Salivary therapeutic drug monitoring has many virtues, for instance, non-invasive and painless sampling techniques, and less infection risk associated with sampling of the blood.\(^1\) This method has been better accepted in the areas of pediatrics and geriatrics. In previous studies, it was demonstrated that saliva analysis was a feasible alternative to plasma analysis for therapeutic monitoring in several agents of various classes including phenytoin,\(^2,3\) carbamazepine\(^2,3\) and theophylline.\(^4\)

Fluoroquinolones are a group of relatively new antibiotics with a broad and effective spectrum of antimicrobial activity against both gram-negative and gram-positive bacteria.\(^5\) Although they are widely chosen for the chemotherapy of various infectious diseases, fluoroquinolones have been known to have severe side effects such as seizures, rhabdomyolysis and photosensitivity. Additionally, the pharmacokinetics of fluoroquinolones is easily affected by various clinical situations. It was reported that blood levels of fluoroquinolones were increased in patients with impaired renal function.\(^5\) In contrast, the interactions of fluoroquinolones with antacids were found to reduce the gastrointestinal absorption of the antibiotics.\(^5\) Therefore, blood level monitoring of fluoroquinolones is important to check the variable pharmacokinetics for the effective and safe application of a drug. Since fluoroquinolones have been reported to penetrate extensively into saliva,\(^9\) the alternative use of saliva rather than plasma for guiding individualized pharmacotherapy and clinical decision-making has been viewed as promising for some quinolones.\(^7,8\) Therefore, it is essential to identify the distribution properties of quinolones into saliva to evaluate the feasibility of salivary therapeutic drug monitoring for this class of drugs.

As a basic approach to identifying the distribution mechanism of quinolone antibiotics into saliva, salivary excretion of five fluoroquinolones, ciprofloxacin (CPFX), norfloxacin (NFLX), lomefloxacin (LFLX), ofloxacin (OFLX) and sparfloxacin (SPFX), was compared in rats. Blood, parotid and mandibular saliva were periodically collected from the anesthetized rats after bolus i.v. administration (10 mg/kg) of the quinolones. Quantification of the fluoroquinolones was performed by HPLC methods. The saliva-to-plasma unbound concentration (S/P\(_u\)) ratios of the fluoroquinolones in parotid saliva were larger than those of mandibular saliva. These five quinolones had considerably different S/P\(_u\) ratios from 0.014 to 1.497, while the S/P\(_u\) ratios theoretically calculated by the pH-partition theory were around 1.0 to 1.3, which showed no relationship to the corresponding measured ratios. Satisfactory linear correlations were observed in the plots of measured S/P\(_u\) ratios against 1-octanol–water partition coefficients of the fluoroquinolones in both types of saliva. These results indicate that fluoroquinolones possess different diffusibility in salivary distribution among the drugs and between parotid and mandibular glands. It was also clarified that the lipophilicity of the fluoroquinolones primarily determines the extent of salivary excretion.

Key words fluoroquinolone; lipophilicity; salivary distribution
floxacin (CPF), norfloxacin (NFLX), lomefloxacin (LFLX), ofloxacin (OFLX) and sparfloxacin (SPFX), into rat saliva. The relationship between salivary distribution and physicochemical properties of these quinolones was examined to identify the major determinant(s) in salivary excretion of fluoroquinolones.

MATERIALS AND METHODS

Chemicals NFLX and LFLX hydrochloride were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). The other fluoroquinolones were generously supplied by the respective manufacturers. CPF hydrochloride was from Bayer AG (Leverkusen, Germany), OFLX from Daiichi Seiyaku (Tokyo, Japan), SPFX from Dainippon Pharmaceuticals (Osaka, Japan). All other reagents were commercially available and of analytical or HPLC grade.

Animals Male Wistar rats (Nippon SLC, Hamamatsu, Japan) weighing 370 to 420 g (12 to 14 weeks old) were used in this study. Animals were housed in the feeding room of the laboratory with a 12-h light–dark cycle, steady temperature (23±2 °C) and relative humidity (50±10%). Under anesthetic with sodium pentobarbital (40 to 50 mg/kg, i.p.), rats were cannulated in the right jugular vein with a silicon polymer tube (i.d. 1.0 mm, o.d. 1.5 mm) and in the left femoral vein with a polyethylene tube (PE-50) after catheterization in the trachea. A polyethylene tube (PE-10) was also inserted into duct orifices of the parotid and mandibular salivary glands in the buccal cavity. Constant-rate infusion of pilocarpine at a dose of 5 mg/kg/h was started immediately after the cannulation and continued throughout the experiment to stimulate salivation. A blanket maintained at 37.5 °C was placed under the back of rat lying supine to maintain body temperature until the end of the experiment.

