Regular Article

Muscle Distribution of Antimicrobial Agents after a Single Intravenous Administration to Rats

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Summary: The purpose of this study was to evaluate the distribution of three fluoroquinolones (pazufloxacin, ciprofloxacin and ofloxacin) and a β-lactam, ceftazidime in the tissue interstitial and intracellular spaces after a single intravenous administration to rats based on muscle microdialysis. The unbound concentration in the tissue interstitial fluid (C_{ist,un}) after administration was estimated from the concentration in the dialysate by muscle microdialysis, the in vitro permeability rate constant, and the previously reported effective dialysis coefficient. The C_{ist,un}S of pazufloxacin, ciprofloxacin, ofloxacin and ceftazidime in the muscle were close to their unbound concentrations in the venous plasma. These results were consistent with ones previously obtained at steady state. Based on these results, the total concentration in the tissue interstitial fluid (C_{ist}) was calculated from the ratio of plasma protein binding, the plasma concentration, and previously reported interstitial-to-plasma albumin ratio in muscle of rats. The calculated C_{ist} was compared with the muscle concentration (C_m) obtained using the homogenized tissue. The C_{ist} of ceftazidime was higher than the C_m. The C_{ist} of pazufloxacin was found to be almost equal to its C_m. The C_{ist}S of ciprofloxacin and ofloxacin were lower than their C_mS with the exception of the values at 5 min after administration. These results indicate that ceftazidime is mainly distributed in the interstitial space of the muscle, that pazufloxacin is distributed equally in both the interstitial space and the tissue cells, and that ciprofloxacin and ofloxacin are mainly distributed in the tissue cells rather than the interstitial space.

Key words: microdialysis; non-steady state; muscle; interstitial fluid; fluoroquinolone; β-lactam

Introduction

The distribution of antimicrobial agents in infectious tissue is usually evaluated from the concentration in homogenized tissue. However, it is important to evaluate the distribution in the tissue interstitial space and the tissue intracellular space separately for analysis of the in vivo efficacy and pharmacokinetic studies. The unbound concentrations in tissue interstitial fluid (C_{ist,un}S) are especially important for the evaluation of antimicrobial agents because bacterial infections are mostly restricted to the extravascular interstitial spaces of organs1-5 and the unbound concentrations are responsible for the antimicrobial effect.2,6-9

Microdialysis is a relatively new technique for sampling interstitial fluid in tissues such as muscle, lung, subcutaneous adipose tissue, etc. It is a method of extrapolating the C_{ist,un} from the concentration in a dialysate sample. It has been reported extensively that it is a suitable method for evaluating the C_{ist,un}S both in experimental animals6,8,10-16 and humans8,15-18 since it does not disturb physiological conditions and permits continuous monitoring of the C_{ist,un}S in a specific tissue. However, to successfully apply this technique, knowledge of the recovery from the microdialysis fiber used is a prerequisite for calculating the C_{ist,un}S, and the analysis of a dialysate sample requires analytical considerations involving sample volume requirements and limited sensitivity of the assay.

The muscle microdialysis technique at steady state has
been previously used to evaluate the $C_{\text{inf,o}}$ of various antimicrobial agents.\textsuperscript{19,20} Muscle was selected as a large volume organ for determination of drug distribution in the body. Deguchi et al.\textsuperscript{19} have reported that the $C_{\text{inf,o}}$ of $\beta$-lactam antibiotics, which were restricted to the interstitial space of the muscle, were close to the unbound concentration in the venous plasma ($C_{p,o}$) by their muscle microdialysis method at steady state. In our previous study,\textsuperscript{20} the $C_{\text{inf,o}}$ of the fluoroquinolones that showed good penetration into cells\textsuperscript{21-24} were also in good agreement with the $C_{p,o}$ by muscle microdialysis using rats under steady state conditions, according to a method by Deguchi et al.\textsuperscript{25} Recently, it has been reported that the application of the muscle microdialysis technique is useful for evaluating the $C_{\text{inf,o}}$ of antimicrobial agents at non-steady state such as after a single intravenous administration.\textsuperscript{3,4,6,7} Thus, this study was an attempt to apply the muscle microdialysis to non-steady state conditions after a single intravenous administration. In order for the muscle microdialysis to evaluate the $C_{\text{inf,o}}$ after a single intravenous administration to rats, the dialysate sample was collected during a 2-min period because the change in concentration in the muscle interstitial space could be made as small as possible during each sampling period. In addition, the flow rate was set at 10 $\mu$L/min and the agent was administered at the relatively high dose (fluoroquinolone: 50 mg/kg, ceftazidime: 200 mg/kg) due to the analytical limitation of a dialysate sample.

