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Effective Monitoring of Concentrations of Ofloxacin in Saliva of Patients with Chronic Respiratory Tract Infections

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To ascertain whether monitoring of the concentrations of ofloxacin in saliva during a course of treatment is more suitable and safer than that of its levels in blood, we simultaneously monitored its concentrations in three body fluids (blood, saliva, and expectorated sputum) after a 300-mg administration in 18 patients with chronic respiratory infection. The mean (± standard error of the mean) half-lives derived from the three drug level-time relationships were similar: 6.04 ± 0.58 h for serum, 6.34 ± 0.63 h for sputum, and 6.61 ± 0.65 h for saliva. The mean peak concentration (4.06 to 4.53 μg/ml) did not differ at the three sites, but the times taken to reach peak concentration in saliva and sputum (3.17 ± 0.46 h) were significantly longer than that in serum (2.22 ± 0.28 h). The ratios of the concentrations in saliva and sputum to the concentration in serum increased during the first 2 h and reached 1.0 between 2 and 8 h after administration. They rose above 1.0 16 h after administration: 1.14 ± 0.11 for saliva and 1.19 ± 0.10 for sputum. The concentration-time relationship for sputum corresponded closely with the concentration-time relationship for saliva, and an overall significant correlation between the concentrations in sputum and saliva was obtained ($P < 0.01$). These results suggest that monitoring concentrations in saliva may be more valid, as well as less invasive, than monitoring of the levels in blood for ensuring that the drug concentration reaches its therapeutic level in bronchial secretions.

Ofloxacin (OFLX) is a fluoroquinolone antimicrobial agent having a broad spectrum of activity against gram-negative and -positive bacteria and some anaerobes in vitro (4, 13). Grassi reported the successful use of OFLX against respiratory tract infections in a multicenter study (6), and many other clinical investigations have proved its effectiveness in the treatment of various systemic bacterial infections (8, 14, 21).

Ofloxacin is widely distributed in the body and penetrates thoroughly into extravascular fluids such as bronchial secretions (8, 10, 20, 21). We consider that its relatively high concentration in such secretions is especially important in chronic infections, since it maintains levels higher than the MICs for various respiratory pathogens. In chronic airway infections, it is necessary to maintain the concentration of an antibiotic in bronchial secretions at a level equal to or higher than the concentration in blood because the bacteria are usually present in and around the airways. On the other hand, since OFLX is mostly eliminated via the kidney and is excreted unchanged in the urine (3, 11), careful regulation of the dosage during chronic administration in patients with impaired renal function has been advised (3, 5).

In the present study, we performed simultaneous monitoring of OFLX concentrations in three fluids, blood, expectorated sputum, and saliva, in patients with chronic respiratory infections and various degrees of renal impairment. Our purpose was to determine whether the measurement of the concentration of this drug in saliva is an accurate and reliable method of safe monitoring in infectious lung diseases and whether the degree of functional impairment of the kidney affects the drug's concentration in sputum or saliva.

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MATERIALS AND METHODS

Eighteen patients, 13 males and 5 females 42 to 86 years of age (mean ± standard deviation, 63.3 ± 11.3 years) with chronic respiratory tract infections and weighing between 37 and 69 kg (mean ± standard deviation, 54.3 ± 7.9 kg), were enrolled in the present study. They were all in the period of recovery from the acute stage of chronic respiratory tract infections and were still producing large amounts of mucopurulent sputum. As the underlying respiratory condition, six had bronchiectasis, three had chronic bronchitis, two had emphysema, two had diffuse panbronchiolitis, two had lung abscesses, two had idiopathic interstitial pneumonia, and one had bronchial asthma. The exclusion criteria for the study were severe hepatic disease, gastroenteric absorption disorders, and serious disease in any organ other than the lung.