Drug Administration and Sampling The rat was given a bolus dose of 10 mg/kg (as a free base) of the fluoroquinolone via the right jugular vein about 2 h after the beginning of pilocarpine infusion. The parotid and mandibular saliva was separately collected at seven periods: 0—10, 10—30, 30—60, 60—90, 90—120, 120—150, and 150—180 min after administration. The flow rate of salivary fluid was determined to be the weight of the collected saliva during the period based on the specific gravity of saliva being 1.0. About 100 μl of the blood was collected in a heparinized microtube at 3, 10, 30 min and the midpoints (5, 20, 45, 75, 105, 135, 165 min) of the above time periods for saliva collection. The plasma was immediately obtained by centrifugation. All biological samples were kept frozen at −34 °C until analyzed.

At the end of the periodical sampling of blood and saliva, about 4 ml of blood was withdrawn and separated immediately into the serum to determine the protein binding of the drugs and the pH value. Part of the serum was centrifuged in a micropartition device (MPS-3; Amicon, Inc., Beverly, MA U.S.A.) to obtain the ultrafiltrate for determination of the uncoupled fractions of the quinolones. Additional samples of the parotid and mandibular saliva from 180 to 240 min were collected using the microtube with liquid paraffin to prevent pH change during collection. The pH values of the serum and saliva were immediately determined with a compact pH meter (B-211; Horiba Seisakusho, Ltd, Kyoto, Japan).

Determination of the Fluoroquinolone Concentrations The concentration of every fluoroquinolone in the biological samples was determined by HPLC. A reversed-phase Wakosil-II SC18 column (150 mm×4.6 mm i.d.: Wako Pure Chemical Industries, Ltd., Osaka), a LC-10AT pump (Shimadzu, Kyoto), a CTO-10A column oven (Shimadzu) heated at 40 °C and a SIL-10Axl auto injector (Shimadzu) were employed for all assay methods. The assay of OFLX or CPF was performed by the method of Ding et al. CPFX was detected at the excitation and emission wavelengths of 277 and 445 nm, respectively. NFLX levels were determined in accordance with the procedure of Katagiri et al. In the assay of LFLX, the previously reported method was utilized with some modifications in the mobile phase of acetonitrile–0.05 M citric acid–1.0 M ammonium acetate (13:86:1) and the flow rate of 1.0 ml/min. SPFX concentrations were determined by a modified method of the previous papers. A 10 or 20 μl aliquot of the sample was added with 100 μl of the internal standard solution (1 μg/ml of OFLX) and 1.0 ml of acetonitrile in a 2.0-ml plastic tube. The mixture was vortexed for 30 s and centrifuged for 5 min at 10000 rpm. The supernatant (0.9 ml) was collected and evaporated to dryness at 40 °C under a nitrogen gas stream. The residue was reconstituted with 100 μl of NaOH (55 : 45). A fluorescence detector was used at excitation and emission wavelengths of 295 and 525 nm, respectively. The mobile phase consisted of acetonitrile–methanol–0.02 M KH₂PO₄ (10:45:45, pH 2.5) containing 2 mM sodium lauryl sulfate and was pumped at a flow rate of 1.0 ml/min. The assay methods for LFLX and SPFX were validated as sufficiently reliable to determine the concentrations of the drugs in blood and saliva samples.

Determination of the Octanol–Water Partition Coefficient (P') The lipophilicity of the fluoroquinolones was characterized by determining the partition between 0.1 M phosphate buffer (pH 7.4) and 1-octanol by the method used previously. Briefly, each fluoroquinolone solution (10 μg/ml, Cₐ) in the phosphate buffer was agitated with an equal volume of 1-octanol at 15 °C for 24 h, and then centrifuged for phase separation. To minimize the volume change due to mutual miscibility, the phosphate buffer and 1-octanol were previously mixed and sufficiently saturated with each other. The concentration of each fluoroquinolone in the aqueous phase (Cₐ) was determined by the HPLC methods described above. The partition coefficient (P’) is expressed as the ratio of the compound concentration in the organic phase, calculated by subtracting Cₐ from Cₒ to that in the aqueous phase (Cₐ).