The aim of the present study was to measure and compare the distribution of three fluoroquinolones (pazufloxacin, ciprofloxacin and ofloxacin) and a $\beta$-lactam, ceftazidime from the plasma compartment into the tissue interstitial and intracellular space of skeletal muscle after a single intravenous administration to rats. Furthermore, there has been no report on the direct measurement of the $\textit{in vivo}$ intracellular concentration of antimicrobial agents in cells of living animals. Penetration into cells is usually evaluated by $\textit{in vitro}$ studies.\textsuperscript{21-24} It is therefore considered that the calculated value of the tissue intracellular concentration provides an estimation of $\textit{in vivo}$ penetration of the agents into tissue cells. It will be useful to employ some extraordinary experimental conditions and some hypotheses for the purposes in this study. The analytical limitation of the muscle microdialysis did not allow for the comparison of the distributions of the agents at a lower dose. If the $C_{\text{inf,o}}$ of the agents in muscle appeared to be close to their $C_{p,o}$ by muscle microdialysis after a single intravenous administration, it was possible to compare the distribution of the agents at a lower dose by calculating the tissue interstitial and intracellular concentrations using the plasma concentration ($C_{p}$), the muscle concentration ($C_{m}$) obtained using the homogenized tissue, the ratio of plasma protein binding, and previously reported values (the ratios for the volume of interstitial fluid space and cell volume in muscle of a rat,\textsuperscript{26} and the interstitial-to-plasma albumin ratio in muscle of rats.\textsuperscript{1,27}).

**Materials and Methods**

**Materials:** The methanesulfonic acid salt of pazufloxacin (PZFX-ME) was synthesized, and ciprofloxacin was extracted from commercial products (Bayer Yakuhin, Ltd., Osaka, Japan) in the Research Laboratories of Toyama Chemical Co., Ltd. (Tokyo, Japan). PZFX-ME was converted to pazufloxacin (active form of PZFX-ME) for use in this study. Ofloxacin (Sigma Chemical Co., USA) and ceftazidime (GlaxoSmithKline, K.K., Tokyo, Japan) are commercially available materials.

**Animals:** Male Wistar rats (SLC Inc., Shizuoka, Japan) weighing 220–300 g (8–9 weeks old) were used in all experiments. The animals were purchased at 7–8 weeks old and were housed in an air-conditioned room at 22 ± 2°C with relative humidity of 60 ± 10% and 12-h light cycle. Prior to the experiments they had free access to food and water.

**Microdialysis fiber:** The microdialysis fiber was purchased from Eicom (Kyoto, Japan) and was prepared as described by Deguchi et al.\textsuperscript{25} using a Cuprophan hollow-fiber (inside diameter 0.2 mm, wall thickness 11 $\mu$m, MW cutoff 12500) and stainless steel tubing (outside diameter 0.2 mm; MT Giken, Tokyo, Japan). The fiber consists of a 22-mm long segment of hollow dialysis fiber with a length of fine stainless steel tubing inserted to a depth of 7 mm in each end and then attached. Dialysis takes place in an 8-mm length of the fiber.

**In vitro microdialysis study:** The $\textit{in vitro}$ microdialysis study was carried out with modifications to a previously described method.\textsuperscript{20,25} The microdialysis fiber was linked with polyethylene tubing (SP10, Natsume Seisakusyo Co., Ltd., Tokyo, Japan) and was soaked in a reservoir medium of Ringer-HEPES buffer (RHB; 141 mM NaCl, 4 mM KCl, 2.8 mM CaCl$_2$·2H$_2$O, 10 mM N-(2-hydroxyethyl)-piperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.40) containing each drug placed in a glass plate at 37°C without mixing. The RHB was perfused through the fiber at a constant flow rate of 10 $\mu$L/min and controlled by means of a syringe infusion pump (Model 230, Neuroscience Ltd., Tokyo, Japan). After an appropriate time lapse to enable the establishment of steady state conditions the dialysate was collected in a tube during five sequential 2-min periods for fluoroquinolone and 4-min periods for ceftazidime. For the accurate calculation of the $\textit{in vitro}$ dialysis flow rate ($F_{\text{vito}}$), the tube was weighed before and after the collection of dialysate. The reservoir medium (10 $\mu$L) was sampled at the midpoint of each collection period. Each fiber was tested in the reservoir medium containing low and high concentrations of each drug.
(fluoroquinolone: 0.5 and 5 μg/mL, ceftazidime: 10 and 100 μg/mL).