All patients were hospitalized, and informed consent was obtained from each before the study. They did not fast, and each was given a single 300-mg dose of oral OFLX (three tablets of 100 mg each, supplied by Daiichi Pharmaceutical Co., Tokyo, Japan) at 9:00 a.m. on the study day after a standard hospital breakfast, which had the same caloric content and the same water and electrolyte contents in each case. Antacid, H_2 receptor blockers, other antimicrobial agents, and any drug that might have affected the pharmacokinetic disposition of the OFLX were avoided from the day before drug administration until the end of the study. Blood (5 ml), sputum, and saliva specimens were obtained before and 1, 2, 4, 8, and 16 h after the administration. The sputum and saliva specimens were collected in sterile glass Petin dishes. Samples (1 ml) taken from the centers of these specimens were placed in small glass tubes and stored frozen at −20°C until analysis. Each sample was then homogenized in a Polytron homogenizer (Shimadzu, Tokyo, Japan) for 2 min, and 200 μl was used for the assay. Conditions were kept as sterile as possible at all times.

Assay for OFLX in biological fluids. OFLX in the biological

fluids was determined by an already-reported high-performance liquid chromatography (HPLC) method (7). Briefly, to 0.2 ml of serum was added 0.2 ml of internal standard solution (pipemidic acid buffered with 0.1 M phosphate buffer [pH 7.0], 1.5 μg/ml). The mixture was extracted into 5 ml of dichloromethane by shaking for 15 min. After centrifugation at 1,600 × *g* for 10 min, the organic phase (4 ml) was transferred to a clean centrifuge tube and evaporated to dryness in a nitrogen gas flow. The residue was dissolved in 0.2 ml of the mobile phase and sonicated for 10 min, and 0.2 ml of the resulting solution was injected into the HPLC system (JASCO 800 series equipped with an 880-PU pump, an 860-CO column oven, an 850-AS autosampler, and an 801-SC system controller; JASCO Co. Tokyo, Japan) and a Hitachi F-1050 fluorescence detector (Tokyo, Japan). The excitation wavelength was set at 285 nm, and the emission wavelength was set at 460 nm. Chromatography was carried out on a reversed-phase column (YMC-Pack ODS-AM; 5 μm, 150 by 6.0 mm internal diameter). The mobile phase was a mixture of acetonitrile and 10 mM phosphoric acid (pH 7.0) containing 20 mM tetrabutylammonium hydrogen sulfate (1:10, vol/vol) at a flow rate of 1.3 ml/min. The retention times were 5 min for the internal standard and 8 min for OFLX. For the measurement of OFLX concentrations in sputum and saliva, the addition of the internal standard, evaporation, injection of the eluent, and HPLC were performed in exactly the same manner as for the serum.

The calibration curves were linear (r = 0.9999) for concentrations up to 2.0 μg/ml in three biological fluids, for each of which a different standard curve (with almost the same slope) was used. The relative standard deviations of the five measurements of each OFLX concentration (within the same day) were 2.85% for 0.125 μg/ml, 1.88% for 0.25 μg/ml, and 0.854% for 1.0 μg/ml. The sensitivity permitted quantitation of levels as low as 0.01 μg/ml in three biological fluids. The between-day coefficient of variation was 6.05% over 5 days for 1.0 μg/ml of OFLX.

Pharmacokinetic and statistical analyses. Ofloxacin concentrations in serum, sputum, and saliva were fitted to a pharmacokinetic model by a nonlinear least-squares method, with the microcomputer program MULTI (22). We used an open one-compartment model with first-order absorption, and the weight for each analysis was 1 (16). The half-life ($t_{1/2}$) was determined as 0.693/K. Peak concentrations (C_{max}s) and time to maximum concentration (T_{max}) values were taken from actual datum points. The area under the concentration-time curve (AUC) after administration was calculated by log-linear trapezoidal approximation from time zero to the time of the last detected concentration, and the $AUC_{0-\infty}$ was calculated by adding the sum of the AUCs obtained from time zero until the final concentration measurement to the last measured concentration (at 16 h), divided by K. The mean residence time (MRT), the average time for the drug to remain in the body, was calculated as follows: MRT = AUMC/AUC, in which AUMC is the area under the curve of the product of time t and the drug concentration from zero to infinity. The relative clearance (CL/F) was estimated as follows: CL/F = dose/$AUC_{0-\infty}$, in which F, the bioavailability, could not be determined in the present study. The volume of distribution was obtained from the following expression: dose/(AUC × K).

