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Application of Muscle Microdialysis to Evaluate the Concentrations of the Fluoroquinolones Pazufloxacin and Ofloxacin in the Tissue Interstitial Fluids of Rats

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Abstract

Muscle microdialysis has been used to determine the unbound concentrations of the fluoroquinolones, pazufloxacin and ofloxacin, in tissue interstitial fluids ($C_{isf,u}$) of rats under steady state conditions.

$C_{isf,u}$ was estimated from the concentration in dialysate and the in-vitro permeability rate constant by the extrapolation method based on the clearance concept. Paper disks were inserted under the abdominal skin of rats, and the drug concentrations in the fluids penetrating into the disks (C_{disk}) were measured and compared with $C_{isf,u}$. The $C_{isf,u}$ of pazufloxacin and ofloxacin in muscle were close to their unbound concentrations in the venous plasma; these were 75·3% and 77·1%, respectively, of the total concentrations in plasma at the steady state. The C_{disk} of pazufloxacin and ofloxacin were also close to their $C_{isf,u}$.

These results indicate that the unbound concentrations of the fluoroquinolones in the tissue interstitial fluids were the same as those in the venous plasma. The disk insertion technique seems to be useful for evaluating drug concentrations in tissue interstitial fluid.

Because bacterial infections are mostly restricted to the extravascular interstitial space of organs, to evaluate the in-vivo efficacy of a drug it is important to know its in-vivo unbound concentration in tissue interstitial fluid ($C_{isf,u}$). Microdialysis has previously been proposed as a method of extrapolating the in-vivo concentration of unbound drug in tissue interstitial fluid from the concentration in a dialysate sample (Deguchi et al 1991), and has been used to evaluate the tissue interstitial concentration of β-lactam or aminoglycoside antibiotics (Deguchi et al 1992; Eisenberg et al 1993). It was reported (Deguchi et al 1992) that the $C_{isf,u}$ of β-lactam antibiotics which were restricted to the interstitial space in a non-eliminating organ were close to the unbound concentrations in the venous plasma ($C_{p,u}$). However, there have been no reports of the $C_{isf,u}$ of fluoroquinolones, compounds reported to show good penetration of tissue cells (Easmon et al 1986; Pascual et al 1989, 1990; Mikami et al 1995).

The purposes of this study were to use muscle microdialysis to evaluate $C_{isf,u}$ for the fluoroquinolones pazufloxacin and ofloxacin in rats, and to compare $C_{isf,u}$ and $C_{p,u}$ values. Furthermore, we attempted to examine the usefulness of paper-disk insertion for evaluating drug concentrations in tissue interstitial fluid.

Materials and Methods

Materials

The methanesulphonic acid salt of pazufloxacin (Lot no 04214) was synthesized; ofloxacin was extracted from commercial products (Daiichi Pharmaceutical, Tokyo, Japan) in the research laboratory of Toyama Chemical. The chemical structures of pazufloxacin and ofloxacin are shown in Fig. 1.

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Animals

Male Wistar rats (Japan SLC, Shizuoka, Japan), 250–350 *g*, were used in all experiments. Before the experiments they had free access to food and water.

Microdialysis fibre

The microdialysis fibre was purchased from Eicom (Kyoto, Japan) and was prepared as described by Deguchi et al (1991) using Cuprophan hollow-fibre (inside diameter 0·2 mm, wall thickness 11 μm, MW cut-off 12 500) and stainless steel tubing (outside diameter 0·2 mm; MT Giken, Tokyo, Japan). The fibre consists of a 22-mm long segment of hollow dialysis fibre 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 fibre.

In-vitro microdialysis

In-vitro microdialysis was performed by the method described by Deguchi et al (1991). The microdialysis fibre was linked with polyethylene tubing (SP10, Natsume Seisakusyo, Tokyo, Japan) and was soaked in reservoir medium, Ringer-HEPES buffer (RHB; 141 mM NaCl, 4 mM KCl, 2·8 mM $CaCl_2 \cdot 2H_2O$, 10 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulphonic acid (HEPES), pH 7·40) containing pazufloxacin or ofloxacin (5 μg mL^{-1}) placed in a glass plate at 37°C without any mixing. RHB was perfused through the fibre at the constant flow rate of 2·5 μL min^{-1} which was controlled by means of a syringe infusion pump (Model 230, Neuroscience, Tokyo, Japan). After an appropriate time lapse to enable the establishment of steady state conditions the dialysate was collected during five sequential 10-min periods. Reservoir medium, (10 μL) was sampled at the midpoint of each collection period.

