Active Uptake of Ulifloxacin from Plasma to Lung That Controls Its Concentration in Epithelial Lining Fluid

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Ulifloxacin is a new quinolone antibiotic and it is effective against pneumonia. We previously showed that it is highly distributed into the epithelial lining fluid (ELF) in rats, which might be resulting from certain active transport. The transport system has not been, however, clarified yet. In this study, we attempted to characterize the distribution mechanism of ulifloxacin into the rat ELF. We also aimed to elucidate the feature of ulifloxacin uptake in rat lung and human lung adenocarcinoma cells (Calu-3). In infusion studies, ulifloxacin concentrations in the ELF and lung were higher than that in the plasma, and decreased by co-administration of sparfloxacin or azithromycin to the level of plasma concentration. Integration plot analysis showed that active uptake of ulifloxacin from the plasma to lung was also inhibited by sparfloxacin and azithromycin. In in vitro studies, time and temperature-dependent uptake into Calu-3 was observed, and this uptake was inhibited by sparfloxacin and azithromycin as observed in the rat lung. Additionally sparfloxacin inhibited the active uptake of ulifloxacin into Calu-3 more strongly than levofloxacin as observed in the rat lung. These results suggest that active uptake of ulifloxacin from the plasma to lung controls the distribution of ulifloxacin from the plasma to ELF, and that the uptake of ulifloxacin into Calu-3 has partly similar characteristics to its uptake into the rat lung. We believe our study will contribute to much better understanding of antibiotic efficacy against pathogens which cause pneumonia.

Key words epithelial lining fluid; bronchoalveolar lavage method; ulifloxacin; rat; uptake; human lung adenocarcinoma cell

Epithelial lining fluid (ELF) exists in the inside surface of the alveolus.1) Staphylococcus aureus and Streptococcus pneumoniae, which cause pneumonia, are located in the ELF and lead to tissue damages.2) Antibiotic concentrations in the ELF have therefore stronger relationship with the efficacy of antibiotics than those in the whole lung tissues.1)

Plasma concentrations of various antibiotics that are effective against pathogens responsible for pneumonia are not equal to ELF concentrations of such antibiotics,3—5 suggesting that there might be a certain active transport system of antibiotics penetration from the plasma to ELF.

Human lung adenocarcinoma cell (Calu-3), which is derived from human airway epithelial cells, is believed to have serous cell properties,6—9) and produces more secretory components compared to other similar epithelial cells, e.g. 16HBE cells.10) Additionally Calu-3 has cystic fibrosis transmembrane conductance regulator (CFTR)8,11) and P-glycoprotein12) as observed in alveolar epithelial cells.13) Therefore Calu-3 has some functions in common with alveolar epithelial cells. Although usefulness of Calu-3 has not been clarified yet as an in vitro model for estimation of in vivo active uptake from plasma to the lung, the estimation of uptake in Calu-3 will give us some significant information.

Ulifloxacin is a new quinolone antibiotic and effective against pneumonia because it has strong antimicrobial activities against Gram-positive and -negative bacteria. It shows reasonable efficacy against infections in various organs including the lung.14) We previously showed that penetration of ulifloxacin into the rat ELF might be induced by a certain active transport.15) The transport system has, however, not been clarified yet.

In this study, we have attempted to characterize the distribution mechanism of ulifloxacin into the rat ELF. We also aimed to elucidate the feature of ulifloxacin uptake in rat lung and human lung adenocarcinoma cells (Calu-3).

Sparfloxacin16) and levofloxacin17) are the quinolones with high and low penetration into the ELF, respectively. Azithromycin is a macrolide which highly penetrates into the ELF.18) We used these compounds as co-administered compounds to investigate the specificity of quinolone transport mechanism from plasma to the ELF and lung.

MATERIALS AND METHODS

Chemicals Ulifloxacin was synthesized in Nippon Shinyaku Co., Ltd. (Kyoto, Japan). Sparfloxacin and levofloxacin were purchased from LKT Laboratories, Inc. (St. Paul, MN, U.S.A.), respectively. Azithromycin was purchased from Sigma-Aldrich Japan (Tokyo, Japan). As a specific inhibitor of organic anion transporter (OAT), p-aminobenzoinic acid (PAB) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). As a specific inhibitor of organic anion-transporting polypeptide (OATP), ouabain (OUA) was purchased from Sigma-Aldrich Japan (Tokyo, Japan). As specific inhibitors of organic cation transporter (OCT), cimetidine (CMT) and tetraethylammonium (TEA) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). As internal standards, enoxacin and ofloxacine were purchased from Sigma-Aldrich Japan (Tokyo, Japan). All the other chemicals were of analytical grade.