Data were expressed as mean ± standard error of the mean (SEM). Correlations between three samples at different sites or between creatine clearance (CL_{CR}) and kinetic parameters were assessed by linear least-squares regression analysis. Statistical comparisons were performed with Student's paired t test and analysis of variance, and the minimum level of statistically significant difference was considered to be $P < 0.05$.

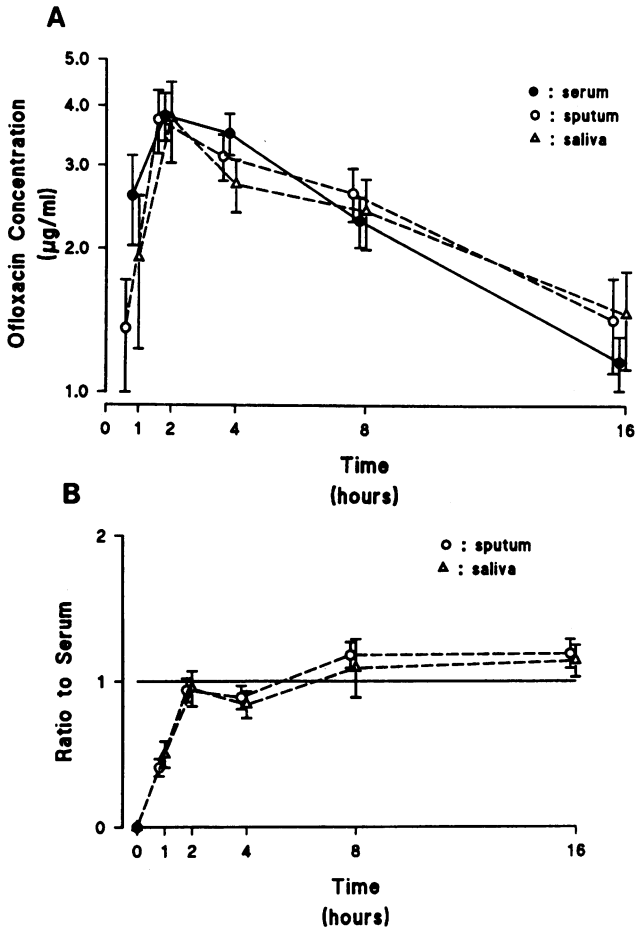

FIG. 1. (A) Mean (± SEM) concentration-time relationship for serum (closed circles), sputum (open circles), and saliva (open triangles) after 300-mg OFLX oral administration. (B) Mean (± SEM) sputum- or saliva-to-serum OFLX concentration ratio–time relationships after the administration.

RESULTS

The mean (± SEM) serum, sputum, and saliva drug concentration-time relationships are depicted in Fig. 1. After the peak levels are attained, the drug concentrations decline with time in a log-linear fashion. The mean (± SEM) $t_{1/2}$s of the three drug levels were very similar: 6.04 ± 0.58 h for serum, 6.34 ± 0.63 h for sputum, and 6.61 ± 0.65 h for saliva (Table 1). The mean observed C_{max}s and T_{max}s ranged from 4.06 to 4.53 μg/ml and 2.22 to 3.17 h, respectively (Table 1). Although the C_{max} did not differ among the three fluids, the mean T_{max} in the secretions (sputum and saliva, 3.17 ± 0.46 h) was significantly longer than that in the serum (2.22 ± 0.28 h) (Table 1). The mean (± SEM) of the volume of distribution of OFLX calculated from the serum data was 1.30 ± 0.08 liters/kg.

The pathogens detected in the sputum were *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Branhamella catarrhalis*, and *Pseudomonas aeruginosa*, and their numbers were between 10^3 and 10^5.

TABLE 1. Pharmacokinetic dispositions of ofloxacin (means ± SEMs) in 18 patients with chronic respiratory tract infection

Sample substance	$t_{1/2}$ (h)	C_{max} (μg/ml)	T_{max} (h)	AUC (μg/ml · h)	MRT (h)	Drug level at 16 h (μg/ml)
Serum	6.04 ± 0.58	4.25 ± 0.41	2.22 ± 0.28	51.5 ± 5.7	11.1 ± 1.0	1.16 ± 0.18
Sputum	6.34 ± 0.63	4.06 ± 0.49	3.17 ± 0.46[a]	61.4 ± 8.4[a]	13.4 ± 1.2[b]	1.42 ± 0.32[a]
Saliva	6.61 ± 0.65	4.53 ± 0.75	3.17 ± 0.46[a]	63.0 ± 8.9[b]	13.2 ± 1.4[a]	1.46 ± 0.34[a]

[a] $P < 0.05$ compared with value in serum.
[b] $P < 0.01$ compared with value in serum.

