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# Determinant of the distribution volume at steady state for novel quinolone pazufloxacin in rats

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**Abstract**

The distribution properties of the novel quinolone pazufloxacin (PZFX) in rats were compared with those of sparfloxacin (SPFX) and ofloxacin (OFLX). Following intravenous administration of these quinolones to rats, the distribution volume at steady state ($Vd_{ss}$) of PZFX was found to be 0.945 L $kg^{-1}$, whereas for OFLX and SPFX it was 1.83 and 3.42 L $kg^{-1}$, respectively. In order to understand this difference in $Vd_{ss}$, first the contribution of each tissue to the $Vd_{ss}$ using pharmacokinetic parameters was estimated and it was found that the type of tissue contributing the most was muscle. Subsequently, we determined the extent of tissue binding and uptake clearance (CLuptake) in the muscle. The ratio of unbound fraction of plasma to unbound fraction of muscle was almost equal for all quinolones tested, with values of 1.60, 1.52 and 1.32 for PZFX, OFLX and SPFX, respectively. In contrast, the tissue CLuptake of PZFX in the muscle (0.012 mL $min^{-1}$ $g^{-1}$) was significantly lower than that of OFLX and SPFX (0.118 and 0.195 mL $min^{-1}$ $g^{-1}$, respectively). These results suggest that the low $Vd_{ss}$ value for PZFX can be attributed to the low CLuptake in the muscle.

## Introduction

Quinolones are widely used against a variety of infections. The dosages and dosing intervals of quinolones should be designed with reference to pharmacokinetic and pharmacodynamic parameters. In order to predict the efficacy, it is important to find appropriate surrogate markers that account for the efficacy by using a combination of pharmacokinetic and microbiology parameters. Quinolones display a concentration-dependent bactericidal effect for most bacteria (Hyatt et al 1995). Thus, the maximum plasma concentration to minimum inhibitory concentration (MIC) ratio can act as a parameter that correlates to efficacy (Stein 1996). The area under the curve of a concentration–time profile (AUC) to MIC ratio is also an important predictor of efficacy for quinolones in clinical practice (Forrest et al 1997). In addition, we previously reported that the unbound concentrations of quinolones in the tissue interstitial fluid, which is the main site of bacterial infection, were the same as those in the venous plasma (Araki et al 1997). Accordingly, a high serum unbound concentration is considered to be an essential factor in deciding the efficacy of quinolones. Serum or plasma concentration is a reflection of serum unbound concentration. In animal and clinical experiments, however, variations in the plasma concentration–time profiles of quinolones were observed. Therefore, it was considered important to investigate the differences in plasma concentration–time profiles when developing novel quinolones. It is likely that the distribution volume at steady state ($Vd_{ss}$) is a crucial parameter for deciding plasma concentration–time profiles. To clarify the mechanism that determines the $Vd_{ss}$ in quinolones, we previously studied the structure–tissue distribution relationship and found that the unbound fraction of quinolones in the plasma determined the $Vd_{ss}$ of the drugs tested (Okezaki et al 1988). However, a new quinolone antibacterial agent, pazufloxacin (PZFX), did not exactly follow this pattern. The target pathogens of PZFX are the bacteria that exist in the interstitial fluid. Therefore, from the viewpoint of drug delivery, PZFX has shown several positive characteristics such as

a lower tissue to plasma concentration ratio and a higher serum concentration compared with other quinolones (Minami et al 1995; Nakashima et al 1995; Hayakawa et al 1999). PZFX can be distinguished from ciprofloxacin (CPFX), ofloxacin (OFLX) and other marketed quinolones by its secondary action of releasing histamine (Furuhata et al 1998). Therefore, in order to understand the distribution mechanism for PZFX, we investigated the important parameters that affect the $Vd_{ss}$ of quinolones in rats. We chose PZFX, OFLX and sparfloxacin (SPFX) as model compounds because their serum concentration–time profiles in rats were almost the same as those observed in humans, that is SPFX displayed low concentration/slow elimination, whereas PZFX displayed high concentration/rapid elimination (Nakamura et al 1990; Minami et al 1995; Nakashima et al 1995). The purpose of this study was to determine the tissue that contributes most to the $Vd_{ss}$, and to investigate the tissue binding and tissue uptake clearance (CLuptake) in that tissue.

