Excretion of Ofloxacin into Saliva in Rats with Renal Failure

GUOHUA DING, KOJI NAORA, SADAO NAGASAKO, HIDENARI HIRANO and KIKUO IWAMOTO
Department of Pharmacy, Shimane Medical University Hospital, 89–1 Enya-cho, Izumo, Shimane 693-8501, Japan
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ABSTRACT
To clarify effects of renal failure on salivary distribution of ofloxacin (OFLX), a quinolone antibiotics, blood, parotid and mandibular saliva were collected from the single-step 5/6th-nephrectomized and sham-operated (control) rats after bolus i.v. administration of OFLX (5 mg/kg). The concentrations of OFLX in these samples were determined by high-performance liquid chromatography. Renal failure induced by the partial nephrectomy significantly elevated plasma levels and cumulative salivary excretion of OFLX when compared to control rats. Total body clearance was significantly decreased by the renal failure, although salivary clearance of the partially nephrectomized rats was about three times larger than that of the control. At the terminal phase, the salivary/plasma concentration ratios of OFLX for parotid and mandibular saliva in control rats was 0.249 ± 0.180 and 0.136 ± 0.024, respectively, and there was a significant difference between both salivary glands. The saliva/plasma concentration ratios in the rats with renal failure were significantly greater than those in the control group in both parotid (about 3.2 times) and mandibular (about 2.5 times) saliva. The results of this study suggest that the salivary excretion of OFLX is significantly increased by renal failure and a glandular difference in the salivary excretion of OFLX exists in both rats with normal and impaired renal function.

OFLX is a synthetic fluoroquinolone antimicrobial agent with a wide spectrum of microorganisms including gram-positive bacteria (Wolfson and Hooper, 1985) and is widely prescribed as the drug of choice to treat various infectious diseases. New quinolone antibiotics including OFLX have been known to have severe adverse effects such as seizures and rhabdomyolysis. It has been reported that OFLX is predominantly excreted in unchanged in urine (Ichihara et al., 1984; Wise and Lockley, 1988) and significant changes in pharmacokinetic parameters were found in renal dysfunction; for example, the prolonged terminal half-life and decreased systemic clearance (Fillastre et al., 1987). Additionally, many investigators have reported the pharmacokinetic interaction that gastrointestinal absorption of new quinolones is inhibited or reduced by antacids (Sörgel et al., 1989). Therefore, OFLX may be a quinolone dosage adjustment of which is required on the basis of the therapeutic drug monitoring.

Drug monitoring using the saliva offers a convenient and noninvasive alternative to blood analysis with particular advantages in geriatric and pediatric cases. For several drugs, it was reported that determination of saliva levels was successfully used for the therapeutic drug monitoring (Drobitch et al., 1989). OFLX is a zwitterionic compound (pKa; 6.05, 8.22) which has a relatively high lipophilicity among new quinolones (Ross and Riley, 1990), and has been demonstrated to show extensive distribution and good penetration into the extravascular compartment (Sörgel et al., 1989; Wolfson and Hooper, 1989). A number of studies have reported a close relationship of OFLX levels in saliva and plasma or serum (Shiiki, 1989; Warlich et al., 1990), and the saliva OFLX concentration may be used as an index of therapeutic drug monitoring guided to the plasma drug concentration in patients (Takagi et al., 1992; Yamaki et al., 1992). However, recent reports have suggested the change in salivary distribution of OFLX in patients with chronic renal impairment. Tsubakihara et al. (1994) described that in patients with severe renal failure saliva OFLX levels were lower than the serum levels, although the saliva-to-vehicle concentration ratio of OFLX was reported to be nearly equal to unity in patients with the normal renal function (Shiiki, 1989). However, Koizumi et al. (1994) demonstrated the negative correlation between the creatinine clearance and saliva-to-serum ratio of the area under OFLX concentration-time curve, suggesting the enhancement of salivary OFLX distribution by decreased renal function. To establish the therapeutic drug monitoring of OFLX with saliva, it is necessary to investigate the alteration of salivary distribution of this quinolone at the disease state of renal insufficiency. However, information on salivary excretion of OFLX in renal failure has been very limited, and no possible mechanism for the change in OFLX penetration into saliva has been discussed yet.

