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Pharmacokinetic and Pharmacodynamic Efficacy of Intrapulmonary Administration of Ciprofloxacin for the Treatment of Respiratory Infections

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Summary: The pharmacokinetic and pharmacodynamic efficacy of intrapulmonary administration of ciprofloxacin (CPFX) for the treatment of respiratory infections caused by pathogenic microorganisms resisting sterilization systems of alveolar macrophages (AMs) was evaluated by comparison with an oral administration. The time-courses of the concentration of CPFX in AMs and lung epithelial lining fluid (ELF) following intrapulmonary administration of CPFX solution to rats (200 μg/kg) were markedly higher than that following oral administration (10 mg/kg). The time-course of the concentrations of CPFX in plasma following intrapulmonary administration was markedly lower than that in AMs and ELF. These results indicate that intrapulmonary administration is more effective in delivering CPFX to AMs and ELF, compared with oral administration, in spite of a low dose and it avoids distribution of CPFX to the blood. In addition, the antibacterial effects of CPFX in AMs and ELF following intrapulmonary administration were evaluated by pharmacokinetics/pharmacodynamics analysis. The concentration of CPFX in AMs and ELF-time curve (AUC)/minimum inhibitory concentration of CPFX (MIC) ratio and the maximum concentration of CPFX in AMs and ELF (Cmax)/MIC ratio were markedly higher than the effective values. The present study indicates that intrapulmonary administration of CPFX is an effective technique for the treatment of respiratory infections.

Key words: ciprofloxacin; intrapulmonary administration; drug delivery; alveolar macrophages; lung epithelial lining fluid; respiratory infections; PK/PD

Introduction

Alveolar macrophages (AMs) treats pathogenic microorganisms in lungs and are associated with biophylaxis. However, intracellular parasites, such as M. tuberculosis, C. pneumoniae, L. monocytogenes, L. pneumophila, and F. tularensis, are taken up by AMs via phagocytosis after reaching the alveolus, however, they are resistant to the biocidal mechanisms of AMs, and survive or multiply intracellularly in AMs.1-5 Also, several microorganisms, such as P. aeruginosa, H. influenzae and S. pneumoniae, avoid uptake and digestion by AMs, and survive or multiply in lung epithelial lining fluid (ELF).6-8 Thus, severe respiratory infections are frequently induced by these pathogenic microorganisms.9-11 For sterilization of these pathogenic microorganisms in AMs and ELF, the antibiotic concentration in AMs must be higher than the minimum inhibitory concentration. Consequently, efficient delivery of antibiotics to AMs and ELF is required in order to produce an antimicrobial effect against pathogenic microorganisms resisting sterilization systems of AMs. Clinically, antibiotics are generally given by the oral route for the treatment of respiratory infections. However, because antibiotics distribute to many different tissues via the blood after oral administration, systemic side effects are frequently induced.12 In contrast, intrapulmonary administration is an efficient method for delivering antibiotics directly to the lung. Therefore, an enhancement of the antimicrobial effect, a reduction in the dose and avoidance of systemic side effects could be achieved by the intrapulmonary administration of antibiotics.

Ciprofloxacin (CPFX), a fluoroquinolone antibiotic, produces its antibacterial effects by inhibition of DNA gyrase and topoisomerase IV;13-15 it also has a wide antibacterial spectrum and is effective against the pathogenic microorganisms resisting sterilization sys-
tems of AMs as described above.24–32 At present, in clinical situations, CPFX is given orally but the development of an intrapulmonary administration system for CPFX would be an important advance if enhancement of its antibacterial effect, a reduction in the dose and avoidance of systemic side effects such as hypoglycemia, QT prolongation and convulsions,33–35 were possible.

Consequently, in the present study, the pharmacokinetic and pharmacodynamic efficacy of intrapulmonary administration of ciprofloxacin for the treatment of respiratory infections caused by pathogenic microorganisms resisting sterilization systems of AMs was evaluated by comparison with oral administration.

Materials and Methods

Materials and animals: CPFX was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other regents were commercially available and of analytical grade. Male SD rats (200–250 g) were purchased from Japan SLC (Shizuoka, Japan). The animal experimental plan used was been approved by the Committee of Laboratory Animal Center (No. 05-004), and conforms to the Guiding Principles for the Care and Use of Experimental Animals in Hokkaido Pharmaceutical University.