## Materials and Methods

### Chemicals

PZFX, SPFX and piperacillin (PIPC) were synthesized by Toyama Chemical Co. Ltd, Japan. OFLX was extracted from Taribbit (Daiichi Pharmaceutical Co. Ltd, Japan). Internal standards for HPLC analysis (temafloxacin (TMFX) and compound 1) were synthesized by Toyama Chemical Co. Ltd. These compounds are illustrated in Figure 1. Penicillin G (PCG) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The solvents and water used were of HPLC grade. All other chemicals were of reagent grade or of the highest purity commercially available.

### Animals

Male Wistar/ST rats (9–10 weeks old) were obtained from Japan SLC Inc., Shizuoka, Japan, and had free access to food and water before the experiments. Animal studies were performed in accordance with the UK Animal (Scientific Procedures) Act 1986 and an associated guideline for the Care and Use of Laboratory Animals published by Toyama Chemical Co. Ltd.

### Plasma concentrations and pharmacokinetic parameters

The femoral artery and bladder of each rat were cannulated under a light ether anaesthetic. After waking, PZFX, SPFX or OFLX (10 mg $kg^{-1}$, dissolved as the sodium salt in a saline solution) was injected into the external jugular vein. A series of blood samples (0.2 mL) were collected from the femoral artery at 2, 5, 15, 30 min and 1, 2, 4 and 6 h after administration, placed in heparinized microtubes and the plasma separated by centrifugation. A volume of saline equal to the volume of blood drawn was injected after collection of each sample. Pooled urine samples were collected at 0.5, 1.5, 2.5, 5.5 and 6.5 h after dosing, and the total volume of urine collected was measured. The plasma and urine samples were stored at −20°C pending analysis.

### Tissue binding

Rats were killed under an ether anaesthetic by cutting the abdominal vena cava, after which the leg muscle was quickly excised. The muscle was stored at −20°C before investigation. The muscles were snipped into small pieces and then homogenized at 0°C with 0.15 M phosphate buffer (pH 7.0) in a Teflon homogenizer (Digital Multi Stirrer, Iuchi, Osaka, Japan) to prepare 10% and 20% (w/v) homogenate. The sodium salt of each quinolone was added to the muscle homogenate to yield final concentrations of 1, 5, 10, 15, 20 $\mu$g $mL^{-1}$, and then incubated at 37°C for 30 min. Under these conditions, the binding of each quinolone to the muscle homogenate reached equilibrium within 20 min. A protein-free fraction was obtained by centrifugation of the homogenate with an ultrafiltration kit (Millipore MPS1 with YMT10 membrane; Millipore, Bedford, MA, USA). Adsorption to the ultrafiltration membrane was predetermined and found to be negligible. The concentration of each test drug in the ultrafiltrate ($C_f$) was determined directly by HPLC. Binding concentrations of each drug ($C_b$) were estimated by subtracting $C_f$ from the nominal concentrations. The unbound fraction of each drug ($f_t$) was estimated according to a previous report by Jiunn et al (1982).

### Serum protein binding

PZFX, SPFX or OFLX (2 $\mu$g $mL^{-1}$) were incubated with rat serum for 30 min at 37°C and the extent of binding was determined by ultrafiltration as described above.

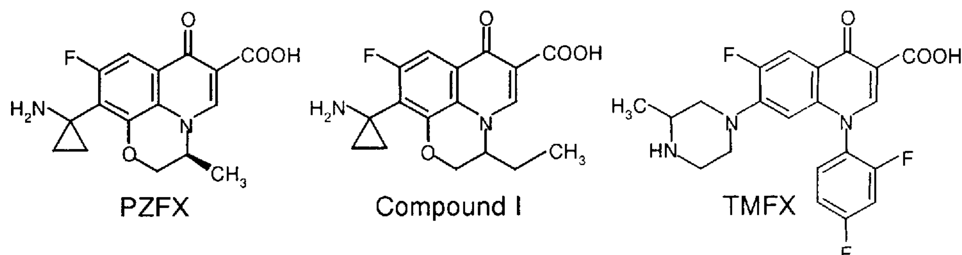


**Figure 1** Chemical structures of pazufloxacin (PZFX) and internal standards for HPLC, compound 1 and temafloxacin (TMFX).

### Tissue CLuptake

The femoral artery of each rat was cannulated under a light ether anaesthetic. After waking, PZFX, SPFX, OFLX (2 mg $kg^{-1}$) or PIPC (40 mg $kg^{-1}$, dissolved in saline) was injected into the external jugular vein and blood samples collected from the femoral artery at 10-s intervals. Immediately after the last sample was taken, the rats were killed by cutting the carotid artery. The leg muscle was quickly excised, washed with ice cold saline, wiped with filter paper, and weighed. The blood samples were centrifuged to separate the plasma. The plasma and muscle samples were stored at −20°C pending analysis. CLuptake was obtained from the slope of $X_{(t)}/C_{p(t)}$ vs $AUC_{(t)}/C_{p(t)}$, where $X_{(t)}$ denotes the concentration of test quinolone in the muscle at time t, $C_{p(t)}$ denotes the concentration of test quinolone in the plasma at time t and $AUC_{(t)}$ denotes the area under the plasma concentration–time curve at time t (Yamazaki et al 1996).