Animal Male Sprague-Dawley (SD) rats, aged 8 weeks, were supplied by Charles River Japan (Tokyo, Japan). They were housed and handled according to the “Principles of Laboratory Animal Care” (NIH publication #85-23, revised in 1985) and the “Guidance for the Care and Use of Laboratory Animals” (Pharmaceutical Research Center, Meiji Seika

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Kaisha Ltd.). The rats were kept in the temperature- and light-controlled environment with the standard food and tap water provided ad libitum.

**In Vivo Experiment. 1. Penetration of Ulifloxacin into the Lung and ELF of Rats** Under pentobarbital anesthesia, the animals were cannulated at the femoral artery and vein for facilitation of blood sampling and intravenous administration. Following intravenous bolus administration of ulifloxacin (7.9 mg/kg), 2.5 mg/h/kg of ulifloxacin was infused to the rats to maintain a steady state plasma concentration of ulifloxacin at about 1 μg/ml. The infusion rate of ulifloxacin was controlled by syringe pump PHD2000 (Harvard Apparatus Inc., South Natick, MA, U.S.A.).

Dosages of sparfloxacin, levofloxacin, and azithromycin were designed in order that the plasma concentration of each compound after the administration was higher than that of ulifloxacin.19—21) Sparfloxacin and levofloxacin (40 mg/kg, respectively) were intravenously administered immediately before ulifloxacin administration to the rats. Azithromycin (20 mg/kg) was intravenously administered to rats immediately before ulifloxacin administration, and at 1 and 2 h after the start of ulifloxacin administration. Blood, bronchoalveolar lavage fluid (BALF), and the lungs were sampled at 3 h after the start of the ulifloxacin infusion. Blood was collected from the femoral artery. The trachea was cannulated, and the lungs were lavaged three times with 5 ml of the Ringer’s solution (2.7 mM potassium chloride, 1.8 mM calcium chloride, 0.5 mM magnesium chloride, 0.7 mM sodium phosphate monobasic, 0.7 mM sodium phosphate dibasic, 147.2 mM sodium chloride, 5.6 mM glucose, pH 7.5 adjusted by 0.1 or 0.01 mM sodium hydroxide). The lung was excised after collection of the BALF. The remaining water in the lung was soaked up with filter papers.

2. Uptake of Ulifloxacin from Plasma to Lung Pulmonary uptake of ulifloxacin was evaluated by integration plot analysis. Under ether anesthesia, the animals were cannulated at the femoral artery and vein for facilitation of blood sampling and intravenous administration. Ulifloxacin (1 mg/kg) was administered to rats via the femoral vein. Sparfloxacin (20 mg/kg) or azithromycin (20 mg/kg) was administered via the femoral vein immediately before ulifloxacin administration. Blood samples from the femoral artery were collected in two different sets of time points such as at 0.16, 0.33, and 0.5 min and at 0.25, 0.5, 0.75, and 1 min. Lung tissues were excised in two different sets of time points such as at 0.5 or 1 min after the administration of ulifloxacin.

**In Vitro Experiment. 1. Cell Culture** Calu-3 were purchased from American Type Culture Collection (Arlington, VA, U.S.A.) and used in passage numbers 20—40. The cells were maintained in a ratio of 200 mg lung tissue/1 ml, the excised lung tissues were homogenized using a Teflon homogenizer. A portion (0.1 ml) of homogenized lung was diluted with 0.1 ml of distilled water or standard solution (0.2—5 μg/ml of ulifloxacin in distilled water), 0.1 ml of internal standard solution (2.5 μg/ml of enoxacin in 10 mM sodium hydroxide), and 2.7 ml of 10 mM phosphate buffered solution (pH 6.0). The diluted samples were loaded on Waters OASIS HLB Extraction Cartridge (60 mg/3 ml). Each cartridge was washed with 3 ml of distilled water and 2 ml of 10% (v/v) methanol, and eluted with 2 ml of acetonitrile. The eluates were evaporated to dryness under nitrogen stream at 50°C. The residue was dissolved in 0.1 ml of solvent mixture A (50 mM sodium hydroxide : methanol = 1 : 2) prior to analysis. A portion (0.02 ml) of each sample was injected into HPLC.