ABBREVIATIONS: OFLX, ofloxacin; t1/2, elimination half-life; CLtot, total body clearance; Vss, distribution volume at the steady state; CLsal, salivary clearance; HPLC, high-performance liquid chromatography; S/P ratio, saliva-to-plasma concentration ratio.

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1 Current address: Department of Pharmacy, Heilongjiang Provincial 2nd Hospital, 159 Diduan Street, Harbin 150010, Heilongjiang, China.
Our study was planned as a fundamental approach in the laboratory animals to elucidate the effect of renal failure on salivary excretion of OXL. OXL distribution into saliva after bolus i.v. administration was compared between the rats which had the normal renal function and experimentally induced renal failure. In addition, the difference in salivary distribution of OXL between parotid and mandibular glands was also examined.

**Materials and Methods**

**Chemicals.** OXL was kindly supplied by Daiichi Seiyaku (Tokyo, Japan) and ciprofloxacin hydrochloride (internal standard) by Bayer AG (Leverkusen, Germany). All other reagents and solvents were commercially available and of analytical grade.

**Animals.** Twelve- to fourteen-week-old male Wistar rats (Nippon SLC, Hamamatsu, Japan), 340 to 400 g, were used in this study. Animals were housed in a laboratory maintained a 12-hr light-dark cycle, and controlled room temperature (23 ± 2°C) and relative humidity (50 ± 10%). Two days before drug administration, the single-step 5/6th-nephrectomy was performed to the rats according to the method of Giacomini et al. (1981). The sham-operation (only incision and sutures on the abdomen) was performed on the controls. Food and water were allowed ad libitum thereafter.

**Pharmacokinetic study.** The rats were anesthetized with i.p. dose of sodium pentobarbital (30–40 mg/kg). After tracheotomy and catheretization, cannulae were made according to the method of Watanabe et al. (1987). The right jugular vein was cannulated with a silicon polymer tubing (i.d. 1.0 mm, o.d. 1.5 mm, Dow Corning, Tokyo, Japan) for constant-rate infusion of pilocarpine hydrochloride by a infusion pump (KN-201; Natsume Seisakusho Co., Ltd., Tokyo, Japan) to stimulate saliva. A polyethylene tubing (PE-10; i.d.0.28 mm, o.d.0.61 mm, length 12 cm, Becton Dickinson Co.) was inserted into the parotid and mandibular duct orifices in the buccal cavity to collect saliva samples separately. Through the experiments, the body temperature of rats was maintained at 37.5°C using a heated pad placed under the supine rats.

OXL was dissolved in 0.1 M sodium hydroxide, and then diluted with normal saline. After the constant-rate infusion of pilocarpine (5 mg/kg/hr) for 2 hr to stabilize the salivation (Watanabe et al., 1987), the rats received a bolus i.v. injection of OXL at a dose of 5 mg/kg. Blood samples were collected just before drug administration (about 200 µl) and at designated times of 3, 5, 10, 20, 30, 40, 60, 80, 100, 120 and 140 min (about 100 µl) after administration, and the plasma was immediately separated by centrifugation after heparinization. Parotid and mandibular saliva samples were separately collected at 30, 60, 120, and 140 min (about 100 µl) after administration. The plasma and saliva collection was calculated from the salivary flow rate and the internal volume of saliva cannula.