### Analytical method

*Preparation of samples for HPLC*

For the measurement of PZFX, OFLX and SPFX in plasma, an equal volume of methanol was added to the plasma, the mixture was centrifuged, and the supernatant used for HPLC analysis.

For the measurement of PZFX, OFLX and SPFX in urine, the urine samples were diluted with an appropriate amount of HPLC mobile phase.

For the measurement of PZFX, OFLX and SPFX in the muscle, a volume of 1 M NaOH was added to the muscle such that the concentration of the resulting solution was 40 mg $mL^{-1}$. The solution was heated at 60°C for 30 min to dissolve the tissue. Decomposition of each quinolone during this procedure was less than 5%. An internal standard (compound 1 for PZFX and OFLX, and TMFX for SPFX) was added, the solution mixed with methanol and centrifuged. The supernatant was neutralized with 1 M $KH_2PO_4$ and HCl. The solution was centrifuged again and the supernatant, which was made up of both organic and aqueous parts, was evaporated until no organic solution remained. To the aqueous solution, 0.1 M phosphate buffer (pH 7.4) was added and the solution extracted in chloroform by solid-phase extraction (Chem Elut; Varian, Harbor City, CA, USA). The eluate was evaporated to dryness, and the residue dissolved in the HPLC mobile phase. The recovery of each quinolone in this sample preparation was approximately 70%.

For the measurement of PIPC in the muscle and plasma, the muscle was homogenized with four times its volume of 0.2 M phosphate buffer (pH 5.5) containing internal standard, PCG. The homogenate was added to an equal volume of acetonitrile and centrifuged. The supernatant was added to an equal volume of chloroform and centrifuged. The upper aqueous layer was then used for HPLC. The plasma was added to four times its volume of 0.2 M phosphate buffer (pH 5.5) containing PCG and then the same procedure as for the muscle sample was carried out. The recovery of PIPC in this sample preparation was over 70%.

*Chromatography conditions*

The concentration of each drug was determined by HPLC using a reversed-phase column and isocratic elution. HPLC conditions were designed to obtain high specificity for the peak of each drug and internal standard. The conditions for each sample were as follows.

For the measurement of PZFX and OFLX in plasma, urine and ultrafiltrate, the analytical column used was a Develosil ODS-HG-5 (150 mm × 4 mm i.d.; Nomura kagaku Co. Ltd, Japan) with a mobile phase of 0.2 M phosphate buffer (pH 7.0)/acetonitrile/water (5:9:86, v/v/v) containing 0.68% (g $mL^{-1}$) tetra-*n*-butyl ammoniumhydrogensulfate (TBA-HS) and UV detection at a wavelength of 330 nm.

For the measurement of SPFX in plasma and the ultrafiltrate, the analytical column used was a Develosil ODS-HG-5 (150 mm × 4 mm i.d.) with a mobile phase of 0.2 M phosphate buffer (pH 7.0)/acetonitrile/water (5:13:82, v/v/v) containing 0.68% (g $mL^{-1}$) TBA-HS and UV detection at a wavelength of 300 nm.

For the measurement of SPFX in urine, the conditions were the same as for plasma except that the mobile phase was 0.2 M phosphate buffer (pH 7.0)/acetonitrile/water (5:9:86, v/v/v) containing 0.34% (g $mL^{-1}$) TBA-HS.

For the measurement of PIPC in plasma and the muscle, the analytical column used was a STR ODS-II (150 mm × 4.6 mm i.d.; GL Science Inc., Tokyo, Japan) with a mobile phase of 0.2 M phosphate buffer (pH 5.5)/acetonitrile/water (10:16:74, v/v/v) and UV detection at a wavelength of 220 nm.

For the measurement of PZFX and OFLX in the muscle, the analytical column used was an Inertsil ODS-II (150 mm × 4.6 mm i.d.; Shimadzu Techno Research Inc., Kyoto, Japan) with a mobile phase of 0.2 M citrate buffer (pH 2.5)/acetonitrile/water (10:245:745, v/v/v) containing 0.08% (g $mL^{-1}$) sodium octanesulfonate (SOS) and measurement by fluorescence detection (excitation 330 nm, emission 394 nm for PZFX; excitation 333 nm and emission 394 nm for OFLX).