2. BALF A portion (0.4 ml) of BALF or standard solution (0.01—0.5 μg/ml of ulifloxacin in distilled water) was diluted with 0.02 ml of solvent mixture A and 0.02 ml of internal standard solution (0.5 μg/ml of enoxacin in solvent mixture A). The solution was evaporated to dryness under nitrogen stream at 50°C, and the residue was dissolved in 0.1 ml of solvent mixture A prior to analysis. A portion (0.05 ml) of each sample was injected into HPLC.

3. Lung After the addition of cold 0.1% formic acid at a ratio of 200 mg lung tissue/1 ml, the excised lung tissues were homogenized using a Teflon homogenizer. A portion (0.1 ml) of homogenized lung was diluted with 0.1 ml of 0.1% formic acid or standard solution (0.05—5 μg/ml of ulifloxacin in 0.1% formic acid), 0.1 ml of internal standard solution (10 μg/ml of ofloxacin in distilled water), and 0.3 ml of 7% (v/v) perchloric acid. The solutions were centrifuged at 7800 × g for 5 min, and the supernatant of the homogenized lung was moved into another tube and diluted with 2 ml of distilled water. The diluted samples were loaded on Waters OASIS HLB Extraction Cartridge (60 mg/3 ml). Each
cartridge was washed with 3 ml of distilled water and dried with a vacuum chamber. After the analytes were eluted with 2 ml of methanol, 0.1 ml of 10% propylene glycol in methanol was added into the eluates. In the case of lung, use of acetonitrile for elution produced many interference peaks on the chromatograms, and thus, we used methanol for acetonitrile. The eluates were evaporated to dryness under nitrogen stream at 50°C. The residue was dissolved in 0.1 ml of solvent mixture A prior to analysis. A portion (0.02 ml) of each sample was injected into HPLC.

**Assay. 1. Ulifloxacin** The concentrations of ulifloxacin in plasma, BALF, and homogenized lung were determined by Shimadzu HPLC system (Kyoto, Japan), consisting of a pump (LC 10AD), an automatic sampler (SIL 10 AXL), a wavelength UV detector (SPD 10AV), and a software system (CLASS-LC10). Chromatographic separation was carried out with TSK-gel ODS-120T 5-μm (4.6 mm i.d.×250 mm, Tosoh, Tokyo, Japan). The mobile phase was composed of 50 mm potassium phosphate monobasic-phosphoric acid (pH 4) and acetonitrile (80:20, v/v). The flow rate was 1.0 ml/min. The column temperature was kept at 40°C, and the UV detector wavelength was set at 275 nm. Peak area of ulifloxacin to the internal standard (enoxacin or the UV detector wavelength was set at 275 nm. Peak area was proportional to the area under the time–plasma concentration curves from 0 to designated times (AUC<sub>t</sub>). By dividing the ulifloxacin concentration in the lung at designated times (C<sub>lung</sub><sup>t</sup>) and AUC<sub>t</sub>/f<sub>i</sub> by the ulifloxacin concentration in plasma at designated times (C<sub>plasma</sub><sup>t</sup>), ulifloxacin uptake clearance (CL<sub>uptake</sub>) was obtained from the initial slope of the plot of C<sub>lung</sub>/C<sub>plasma</sub> vs. AUC<sub>t</sub>/f<sub>i</sub>.<sup>27,28</sup>

The intrinsic pulmonary uptake clearance (CL<sub>int</sub>) in vivo was calculated using the following equation according to well-stirred model:

\[
CL_{int} = CL_{uptake} \times R_B \times Q_{lung} / (f_p \times (Q_{lung} - CL_{uptake}))
\]

where R<sub>B</sub> is the blood-to-plasma concentration ratio (R<sub>B</sub> = 2.0; measured in our laboratory), f<sub>p</sub> is the plasma unbound fraction (f<sub>p</sub> = 0.50; measured in laboratory), and Q<sub>lung</sub> is the lung blood flow rate (Q<sub>lung</sub> = 178 ml/min/kg).<sup>29</sup>

Statistical analysis was performed using Dunnett’s multiple comparison following two-way ANOVA by SAS (SAS Institute Japan). The p value for statistical significance was set at <0.05. All data are expressed as the mean ± standard error (S.E.).