Binding of OXL to the rat serum protein was determined ex vivo in the individual rat after the periodical blood and saliva collections. About 2.5 ml of the blood were withdrawn at 150 min after drug administration, and serum was immediately separated by centrifugation with the serum separator (Fibrichin; Takazono Sangyo Co., Ltd., Osaka, Japan) and a portion of the serum was ultrafiltered by using a micropartition system (MPS-3; Amicon, Inc., Beverly, MA) with a membrane filter at 4000 rpm for 20 min at room temperature. The concentration of OXL bound to serum protein was calculated by subtracting the drug concentration in the ultrafiltrate from the total concentration in serum. Nonspecific adsorption of the drug to the membrane was negligible. Plasma, serum, ultrafiltrate and saliva samples were frozen and kept until the analysis.

**The pH of plasma and saliva.** To determine the pH of plasma and saliva of the sham-operated and nephrectomized rats, the other rats received the surgical operation and saliva stimulation in the same manner as for the pharmacokinetic study. Parotid and mandibular saliva samples were separately collected under a liquid paraffin layer (about 0.15 ml) in a microtube during consecutive two 75-min periods from 2 hr after the beginning of constant-rate infusion of pilocarpine. Blood samples were collected immediately before the saliva collection and midway through the collection period, and the plasma were obtained by centrifugation after heparinization. Immediately after the collection of plasma and saliva, the pH of these samples were determined by a compact pH meter with combined electrode (B-212; Horiba Seisakusho, Ltd., Kyoto, Japan).

**Assay.** Concentrations of OXL in the plasma, serum, ultrafiltrate and saliva were determined by HPLC. Sample preparation was carried out in accordance with our previous method (Katagiri et al., 1988) except that the initial sample size was 10 µl, the reconstitution volume was 200 µl, a 20-µl aliquot was injected into the chromatograph and ciprofloxacin was used as an internal standard.

The HPLC apparatus was a Shimadzu LC-10A system (Shimadzu, Kyoto, Japan) consisting of a LC-10AT pump, a RF-10Axl fluorescence detector and a SIL-10Axl auto injector. A reversed-phase Wakoski-II C18 column (150 mm × 4.6 mm i.d.; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used with a CTO-10A column oven heated at 40°C. The mobile phase was CH3OH-0.02 M KH2PO4 (55:45, v/v) containing 2 mM sodium lauryl sulfate which was adjusted to pH 2.5 with phosphoric acid. The flow rate was 1.0 ml/min. The excitation and emission wavelengths of the detector were set at 340 and 460 nm, respectively. The chromatographic data were calculated with a Shimadzu CLASS-LC10 HPLC workstation.

Creatinine and urea nitrogen in the plasma collected before drug administration were measured using a biochemical assay system, Reflotron (Yamanouchi Seiyaku, Tokyo, Japan). Serum albumin was measured at 150 min after drug administration by a BCG method (Albunin B-Test Wako; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

**Data analysis.** Measured plasma concentration (Cp)-time (t) data for OXL were analyzed on the basis of a two-compartment model expressed as the following equation:

\[ C_p = A \cdot e^{-at} + B \cdot e^{-bt} \]

where A, B, α, β are hybrid parameters. The nonlinear least-squares regression program WinNonlin (Scientific Consulting, Inc., Cary, NC) was used for the regression analysis to obtain the hybrid parameters and secondary parameters, i.e., the elimination half-life (t1/2), total body clearance (CLtot) and distribution volume at the steady state (Vd). The flow rate of saliva was determined gravimetrically assuming the specific gravity to be approximately 1.0 (Watanabe et al., 1981).

The S/P ratio of OXL was calculated as follows: 1) the lag time for saliva collection was calculated from the salivary flow rate and the internal volume of saliva cannula; 2) the true midpoint of the saliva collection period was presumed from the lag time; 3) the plasma OXL concentration at the true midpoint was predicted by the two-compartment model equation for each rat; 4) the S/P ratio was expressed as the measured salivary concentration divided by the predicted plasma concentration.

The salivary clearance (CLsal) of OXL was calculated by multiplying the S/P ratio by the total salivary flow rate (per body weight) into which the measured flow rate of the single side was doubled, assuming that salivary OXL concentrations and salivary flow rates in the both sides would be equal.