For the measurement of SPFX in the muscle, the analytical column used was an Inertsil ODS-II (150 mm × 4.6 mm i.d.) with a mobile phase of 0.2 M citrate buffer (pH 3.5)/acetonitrile/water (1:28:71, v/v/v) containing 0.08% (g $mL^{-1}$) SOS and UV detection at a wavelength of 300 nm.

### Pharmacokinetic analysis

Systemic clearance (CL) was calculated as the intravenous dose divided by the AUC from zero to infinity calculated by the trapezoidal method. The renal clearance ($CL_r$) was calculated as the slope in the following equation:

$$Xu_{(t)} = CL_r AUC_{(t)} \quad (1)$$

where $Xu_{(t)}$ is the amount of drug excreted in the urine at time t calculated from the pooled urine volume and its concentration, and $AUC_{(t)}$ is the AUC from zero to time t.

**Table 1** Pharmacokinetic parameters of pazufloxacin, ofloxacin and sparfloxacin in rats.

| Parameter | Pazufloxacin | Ofloxacin | Sparfloxacin |
|---|---|---|---|
| t1/2 (h) | 1.08±0.11* | 1.50±0.18 | 2.58±0.42 |
| AUC ($\mu$g min $mL^{-1}$) | 562±89 | 565±80 | 575±51 |
| CL (mL $min^{-1}$ $kg^{-1}$) | 18.1±2.9 | 18.0±2.8 | 17.5±1.6 |
| $CL_r$ (mL $min^{-1}$ $kg^{-1}$) | 12.6±1.6* | 8.72±1.64 | 0.704±0.165 |
| $CL_h$ (mL $min^{-1}$ $kg^{-1}$) | 5.52±1.38* | 9.25±1.17 | 16.8±1.7 |
| $Vd_{ss,plasma}$ (L $kg^{-1}$) | 0.945±0.152* | 1.83±0.27 | 3.42±0.67 |
| $f_p$ | 0.78±0.06 | 0.77±0.10 | 0.55±0.06 |
| $CL_{int,kidney}$[a] (mL $min^{-1}$ $kg^{-1}$) | 17.4 | 12.9 | 0.901 |
| $CL_{int,liver}$[a] (mL $min^{-1}$ $kg^{-1}$) | 6.39 | 13.3 | 26.6 |

Pazufloxacin, ofloxacin and sparfloxacin were administered to rats intravenously at a dose of 10 mg $kg^{-1}$, and the drug concentration in the plasma and urine was determined by HPLC. In-vitro binding to serum protein was measured by ultrafiltration. Each result is represented as the mean (n = 3) or mean±s.d. (n = 3; or n = 4 in the case of SPFX). The analysis of significant differences was achieved using non-parametric Tukey's test. *$P < 0.05$, significantly different compared with sparfloxacin. [a]Values are calculated using Rb values that were calculated from distribution data reported previously (Okazaki 1984; Matsunaga 1991; Hayakawa 1999).

The hepatic clearance ($CL_h$) was obtained by subtracting $CL_r$ from CL. $Vd_{ss,plasma}$ denotes the $Vd_{ss}$ calculated from the plasma concentration data (observed $Vd_{ss}$). The $Vd_{ss,plasma}$ was calculated using the following equation:

$$Vd_{ss,plasma} = \text{dose}MRT/AUC \qquad (2)$$

where MRT is the mean residence time. The tissue to plasma concentration ratio, $K_p$, was calculated using the following equations (3–6) according to the method previously reported (Okezaki et al 1988). The apparent tissue-to-plasma concentration ratio ($K_{p,app}$) and whole blood-to-plasma concentration ratio (Rb) were obtained as the tissue concentration or whole blood concentration divided by the serum or plasma concentration. The distribution data used for the $K_{p,app}$ and Rb and the slope of the terminal phase ($\beta$) were obtained from previously published reports (Okazaki et al 1984; Matsunaga et al 1991; Hayakawa et al 1999). The tissue volume (V) and blood flow rate (Q) used for the calculation were cited from a previously published report (Okezaki et al 1988).

