k_{12} X_l - (k_{10} + k_{12}) V_i C_p
\]  

(2)
Fig. 2. A hybrid pharmacokinetic model to describe the distribution of GPFX in ELF and AM.

Peripheral compartment
\[ \frac{dX_2}{dt} = k_{12}V_1C_p - k_{21}X_2 \]  

ELF compartment
\[ V_{ELF} \frac{dC_{ELF}}{dt} = CL_1C_p + CL_4f_TCAM - (CL_2 + CL_3)C_{ELF} \]

AM compartment
\[ V_{AM} \frac{dC_{AM}}{dt} = CL_3C_{ELF} - CL_4f_TCAM \]

where \( X_a \) and \( X_2 \) are the drug amounts in the intestine and the peripheral compartment, respectively. \( C_p \), \( C_{ELF} \) and \( C_{AM} \) are the concentrations in the plasma, ELF and AM cells, respectively. \( V_1 \), \( V_{ELF} \) and \( V_{AM} \) are the volumes of the central-, ELF- and AM compartments, respectively. The unbound fraction of antibiotics in AM cells is denoted as \( f_T \).

In the first stage, plasma concentrations were fitted to eqs. (1) to (3) by nonlinear regression analysis using MULTI(RUNGE)\(^{10}\) to estimate the parameters \( k_{10}, k_{12}, k_{21} \) and \( V_1 \). In the second stage, the plasma profile \( (C_p) \) generated by these parameters was used as a forcing function to describe antibiotic concentration in ELF and AM cells. The concentrations of GPFX in ELF and AM compartments were fitted to eqs. (4) and (5) by nonlinear regression analysis to estimate \( CL_3 \) and \( f_T \).

The uptake and efflux of quinoline antibiotics in the \textit{in vitro} cultured AM cells were kinetically analyzed according to the same relationship as Eq. (5).

\[ V_{AM} \frac{dC_{AM}}{dt} = CL_{3,vitro}C_{medium} - CL_{4,vitro}f_TCAM \]

where \( CL_{3,vitro} \) and \( CL_{4,vitro} \) are the uptake and efflux clearances across the \textit{in vitro} cultured AM cell membrane, and \( C_{medium} \) represents the antibiotic concentration in the incubation medium. The experimental results shown in Figs. 3(A) and (B) were simultaneously fitted to the integral form of eq. (6) using the program MULTI\(^{10}\) to estimate \( CL_{3,vitro} \) and \( f_TCAM \).

If it is assumed that any active transport system does not involved in the transport of quinolone antibiotics across the AM cell membrane, \( CL_{3,vitro} \) would be equal to \( CL_{4,vitro} \). Then the following relationship can be given.
Fig. 3. Time profiles for uptake (A) and efflux (B) of quinolone antibiotics in the cultured AM cells. (A) The cultured AM cells were incubated with a medium containing 500 μM GPFX (●), 500 μM CPFX (○) and 500 μM LVFX (▲) at 37°C for 1 to 120 min. (B) After the AM cells were equilibrated with a medium containing GPFX, CPFX or LVFX (each 500 mM) at 37°C for 30 min, the cells were washed twice with a SFM medium, and then, the cells were incubated in the drug-free medium at 37°C for 0 to 30 min. The antibiotic concentration in AM cells was estimated by dividing the cell-medium ratio (mmol/mg protein) by the cell volume (4.2 μL/mg protein). Each point represents the mean ± S.E. of three to six determinations.

Table 1. AUC ratios for GPFX and CPFX in rats

<table>
<thead>
<tr>
<th>AUC ratioa</th>
<th>GPFX</th>
<th>CPFX</th>
</tr>
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<tbody>
<tr>
<td>ELF/plasma</td>
<td>5.69 ± 1.00</td>
<td>0.477 ± 0.075</td>
</tr>
<tr>
<td>AM/plasma</td>
<td>352 ± 57</td>
<td>15.1 ± 3.0</td>
</tr>
<tr>
<td>AM/ELF</td>
<td>62.0 ± 12.0</td>
<td>31.6 ± 7.1</td>
</tr>
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</table>

*Each AUC ratio is calculated by the area under curve, 16 h after the oral administration of GPFX and 6 h after the administration of CPFX to the rats. The AM concentration of CPFX 6 h after the administration was dealt with as 0 μM. Values are the mean ± S.D.

\[
\frac{C_{AM}}{C_{medium}} = 1 + \alpha = \frac{1}{T_f} \tag{7}
\]

where \( \alpha \) is the binding constant in the AM cell.