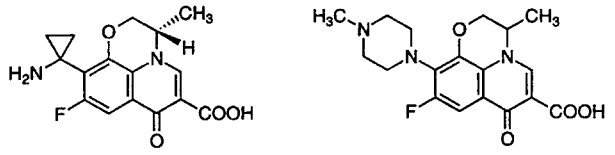

FIG. 1. The chemical structures of pazufloxacin and ofloxacin.

Microdialysis data were analysed by use of the steady state clearance concept, as described previously in detail by Deguchi et al (1991). The in-vitro permeability rate constant (PA_{vitro}) was calculated by use of equation 1 using the in-vitro dialysis flow rate (F_{vitro}), the dialysate concentration of drug at steady state (C_d) and the reservoir concentration of drug (C_r) in RHB.

$$PA_{vitro} = -F_{vitro} \times \ln(1 - C_d/C_r) \quad (1)$$

In-vivo microdialysis study

Rats were intramuscularly anaesthetized with ketamine (235 mg kg^{-1}; Sankyo, Tokyo, Japan) and xylazine (2·3 mg kg^{-1}; Sigma, St Louis, MO) and were warmed with lamps to maintain the body temperature at 37°C. The left femoral vein was cannulated with polyethylene tubing filled with heparin-saline solution (100 units mL^{-1}) for constant infusion of a maintenance dose (see below). The microdialysis fibre was implanted in the right hind leg muscle and RHB was perfused throughout the fibre at the constant flow rate of 2·5 $\mu L\ min^{-1}$ controlled by means of an infusion pump. The fibre was the same as that used in the corresponding in-vitro experiments.

Fifteen minutes after the start of dialysis, bolus doses of the drugs (pazufloxacin 10·6 mg kg^{-1}, ofloxacin 22·8 mg kg^{-1}) were administered intravenously via the right jugular vein, immediately followed by the constant infusion of a maintenance dose (pazufloxacin 17·0 mg $kg^{-1}\ min^{-1}$, ofloxacin 20·2 mg $kg^{-1}\ min^{-1}$) at a constant flow rate of 10 $\mu L\ min^{-1}$ by use of an infusion pump. Each bolus dose and maintenance dose of the drug was calculated on the basis of the pharmacokinetic data in serum after intravenous administration to rats. Forty-five minutes after bolus injection of each substance, dialysate samples were collected during five sequential 10-min periods. Blood samples (0·3 mL) were collected into heparinized tubes at the midpoint of each collection period. Plasma was separated from blood by centrifugation at 1000 *g* for 10 min at 4°C.

As described previously in detail (Deguchi et al 1991), $C_{isf,u}$ was defined as follows:

$$C_{isf,u} = C_{d,vivo}/(1 - \exp(-RD \times PA_{vitro}/F_{vivo})) \quad (2)$$

where $C_{d,vivo}$ is the in-vivo dialysate concentration of the drug at steady state and F_{vivo} is the in-vivo dialysis flow rate. In addition, RD is the effective dialysis coefficient which is defined as the ratio PA_{vivo}/PA_{vitro}, and is independent of molecular weight and plasma membrane permeability. The RD value used, 0·367, was that obtained previously (Deguchi et al 1991).

Insertion of disk

Before the start of the in-vivo microdialysis study, paper disks (diameter 8 mm, thickness 1·5 mm, Toyo Roshi Kaisha, Tokyo, Japan) were inserted into the subdermal cavity under the abdominal skin of rats (3 disks per rat).

Immediately after final sampling of the dialysate, the disks were removed from the subdermal cavity. The volume of fluid which had penetrated into the disk was calculated from the difference between the weights of the disk before and after insertion. The concentrations of drug in the fluids which had penetrated into the disks (C_{disk}) were measured by HPLC.

In-vivo plasma unbound fraction under steady state conditions

Forty-five minutes after bolus injection then constant infusion of a maintenance dose of each substance, as described above, blood samples were collected into heparinized tubes for three sequential 10-min intervals and the plasma was separated. Ultrafiltration of the plasma was performed at 1000 *g* for 10 min at 4°C using a micropartition system (MPS-1; Amicon, Danvers, MA). The concentrations of pazufloxacin and ofloxacin in the plasma (total concentration, C_p) and filtrate (unbound concentration, $C_{p,u}$) were assayed by HPLC, and the ratio of the plasma unbound fraction was calculated from $(C_{p,u}/C_p) \times 100$.