The microdialysis data was analyzed by an extrapolation method based on steady state clearance concept, described previously in detail by Deguchi et al. The \( \text{in vitro} \) permeability rate constant (\( \text{PA}_{\text{vivo}} \)) was calculated by equation 1 using the \( \text{in vitro} \) dialysis flow rate (\( F_{\text{vivo}} \)), the dialysate concentration of drug at steady state (\( C_d \)), and the reservoir concentration of drug (\( C_r \)) in RHB.

\[
\text{PA}_{\text{vivo}} = -F_{\text{vivo}} \times \ln (1 - \frac{C_d}{C_r}).
\]  

(1)

**In vivo microdialysis study:** Rats were intramuscularly anesthetized with ketamine (235 mg/kg; Sankyo Co., Ltd., Tokyo, Japan) and xylocaine (2.3 mg/kg; Sigma Chemical Co.) and were warmed with lamps to maintain the body temperature at 37°C. A microdialysis fiber was implanted in the right hind leg muscle and then RHB was perfused through the fiber at a constant flow rate of 10 μL/min controlled by means of an infusion pump. The fiber was the same as that used in the corresponding \( \text{in vitro} \) experiments.

About fifteen minutes after the start of dialysis, each bolus dose of the drug (fluoroquinolone: 50 mg/kg, ceftazidime: 200 mg/kg) was administered intravenously via the left femoral vein. Dialysate samples were collected during 2 min periods at sampling times of 0 (just before drug administration), 15, 30, 60, 120, 240 and 360 min for fluoroquinolone, and 0, 15, 30, 60, 120, 180 and 240 min for ceftazidime after drug administration. Blood samples (0.3 mL) were taken with syringes via the jugular vein and were immediately collected into heparinized tubes at the midpoint of each collection period of dialysate. Plasma was separated from the blood by centrifugation at 1000 \( \times \) g for 10 min at 4°C.

As described previously in detail, \( C_{\text{inf},a} \) was defined as follows.

\[
C_{\text{inf},a} = C_{d,\text{vivo}} / \{1 - \exp (-RD \times \text{PA}_{\text{vivo}} / F_{\text{vivo}})\}.
\]  

(2)

\( C_{d,\text{vivo}} \) is the \( \text{in vivo} \) dialysate concentration measured by HPLC and \( F_{\text{vivo}} \) is the \( \text{in vivo} \) dialysis flow rate calculated from the weights of a tube before and after the collection of dialysate. RD is the effective dialysis coefficient that is defined as the ratio \( \text{PA}_{\text{vivo}} / \text{PA}_{\text{vivo}} \), and is independent of molecular weight and plasma membrane permeability. The RD value used, 0.367 for muscle tissue of a rat, was obtained previously.

**In vivo plasma unbound fraction:** Ultrafiltration of the plasma was performed at 1000 \( \times \) g for 10 min at 4°C using a micropartition system (MPS-1, Amicon, Danvers, MA). The concentrations of ciprofloxacin and ceftazidime in the plasma (total concentration in plasma, \( C_p \)) and the filtrate (unbound concentration in plasma, \( C_{p,a} \)) were assayed by HPLC. The ratio of the plasma unbound fraction was calculated using the equation \( (C_{p,a} / C_p) \times 100. \)

**Determination of muscle concentration using homogenized tissue:** After an intravenous bolus dose of each drug at 10 mg/kg via the left femoral vein, blood samples were taken with heparinized syringes via the jugular vein at 5, 15, 30, 60, 120, 180, and 240 min. Rats were sacrificed by exsanguination from the abdominal aorta under ether anaesthesia immediately after blood sampling. Plasma was separated in the same manner as described above. The right hind leg muscle was removed, weighed, and homogenized in 1/15-M phosphate buffer (pH 7.0). The homogenized samples (final 10 w/v%) were centrifuged at 1000 \( \times \) g for 10 min at 4°C. Each group comprised 5 animals.