The mean (± SEM) CL_{CR} was 63.5 ± 7.3 ml/min/1.73 m^2, the range being from 20.3 to 113.0 ml/min/1.73 m^2.

The OFLX concentrations in the two secretions were lower 1 h after administration than that in the serum ($P < 0.05$) but were higher 16 h after ($P < 0.05$). Figure 1 shows the secretion-to-serum OFLX concentration ratio–time relationships. Both ratios increased during the first 2 h, reaching 1.0 from 2 to 8 h after administration. At 16 h after administration, the ratios were higher: 1.19 ± 0.10 for sputum and 1.14 ± 0.11 for saliva.

Pharmacokinetic analyses demonstrated that the AUCs and MRTs in the secretions were significantly greater and longer than those in the serum (Table 1). The ratios between the AUC and MRT values in the secretions and those in the serum were 1.19 ± 0.10 and 1.22 ± 0.08 for sputum and 1.18 ± 0.06 and 1.25 ± 0.12 for saliva.

The correlation between serum and sputum ($r = 0.854$) or saliva ($r = 0.882$) OFLX concentrations was statistically significant ($P < 0.001$). The correlations between CL_{CR} and the concentrations in serum 16 h after administration reached statistical significance ($r = -0.64$, $P < 0.05$), and there was a significant correlation between CL_{CR} and the AUCs for serum ($r = -0.561$), sputum ($r = -0.618$), and saliva ($r = -0.62$) ($P < 0.05$ to 0.01). In addition, there were negative linear relationships between CL_{CR} and both the sputum AUC-to-serum AUC ratio ($r = -0.643$) and the saliva AUC-to-serum AUC ratio ($r = -0.613$) ($P < 0.05$) (Fig. 2).

DISCUSSION

Tissue penetration of antibiotics into the target organs in the treatment of bacterial infections is sometimes limited and thus limits the success of therapy. Antibiotics such as aminoglycosides and β-lactams are distributed in small amounts, mainly to the blood and to some extracellular fluids (2, 18). Aminoglycoside levels in bronchial secretions are unpredictable but are usually 40% or less of the corresponding concentrations in blood (2).

On the other hand, the fluoroquinolone antibiotics have been demonstrated to show extensive distribution and good penetration of the extravascular compartment (8, 15, 18, 19, 21). This characteristic is probably due to their low protein binding, high lipophilicity, and relatively small molecular size. The present study demonstrated that the fluoroquinolone ofloxacin penetrated into the sputum to an excellent degree and had higher concentrations in sputum at 8 to 16 h after administration and greater AUC values in the sputum. The ratios of concentrations and AUCs for sputum to those for serum 8 to 16 h after administration were greater than one, suggesting excellent diffusion into the bronchial space.

The mean volume of distribution in our patients was 1.30 liters/kg, a much greater value than those for aminoglycoside (0.21 to 0.26 liter/kg) and the β-lactams (0.12 to 0.26 liter/kg) (1). In addition, the mean concentrations in sputum of 2.63 and 1.42 μg/ml at 8 and 16 h after administration, respectively, were sufficiently high to inhibit many respiratory pathogens (4, 8, 13, 21): *S. aureus* (MIC for 90% of strains tested [MIC_{90}], 0.4 μg/ml), *H. influenzae* (MIC_{90}, 0.03 μg/ml), *K. pneumoniae* (MIC_{90}, 0.2 μg/ml), and *B. catarrhalis* (MIC_{90}, 0.1 μg/ml). These high concentrations in sputum, coupled with the bactericidal activity, may explain the excellent clinical record of this agent in the treatment of bacterial pneumonia. Moorhouse and his colleagues have reported a study of 98 outpatients with lower respiratory tract infections, in which treatment with OFLX was successful in 92 cases (14), and other investigators demonstrated its therapeutic success in patients with acutely exacerbated chronic bronchitis (9, 12).