**Statistical analysis.** The results were expressed as the mean ± S.D. for the indicated numbers of experiments. The significance of differences between the mean observations for two groups was determined using Student’s t test or Mann-Whitney test. Repeated measures analysis of variance was used to test for differences in the
plasma concentration-time profiles between the treatments. Statistical significance was defined as $P < .05$.

**Results**

**OFLX assay.** Calibration curves for OFLX in the plasma and saliva were satisfactorily linear over the concentration ranges from 0.4 to 20 and 0.05 to 7 $\mu$g/ml, respectively. The coefficients of variation for the assay were 2.1% at 0.4 $\mu$g/ml of the plasma concentration and 3.3% at 0.05 $\mu$g/ml of the saliva concentration. The respective regression equations for plasma and saliva were $y = 0.246x + 0.011$ (r = 0.999) and $y = 1.25x + 0.022$ (r = 0.992), where $y$ is the peak-area ratio of the drug to the internal standard, $x$ is the concentration in plasma or saliva ($\mu$g/ml) and $r$ is the coefficient of correlation. The limits of determination were established at 0.4 $\mu$g/ml in plasma and at 0.05 $\mu$g/ml in saliva. Blank plasma and saliva samples did not interfere with the peaks for either OFLX or the internal standard, ciprofloxacin.

**Pathophysiological data.** Body weights and concentrations of plasma creatinine, urea nitrogen and serum albumin in the sham-operated and 5/6th-nephrectomized rats are summarized in table 1. Plasma creatinine levels were less than the detection limit of 0.5 mg/dl in sham-operated rats, whereas nephrectomized rats had higher creatinine levels of about 3.4 mg/dl. Plasma urea nitrogen levels in the nephrectomized rats were about five times as high as those in the sham-operated rats. In serum albumin levels, there is no significant difference between the two groups.

**Plasma and saliva pH.** The pH of plasma obtained from the sham-operated and nephrectomized rats ranged from 7.4 to 7.6 through the experiment. Parotid and mandibular saliva had the pH of 7.9 to 8.2 and 8.3 to 8.6, respectively, in both pretreatment groups. In the plasma and saliva pH, there was no difference between the sham-operated and nephrectomized groups, and between the initial and latter halves of the sampling periods. A consistent tendency that the pH of parotid saliva was lower than that of mandibular saliva was observed in both groups.

**Plasma concentration-time profile.** The mean plasma concentration-time curves for OFLX after single i.v. administration at a dose of 5 mg/kg in the sham-operated and nephrectomized rats are summarized in figure 1. In the nephrectomized rats, significantly higher plasma OFLX concentrations were observed when compared to the sham-operated rats. In both groups, plasma concentrations of OFLX were found to decline biexponentially with time. Mean plasma concentration vs. time data were fitted to exponential functions by the nonlinear least-squares regression method. Among several compartmental models which were attempted to analyze the data, a two-compartment model was the most adequate to describe the timecourses on the basis of the minimum AIC estimation (Akaike, 1974).

The corresponding pharmacokinetic parameters of OFLX are summarized in table 2. The $CL_{tot}$ was significantly decreased to about 50% by the partial nephrectomy and the $t_{1/2b}$ of the nephrectomized rats tended to be longer than that of sham-operated control. No difference was observed in the $V_{ss}$ of the two groups. At 150 min after drug administration, the fractions of OFLX bound to the serum protein for the sham-operated and nephrectomized rats were 11.8 ± 2.6 and 16.4 ± 3.9%, respectively. There was no significant difference between them.

**Salivary excretion.** The total cumulative OFLX excretion into parotid and mandibular saliva of sham-operated and nephrectomized rats is shown in figure 2. In sham-operated rats, the cumulative salivary excretion of OFLX up to 150 min after administration was less than 0.02% of the dose in both parotid and mandibular saliva. The nephrectomy induced a significant increase in the cumulative salivary excretion of OFLX in both types of saliva. Cumulative amounts of OFLX excreted into saliva in the nephrectomized rats until 150 min were about five to six times as large as those in the sham-operated rats.