Experiment involving intrapulmonary administration: CPFX solution prepared by dissolution of CPFX in 50 mM phosphate buffer (pH 5.5) was administered to rat lungs at a dose of 200 μg/250 μL/kg via the nasal cavity using Liquid MicroSprayers® (Model IA-1C, PennCentury, Inc., Philadelphia, PA, USA) under pentobarbital anesthesia. The dose of CPFX (200 μg/kg) used in the present study was approximately a one-fifth of the clinical dose. At the indicated time-points after administration, blood was collected from the jugular vein under pentobarbital anesthesia. The trachea was immediately cannulated and the lungs were lavaged three times with 5 mL ice-cold phosphate buffered saline (PBS, pH7.4). The bronchoalveolar lavage fluid was immediately centrifuged at 4°C (650 × g for 10 min) to separate AMs from the epithelial lining fluid (ELF). AMs were extracted with 1 mL 0.1 M NaOH solution for HPLC analysis. The apparent volume of ELF was estimated by the method using urea, an endogenous marker of ELF dilution.37 The mean value estimated in the present study was 395 μL/225 g rat. The intracellular volume of the AMs was determined by a velocity-gradient centrifugation technique using 3H-water and was estimated to have a mean value of 4.2 μL/mg cell protein. The protein concentration in the cell extracts was determined using Coomassie Protein Assay reagent (Pierce Chemical Company, Rockford, IL, USA) with bovine serum albumin as a standard.39

Data analysis: For the pharmacokinetic analysis, the area under the concentration of CPFX-time curve in AMs, ELF and plasma from time 0 to final time (AUC) was calculated by the trapezoidal rule. The antibacterial effects of CPFX in AMs and ELF following intrapulmonary administration were evaluated by pharmacokinetics/pharmacodynamics (PK/PD) analysis. The concentration of CPFX in AMs and the ELF-time curve (AUC)/minimum inhibitory concentration of CPFX (MIC) ratio and the maximum concentration of CPFX in AMs and ELF (Cmax)/MIC ratio were calculated as the PK/PD parameters of an antibacterial effect. The MIC values against pathogenic microorganisms resisting sterilization systems of AMs were taken from the literature. The effective values of AUC/MIC and Cmax/MIC were larger than 125 and 12, respectively.40–45

Determination of CPFX by HPLC: The concentration of CPFX in AMs, ELF and plasma was measured by HPLC as follows. The biological samples (30 μL), ofloxacin solution (as an internal standard, 30 μL) and CH3OH (30 μL) were mixed and centrifuged at 4°C (650 × g for 10 min), and then supernatant (20 μL) was subjected to HPLC using a system (Shimadzu Co., Kyoto, Japan) involving a 5-μm STR ODS-II column (4.0 × 250 mm, Shinwa Chemical Industries, Ltd., Kyoto, Japan). The mobile phase was 50 mM phosphate buffer (pH2.8)/CH3OH/tetrahydrofuran (667/325/8) and the flow rate was 0.6 mL/min. The eluate was monitored at 277 nm and quantified using a model C-R6A Chromatopac integrator (Shimadzu). The concentrations were determined with respect to a standard curve of CPFX.

Results

Pharmacokinetics of CPFX in rats: The time-courses of the concentrations of CPFX in AMs, ELF and plasma after intrapulmonary and oral administration of CPFX solution to rats are shown in Fig. 1. The time-courses of the concentrations of CPFX in AMs and ELF following intrapulmonary administration to rats (200 μg/kg) were markedly higher than those following oral administration (10 mg/kg). On the other hand, the time-courses of the concentrations of CPFX in plasma following intrapulmonary administration were lower than those following oral administration. In the case of intrapulmonary administration, the time-course of the concentrations of CPFX in AMs and ELF were markedly higher than those in plasma. The pharmacokinetic parameters of CPFX in AMs, ELF and plasma following intrapulmonary and oral administration are summarized in Table 1. The AUC of CPFX in AMs and ELF following intrapulmonary administration were 244 and 103 μg·h/mL, respectively, and the AUC ratios of AMs and ELF to plasma were 290 and 122, respectively. The AUC of AMs and
Fig. 1. Time-courses of concentrations of CPFX in AMs (A), ELF (B) and plasma (C) after intrapulmonary (●) and oral (○) administration to rats. CPFX solution (200 μg/kg) was administered to rat lungs using Liquid MicroSprayers. At each time-point after administration, AMs, ELF and plasma were collected. The CPFX in AMs, ELF and plasma were determined as described in Materials and Methods. Each value represents the mean ± S.E. (n = 4). The time-courses of CPFX after oral administration (10 mg/kg as a clinical dose) were taken from the published literature.

Evaluation of antibacterial effect: The antibacterial effects of CPFX in AMs and ELF following intrapulmonary and oral administration to rats are summarized in Tables 2 and 3. In the case of intrapulmonary administration, the AUC/MIC and Cmax/MIC against the intracellular parasites in AMs and pathogenic microorganisms avoiding uptake by AMs in ELF were larger than the effective values (AUC/MIC: 125, Cmax/MIC: 12). However, in the case of oral administration, several parameters were smaller than the effective values.

Discussion

In the present study, the pharmacokinetic and pharmacodynamic efficacy of intrapulmonary administration of CPFX for the treatment of various respiratory infections caused by pathogenic microorganisms resisting sterilization systems of AMs was investigated.