Pharmacokinetic Analysis and Statistics A two-compartment model was applied to describe pharmacokinetics of the fluoroquinolones. The pharmacokinetic parameters were calculated by a nonlinear least-squares regression program, WinNonlin (Pharsight Corp., Mountain View, CA, U.S.A.). The saliva-to-plasma concentration ratios (S/P ratios) were used to evaluate the salivary distribution of the drugs and were obtained from the measured saliva levels and the corrected plasma levels. To compensate for the lag time of saliva collection by the cannulae, the midpoint of the saliva collection period was corrected by the salivary flow rate, and then the plasma level at the true midpoint was calculated from the two-compartment model equation obtained by the measured
The saliva-to-plasma unbound concentration ratios (S/Pu ratios) were calculated by dividing S/P ratios by the unbound fractions of the quinolone.

Fluoroquinolones are amphoteric compounds with pK1 and pK2. For the quinolones with pK values listed in Fig. 1, the molecules are believed to exist as zwitterionic, cationic and anionic species and percentages of the uncharged species are very low. On the basis of the pH-partition theory in which it is assumed that the zwitterionic forms being electrically neutral can primarily diffuse across the membrane, the theoretical S/Pu ratios of the fluoroquinolones can be expressed as the following equation which was modified from Matin's equation:

\[
S/P = \frac{1 + 10^{p\text{K}1 - p\text{H}1} + 10^{p\text{K}2 - p\text{H}2}}{1 + 10^{p\text{K}1 - p\text{H}1} + 10^{p\text{K}2 - p\text{H}2}} \times \frac{f_{u,p}}{f_{u,s}}
\]

where pH1 and pH2 represent measured pH values of saliva and plasma, respectively, and fu,p and fu,s represent unbound fractions of the fluoroquinolones in saliva and plasma. Since the protein content in saliva is so low as to be negligible compared to the plasma protein concentration, fu,s was assumed to be 1.20)

The results were represented as the mean±S.D. of the data from individual rats. Statistical significance was considered as p<0.05.

**RESULTS**

**Fluoroquinolone Concentration–Time Profile**

Figure 2 illustrates the mean concentration–time profiles of CPFX, NFLX, LFLX, OFLX and SPFX in plasma and saliva after a single i.v. injection at a dose of 10 mg/kg to rats. The plasma concentration for every quinolone was observed to decline in a biexponential fashion with time. Plasma concentration ranges in the elimination phase were from 0.9 to 1.1 μg/ml for CPFX, from 1.5 to 2.0 μg/ml for NFLX, from 3.1 to 3.4 μg/ml for LFLX, from 2.1 to 2.6 μg/ml for OFLX, and from 2.2 to 2.6 μg/ml for SPFX. The saliva levels of these fluoroquinolones rose quite rapidly after administration, followed by a parallel decline of these levels with the plasma levels. The time required to reach the maximum concentration in mandibular saliva was shorter than that in parotid saliva for every drug. Fractions bound to serum protein were 0.246±0.063, 0.223±0.032, 0.247±0.012, 0.225±0.059 and 0.307±0.063 (n=4) for CPFX, NFLX, LFLX, OFLX and SPFX, respectively. There was no significant difference among the bound fractions for these quinolones (one-way analysis of variance).

**Flow Rate and pH**

The measured pH values were from 7.5 to 7.7 in serum, 7.7 to 8.1 in parotid saliva and 7.8 to 8.1 in mandibular saliva. No significant difference was found among the pH values in rats injected with any quinolone, except that animals given NFLX had slightly but significantly higher pH values of parotid saliva than did other groups. The pH values of plasma samples tended to be lower than those of parotid or mandibular saliva samples.

Saliva flow rates of the rats given each quinolone were 1.81±1.76 μl/min (n=20) and 6.32±4.42 μl/min (n=20) for parotid and mandibular saliva, respectively, the rate of the latter being significantly faster (p<0.01, Student’s t-test).