The salivary flow rates and $CL_{sal}$ of OFLX were calculated from the mean observations of three collection periods from

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### Table 1

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<thead>
<tr>
<th>Measurement</th>
<th>Sham-Operated</th>
<th>Nephrectomized</th>
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<tbody>
<tr>
<td>Body weight before treatment$^b$</td>
<td>378 ± 22</td>
<td>369 ± 13</td>
</tr>
<tr>
<td>Body weight before dosing$^c$</td>
<td>376 ± 26</td>
<td>346 ± 9</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)$^f$</td>
<td>&lt;0.5</td>
<td>3.39 ± 1.29*</td>
</tr>
<tr>
<td>Plasma urea nitrogen (mg/dl)$^f$</td>
<td>25.7 ± 3.8</td>
<td>131 ± 41**</td>
</tr>
<tr>
<td>Serum albumin (g/dl)$^f$</td>
<td>3.10 ± 0.10</td>
<td>3.28 ± 0.17</td>
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$^a$ Data are expressed as the mean ± S.D. of five rats. There are significant differences from sham-operated rats ($^* P < .01$ by Mann-Whitney test; $^{**} P < .001$ by Student’s t test).

$^b$ The treatment involves sham-operation or 5/6th-nephrectomy.

$^c$ Measured immediately before OFLX administration.

$^d$ Measured at 150 min after OFLX administration.

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### Table 2

<table>
<thead>
<tr>
<th>Parameter$^a$</th>
<th>Sham-Operated</th>
<th>Nephrectomized</th>
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<tr>
<td>$A$ ($\mu$g/ml)</td>
<td>11.1 ± 1.8</td>
<td>10.4 ± 2.0</td>
</tr>
<tr>
<td>$B$ ($\mu$g/ml)</td>
<td>1.76 ± 0.28</td>
<td>2.74 ± 0.33*</td>
</tr>
<tr>
<td>$\alpha$ ($10^{-1}$ min$^{-1}$)</td>
<td>1.52 ± 0.12</td>
<td>1.52 ± 0.20</td>
</tr>
<tr>
<td>$\beta$ ($10^{-3}$ min$^{-1}$)</td>
<td>6.27 ± 2.70</td>
<td>4.02 ± 0.67</td>
</tr>
<tr>
<td>$t_{1/2b}$ (min)</td>
<td>127 ± 49</td>
<td>176 ± 25</td>
</tr>
<tr>
<td>$CL_{sal}$ (ml/min/kg)</td>
<td>13.9 ± 4.2</td>
<td>6.71 ± 1.37**</td>
</tr>
<tr>
<td>$V_{ss}$ (ml/kg)</td>
<td>1897 ± 355</td>
<td>1514 ± 117</td>
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</table>

$^a$ A, B, $\alpha$ and $\beta$: the treatment involves sham-operation or 5/6th-nephrectomy. $CL_{sal}$ total body clearance; $V_{ss}$: distribution volume at the steady-state.

$^b$ Data are expressed as the mean ± S.D. of five rats. There are significant differences from sham-operated rats (Student’s t test: $^* P < .001$, $^{**} P < .05$).
90 to 150 min after administration, and were compared between two types of saliva and between two groups of the rats. The salivary flow rates in the sham-operated rats were 5.49 ± 4.48 and 9.11 ± 1.84 ml/min/kg for parotid and mandibular saliva, respectively. The nephrectomy tended to increase the flow rates of parotid and mandibular saliva to 6.78 ± 4.45 and 14.2 ± 6.2 ml/min/kg, respectively, although there was no significant difference between sham-operated and nephrectomized rats. In addition, the flow rate of mandibular saliva tended to be larger than that of parotid saliva in both groups. The CL_{sal} of OFLX was 3.30 ± 3.49 ml/min/kg for parotid saliva and 2.52 ± 0.77 ml/min/kg for mandibular saliva. In the nephrectomized rats, the CL_{sal} in mandibular saliva was significantly increased by about four times (9.34 ± 5.54 ml/min/kg). Similar tendency was observed in parotid saliva (10.4 ± 5.8 ml/min/kg).