The pharmacokinetics of CPFX in AMs, ELF and plasma following intrapulmonary and oral administration to rats was examined (Fig. 1 and Table 1). In the case of oral administration, CPFX distributes in the alveolus through vascular endothelial cells and alveolar epithelial cells from the blood side. The alveolar barrier consists of three layers, the capillary lumen, connected tissue and alveolar epithelial cells. The alveolar epithelial cells that are tightly connected by numerous zonulae occludins is considered to provide a significant barrier between plasma and ELF. There are several efflux transporters expressed in alveolar epithelial cells in the human and rat lung, including P-glycoprotein, MDR and breast cancer-resistant protein. CPFX is an MDR substrate and, thus, CPFX may cross from the alveolar epithelium to ELF via an MDR transporter. However, transport of CPFX to ELF across the alveolar barrier after oral administration is not efficient. In contrast, in the case of intrapulmonary administration, CPFX distributes in the alveolus directly. Thus, a reduction in the dose and avoidance of distribution to the blood is possible by intrapulmonary administration. Consequently, the intrapulmonary administration of CPFX results in efficient delivery to AMs and ELF and avoids distribution to the blood.

The AUC of CPFX in AMs following intrapulmonary administration was 2.4-fold greater than that in ELF (Table 1). This result indicates that CPFX is able to concentrate intracellularly in AMs. In fact, most fluoroquinolones are concentrated in phagocytic cells. There is little information about the uptake mechanism of CPFX by AMs. Similar to the purines, CPFX is a multiringed heterocyclic compound and an...
Table 1. The pharmacokinetic parameters of CPFX in AMs, ELF and plasma following intrapulmonary and oral administration to rats

<table>
<thead>
<tr>
<th>Administration</th>
<th>Tissues</th>
<th>AUC (µg*h/mL)a)</th>
<th>Cmax (µg/mL)b)</th>
<th>Tmax (h)c)</th>
<th>AUC ratiod)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrapulmonary</td>
<td>AMs</td>
<td>244 ± 99</td>
<td>39.6 ± 8.2</td>
<td>2</td>
<td>290</td>
</tr>
<tr>
<td></td>
<td>ELF</td>
<td>103 ± 26</td>
<td>17.6 ± 1.4</td>
<td>0.25</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>0.81 ± 0.22</td>
<td>0.14 ± 0.04</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>Oral</td>
<td>AMs</td>
<td>14.1 ± 6.5</td>
<td>5.8 ± 1.9</td>
<td>2</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>ELF</td>
<td>0.74 ± 0.20</td>
<td>0.15 ± 0.02</td>
<td>0.75</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>1.75 ± 0.49</td>
<td>0.35 ± 0.03</td>
<td>0.75</td>
<td>1</td>
</tr>
</tbody>
</table>

Each pharmacokinetic parameters were obtained from data shown in Fig. 1.
a), AUC from time 0 to final time
b), The maximum concentration of CPFX
c), The time to reach Cmax after administration
d), The ratio to AUC in plasma
AUC are represented as mean± calculated SD.
Cmax are represented as mean±SE.

Table 2. The antibacterial effects of CPFX against the intracellular parasites in AMs

<table>
<thead>
<tr>
<th>Administration</th>
<th>Intracellular parasites (MIC)</th>
<th>AUC/MIC (h)</th>
<th>Cmax/MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrapulmonary</td>
<td>M. tuberculosis (0.5 µg/mL)a)</td>
<td>488</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>C. pneumoniae (2 µg/mL)b)</td>
<td>122</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes (1 µg/mL)c)</td>
<td>244</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>L. pneumophila (0.06 µg/mL)d)</td>
<td>4067</td>
<td>660</td>
</tr>
<tr>
<td></td>
<td>F. tularensis (0.015 µg/mL)e)</td>
<td>16266</td>
<td>2640</td>
</tr>
<tr>
<td>Oral</td>
<td>M. tuberculosis (0.5 µg/mL)a)</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>C. pneumoniae (2 µg/mL)b)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>L. monocytogenes (1 µg/mL)c)</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>L. pneumophila (0.06 µg/mL)d)</td>
<td>235</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>F. tularensis (0.015 µg/mL)e)</td>
<td>940</td>
<td>387</td>
</tr>
</tbody>
</table>

AUC and Cmax in AMs as described Table 1 were used for calculation of PK/PD parameters.
The MIC values were taken from the literature. a), Ref. 28; b), Ref. 26; c), Ref. 31; d), Ref. 25; e), Ref. 27.