Results

In vivo distribution of GPFX and CPFX in ELF and AM: The concentration-time profiles of GPFX and CPFX in plasma, ELF and AM after the oral administration to rats are illustrated in Figs. 4(A) and (B). For GPFX, the plasma concentration increased rapidly with time and reached the maximal level after 1 h, and then, decreased bi-exponentially. The concentrations of GPFX, both in ELF and AM, were higher than that in the plasma at all times after the administration. As shown in Table 1, the AUC ratio of ELF to plasma (ELF/plasma) for GPFX was 5.69 ± 1.00, which was approximately 12-fold greater than that for CPFX. In addition, the AM/plasma ratio for GPFX (352 ± 57) was 23-fold greater than that for CPFX, suggesting that GPFX is highly concentrated in ELF and AM compartments.

In order to evaluate the distribution of GPFX in ELF and AM quantitatively, the concentration profiles shown in Fig. 4(A) were analyzed by the hybrid pharmacokinetic model of Fig. 2. The solid lines drawn on Fig. 4(C) represent the computer-generated simulation curves using the pharmacokinetic parameters estimated by the present model (Table 2), which were in fairly good agreement with the observed data. The value of \( CL_1 \) was 5-fold greater than \( CL_2 \), indicating the asymmetrical transport of GPFX across the alveolar barrier.

In vitro uptake of GPFX by the cultured AM cells: The results of uptake and efflux of quinolone antibiotics in cultured AM cells are shown in Figs. 3(A) and (B). The uptake of GPFX by AM cells was rapid and concentrative. The ratio of the concentration of GPFX in AM cells to the concentration in the medium (\( \frac{C_{AM}}{C_{medium}} \)), 60 min after the start of incubation, reached 55.4 ± 1.4, which was 4.5-fold and 6.5-fold greater than the ratio for CPFX (11.9 ± 0.4) and LVFX (8.48 ± 0.26), respectively. The efflux of GPFX, CPFX and LVFX from AM cells was rapid and the percentage remaining in AM cells was less than 1% for GPFX, 24% for CPFX and 7% for LVFX, 30 min after the start of incubation in a drug-free medium. As shown in Table 3, the estimated uptake clearance (\( CL_{uptake} \)) for GPFX was 7-fold and 11-fold higher than that for LVFX and CPFX, respectively. Furthermore, the intracellular accumulation of GPFX in AM cells was significantly decreased; by 94% with 10 mM quinidine and by 62% with 10 mM propranolol.

Relationship between in vitro binding constant (\( \alpha \)) and \( K_{IAM} \): The intracellular binding constant (\( \alpha \)) was estimated from the observed \( \frac{C_{AM}}{C_{medium}} \) ratio accord-
Fig. 4. (A) Concentration-time profiles of GPFX in plasma (○), ELF (●) and AM (▲). (B) Concentration-time profiles of CPFX in plasma (○), ELF (●) and AM (▲). Either GPFX or CPFX was orally administered to rats at a dose of 24 μmol/kg. Plasma samples were collected from the superior vena cava, and ELF and AM samples were collected by the bronchoalveolar lavage (BAL) method. (C) Concentration-time profiles of GPFX in plasma (○), ELF (●) and AM (▲). Solid lines represent the computer-generated simulation curves using the parameters estimated by the hybrid pharmacokinetic model. Each point represents the mean ± S.E. of three to six rats.