**Analytical procedure:** The concentrations of each fluoroquinolone in the samples were determined by HPLC. Samples from plasma, filtrate, dialysate and supernatant of homogenized muscle were prepared for analysis by the addition of an appropriate volume of the mobile phase solution, vigorously mixed, and centrifuged at 1000 \( \times \) g for 10 min at 4°C. HPLC was performed with an L-6200 Intelligent pump (Hitachi, Tokyo, Japan), an F-1050 Fluorescence spectrophotometer (Hitachi), and a D-2500 Chromato-integrator (Hitachi). The analytical column (150 mm length \( \times \) 4.0 mm I.D.; Chemco Scientific, Tokyo, Japan) was packed with STR ODS-II (Shimadzu, Kyoto, Japan) in this laboratory. The mobile phase comprised 15% acetonitrile in 10 mM phosphate buffer (pH 7.0) containing 0.68% tetra-n-butylammonium hydrogensulfate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) for pazufoxacin, and 30% acetonitrile in 10 mM citrate buffer (pH 3.5) containing 0.15% sodium 1-octanesulfonate (Tokyo Kasei Kogyo Co., Ltd.) for ciprofloxacin and ofloxacin. The HPLC conditions used were: room temperature, flow rate 1.0 mL/min, excitation and emission wavelength 327 nm and 394 nm for pazufoxacin, 279 nm and 452 nm for ciprofloxacin, and 293 nm and 495 nm for ofloxacin.

In the case of ceftazidime, HPLC was performed with a PG-150:250p006 pump (Chemco Scientific), an L-4200 UV-VIS detector (Hitachi), and a D-7500 integrator (Hitachi) using the analytical column packed with STR ODS-II. Detection was performed at wavelength 254 nm using the mobile phase that comprised 6.5% acetonitrile containing 5 mM acetic acid and 20 mM sodium acetate. The concentrations of the samples obtained from the rats administered at 10 mg/kg of ceftazidime were determined by bioassay using Proteus mirabilis ATCC21100. The detection limit of the bioassay was 0.1 μg/mL and linearity was recognized from 0.1 μg/mL to 100 μg/mL.

**Distribution in muscle:** The distribution in the muscle was estimated from the concentration in the muscle cells (intracellular concentration; \( C_m \)) and the concentration in the muscle interstitial fluid (total amount of
unbound and bound drug in the muscle interstitial space; \( C_{\text{inf}} \) of each drug after the intravenous administration of 10 mg/kg. The \( C_{\text{in}} \) was calculated using the muscle concentration obtained from the homogenized tissue (\( C_{\text{m}} \)) and the \( C_{\text{inf}} \) by the following equation.

\[
C_{\text{in}} = \frac{(C_{\text{m}} - C_{\text{inf}} \times 0.119)}{0.881}.
\]  

(3)

The constants of 0.119 and 0.881 were cited from the ratios previously reported for the volume of interstitial fluid space and cell volume in muscle of a rat, respectively.26

The \( C_{\text{inf}} \) was calculated using \( C_{\text{p,a}} \) (unbound concentration in plasma) and \( C_{\text{p,b}} \) (bound concentration in plasma) by the equation \( C_{\text{inf},a} + C_{\text{inf},b} = C_{\text{p,a}} + 0.6 \times C_{\text{p,b}} \). The \( C_{\text{inf},a} \) was estimated to be equal to \( C_{\text{p,a}} \) based on the results of the muscle microdialysis in this report, and the \( C_{\text{inf},b} \) was estimated to be \( 0.6 \times C_{\text{p,b}} \), where the constant of 0.6 was cited from the previously reported interstitial-to-plasma albumin ratio in muscle of rats.1,27

Results

**In vitro microdialysis:** The \( PA_{\text{vivo}} \) value of each fiber was estimated from *in vitro* microdialysis study and showed no difference between low and high reservoir concentrations of each drug. The \( PA_{\text{vivo}} \) values of paezufloxacin, ciprofloxacin, ofloxacin and ceftazidime were \( 0.450 \pm 0.034 \) (mean \( \pm \) S.D. of five fiber), \( 0.390 \pm 0.031 \), \( 0.469 \pm 0.077 \), and \( 0.206 \pm 0.016 \), respectively. Comparative values for \( PA_{\text{vivo}} \) were obtained for the three fluoroquinolones, but the values for ceftazidime were about half of those for fluoroquinolones.