$$K_{p,lung} = K_{p,app,lung}Q_{lung}/(Q_{lung} - \beta V_{artery}) \qquad (3)$$

$$K_{p,liver} = K_{p,app,liver}(RbQ_{liver} + f_pCL_{int,liver}) / (\beta V_{liver}K_{p,app,liver} + Rb[(Q_{liver} - Q_{spleen} - Q_{git}) + Q_{spleen}(K_{p,app,spleen}/K_{p,spleen}) + Q_{git}(K_{p,app,git}/K_{p,git})]) \qquad (4)$$

$$K_{p,kidney} = K_{p,app,kidney}(RbQ_{kidney} + f_pCL_{int,kidney}) / (RbQ_{kidney} + \beta V_{kidney}K_{p,app,kidney}) \qquad (5)$$

$$K_{p,other} = K_{p,app,other}RbQ_{other}/(RbQ_{other} + \beta V_{other}K_{p,app,other}) \qquad (6)$$

In equations 3–6, $f_p$ is the fraction not bound to the serum protein, $CL_{int}$ is the intrinsic clearance calculated using Q, $f_p$, Rb and $CL_r$ or $CL_h$. Subscripts indicate the types of tissue: lung, liver, kidney, spleen, gastrointestinal tract (git), artery and other tissues.

$Vd_{ss,tissue}$ denotes the $Vd_{ss}$ calculated from the $K_p$ value of each tissue and each tissue volume (total $Vd_{ss}$). The $Vd_{ss,tissue}$ was calculated by the following equation:

$$Vd_{ss,tissue} = V_p + \sum K_pV \qquad (7)$$

where $V_p$ is the plasma volume.

### Statistical analysis

The analysis of significant differences between pharmacokinetic parameters was achieved using the non-parametric Tukey's test. The level of significance was set at $P < 0.05$. For Figures 3 and 4, linear regression analysis was used to determine the slope. The test for significance of the regression was performed. For comparison of CLuptake, the comparison of two regression slopes in Figure 4 was performed. The level of significance was set at $P < 0.05$.

## Results

### Plasma concentrations of PZFX, SPFX, OFLX and pharmacokinetic parameters

PZFX had a higher initial plasma concentration, but it decreased faster, with a 1.4- to 2.4-fold shorter half-life compared with that of OFLX and SPFX following intravenous administration at a dose of 10 mg $kg^{-1}$. The estimated pharmacokinetic parameters are listed in Table 1. Although the contribution of the renal clearance to the systemic clearance for PZFX was larger than that of OFLX and SPFX, there was no significant difference between the systemic clearance values. The $Vd_{ss,plasma}$ value of PZFX was significantly smaller than that of SPFX (Table 1).

### Extent of the contribution of each tissue to the $Vd_{ss}$

When a drug was administered by an intravenous bolus injection, as reported for PZFX and OFLX, a pseudo-distribution equilibrium was reached following an initial distribution. During this terminal phase, $K_p$ values for the lung, liver, kidneys, and other tissues were calculated using equations 3–6. Then, $Vd_{ss,tissue}$ was estimated by equation 7. In the case of SPFX, the only reported distribution data was from a study in which the drug was administered orally. Therefore, mean values of $K_{p,app}$ during the terminal phase were used instead of $K_p$. $Vd_{ss,tissue}$ values (expressed as total $Vd_{ss}$ in Figure 2) for PZFX and OFLX calculated using equation 7 were in good agreement with the $Vd_{ss,plasma}$ obtained from the plasma concentration–time curves (expressed as observed $Vd_{ss}$ in Figure 2). The $Vd_{ss,tissue}$ for SPFX was also comparable with the $Vd_{ss,plasma}$, considering the $K_{p,app}$ was only estimated for the calculation. It is possible to evaluate the extent of contribution to $Vd_{ss}$ in each tissue using $K_pV$. As shown in Figure 2, quinolones are mainly distributed in muscle.

### Binding in the muscle

Quinolone muscle binding was directly proportional to the protein concentration in the muscle homogenate, indicating that the binding parameter was not affected by dilution and that Kd was much larger than $C_f$ (Figure 3). Therefore, according to equation 8, it is possible to calculate nP/Kd from the slope of the curve of $C_b$ vs $C_f$ by extrapolating to 100% homogenate.

$$C_b = (1/d)nPC_f/(Kd+C_f) \qquad (8)$$

where d is the dilution factor, nP is the maximum binding capacity, Kd is the dissociation constant, $C_b$ is the concentration of bound quinolones, and $C_f$ is the concentration of unbound quinolones. The unbound fractions of