**Salivary Distribution of the Fluoroquinolones**

Table 1 demonstrates the S/Pu ratios of the five quinolones based on the measured plasma and saliva levels and the S/Pu ratios theoretically calculated with the plasma and saliva pH values and unbound fractions of quinolones. From the plasma concentration–time profiles depicted in Fig. 2, it was obvious that, in every fluoroquinolone, the period after 90 min could be considered as an elimination phase. In this period, the ratios of plasma and saliva levels were almost constant so that the drug distribution between blood and saliva was considered to be in equilibrium. Therefore, the measured S/Pu ratios of the fluoroquinolones were defined as the mean of the ratios in the three sampling periods from 90 to 180 min following drug administration. In both parotid and mandibular saliva, S/Pu ratios widely varied among quinolones. The largest parotid S/Pu ratio for SPFX (1.5) was about sixteen times as large as the smallest one for CPFX. The tendency for the parotid S/Pu ratios to be larger than the mandibular ones was observed and there were significant differences between these two ratios in CPFX, LFLX and SPFX.

The theoretical S/Pu ratios calculated by Eq. 2 are also listed in Table 1. In contrast with the measured ratios, the theoretical ratios of the five quinolones were from about 1.0 to 1.3, and no obvious difference of S/Pu ratios was found between parotid and mandibular saliva.

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**Fig. 2. Time-Courses of Fluoroquinolone Levels in Plasma, Parotid and Mandibular Saliva after Bolus i.v. Administration (10 mg/kg) to Rats**

- **CPFX**: plasma; ▲, parotid saliva; ■, mandibular saliva. Each point and vertical bar represent the mean and S.D. of 3 to 4 rats, respectively. The solid lines of plasma indicate the simulated concentration–time curves obtained by WinNonlin.
Correlation between Lipophilicity and Salivary Distribution of Fluoroquinolones To investigate the correlation between lipophilicity and salivary distribution, the measured S/Pu ratios were plotted against the $P^r$ values (Fig. 3). The quinolones employed in this experiment had widely varied $P^r$ values, and more than a ten fold difference was obtained between CPFX and SPFX. In both saliva specimens, the S/Pu ratios and $P^r$ values showed a positive linear relationship. Regression analysis for parotid and mandibular saliva resulted in the intercepts of $-0.091$ and $-0.0006$, the slopes of $2.84$ and $0.85$, and the coefficients of determination of $0.917$ and $0.977$, respectively. In the parotid saliva, the data of OFLX tended to be considerably below the regression line.

DISCUSSION

Saliva concentration–time profiles of the quinolones were found to be substantially different between parotid and mandibular saliva (Fig. 2). For every quinolone, mandibular saliva showed the maximum level soon after drug administration similar to the plasma level, while the drug levels in parotid saliva rose during the initial 15 min. These results suggest that the permeation rate from blood to salivary fluid differed between parotid and mandibular salivary glands for the quinolones. The flow rate of mandibular saliva was much faster than that of the parotid. Rapid production of the salivary fluid in the mandibular gland could cause better response of salivary quinolone levels to the change in plasma levels. Another gland-type difference is obtained in the S/Pu ratios. Table 1 demonstrates less distribution of quinolones into mandibular saliva compared with parotid saliva. It is well known that many kinds of drugs are translocated from the blood to saliva through the lipid membrane of the acinar cells in the salivary glands. Parotid glands contain many more lipocytes than other salivary glands.21) Histological differences between the salivary glands may be one reason for the more extensive penetration of quinolones in parotid saliva.

Molecular mass, salivary pH, salivary flow rate, degree of ionization, lipid solubility and protein binding are known to be factors that can influence the diffusion of substances into saliva22); therefore, these factors should be considered when salivary distribution of quinolones is discussed. There was no great difference among molecular weights of the quinolones used in this study. In addition, salivary pH values were almost the same in rats administered each quinolone. In each quinolone, the S/Pu ratio had no significant relationship with the flow rate of saliva in either gland (data not shown). Consequently, the effect of salivary flow rate on the S/Pu ratios was viewed as negligible under the experimental conditions in this study. In general, it is believed that only the protein-unbound fraction in the blood can permeate the tissues. Therefore, serum protein binding of quinolones was determined in this study. Bound fractions of the quinolones were relatively small (less than 30%), which was compatible with the findings of previous investigators.23–25) To compensate the variation of protein binding, S/Pu’ ratios were utilized for comparison of salivary distribution among quinolones.