**RESULTS**

**In Vivo Experiment. 1. Penetration of Ulifloxacin from Plasma to Lung and ELF of Rats** Ulifloxacin was highly penetrated into rat ELF and lung after single-administration of ulifloxacin. The ratio of ulifloxacin concentration in the ELF to that in plasma (C<sub>ELF</sub>/C<sub>plasma</sub>) and the ratio of ulifloxacin concentration in the lung to that in plasma (C<sub>lung</sub>/C<sub>plasma</sub>) were significantly decreased by co-administration of sparfloxacin, levofloxacin, or azithromycin. Sparfloxacin and azithromycin decreased C<sub>plasma</sub> and C<sub>lung</sub>/C<sub>plasma</sub> values more strongly than levofloxacin (Fig. 1).

**2. Uptake of Ulifloxacin into Rat Lung** The CL<sub>int</sub> values of ulifloxacin were 2.89 and 5.82 ml/min/kg, respectively, and much lower than the pulmonary blood flow.

![Fig. 1. Penetration of Ulifloxacin into Epithelial Lining Fluid or Lung in Rats](image)
Table 1. Effect of Treatment with Sparfloxacin (20 mg/kg) or Azithromycin (20 mg/kg) on the Pulmonary Uptake of Ulifloxacin after Intravenous Administration (1 mg/kg) to Rats

<table>
<thead>
<tr>
<th>UFX mg/kg</th>
<th>SPFX mg/kg</th>
<th>AZM mg/kg</th>
<th>CL_{uptake}</th>
<th>Ratio</th>
<th>CL_{int}</th>
<th>Ratio</th>
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</thead>
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<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5.82</td>
<td>1</td>
<td>2.89</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>0</td>
<td>0.44</td>
<td>0.08</td>
<td>0.22</td>
<td>0.08</td>
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<tr>
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<td>20</td>
<td>1.59</td>
<td>0.27</td>
<td>0.80</td>
<td>0.28</td>
</tr>
</tbody>
</table>

UFX: ulifloxacin. SPFX: sparfloxacin. AZM: azithromycin. CL_{uptake}: pulmonary uptake clearance. CL_{int}: pulmonary intrinsic clearance. Values are expressed as mean, n=4.

Fig. 2. Effect of Co-administration of Sparfloxacin (20 mg/kg) or Azithromycin (20 mg/kg) on the Pulmonary Uptake of Ulifloxacin after Intravenous Administration (1 mg/kg) to Rats

UFX: experiments in absence of any inhibitor. + SPFX and + AZM: experiments in presence of sparfloxacin and azithromycin, respectively. C_{lung}: ulifloxacin concentration in the lung at 0.5 and 1 min. C_{plasma}: ulifloxacin concentration in plasma at 0.5 and 1 min. AUC_{lung}: area under the time–plasma concentration curve of ulifloxacin during 0 to 0.5 and 1 min. Values are expressed as mean±S.E., n=3.

Fig. 3. Time and Temperature-Dependent Uptake of Ulifloxacin into Calu-3

Filled circles: experiments at 37 °C. Open circles: experiments at 4 °C. Values are expressed as mean±S.E., n=3—6.

The result showed that the pulmonary uptake of ulifloxacin was intrinsic clearance-limited. The CL_{uptake} and CL_{int} values of ulifloxacin were decreased by co-administration of sparfloxacin or azithromycin, which strongly decreased C_{ELF}/C_{plasma} and C_{lung}/C_{plasma} values in the infusion study (Fig. 2, Table 1).

In vitro Experiment. 1. Uptake of [14C]-Ulifloxacin into Calu-3 The uptake of [14C]-ulifloxacin at 37 °C was linear up to 2 min and reached equilibrium at 30 min after addition of [14C]-ulifloxacin. At 4 °C, [14C]-ulifloxacin was not linear up to 2 min and reached equilibrium at 30 min after administration study (Fig. 2, Table 1). The results suggest the existence of a certain active transport of ulifloxacin on the penetration from plasma to the lung in which the contribution of the active transport of ulifloxacin on the penetration from plasma to the lung is higher than that from plasma to ELF.

2. Effect of Various Transporter Inhibitors on Uptake of [14C]-Ulifloxacin Sparfloxacin and azithromycin, which strongly inhibited [14C]-ulifloxacin uptake into the rat lung, also significantly inhibited [14C]-ulifloxacin uptake into Calu-3, and the inhibition by levofloxacin was weaker than that by sparfloxacin or azithromycin. PAH, CMT, and TEA significantly inhibited the [14C]-ulifloxacin uptake into Calu-3. The presence of OUA did not affect the [14C]-ulifloxacin uptake into Calu-3 (Fig. 4).