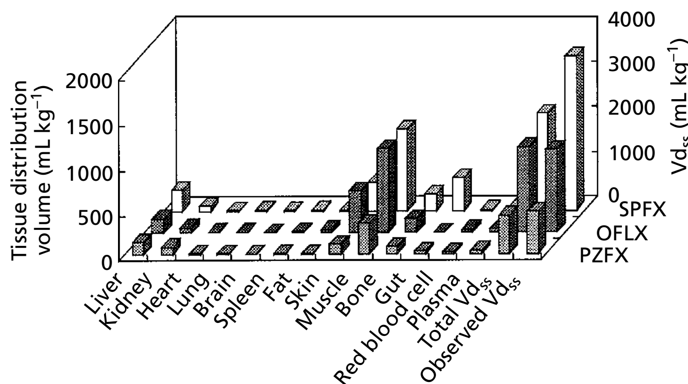


**Figure 2** The contribution of each tissue to the distribution volume at steady state ($Vd_{ss}$). The distribution volume of each tissue was calculated as $K_pV$; $K_p$ was calculated from the data listed in Table 1 and previously reported distribution data (Okazaki 1984; Matsunaga 1991; Hayakawa 1999). The total $Vd_{ss}$ ($Vd_{ss,tissue}$) is the summation of $K_pV$ for each tissue and the plasma volume. The observed $Vd_{ss}$ are $Vd_{ss,plasma}$ listed in Table 1. The y axis on the right corresponds to the total and the observed $Vd_{ss}$, whereas the y axis on the left corresponds to the distribution volume of each tissue.

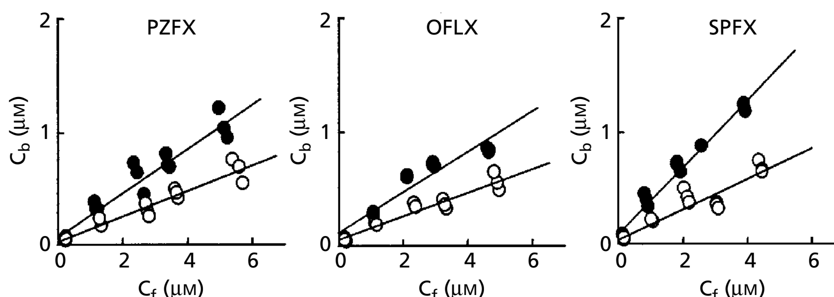


**Figure 3** Binding of pazufloxacin (PZFX), ofloxacin (OFLX) and sparfloxacin (SPFX) in rat muscle. Each drug was incubated with 20% (●) and 10% (○) muscle homogenate, and then the concentrations of unbound drug ($C_f$) were measured by HPLC after ultrafiltration. The lines represent the linear regression of bound drug, $C_b$, vs $C_f$. The *P* values for testing the slope of the regression line were less than 0.05 for all regressions. The regression lines were as follows (95% confidence limits in parentheses); PZFX (10%): y = 0.111 (0.092–0.131)x+0.024 (−0.041–0.089), PZFX (20%): y = 0.196 (0.163–0.229)x+0.062 (−0.038–0.163), OFLX (10%): y = 0.104 (0.086–0.121)x+0.050 (−0.001–0.101), OFLX (20%): y = 0.177 (0.143–0.211)x+0.108 (0.017–0.199), SPFX (10%): y = 0.135 (0.103–0.167)x+0.039 (−0.045–0.123), SPFX (20%): y = 0.296 (0.271–0.321)x+0.085 (0.028–0.141).

quinolones, $f_t$, in the muscle were then calculated from the following equation:

$$f_t = C_f/(C_f+nPC_f/Kd) \quad (9)$$

The ranges of $f_t$ values were calculated from equation 9 using 95% confidence limits of the slope of the curve of $C_b$ vs $C_f$ by extrapolating to 100% homogenate. The $f_t$ values of tested quinolones and the range calculated from 95% confidence limits (in parentheses) were 0.486 (0.458–0.517) for PZFX, 0.507 (0.478–0.540) for OFLX, and 0.417 (0.390–0.449) for SPFX. PZFX had almost the same $f_t$ as OFLX. SPFX had a slightly lower $f_t$ than PZFX and OFLX. The same results were observed for $f_p/f_t$. The $f_p/f_t$ value of PZFX (1.60, range 1.51–1.70) was almost the same as OFLX (1.52, range 1.43–1.61), and slightly higher than that of SPFX (1.32, range 1.22–1.41).