Analytical procedure

The concentrations of pazufloxacin or ofloxacin in the samples were determined by HPLC. Samples from plasma, dialysate, and disks were prepared for analysis by addition of an appropriate volume of the mobile phase solution, vigorous mixing, and then centrifugation at 1000 *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 × 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 30% acetonitrile in 10 mM citrate buffer (pH 3·5) containing 0·15% sodium 1-octanesulphonate (Tokyo Kasei Kogyo Co. Ltd, Tokyo, Japan). The HPLC conditions used were: room temperature, flow rate 1·0 mL min^{-1}, excitation and emission wavelengths 330 nm and 394 nm, respectively, for pazufloxacin and 333 nm and 494 nm, respectively, for ofloxacin.

Results

In-vitro microdialysis

The PA_{vitro} value for each fibre was estimated by in-vitro microdialysis. The values for pazufloxacin and ofloxacin were 0·350 − 0·447 (mean ± s.e. 0·396 ± 0·017) and 0·342 − 0·472 (0·390 ± 0·025), respectively, i.e. almost the same PA_{vitro} values were obtained for the two fluoroquinolones.

In-vivo plasma unbound fraction under steady state conditions

Table 1 lists the values obtained for C_p and $C_{p,u}$ under the steady state plasma concentrations, along with the ratios of $C_{p,u}$ to C_p. The C_p values (mean ± s.e.) of pazufloxacin and ofloxacin were 14·4 ± 1·5 $\mu g\ mL^{-1}$ and 16·5 ± 1·1 $\mu g\ mL^{-1}$, respectively. The ratio of $C_{p,u}$ to C_p for pazufloxacin (mean ± s.e. 75·3 ± 1·2%) was close to that for ofloxacin (mean ± s.e. 77·1 ± 1·2%).

Muscle microdialysis

Fig. 2 shows the plots of C_p and $C_{d,vivo}$ against time obtained for pazufloxacin and ofloxacin by the muscle-microdialysis

Table 1. Plasma unbound fractions of pazufloxacin and ofloxacin in rats under conditions of steady-state plasma concentration.

	Concentration in rat plasma ($\mu g\ mL^{-1}$)		Unbound fraction (%)*
	Total	Unbound	
Pazufloxacin	14·4 ± 1·5	10·9 ± 1·2	75·3 ± 1·2
Ofloxacin	16·5 ± 1·1	12·8 ± 1·0	77·1 ± 1·2

Each value is the mean ± s.e. (n = 9). Steady-state conditions were achieved during the sampling intervals by infusing pazufloxacin or ofloxacin (17·0 mg kg^{-1} min^{-1} or 20·2 mg kg^{-1} min^{-1}, respectively) after a priming intravenous bolus dose (pazufloxacin: 10·6 mg kg^{-1}, ofloxacin 22·8 mg kg^{-1}). *$C_{p,u}/C_p \times 100$.

studies. From 50 to 90 min after bolus injection, C_p in each rat was kept at a steady state of 10–21 $\mu g\ mL^{-1}$. The steady state dialysate concentration ($C_{d,vivo}$) in each rat was approximately 0·3–0·9 $\mu g\ mL^{-1}$.

The $C_{isf,u}$ value in muscle was estimated from the values of $C_{d,vivo}$, PA_{vitro} and F_{vivo} at steady state by use of equation 2. The $C_{p,u}$ value was predicted from the C_p value and the ratio of plasma unbound fraction (pazufloxacin 75·3%, ofloxacin 77·1%). The results are listed in Table 2, with the values of C_{disk}. The ratios of $C_{isf,u}$ and C_{disk} to C_p or $C_{p,u}$ are shown in Table 3. The values of $C_{isf,u}$ were close to those of $C_{p,u}$, and were lower than those of C_p. The values of C_{disk} were also close to those of $C_{p,u}$ and $C_{isf,u}$, and were lower than those of C_p. The ratios of $C_{isf,u}$ and C_{disk} to C_p were, respectively, 0·78 and 0·86 for pazufloxacin and 0·74 and 0·72 for ofloxacin. The ratios of $C_{isf,u}$ and C_{disk} to $C_{p,u}$ were, respectively, 1·04 and 1·15 for pazufloxacin and 0·97 and 0·94 for ofloxacin.

Discussion

Because there have been no reports of studies of the distribution of fluoroquinolones in the extravascular interstitial space which is the main site of bacterial infection, we examined the concentrations of these compounds in the interstitial fluid by the muscle microdialysis technique, under steady state conditions, according to the method of Deguchi et al (1991). Muscle was selected as a large volume organ for determination of drug distribution in the body.