DISCUSSION

The ELF exists in the inside surface of the alveolus. When antibiotics that are effective against pneumonia-causative pathogens are given in oral or intravenous administration, they penetrate into the ELF through the lung. In infusion studies, C_{ELF}/C_{plasma} and C_{lung}/C_{plasma} values of ulifloxacin after single-administration of ulifloxacin to rats were 2.3 and 9.2, respectively, suggesting the existence of a certain active transport system of ulifloxacin penetration from the plasma to lung and ELF (Fig. 1). In rats, C_{lung}/C_{plasma} values of ciprofloxacin, ulifloxacin, HSR903, and grepafloxacin were 0.8, 20) 9.2, 12.7, 20) and 15.1, 20) respectively, and AUC_{ELF}/AUC_{plasma} values of them were 0.477, 21) 2.3, 3.03, 21) and 5.69, 21) respectively, showing that quinolone, whose concentration in the lung was high, highly penetrated into the ELF. These quinolone concentrations in the lung were therefore quite relevant to those in ELF. Additionally, sparfloxacin and azithromycin, which inhibited distribution of ulifloxacin from the plasma to ELF in infusion study, strongly inhibited active uptake of ulifloxacin from the plasma to lung in integration plot analysis (Fig. 2, Table 1). The results suggest that active uptake of ulifloxacin from the plasma to lung controls the distribution of ulifloxacin from the plasma to ELF.

In infusion studies, C_{lung}/C_{plasma} and C_{ELF}/C_{plasma} values of ulifloxacin were 9.2 and 2.3, respectively, indicating that contribution of the active transport of ulifloxacin on the penetration from plasma to the lung is higher than that from plasma to ELF. Additionally intravenous administration of sparfloxacin or azithromycin decreased C_{ELF}/C_{plasma} and C_{lung}/C_{plasma} values of ulifloxacin to approximately 1, suggesting that sparfloxacin and azithromycin were administered under the condition that they almost perfectly inhibit the penetration of ulifloxacin from the plasma to the lung in which an active transport contributed significantly. The results supported the theory that inhibition of the penetration from plasma to lung brings decrease of ulifloxacin concentration in the ELF.

In vitro studies, active uptake of ulifloxacin into Calu-3 was observed and the uptake was inhibited by azithromycin.
or sparfloxacin as observed in the rat lung. Additionally sparfloxacin inhibited the active uptake of ulifloxacin into Calu-3 more strongly than levofloxacin as observed in the rat lung. These results suggested that the uptake of ulifloxacin into Calu-3 has partly similar characteristics to its uptake into the rat lung. On the other hand, levofloxacin inhibited the penetration of ulifloxacin into the rat lung and the ELF, although such inhibition was not found in the uptake of ulifloxacin into Calu-3. The result suggested that the inhibition levels by sparfloxacin and levofloxacin in the rat lung and ELF did not quantitatively correlate with those in Calu-3.