Table 3. The antibacterial effects of CPFX against the pathogenic microorganisms avoiding uptake by AMs in ELF

<table>
<thead>
<tr>
<th>Administration</th>
<th>Microorganisms (MIC)</th>
<th>AUC/MIC (h)</th>
<th>Cmax/MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrapulmonary</td>
<td>P. aeruginosa (0.5 µg/mL)a)</td>
<td>206</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>H. influenzae (0.008 µg/mL)b)</td>
<td>12875</td>
<td>2200</td>
</tr>
<tr>
<td></td>
<td>S. pneumoniae (0.5 µg/mL)c)</td>
<td>206</td>
<td>35</td>
</tr>
<tr>
<td>Oral</td>
<td>P. aeruginosa (0.5 µg/mL)a)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>H. influenzae (0.008 µg/mL)b)</td>
<td>93</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>S. pneumoniae (0.5 µg/mL)c)</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

AUC and Cmax in ELF as described Table 1 were used for calculation of PK/PD parameters.
The MIC values were taken from the literature. a), Ref. 27; b), Ref. 29; c), Ref. 30.

amphoteric compound with a carboxylic group pKa1 of 6.18 and an amino group pKa1 of 8.76. CPFX is taken up by neutrophils via active transport pathways shared by adenine and a wide range of amino acids and other phagocytic, cells such as peritoneal macrophages and polymorphonuclear leukocytes. Also uptake of CPFX by AMs may mediate transport pathways of nucleo-bases and amino acids in addition to passive diffusion. The efflux rate of CPFX from AMs is slower than that of other fluoroquinolones. Active efflux of CPFX from J774 macrophages is achieved via a multidrug resistance proteins (MDR) transporter. However, the susceptibility of CPFX to efflux transport is lower than that of other fluoroquinolones. Thus, CPFX may be able to concentrate intracellularly in AMs. The AUC ratio of ELF to plasma following intrapulmonary administration was 122 (Table 1). This significant asymmetry may be based on the fact that distribution of CPFX to blood after intrapulmonary administration is inhibited by an MDR transporter on the alveolar epithelium. The AUC ratio of ELF to plasma following oral administration was 0.4 (Table 1). It may be difficult for CPFX to cross pulmonary vascular endothelial cells. For sterilization of pathogenic microorganisms
resisting sterilization systems of AMs, it is required that CPFX is stable in AMs and ELF following intrapulmonary administration. Our unofficial study showed that CPFX was remarkably stable in AMs and ELF for a long time (data not shown). Although AMs produce and secrete various bioactive substances, such as enzymes, cytokines, complements, proteins, lipids and reactive oxygen species, these bioactive substances may not affect the stability of CPFX in AMs and ELF. In addition, it is important that the CPFX administered does not injure lung tissues. The CPFX solution was found to be non-toxic following intrapulmonary administration because release of lactate dehydrogenase from lung tissue was not observed (data not shown). This indicates that the intrapulmonary administration of CPFX does not injure lung tissues, at least at the dose used in the present study.

The antibacterial effects of CPFX in AMs and ELF following intrapulmonary and oral administration to rats were evaluated. (Tables 2 and 3). Recently, there has been increasing interest in the relationship between the PK and PD of antibiotics and, therefore, the use of PK/PD parameters is now widespread. It is proposed that the PK/PD analysis of antibiotic treatment is important for selecting a dose and optimizing the treatment of individual patients. The effects of antibiotics are concentration- and/or time-dependent and the PK/PD parameters used generally are Cmax/MIC, AUC/MIC and the time above MIC. Because the antibacterial effects of fluoroquinolones such as CPFX depend on AUC/MIC or Cmax/MIC, the values of these in AMs and ELF following administration of CPFX were calculated in the present study. In the case of intrapulmonary administration, it was shown that the values of AUC/MIC and Cmax/MIC against intracellular parasites in AMs and pathogenic microorganisms avoiding uptake by AMs in ELF were markedly greater than the effective values in spite of the use of one-50th of the clinical doses (Table 2 and 3). Because the values of AUC/MIC and Cmax/MIC against L. pneumophila, F. tularensis and H. influenzae were so great, it may be possible to reduce the intrapulmonary dose of CPFX for sterilization of these microorganisms. Consequently, the present study indicates that efficient antibacterial effects of CPFX are obtained by intrapulmonary administration of a dose lower than that used clinically. The antibacterial effect of CPFX following intrapulmonary administration to animals used as models of respiratory infection should be investigated in the future since it was not examined in the present study.

In conclusion, the present study evaluated the pharmacokinetic and pharmacodynamic efficacy of intrapulmonary administration of CPFX for the treatment of respiratory infections. We have shown that efficient delivery of CPFX to AMs and ELF is possible by using intrapulmonary administration. Furthermore, it was shown that the antibacterial effect of CPFX following intrapulmonary administration against various pathogenic microorganisms is exhibited at a dose lower than that used clinically. These findings suggest that intrapulmonary administration of antibiotics is an efficient method for the treatment of a variety of respiratory infections.

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