As shown in Tables 2 and 3, there was good coincidence between the values of $C_{isf,u}$ and the $C_{p,u}$ in our muscle-microdialysis studies for the fluoroquinolones pazufloxacin and ofloxacin. Similar results have been reported for β-lactam antibiotics (Deguchi et al 1992). These results suggest that the unbound concentrations of fluoroquinolones in the tissue interstitial fluids are the same as those in the venous plasma. It has been reported that drug distribution to the interstitial fluid is an important factor determining the in-vivo efficacy and the pharmacokinetics of a drug (Tsuji et al 1985, 1990). However, the efficacy of the drug in the infectious tissue has usually been predicted from the total concentration in the homogenized tissue. It is believed that fluoroquinolones are particularly effective against infection because higher concentrations are found in homogenized tissue than in plasma. The concentration in the tissue interstitial fluid does not always seem to parallel that in homogenized tissue. From our results, drug

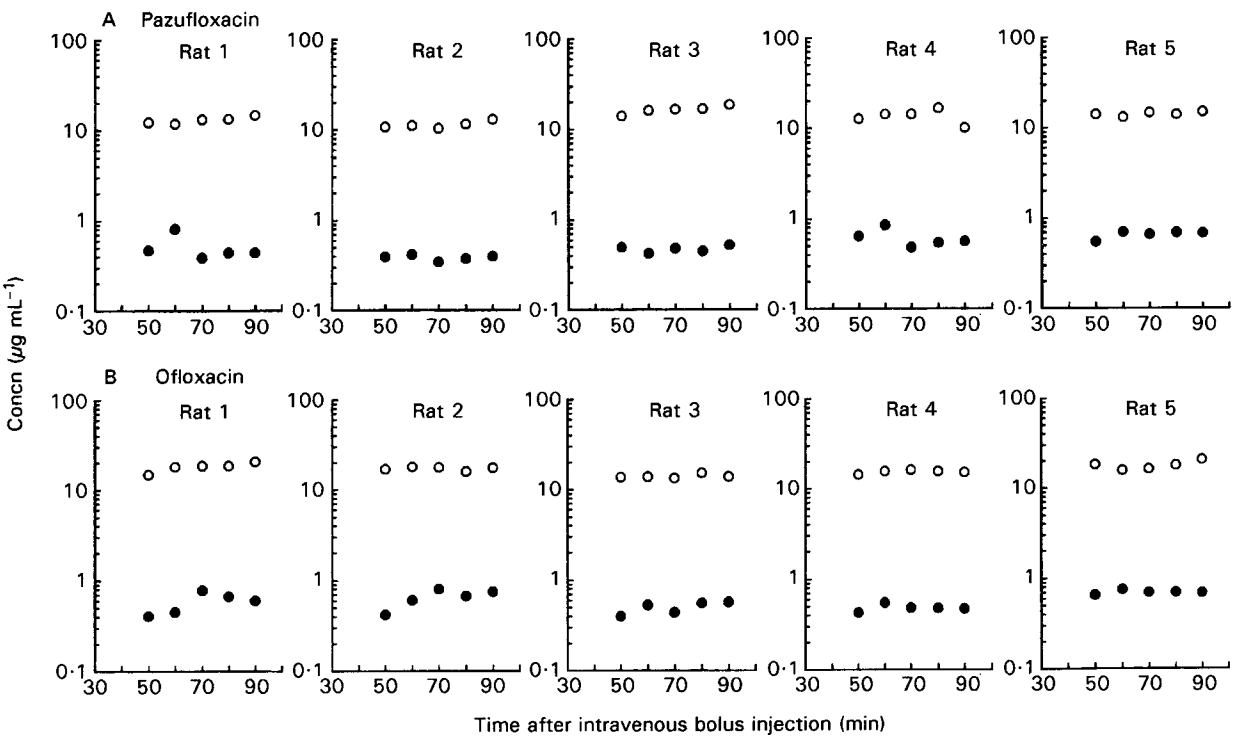

FIG. 2. Time-courses of (A) pazufloxacin and (B) ofloxacin concentrations in plasma (○) and dialysate (●). Each bolus dose of the drug (pazufloxacin 10·6 mg kg^{-1}, ofloxacin 22·8 mg kg^{-1}) was administered intravenously via the right jugular vein of a rat implanted with the microdialysis fibre, immediately followed by constant infusion of a maintenance dose (pazufloxacin 17·0 mg kg^{-1} min^{-1}, ofloxacin 20·2 mg kg^{-1} min^{-1}) into the left femoral vein, cannulated with polyethylene tubing, at the constant flow of 10 $\mu L\ min^{-1}$, by use of an infusion pump. Dialysate samples were collected over 10-min intervals and blood samples were collected into heparinized tubes at the midpoint of each dialysate collection period.