Table 2. Pharmacokinetic parameters of GPFX for 240-g rats

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Value</th>
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<tbody>
<tr>
<td>Dose (nmol)</td>
<td>5760</td>
</tr>
<tr>
<td>(k_a (h^{-1}))</td>
<td>0.330 ± 0.021</td>
</tr>
<tr>
<td>(k_{12} (h^{-1}))</td>
<td>1.40 ± 0.03</td>
</tr>
<tr>
<td>(V_1 (mL))</td>
<td>1174 ± 12</td>
</tr>
<tr>
<td>(CL_1 (mL/h))</td>
<td>51.0 ± 3.2</td>
</tr>
<tr>
<td>(CL_2 (mL/h))</td>
<td>10.1 ± 2.8</td>
</tr>
<tr>
<td>(CL_3 (mL/h))</td>
<td>0.0762 ± 0.036</td>
</tr>
<tr>
<td>(f/CL_1 (mL/h))</td>
<td>0.00112 ± 0.000066</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters for GPFX were estimated using nonlinear least squares regression program MULTI (RUNGE). Physiological parameters of \(V_{ELF}\) and \(V_{AM}\) were estimated to be 0.395 mL/240-g rats and 0.000723 mL/240-g rats, respectively, in the separate experiments. Values are the mean ± calculated S.D.

Discussion

The present study demonstrates that GPFX is highly distributed both in ELF and AM, which are potential infection sites in respiratory tract infections. Recently, Suzuki et al. showed that approximately 10% of the intravenously injected dose of GPFX was trapped by the lung tissue of rats, and that this efficient distribution was due to the avid binding to the phospholipids, mainly phosphatidyserine (PhS), in the nucleus and plasma membrane fraction of the lung. As shown in Table 1, the AUC ratio of ELF to plasma of GPFX was 5.7, which was much larger than unity. One possible mechanism that accounts for this result is that GPFX binds avidly to the phospholipids in ELF, because it is reported that various phospholipids exist in the ELF. However, the unbound fraction of GPFX in plasma and
ELF was 0.593 (see reference 2) and 0.78 ± 1.2 (measured in the present study), respectively. This result indicates that the free-ligand hypothesis that the unbound concentration in plasma is equal to the unbound concentration in the ELF is not established for the distribution of GPFX in the ELF. An alternative mechanism that interprets the high concentration of GPFX in ELF would be the asymmetrical transport of GPFX between the plasma and the ELF, across the alveolar barrier.

Kinetic analyses of the GPFX concentrations in ELF and AM using the present hybrid pharmacokinetic model showed that influx clearance from the plasma to the ELF (CL1 = 51.0 mL/h/240-g rats) is 5-fold greater than the efflux clearance from ELF to plasma (10.1 mL/h/240-g rats). In addition, the CL1 value was much smaller than the lung plasma flow (1410 mL/h/240-g rats), indicating that the distribution of GPFX into the ELF is limited by the permeability across the alveolar barrier. The alveolar barrier consists of three layers, the capillary lumen, the connective tissue and the alveolar epithelial cells. The alveolar epithelial cells that are tightly connected by numerous zonulae occludens had been considered to provide a significant barrier between plasma and ELF. Therefore, the present kinetic analyses demonstrated the existence of asymmetry in the transport of GPFX across the alveolar barrier. Interestingly, Campbell et al. demonstrated that MDR/mdr-1 P-glycoprotein was expressed in alveolar epithelial (AE) type I epithelium within human and rat lung tissue, and plays a significant role in the efflux transport in AE type I cells. Therefore, GPFX may undergo the efficient efflux from the alveolus to the ELF via this ATP-dependent primary active transporter. This possibility is supported by the results that P-gp mediates the efflux transport of GPFX in the intestine and the blood-brain barrier.