The S/P ratios of OFLX for each saliva are shown in figure 3. In each group, the S/P ratios were nearly constant during the periods which corresponded to the elimination phase of the plasma concentration-time profile. The S/P ratios in the nephrectomized rats were significantly higher than those in the sham-operated control during 70 to 150 min after administration. Table 3 shows the S/P ratios of OFLX at the elimination phase, which were calculated as the mean of the ratios of three saliva collection periods from 90 to 150 min after drug administration, and the S/P ratios predicted with the measured values for saliva and plasma pH and unbound fractions of OFLX. In both parotid and mandibular saliva, nephrectomized rats had about two to three times larger S/P ratios than sham-operated rats. In the sham-operated rats, significantly larger S/P ratios were observed in parotid saliva when compared to mandibular saliva. Similar results were obtained in the nephrectomized rats.

OFLX is a dipolar quinolone which possesses pK_{a1} (6.05) for the carboxyl group and pK_{a2} (8.22) for the methylpiperazinyl group. Because pH values of plasma and saliva were extremely higher than the pK_{a1}, the carboxyl group is almost charged negatively in the plasma and saliva. Consequently, the prediction of the S/P ratio of OFLX was performed for pK_{a2}. At some pH values, the methylpiperazinyl moiety may be also ionized and OFLX can exist as a zwitterion. The zwitterionic species is electrically neutral and is considered to be more hydrophobic in comparison to the negatively charged one. When it is assumed that zwitterionic species can diffuse across the membrane (Furet et al., 1992), on the basis of the pH-partition theory, the S/P ratio of OFLX can be

Fig. 2. Cumulative excretion of OFLX into parotid (A) and mandibular (B) saliva after bolus i.v. administration to sham-operated and nephrectomized rats. △, Sham-operated; ●, nephrectomized. Each point represents the mean and S.D. of three to five rats. There are significant differences from sham-operated rats (Student’s t test: *P < .05; **P < .01).

Fig. 3. S/P ratios of OFLX for parotid (A) and mandibular (B) saliva after bolus i.v. administration to sham-operated and nephrectomized rats. Columns: opened, sham-operated; dotted, nephrectomized. Each column represents the mean and S.D. of three to five rats. There are significant differences from sham-operated rats (Student’s t test: *P < .05; **P < .01).
the discrepancy between measured and theoretical S/P ratios was observed in the theoretical ratios. Furthermore, the measured S/P ratios were extremely less than the values predicted according to the pH-partition theory where the zwitterionic species were assumed to predominantly penetrate into salivary glands by passive diffusion. Thus, OFLX distribution into saliva could not be apparently explained by the pH-partition theory itself. In this theory, it is assumed that a nonionized (or zwitterionized) species is sufficiently lipophilic and rapidly diffuse across the membrane. If a zwitterionic form of OFLX was much less lipophilic than the uncharged form, lower S/P ratios compared with the theoretical values would not be unexpected. Another explanation for the discrepancy between measured and theoretical S/P ratios can be discussed. Passive diffusion through the membrane may not be only the mechanism for OFLX penetration into saliva. Possible active transport system(s) which could pump out this quinolone from saliva to the circulation may operate.

Discussion

We have previously described the HPLC method for determination of OFLX in rat plasma (Ichikawa et al., 1992). In the study, the blood samples in the volume as small as possible should be withdrawn from the individual rat in many times to make the pharmacokinetic analysis possible. Additionally, the saliva volume that could be collected during 20 min was quite small (about 30–60 μl). From these reasons, the previous assay method was made some modification to be more sensitive. As the results, the present assay method had coefficients of variation less than 4% in a wide concentration range despite a small sample size of both plasma and saliva. These results indicate that this method provides a highly sensitive, accurate and reproducible analytical procedure for determining OFLX in rat plasma and saliva.