For weakly acidic and basic drugs, only the unionized and unbound form in the blood is thought to penetrate into salivary fluid. Based on the pH-partition theory, the penetrability is determined by the pH of blood and saliva, the $pK_a$ and protein binding of drugs. In regard to quinolones, it was reported that salivary distribution of enoxacin was dependent on the saliva pH.26) To learn whether the degree of ionization is a main factor in the salivary distribution of quinolones, the the-
toretical values of S/Pu ratios were compared with the actually measured ratios in each quinolone. As shown in Table 1, considerable disparities between measured and theoretical S/Pu ratios were observed in both parotid and mandibular saliva. This finding suggests that salivary distribution of fluoroquinolones cannot be explained by the pH-partition theory and that other factor(s) may determine the drug penetration into saliva.

Oil–water partition coefficients of NFLX, CPFX, LFLX, OFLX and SPFX, which are a common index for the lipid solubility of drugs, have been reported in previous studies. However, there has been no report that lipophilicities of these five quinolones were examined in an experiment. Therefore, we determined Pʾ values of the quinolones under the same conditions, and found that the Pʾ values varied greatly. Though the Pʾ value of LFLX measured in our study was higher than that reported, the other quinolones had Pʾ values consistent with the previously published data. Thus, our lipophilicity data were used to characterize salivary distribution of quinolones.

As shown in Fig. 3, linear relationships were obtained between the S/Pu ratios and Pʾ values of these quinolones in parotid and mandibular saliva, suggesting that the lipid solubility is one of the determinants for penetrability of quinolones into saliva. This also implies the possibility that the extent of salivary distribution for quinolones can be predicted by their lipophilicities and protein binding.

In other organs, lipophilicity is reported to be closely related with tissue distribution of quinolones. For examples, it was demonstrated that higher lipophilic quinolone, nalidixic acid, well distributed into cerebrospinal fluid (CSF) despite its high protein binding to serum and smaller portion of non-ionic species; additionally, the penetration of the fluoroquinolones OFLX, SPFX, NFLX and CPFX into brain, CSF and vitreous humor was previously described to be strongly dependent on the individual lipophilicity. Thus, the quinolones are considered to possess a common characteristics in their transport across the biomembranes existing between blood and brain, blood and CSF, and blood and ocular tissue. Obviously, these previous findings support the present suggestion that lipophilicity is one of the determinants of salivary distribution for the fluoroquinolones in rat plasma.

Correlation between salivary distribution and lipophilicity of quinolones in human was studied by Kozjek et al. They demonstrated no relationship between logarithm of the distribution coefficients and S/P ratios of CPFX, NFLX, OFLX and pefloxacin, contradicting the present results. In the study by Kozjek et al., the mixed saliva in the absorption phase was used as samples and protein binding was not taken into account in calculation of the S/P ratios. These differences in experimental methods may explain the discrepancies between the results in the two studies. Species differences, however, should also be considered.

As is clear from Fig. 3, only the S/Pu ratio of OFLX in parotid saliva was substantially smaller than the value obtained from the regression equation, implying that OFLX may not be part of the relationship between the lipophilicity and salivary distribution in parotid saliva. Salivary distribution of this fluoroquinolone may be also influenced by some factor(s) other than lipophilicity. OFLX was reported to be the substrate for the specific transport systems in several kinds of organs, such as kidney, small intestine and the central nervous system. Specific systems transporting various drugs have also been known to play a remarkable role in the salivary glands. In addition, Ding et al. suggested that there was a concentration-dependency of salivary distribution of OFLX in rats. Thus, it is likely that specific transport systems may function in OFLX penetration in the salivary gland. The lower parotid distribution of OFLX presented in Fig. 3 may be induced by this putative specific system, presumably functioning as a reabsorption mechanism from saliva to the circulation. However, the possibility that the transport mechanism may be involved in salivary distribution of the quinolones other than OFLX could not be denied. Further studies are necessary to identify the specific transport mechanism for fluoroquinolones in salivary glands.

In conclusion, this study clarified that the distribution of fluoroquinolones into saliva was mainly governed by their lipophilicities. Fluoroquinolones possessed a different extent of salivary distribution among the drugs from CPFX to SPFX. Furthermore, these drugs can penetrate more easily into parotid saliva than into mandibular saliva.

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