### Tissue CLuptake in muscle

The integration plot of $X_{(t)}/C_{p(t)}$ vs $AUC_{(t)}/C_{p(t)}$ showed a linear relationship until the terminated time, which did not exceed 80 s (Figure 4). The CLuptake was obtained from the slope of the plots. The CLuptake and its 95% confidence limits (in parentheses) were 0.012 mL $min^{-1}$ $g^{-1}$ (0.0002–0.023) for PZFX, 0.118 mL $min^{-1}$ $g^{-1}$ (0.039–0.197) for OFLX, 0.195 mL $min^{-1}$ $g^{-1}$ (0.004–0.386) for SPFX and 0.008 mL $min^{-1}$ $g^{-1}$ (−0.013–0.029) for PIPC. The CLuptake of PZFX in muscle was significantly different from that of OFLX and SPFX ($P < 0.01$), which showed a low CLuptake similar to that of the $\beta$-lactam antibiotic PIPC.

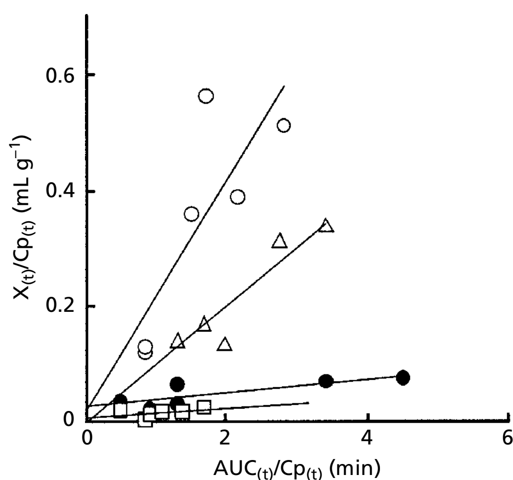


**Figure 4** The integration plot for the determination of muscle uptake clearance: ○, 2 mg $kg^{-1}$ sparfloxacin (SPFX); △, 2 mg $kg^{-1}$ ofloxacin (OFLX); ●, 2 mg $kg^{-1}$ pazufloxacin (PZFX); □, 40 mg $kg^{-1}$ piperacillin (PIPC). After intravenous administration of each drug tested, both plasma concentration–time profiles and concentrations in the muscle were measured by HPLC. The lines represent the linear regression of $AUC_{(t)}/C_{p(t)}$ vs $X_{(t)}/C_{p(t)}$. The $P$ values for testing the slope of the regression line were less than 0.05 for all regressions except for PIPC. The slope of the regression lines and 95% confidence limits (in parentheses) were as follows; PZFX: 0.012 (0.0002–0.023), OFLX: 0.118 (0.039–0.197), SPFX: 0.195 (0.04–0.386), PIPC: 0.008 (−0.013–0.029). The results of comparison of two regression slopes were significant for PZFX vs OFLX ($P < 0.01$) and PZFX vs SPFX ($P < 0.01$).

## Discussion

We previously reported that there was a linear relationship between $Vd_{ss}$ and $f_p$ (Okezaki et al 1988) for certain quinolones tested, and that the $Vd_{ss}$ could be predicted from the $f_p$. However, the novel quinolone, PZFX, does not follow this rule. SPFX is another quinolone that does not follow the rule. From the results of a previous report (Okezaki et al 1988), estimated values of $Vd_{ss}$ for PZFX and SPFX were 1.6 L $kg^{-1}$ and 1.1 L $kg^{-1}$ respectively. In the case of PZFX, the observed $Vd_{ss}$ (Table 1) was considerably lower than the estimated value of 1.6 L $kg^{-1}$. Conversely, that of SPFX was higher than the estimated value of 1.1 L $kg^{-1}$. In order to account for these deviations, we decided to investigate the distribution properties of the above mentioned quinolones in comparison with that of OFLX, whose $Vd_{ss}$ can be estimated from its $f_p$ value. The $Vd_{ss,plasma}$ calculated from plasma concentration was comparable with the $Vd_{ss,tissue}$ for each quinolone. Thus, the extent of the contribution to the $Vd_{ss}$ in several types of tissue was evaluated using the equation: $K_pV/Vd_{ss,tissue}$ where V is the volume of tissue tested. PZFX, OFLX and SPFX were all mainly distributed in muscle (Figure 2), with contribution values of 40, 49 and 39% for each respective quinolone tested. These results indicate that muscle plays the most important role in determining the value of $Vd_{ss}$ for the quinolones tested. Therefore, we investigated the distribution properties of the quinolones in muscle. The $Vd_{ss}$ in muscle can be evaluated using the following equation:

$$Vd_{ss,muscle} = qVf_p/f_t \quad (10)$$

where V is muscle volume and q is the accumulation factor in muscle cells. The ratio of intracellular to plasma unbound concentration and/or accumulation in a cell by the pH partition theory contribute to the q value. The variables in equation 10 that could cause deviations from an estimated value of $Vd_{ss}$ using $f_p$ are q and $f_t$. PZFX, OFLX and SPFX have $Vd_{ss}$ values that are larger than the extracellular space volume ($Vd_{ss}$ of inulin was 0.34 L $kg^{-1}$), which suggests that the quinolones penetrate the cells and bind to intracellular fluid protein (Table 1). This binding to intracellular protein could be responsible for the change in $Vd_{ss}$, and therefore the difference in binding to intracellular protein was investigated. The $f_t$ values were almost comparable among the three quinolones tested, consistent with the assumption that tissue binding of quinolones would not be significantly different (Okezaki et al 1988). Additionally, the $f_p/f_t$ values of PZFX, OFLX and SPFX were comparable; thus the tissue binding in the muscle and plasma protein binding cannot be solely accountable for the low $Vd_{ss}$ value of PZFX. According to pH partition theory, a pH gradient across cell membranes could give rise to the accumulation of certain compounds within cells, thus contributing to the other variable, q. Quinolones, being amphoteric by nature, have both acidic and basic properties, and at a given pH they can exist as a mixture of

three species (acidic, basic and neutral). Thus, the accumulation factor ($q_{pH}$) in a cell caused by pH gradient can be expressed as follows:

$$q_{pH} = (1+10^{pK_a1-pH_i}+10^{pH_i-pK_a2})/(1+10^{pK_a1-pH_e}+10^{pH_e-pK_a2}) \quad (11)$$

where $pK_a1$ and $pK_a2$ are the acid dissociation constant for each quinolone, $pH_e$ and $pH_i$ are the pH values in the extracellular and intracellular fluids, respectively. The $pK_a1$ and $pK_a2$ values for OFLX were 5.9 and 8.8 (Imanura et al 1991), and those of SPFX were 6.27 and 8.8 (Segawa et al 1994), respectively. We determined the $pK_a1$ and $pK_a2$ of PZFX (5.5 and 7.8, respectively) and compared the $q_{pH}$ value of PZFX with those of OFLX and SPFX. Values of 7.4 and 7.0 were used for $pH_e$ and $pH_i$, respectively, as reported previously (Roos & Boron 1981). The $q_{pH}$ values obtained were comparable, having values of 0.84 for PZFX, 1.02 for OFLX, and 1.08 for SPFX. Therefore, the difference in $pK_a$ values did not account for the low $Vd_{ss}$ of PZFX.

The q value could also be affected by the ratio of influx and efflux clearance in the muscle cells if there exists an active-transport mechanism. Interestingly, the CLuptake of PZFX in muscle was significantly low in comparison with that of OFLX and SPFX (Figure 4), and was in fact more comparable with that of penicillin. It is generally accepted that the apparent transfer rate from the capillary vessel to the interstitial fluid for a protein bound drug is slower than that for an unbound drug. The $f_p$ of PZFX was comparable with that of OFLX and higher than that of SPFX (Table 1). Thus, even if considering the protein binding, the CLuptake of PZFX was lower than other quinolones tested. Therefore, the difference between PZFX and the other quinolones in muscular CLuptake is believed to be reflected in the permeation properties of the cell. Recently, it has become known that certain transporters are expressed in muscle plasma membrane in rats and humans (Tamai et al 1998; Berardi et al 2000; Zorzano et al 2000). In addition, we have reported that quinolones (OFLX and lomefloxacin) were taken up by rat erythrocytes via a transport system common to that of nicotinic acid (Simanjuntak et al 1991). At the time, it was not clear whether the efflux clearances of those quinolones were different or not; however, there is a possibility that the low CLuptake into the muscle could be a determinant for a low $Vd_{ss}$ of PZFX. Our results may be the first to observe that a transporter or a number of transporters could be the determinant of $Vd_{ss}$ for some quinolones.

## Conclusion

In this study, in order to clearly understand the difference in $Vd_{ss}$, we investigated the distribution properties of PZFX in comparison with OFLX and SPFX. The quinolones were mainly distributed in the muscle, which was the tissue that contributed the most to the $Vd_{ss}$. Binding to intracellular proteins of the muscle and the existence of a pH gradient across cell membranes did not account for the significantly lower value of $Vd_{ss}$ for PZFX when compared with the other quinolones tested. However, the CLuptake of PZFX in the muscle was significantly lower than that of OFLX and SPFX. Therefore, there is a possibility the low $Vd_{ss}$ value of PZFX can be attributed to its low CLuptake in the muscle.

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