The AUC ratio of AM/ELF for GPFX (352 ± 57) after the oral administration was 23-fold greater than that for CPFX (15.1 ± 3.0), suggesting that GPFX is highly concentrated into the cultured AM cells. In order to elucidate the mechanism for the concentration distribution of GPFX, firstly, the uptake and efflux of three antibiotics (GPFX, CPFX and LVFX) in the AM cells were kinetically analyzed using the in vitro model (eq. (6)). The model consists of uptake and efflux rate processes, which were denoted by the clearance dimensions (CLU,iv and CLU,iv), and the binding to the constituents in the AM cell, which is represented by the binding constant (α). As shown in Figure 3, the observed concentration in AM cells for these quinolone antibiotics were in good agreement with the predicted concentration from the model. The ratio of CAM/Cmedium of GPFX and CPFX (45.6 and 11.7, respectively), which are calculated by using Eq. (7) and the estimated α values, was comparable with the AUC ratio of AM/ELF in vivo (62.0 and 31.6), indicating that the results of the functional analysis with the in vitro cultured AM cells reflect the distribution mechanism of quinolone antibiotics in vivo.

In addition, the CLU,iv values significantly correlated with the KIAM values (Table 3). The IAMS consists of PhC residues covalently bound to silica and form the monolayer of PhC residues in the column. Pidgeon et al. have shown that partitioning of a compound into IAMS comprises both hydrophobic and hydrophilic as well as electrostatic forces, all of which are involved in the process of solute partitioning into cell membrane. In fact, it has been reported that there is a good correlation between the membrane permeability and KIAM value of various drugs. Therefore, fairly good correlation between CLU,iv and KIAM (Table 3) indicate that the permeability of quinolone antibiotics across the AM cell membrane could be predicted from the partition to IAMS. This result also suggests that the transport of quinolone antibiotics across the AM cell membrane may be mediated by the lipid-mediated simple diffusion process. Furthermore, the binding constant (α) was calculated from the CAM/Cmedium ratio at equilibrium state. The values of α for quinolone antibiotics as well as quinidine and propranolol correlated well with the respective KIAM values, suggesting that the binding of quinolone antibiotics in AM cells is involved in the binding to phospholipids. This is consistent with the results that have shown a good correlation between the extent of binding of GPFX and PhS contents. On the other hand, PhC has been reported to comprise 30.5% of phospholipids contents in the rat AM cells. Therefore, the binding of quinolone antibiotics in the AM cells should be taken into consideration of the quality and quantity of phospholipid components.

In conclusion, it was found that GPFX is highly distributed in ELF and AM, possible sites for pulmonary infections. Kinetic analysis revealed the existence of asymmetrical transport of GPFX across the alveolar barrier.

**Table 3.** Kinetic parameters for membrane transport and the intracellular binding in the cultured AM cells

<table>
<thead>
<tr>
<th></th>
<th>CLU,iv (µL/min/mg protein)</th>
<th>α</th>
<th>KIAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPFX</td>
<td>28.5 ± 4.3</td>
<td>44.6 ± 1.43</td>
<td>43.6</td>
</tr>
<tr>
<td>CPFX</td>
<td>2.44 ± 0.20</td>
<td>10.7 ± 0.53</td>
<td>7.24</td>
</tr>
<tr>
<td>LVFX</td>
<td>4.11 ± 0.44</td>
<td>6.9 ± 0.12</td>
<td>9.77</td>
</tr>
<tr>
<td>Quinidine</td>
<td>n.d.</td>
<td>50.8 ± 1.9</td>
<td>75.8</td>
</tr>
<tr>
<td>Propranolol</td>
<td>n.d.</td>
<td>48.1 ± 1.3</td>
<td>97.7</td>
</tr>
</tbody>
</table>

CLU,iv was estimated by nonlinear least-squares regression analysis of the integral form of Eq. (6) using program MULTI. The α values were estimated from Eq. (7). Values are the mean ± calculated S.D. Values are the mean ± S.E. of three determinations. n.d.: not determined.
barrier, that is, the influx clearance from plasma to ELF is greater than the efflux clearance in the opposite direction. In addition, it was suggested that both rapid permeability across the AM cell membrane and avid binding to the membrane phospholipids may be responsible for the high accumulation of GPPX in AM.

References