Table 2. Comparison of unbound concentrations in muscle interstitial fluids, in plasma, and in the fluids penetrated into the disks.

Fibre no.	Unbound concentration ($\mu g\ mL^{-1}$)		Total concentration in plasma ($\mu g\ mL^{-1}$)*	Concentration in the fluid penetrated into the disk ($\mu g\ mL^{-1}$§)
	Muscle inerstitial dluid*†	Plasma*‡		
Pazufloxacin				
T-1	9·2 ± 1·2	9·8 ± 0·4	13·1 ± 0·5	11·9 ± 0·5
T-2	7·7 ± 0·2	8·6 ± 0·3	11·4 ± 0·4	9·7 ± 0·7
T-3	9·6 ± 0·3	12·4 ± 0·5	16·5 ± 0·7	13·2 ± 0·8
T-4	13·2 ± 0·5	10·3 ± 0·8	13·6 ± 1·1	12·7 ± 0·5
T-5	13·9 ± 0·5	10·8 ± 0·3	14·3 ± 0·3	11·8 ± 0·3
Mean	10·7 ± 1·2	10·4 ± 0·6	13·8 ± 0·8	11·8 ± 0·6
Ofloxacin				
0-1	13·6 ± 1·1	14·1 ± 0·7	18·3 ± 0·9	12·5 ± 0·2
0-2	12·7 ± 1·2	13·3 ± 0·3	17·3 ± 0·4	11·5 ± 0·5
0-3	11·4 ± 0·6	10·8 ± 0·2	14·0 ± 0·3	12·0 ± 0·7
0-4	11·4 ± 0·4	11·9 ± 0·2	15·4 ± 0·3	9·8 ± 0·6
0·5	12·5 ± 0·2	13·9 ± 0·7	18·0 ± 0·9	13·9 ± 0·2
Mean	12·3 ± 0·4	12·8 ± 0·6	16·6 ± 0·8	11·9 ± 0·7

Values are means ± s.e. Steady-state conditions were achieved during the sampling intervals by infusing pazufloxacin or ofloxacin (17·0 mg kg^{-1} min^{-1} or 20·2 mg kg^{-1} min^{-1}, respectively) after a priming intravenous bolus dose (pazufloxacin 10·6 mg mL^{-1}, ofloxacin 22·8 mg kg^{-1}). *n = 5. †Calculated from equation 2 by use of dialysate concentrations obtained in the muscle-microdialysis studies. ‡Calculated from the total concentrations. §n = 3.

Table 3. Comparison of concentration ratios.

	Pazufloxacin	Ofloxacin
(Unbound concentration in muscle interstitial fluid)*/(Total concentration in rat plasma)	0·78 ± 0·08	0·74 ± 0·02
(Concentration in the fluid penetrating the disk)/(Total concentration in rat plasma)	0·86 ± 0·03	0·72 ± 0·04
(Unbound concentration in muscle interstitial fluid)*/(Unbound concentration in rat plasma)†	1·04 ± 0·11	0·97 ± 0·03
(Concentration in the fluid penetrating the disk)/(Unbound concentration in rat plasma)†	1·15 ± 0·03	0·94 ± 0·05

Values are means ± s.e. (n = 5). *Calculated from equation 2 by use of dialysate concentrations obtained in the muscle-microdialysis studies. †Calculated from the total concentrations in rat plasma.

concentrations in plasma rather than in homogenized tissue, which reflect the concentrations in the interstitial fluids, would be more important for chemotherapy of infections other than intracellular infections. C_{disk} values were close to the $C_{p,u}$, as is shown in Table 3. Consequently the C_{disk} values were also close to $C_{isf,u}$. This suggests that evaluating the concentration of drug in the fluid penetrating into a disk inserted under the skin is also useful for determining the concentration of unbound drug in the tissue interstitial fluid, although this disk insertion technique is applicable to limited tissues owing to the relatively large size of the disk.

In conclusion, the concentrations of unbound fluoro-quinolones in tissue interstitial fluids are in good agreement with those in plasma and in the fluids penetrating into disks under steady state conditions.

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