**In vivo plasma unbound fraction:** The ratio of \( C_{\text{p,a}} \) to \( C_{\text{p,b}} \) of paezufloxacin, ciprofloxacin, ofloxacin and ofloxacin and ceftazidime were \( 75.3 \pm 1.2\% \), \( 79.2 \pm 4.9\% \), \( 77.1 \pm 1.2\% \) and \( 95.2 \pm 10.4\% \), respectively. The values for paezufloxacin and ofloxacin were taken from a previous report.26

**Muscle microdialysis:** The \( C_{\text{inf},a} \) value in muscle was estimated from the values of \( C_{\text{d,vivo}} \), \( PA_{\text{vivo}} \) and \( F_{\text{vivo}} \) according to Eq. (2). The \( C_{\text{p,a}} \) was predicted from the \( C_{\text{p,b}} \) and the ratio of plasma unbound fraction. Results are shown in Fig. 1 (A, B, C, and D). The \( C_{\text{inf},a} \)s for each drug were comparable to those of \( C_{\text{p,a}} \)s at every point.

**Distribution in the muscle:** The \( C_{\text{m}} \)s and the \( C_{\text{inf}} \)s of each drug in the muscle were obtained by the calculation described in the materials and methods, and are shown in Table 1. Ceftazidime was scarcely distributed into the intracellular space. Fluoroquinolones were well distributed in the intracellular space, while ciprofloxacin and ofloxacin were more distributed in the intracellular space in comparison to paezufloxacin. The ratios of \( C_{\text{m}} \) to \( C_{\text{inf}} \) of fluoroquinolones at 5 min were smaller in comparison to those at other times. Some time lag was observed for the distribution of the fluoroquinolones into the intracellular space.

**Muscle concentration and total interstitial concentration:**

<table>
<thead>
<tr>
<th>Drug</th>
<th>( C_{\text{inf}}/C_{\text{inf}} ) at following time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Paezufloxacin</td>
<td>0.79</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.70</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0.76</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

Each value is the mean (\( n = 5 \) for paezufloxacin, ciprofloxacin, and ceftazidime, and \( n = 3 \) for ofloxacin).

ND: not determined.

\( C_{\text{inf}} \): concentration in muscle interstitial fluid calculated from \( C_{\text{p,a}} \) (unbound concentration in plasma) and \( C_{\text{p,b}} \) (bound concentration in plasma) as described in the text.

\( C_{\text{inf}} \): intracellular concentration in muscle cells calculated from the muscle concentration obtained using homogenized tissue (\( C_{\text{in}} \)) and the \( C_{\text{inf}} \) as described in the text.

**Discussion**

In our previous report,20 we showed that there was good correlation between the values of \( C_{\text{inf},a} \) and \( C_{\text{p,a}} \) in the muscle microdialysis studies for the fluoroquinolones, paezufloxacin and ofloxacin, as reported for \( \beta \)-lactam antibiotics19 under steady state conditions, according to a method by Deguchi et al.25 The dialysis clearance was related to the dialysis flow rate (F) and the permeability rate constant (PA) for microdialysis performed with a linear microdialysis fiber. Good correlation was observed between the reciprocal of the PA and the square root of molecular weight (MW) in the range of 18-1039 D.25 This correlation was also apparent in the present study since the \( PA_{\text{vivo}} \) value of ceftazidime (MW: approximately 547) was larger than the values of the fluoroquinolones (MW: approximately 318–361). In addition, the \( PA_{\text{vivo}} \) values of paezufloxacin and ofloxacin in this study were larger than values in the previous study,20 more than likely due to the difference in flow rate. In this study, it was thought that the \( PA_{\text{vivo}} \) value was independent of outside concentration since the \( PA_{\text{vivo}} \) values of each fiber were comparable between the low and high concentrations of each agent in reservoir medium.