The accumulation of \[^{14}\text{C}\]-TEA in HEK293 cells transfected with hOCT1 was decreased by TEA and CMT, but not by levofloxacin.\(^{24}\) Our study also showed that the uptake of ulifloxacin into Calu-3 was significantly decreased by TEA and CMT, but not by levofloxacin. Ulifloxacin is a zwitterionic compound, and has possibility of a substrate of OCT. Additionally accumulation of \[^{14}\text{C}\]-guanidine into rabbit type II alveolar epithelial cell was inhibited by various OCT inhibitors (amiloride, cimetidine, clonidine, procainamide, propranolol, tetraethylammonium, and verapamil).\(^{33}\) The results indicate the participation of OCT to the uptake of ulifloxacin into Calu-3. The uptake of \[^{14}\text{C}\]-PAH by \textit{Xenopus} oocytes injected with OAT1 RNA was markedly inhibited by unlabeled PAH.\(^{34}\) In this study, the uptake of ulifloxacin into Calu-3 was significantly decreased by PAH. Additionally OAT2 was highly detected in the lung of CD-1 mice.\(^{35}\) The results indicate the participation of OAT to the uptake of ulifloxacin into Calu-3. In our \textit{in vitro} study with Calu-3, we used TEA, CMT, and PAH at the final concentration of 1 mM, respectively, and they reduced uptake of ulifloxacin by 61.8, 47.1, and 54.2%, respectively. Additionally they inhibited the uptake by 47.1, 44.3, and 22.8% at the final concentration of 1 mM in our preliminary study, respectively. These results suggested that the inhibition by TEA and PAH were concentration dependent. However, the inhibition by CMT was not concentration dependent. The IC\(_{50}\) values of TEA for hOCT1 and hOCT2 were 158 and 156 \(\mu\text{M}\), respectively.\(^{36}\) The IC\(_{50}\) values of CMT for rOCT1 and rOCT2 were 329 and 373 \(\mu\text{M}\), respectively.\(^{37}\) The IC\(_{50}\) value of PAH for hOAT1 was 8.8 \(\mu\text{M}\).\(^{38}\) CMT inhibited not only rOCT1/2 but also hOAT1 (IC\(_{50}=492 \mu\text{M}\)).\(^{36}\) These results indicate the participation of OCT and/or OAT to the uptake of ulifloxacin into Calu-3. On the other hand, grepafloxacin is a zwitterionic compound, and binds to phosphatidylserine.\(^{30}\) Ulifloxacin, sparfloxacin, and levofloxacin are also zwitterionic compounds. Azithromycin is a basic compound, suggesting the binding of azithromycin to phosphatidylserine. The features indicated that inhibition of binding of ulifloxacin to phosphatidylserine by sparfloxacin, levofloxacin, and azithromycin may possibly result in inhibition of penetration of ulifloxacin from plasma to the lung. However, the zwitterionic levofloxacin did not inhibit the uptake of ulifloxacin into Calu-3. This discrepancy should be investigated in more detail.

Delivering of azithromycin by macrophages results in high azithromycin concentration in the ELF and macrophages.\(^{39}\) The concentrations of various quinolones in the macrophages are higher than that in rat plasma.\(^{32,40}\) suggesting the possibility that macrophages are also important vehicles for delivering ulifloxacin to the ELF.

We calculated log \(D\) (neutral form) values with ACD/ChemSketch (Version 7.05, ACD Labs, Toronto, Canada). The log \(D\) value of sparfloxacin (3.26) was higher than that of levofloxacin (1.61). Additionally log \(D\) value of azithromycin was 5.68, and higher than that of levofloxacin. Murata \textit{et al.} discussed that several quinolone antibiotics are taken up by liver cells \textit{via} a common transporter and that the lipophilicity may contribute to the affinity for the transporter.\(^{27}\) The detail has not been clarified yet, but the log \(D\) values indicated that lipophilicity of antibiotics is related to the inhibition of penetration of ulifloxacin from plasma to the lung and the ELF. Accordingly OCT, OAT, phosphatidylserine, macrophage, and/or lipophilicity of antibiotics contribute to the uptake of ulifloxacin into the lung and Calu-3.

In clinical practice, plasma concentrations after oral administrations of ulifloxacin (600 mg/man/d), sparfloxacin (100 mg/man/d), and azithromycin (500 mg/man for the ini-
tial dose and then 250 mg/man once daily for maintenance dose) were 1.5 μM (4 h after the administration), 1.1 μM (4 h after the administration), and 0.1 μM (4 h after the final administration), respectively. Our preliminary study showed that the uptake of 3 μM ulifloxacin was not inhibited by the presence of sparfloxacin and azithromycin at the same concentrations. Sparfloxacin inhibited transport of 2,4-dinitrophenyl-S-glutathione (DNP-SG) by multidrug resistance-associated protein (MRP2) and transport of doxorubicin by multidrug resistance (mdr1a) at the concentrations of 3 mM and 0.5 mM, respectively. Azithromycin inhibited transport of doxorubicin by MDR1 at the concentration of 0.3 mM. The inhibitory concentrations of these drugs were from 470 to 8400 folds higher than the plasma concentrations as described above in clinical practice. We therefore can consider that combination therapy with ulifloxacin and sparfloxacin or azithromycin does not bring about transporter-mediated drug–drug interaction during the penetration of ulifloxacin into the lung and the ELF.

In conclusion, our results suggested that active uptake of ulifloxacin from the plasma to the lung controls distribution of ulifloxacin from the plasma to the ELF in rats, and that the uptake of ulifloxacin into Calu-3 has partly similar characteristics to its uptake into the rat lung. Our study will surely contribute to much better understanding of antibiotic efficacy against pathogens which cause pneumonia.

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