Ofloxacin also penetrated well into the saliva in this study, its

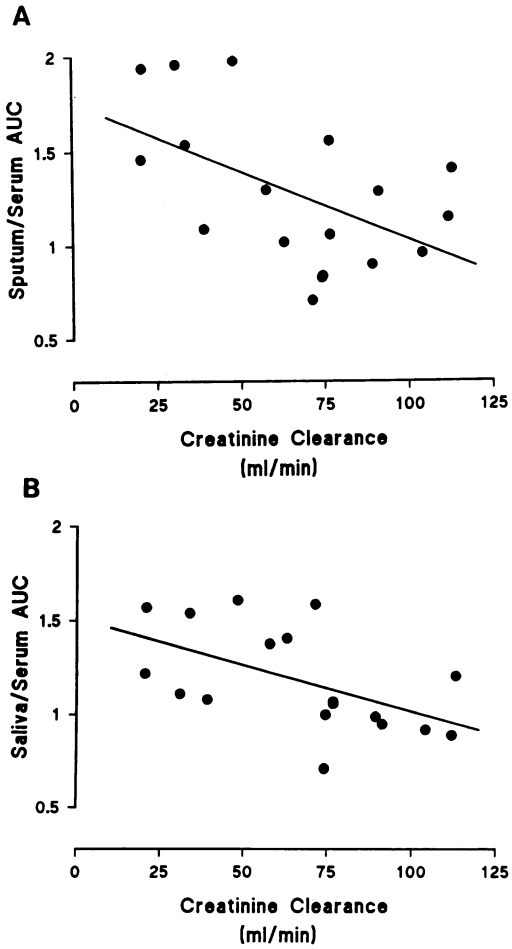

FIG. 2. Relationships between CL_{CR} and sputum AUC-to-serum AUC ratio ($r = -0.643$, $P < 0.05$) (A) and saliva AUC-to-serum AUC ratio ($r = -0.613$, $P < 0.05$) (B) for ofloxacin.

concentration almost paralleling that in the sputum. The 16-h postdose concentrations, the AUCs, and other pharmacokinetic parameters in these secretions were similar. From the viewpoint of OFLX level monitoring during therapy, therefore, the concentration in the saliva appears to be a more accurate index of the level in bronchial secretions than that in the serum in patients with respiratory infections. Furthermore, drug monitoring in the saliva offers a convenient and noninvasive alternative to serum analysis, with particular advantages in geriatric and pediatric cases. However, expectorated sputum is frequently contaminated with saliva, and there have been no reports indicating whether the sputum drug level accurately reflects the level in bronchial secretions; it is also unknown whether the level in sputum is a reliable indicator of the intracellular level in lung tissue. Further study of these matters is needed.

Negative correlations between CL_{CR} and the sputum- and saliva-to-serum AUC ratios suggest that decreased renal function enhanced the distribution to sputum and saliva from the blood, but it is unclear which mechanism(s) might be involved. It is unlikely that impaired renal function would alter the amount of unbound drug necessary for penetration of the tissues, because protein binding has been reported to be 25% (11), which is not high enough to alter the percentage of free OFLX markedly. One possible explanation for this is as follows. An animal study with labeled ^{14}C-OFLX clearly demonstrated greater accumulations in excretory organs such as the salivary gland and the trachea, in comparison with those in the serum (17). From 0.5 to 24 h after the single oral dose, the drug distribution to these organs was two to four times higher than that to the serum, and these higher distributions were maintained after multiple (once a day for 21 days) doses (16). These results suggested that OFLX may be particularly well distributed to the excretory organs and may be secreted into the extravascular fluids, by a process(es) which probably involves not only passive but also active transport. This active transport may be facilitated by renal function impairment. However, these are highly speculative ideas and further study will be needed.

The present study was performed after a single oral administration, and similar observation will be required after multiple dosing, especially since the renal function of the present patients may not have been sufficiently depressed to allow investigation of the relationship between renal impairment and the enhancement of this drug's penetration. Further investigations of whether the concentration in saliva can act as a valid index of the concentration in the bronchial secretion after multiple dosing are required.

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