An effective dialysis coefficient (RD), was defined as the ratio of the *in vivo* PA (\( PA_{\text{vivo}} \)) and *in vitro* PA (\( PA_{\text{vivo}} \)), and used to explain the differences between in
Fig. 1. Unbound concentrations of pazufloxacin, ciprofloxacin, ofloxacin, and ceftazidime in muscle interstitial fluids and plasma of rats. About fifteen minutes after the start of dialysis, each drug was administered intravenously via the left femoral vein of rats implanted with a microdialysis fiber at a single dose of 50 mg/kg for pazufloxacin, ciprofloxacin and ofloxacin, or 200 mg/kg for ceftazidime. The dialysate samples were collected during 2-min periods at 0 (just before drug administration), 15, 30, 60, 120, 240, and 360 min for pazufloxacin, ciprofloxacin and ofloxacin, and 0, 15, 30, 60, 120, 180, and 240 min for ceftazidime after drug administration. Blood samples (0.3 mL) were taken with syringes via the jugular vein and immediately collected into heparinized tubes of dialysate at the midpoint of each collection period. Unbound concentrations of pazufloxacin (A), ciprofloxacin (B), ofloxacin (C), and ceftazidime (D) in muscle interstitial fluid (C_{ist, u} ○) and plasma (C_{p,u} ●) were determined as described in the text. Points represent mean ± S.D. (n = 5).
Fig. 2. Muscle concentrations and total interstitial concentrations of pafloxacain, ciprofloxacin, ofloxacin, and ceftazidime in rats. After an intravenous bolus dose of each drug at 10 mg/kg via the left femoral vein of rats, blood samples were taken with heparinized syringes via a jugular vein at 5, 15, 30, 60, 120, 180, and 240 minutes. Rats were sacrificed by exsanguination from the abdominal aorta under ether anaesthesia immediately after the blood sampling. The right hind leg muscle was removed, weighed, and homogenized in 1/15-M phosphate buffer (pH 7.0). The homogenized samples were centrifuged at 1000×g for 10 min at 4°C. The muscle concentration was obtained from the homogenized tissue (C_m, △) and total concentration in muscle interstitial fluid (C_isf, ■) of pafloxacain (A), ciprofloxacin (B), ofloxacin (C), and ceftazidime (D) were calculated as described in the text. Points represent mean ± S.D. (n = 5 for pafloxacain, ciprofloxacin and ceftazidime, and n = 3 for ofloxacin).
vitro and in vivo microdialysis recoveries. The RD has been demonstrated to be independent of MW and plasma membrane permeability.\textsuperscript{25} Moreover, it was suggested that the RD was ascribed to the difference of the diffusion distance of the outer surface of the fiber, the difference of the effective surface area between in vivo and in vitro, and the interstitial fluid space.\textsuperscript{19,25} By using the RD value, a useful equation at steady state has been proposed to extrapolate the $C_{\text{inf,s}}$ from the dialysate concentration.\textsuperscript{19,25} As shown in Fig. 1, it was considered that the $C_{\text{inf,s}}$ of pafloxacin, ciprofloxacin, ofloxacin (three fluoroquinolones) and ceftazidime (a $\beta$-lactam) were the same as their $C_{\text{p,a}}$ after the administration, as well as at steady state previously reported.\textsuperscript{19,20} Our results of the muscle microdialysis also indicate that the RD value (0.367 for muscle tissue of a rat) and the equation proposed by Deguchi et al.\textsuperscript{25} is appropriate for the estimation of $C_{\text{inf,s}}$ by muscle microdialysis. The results using the point-of-no-net-flux method\textsuperscript{20} for the determination of the in vivo recovery also showed that the free drug concentrations in plasma and in muscle interstitial fluid after a single intravenous administration were in good agreement.\textsuperscript{6,7}