A lot of methods are available for producing renal failure in experimental animals, which include the administration of various nephrotoxic agents such as uranyl nitrate, mercurials and cisplatin, ureteral ligation and partial nephrectomy. Although the chemical methods such as uranyl nitrate injection are simple and reliable (Giacomini et al., 1981), it is feared that the nephrotoxic agents could also damage salivary glands. Therefore, in this experiment, the single-step 5/6th-nephrectomy was used to induce the experimental renal failure. This surgical method can damage only the kidneys, so that it is possible to investigate the effects of renal failure per se on salivary excretion of the drug.

As summarized in table 1, plasma creatinine and urea nitrogen levels were remarkably raised by the partial nephrectomy, indicating that severe renal failure was produced. Because the flow rates for both parotid and mandibular saliva in the rats with renal failure tended to be larger than those of the control, impairment of saliva secretion was not induced by renal failure in rats. The elevated secretion of saliva may be in compensation for decreased urine production.

In the control rats, both of the salivary excretion and S/P ratio of OFLX in parotid saliva were significantly higher than those in mandibular saliva (fig. 2,3; table 3), indicating the gland-type difference in salivary excretion of OFLX. Generally, it is known that the S/P ratio of weakly acidic or basic drugs depends on the salivary pH (Watanabe et al., 1985, 1987). As for quinolone antibiotics, it was reported that salivary penetration of enoxacin was pH-dependent and higher saliva levels of enoxacin were found in the more acidic samples (Sörgel et al., 1989). Therefore, in this study, theoretical S/P ratios of OFLX were calculated according to the pH-partition theory and were compared to the measured ratios. The parotid saliva showed larger S/P ratios than the mandibular saliva in the measured ratios although the contrary relationship was observed in the theoretical ratios. Furthermore, the measured S/P ratios were extremely less than the values predicted according to the pH-partition theory where the zwitterionic species were assumed to predominantly penetrate into salivary glands by passive diffusion. Thus, OFLX distribution into saliva could not be apparently explained by the pH-partition theory itself. In this theory, it is assumed that a nonionized (or zwitterionized) species is sufficiently lipophilic and rapidly diffuse across the membrane. If a zwitterionic form of OFLX was much less lipophilic than the uncharged form, lower S/P ratios compared with the theoretical values would not be unexpected. Another explanation for the discrepancy between measured and theoretical S/P ratios can be discussed. Passive diffusion through the membrane may not be only the mechanism for OFLX penetration into saliva. Possible active transport system(s) which could pump out this quinolone from saliva to the circulation may operate.

It has been known that there are the specific transport systems in the salivary glands (Haeckel and Hänecke, 1996). The transport systems could actively carry not only endogenous substrates but also exogenous materials including various drugs through the salivary gland epithelium membranes (Allen et al., 1976; Dawes et al., 1978). Recently, it has been reported that the specific transport systems for quinolone antibacterial agents including OFLX exist in the epithelium membranes of rat choroid plexus (Ooie et al., 1996a, 1996b) and kidney cortex (Okano et al., 1990). Since salivary glands also have the epithelial membrane which is morphologically

<table>
<thead>
<tr>
<th>Saliva</th>
<th>Measured</th>
<th>Predicted</th>
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<tr>
<td></td>
<td>Sham-operated</td>
<td>Nephrorctomized</td>
</tr>
<tr>
<td>Parotid</td>
<td>0.249 ± 0.180 (4)</td>
<td>0.799 ± 0.139 (3)**</td>
</tr>
<tr>
<td>Mandibular</td>
<td>0.136 ± 0.024 (4)*</td>
<td>0.336 ± 0.118 (5)**</td>
</tr>
</tbody>
</table>

* Data are expressed as the mean ± S.D. The number of rats is indicated in the parentheses. They are based on the mean of the values obtained from 90 to 150 min after drug administration in individual rats. There are significant differences from sham-operated rats (Student’s t test: * P < .05; ** P < .01) or from parotid saliva (Student’s t test; † P < .05; †† P < .001).