This study focused on evaluating the distribution of antimicrobial agents in tissues after a single intravenous administration using the muscle microdialysis technique. As the $C_{\text{inf,s}}$ of the agents were indicated to be close to their $C_{\text{p,a}}$ by muscle microdialysis after a single intravenous administration, it was attempted to compare the distribution of the agents in the muscle interstitial and intracellular space after the administration. The plasma concentrations ($C_{\text{p,s}}$) and the muscle concentrations ($C_{\text{m,s}}$) from the homogenized tissue were then measured after a single intravenous administration of each agent at a dose of 10 mg/kg. Based on the muscle microdialysis results, the $C_{\text{inf,s}}$ of each agent were calculated using the $C_{\text{p,s}}$ and the plasma protein binding. Moreover, in order to compare the comparison of the distribution of the agents, the $C_{\text{inf,s}}$ after the intravenous administration of 10 mg/kg were calculated using the $C_{\text{inf,s}}$, the $C_{\text{p,s}}$, the plasma protein binding, and the interstitial-to-plasma albumin ratio in the muscle of rats.\textsuperscript{1,23} In this calculation, we assumed that the agents in the interstitial fluid bound mainly with albumin existing in this compartment and all the rats used showed comparable interstitial-to-plasma albumin ratios of 0.6.

In order to evaluate the distribution in muscle, the calculated $C_{\text{inf,s}}$ were compared with $C_{\text{m,s}}$ (Fig. 2). Because the $C_{\text{inf,s}}$ reflect the $C_{\text{inf,s}}$, it has been reported that the free drug concentrations in the plasma rather than in the homogenized tissue would be more important for chemotherapy of infections other than intracellular infections.\textsuperscript{6,7} Our results indicate that ceftazidime is distributed mainly in the interstitial space of the muscle, that pafloxacin is distributed equally in both the interstitial space and the tissue cells, and that ciprofloxacin and ofloxacin are mainly distributed in the tissue cells rather than the interstitial space. This was also indicated by the values of the $C_{\text{m}}/C_{\text{inf}}$ for each agent (Table 1). The $C_{\text{m,s}}$ were calculated using the $C_{\text{m,s}}$, the $C_{\text{inf,s}}$, and the previously reported values of the ratios for the volume of interstitial fluid space and cell volume in muscle of rats.\textsuperscript{20} In this calculation, we assumed that all the rats used had approximately the same volume ratio of interstitial fluid space in muscle (0.119) because all rats used were of a similar age. The $C_{\text{m}}/C_{\text{inf}}$ of ceftazidime was almost equal to zero. This indicates that ceftazidime was scarcely distributed in the intracellular space in the muscle. The $C_{\text{m}}/C_{\text{inf}}$ of the fluoroquinolones were approximately equal 5 min after administration. However, with the exception of the values at 5 min after administration, the $C_{\text{m}}/C_{\text{inf}}$ of ciprofloxacin and ofloxacin were 2.10–3.54 and 2.02–2.80, respectively, higher in comparison to those of pafloxacin (0.94–1.55). It has been reported in the in vitro study that penetration of pafloxacin into cells was lower than that of ciprofloxacin and ofloxacin.\textsuperscript{22} Our results suggest that in vivo penetration of pafloxacin into cells was lower than that of ciprofloxacin and ofloxacin as well as in vitro penetration, and that it took a little time to reach the equilibrium for the $C_{\text{m}}/C_{\text{inf}}$ of each fluoroquinolone.

In conclusion, the unbound concentrations of the fluoroquinolones and the $\beta$-lactam in the tissue interstitial fluids was consistent with those in plasma even under non-steady state conditions. From the results obtained in this study, it was shown that it was possible to evaluate the distribution of an antimicrobial agent in the tissue interstitial space and tissue cells using the ratio of plasma protein binding, the plasma concentrations, and the muscle concentrations from the homogenate. It was suggested that using the drug concentrations in the homogenized tissue overestimated the antibacterial activity of ciprofloxacin and ofloxacin and underestimated that of ceftazidime. In the case of pafloxacin, however, both concentrations could be used to estimate its activity against bacterial infections.

References


dures affect the target site distribution of piperacillin. 


13) Huang, Y., Ji, P., Inano, A., Yang, Z., Giebink, G. S. and Sawchuk, R. J.: Microdialysis studies of the middle ear distribution kinetics of amoxicillin in the awake chinchilla. 


14) Smolders, I., Gousseau, C., Marchand, S., Couet, W., Ebinger, G. and Michotte, Y.: Convulsant and subconvulsant doses of norfloxacin in the presence and absence of biphenylacetic acid alter extracellular hippocampal glutamate but not gamma-aminobutyric acid levels in conscious rats. 