S/P ratio = $[(1 + 10^{pHs-pKa}) f_s]/[(1 + 10^{pHp-pKa}) f_p]$

where $pH_s$ and $pH_p$ represent saliva and plasma pH values, respectively; $f_s$ and $f_p$ represent unbound fractions of OFLX in saliva and plasma, respectively; $f_s$ is assumed to be 1.0; $pKa_2$ for the methylpiperazinyl group of OFLX is 8.22.
similar to choroidal and renal tubular epithelium (Tamarin and Sreebny, 1965), it is likely that a specific system for OFLX may function in the salivary glands and OFLX may be reabsorbed from saliva via this system. Relatively low salivary distribution of this quinolone in rats may be due to this putative efflux mechanism which could transport OFLX from saliva to blood.

Interesting results were obtained concerning the effect of nephrectomy on salivary excretion of OFLX. The measured S/P ratios in the group of renal failure were greater than the control for both saliva, suggesting that OFLX distribution between the blood and saliva may be altered in the rats with renal failure. These results obtained from rats coincide the fact that patients with reduced creatinine clearance had higher salivary distribution of OFLX (Koizumi et al., 1994). In contrast, Basshees and DiGregorio (1982) reported that saliva and plasma levels of procainamide, a basic drug, were higher in renal-impaired rats by means of two-step subtotal nephrectomy but the S/P ratio was unchanged in comparison to the controls. Thus, renal failure may cause different changes in the salivary distribution of different types of drugs.

To assess whether increased salivary distribution of OFLX in renal failure could be explained qualitatively by pH-partition theory, the pH of plasma and saliva and OFLX fraction bound to the plasma protein in the nephrectomized rats were also measured. However, there was no influence of the partial nephrectomy on the pH and protein binding, resulting in almost the same predicted S/P ratios observed in control and nephrectomized rats. Consequently, other mechanisms should be considered for the change in the S/P ratio by renal failure.

A few possibilities could be discussed on the mechanisms for enhanced salivary distribution of OFLX in renal failure. In salivary gland, acinus cells form the barrier for drug translocation between blood and saliva. Renal insufficiency may induce destruction of the blood-saliva barrier leading to increase of drug distribution into saliva. In fact, Kinashi et al. (1989) detected amyloid-like fibrils of a salivary gland in patients with renal insufficiency. Another possibility is related with the putative-specific transport in salivary glands. The activity of the possible efflux system for OFLX may be inhibited by uremic toxins which increase in renal failure, resulting in accumulation of OFLX in saliva. Alternately, the possibility of the secondary change in the S/P ratio by elevated OFLX levels in plasma and saliva induced by renal failure could be considered. Salivary distribution of OFLX might be operated by a concentration-dependent system affected by elevation of the plasma and/or saliva concentration of the drug. In humans, however, the S/P ratio of OFLX was reported to be almost constant in the plasma concentration range of 1 to 7 μg/ml (Takagi et al., 1992). Therefore, the influence of the OFLX concentration on the distribution into saliva seems to be small, although the difference in the species should be considered. To elucidate the mechanism for the enhanced distribution of OFLX in renal failure, further detailed studies will be needed.

In conclusion, our study showed that the salivary excretion of OFLX significantly increased by renal dysfunction in rats. Furthermore, remarkable difference in the salivary excretion of OFLX was found between parotid and mandibular glands in both rats with normal and impaired renal function.

References


Send reprint requests to: Dr. K. Iwamoto, Department of Pharmacy, Shimane Medical University Hospital, 89–1 Eny-cho, Izumo, Shimane 693-8501, Japan.