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# Multiple-Dose Pharmacokinetics of Ofloxacin in Serum, Saliva, and Skin Blister Fluid of Healthy Volunteers

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**The pharmacokinetics of ofloxacin were determined in six healthy volunteers after oral administration of 200 mg twice daily for 3.5 days. To study the pharmacokinetic behavior at the target site in bacterial infection of the skin, drug concentrations were determined in suction blister fluid (SBF) and cantharides blister fluid (CBF), as well as in serum and saliva. Ofloxacin was measured by a high-performance liquid chromatographic assay. Ofloxacin concentrations in saliva amounted to 61 ± 3% of levels in serum. After the final dose, ofloxacin concentrations in blister fluid and serum declined in parallel. Terminal half-lives of ofloxacin in blister fluids (SBF, 7.0 h; CBF, 6.3 h) were in accordance with serum half-life (6.6 h). Favorable penetration into the skin is suggested by high area under the concentration-time curve ratios for blister fluid and serum (CBF, 1.1; SBF, 1.3). During repeated ofloxacin intake, drug levels in SBF and CBF at 12 h amounted to 0.94 and 1.10 μg/ml. Thus, ofloxacin levels in the skin are well above the MIC for 90% of strains tested for, e.g., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Neisseria gonorrhoeae*, and various members of the family *Enterobacteriaceae*. This should also hold true with respect to other tissues.**

Ofloxacin is a fluorinated quinolone exhibiting a marked bactericidal effect by inhibiting DNA gyrase (13, 21). Ofloxacin is active against a wide spectrum of microorganisms, including gram-positive bacteria (11, 23, 24). Moreover, it is one of the first drugs allowing oral treatment of infections caused by *Pseudomonas aeruginosa* (4).

Ofloxacin shows therapeutically valuable pharmacokinetic properties such as a medium-range elimination half-life, almost complete bioavailability, negligible metabolism, and predominant renal excretion (9, 11).

Since the target sites of antibiotics are predominantly located in the tissues, suitable tissue penetration is also mandatory. Lockley et al. (8) determined ofloxacin cantharides blister fluid (CBF) levels after a single oral dose of 600 mg. Kalager and co-workers (6) used the suction blister method to compare tissue levels in the fasting and nonfasting state (ofloxacin, 300 mg orally).

In general, however, ofloxacin is not administered as a single dose but is administered repeatedly for 7 to 10 days. Thus, ofloxacin levels in serum and blister fluid after repeated drug intake are of particular interest. It was the aim of this study to compare ofloxacin levels in the fluids of noninflamed tissues (suction blister fluid [SBF]) (7, 20) and inflamed tissues (CBF) (1, 16, 19) under steady-state conditions. Moreover, levels in BF were related to levels in serum and saliva. A frequently used dosage regimen, 200 mg of ofloxacin twice daily, was chosen.

(This research formed part of the Ph.D. thesis of R. Warlich from Johann Wolfgang Goethe-Universität, Frankfurt am Main, Federal Republic of Germany, 1990. Part of this research was presented at the 4th World Conference on Clinical Pharmacology and Therapeutics, Mannheim-Heidelberg, Federal Republic of Germany, 23 to 28 July 1989.)

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## MATERIALS AND METHODS

**Study design.** Six healthy young adults (five female, one male) with a mean body weight of 71.6 kg (range, 70 to 75 kg) and a mean height of 172 cm (range, 165 to 181 cm) participated in the study. The volunteers had no renal, hepatic, or hematological diseases, and there was no record of an allergy to quinolone carboxylic acids. The study protocol was approved by the local ethics committee, and written informed consent was obtained from each volunteer. During the investigation period, the intake of other drugs and the ingestion of alcohol or caffeine were not allowed.

A 200-mg dose of ofloxacin (Hoechst, Frankfurt am Main, Federal Republic of Germany) was administered twice daily (9:30 a.m. and 9:30 p.m.) for 3 days. After ingesting the last evening dose, each volunteer fixed seven cantharides plasters to the abdomen (for details, see reference 17). Then the participants restrained from food. The next morning suction blistering was performed. By using two suction cups with, together, 10 holes (8 mm in diameter), a pressure of −250 mm Hg was slowly built up and maintained for 2.5 h (for experimental details, see references 15 and 17).

After completing suction blistering, the volunteers received the final ofloxacin dose together with a standardized breakfast. Blood samples for serum and saliva were collected prior to and 0.25, 0.5, 1, 1.5, 2, 3, 6, 12, 27, and 36 h after drug administration. Saliva production was stimulated by chewing Parafilm (American Can Company, Greenwich, Conn.). At 1 h, SBF was collected by pooling the fluid of all blisters. Suction blistering started again at 0.5, 9.5, and 24.5 h, and SBF was collected at 3, 12, and 27 h. CBF was obtained at 0.5, 1, 2, 3, 6, and 12 h after the final ofloxacin dose and once more at 36 h from another blister which had developed from 24 to 36 h after drug intake.

Samples were stored at −20°C until analyzed. By storing biological samples under these conditions, no detectable drug decomposition was found in a recent study (22).

**Determination of ofloxacin.** Ofloxacin levels in serum, saliva, SBF, and CBF were determined by using a new

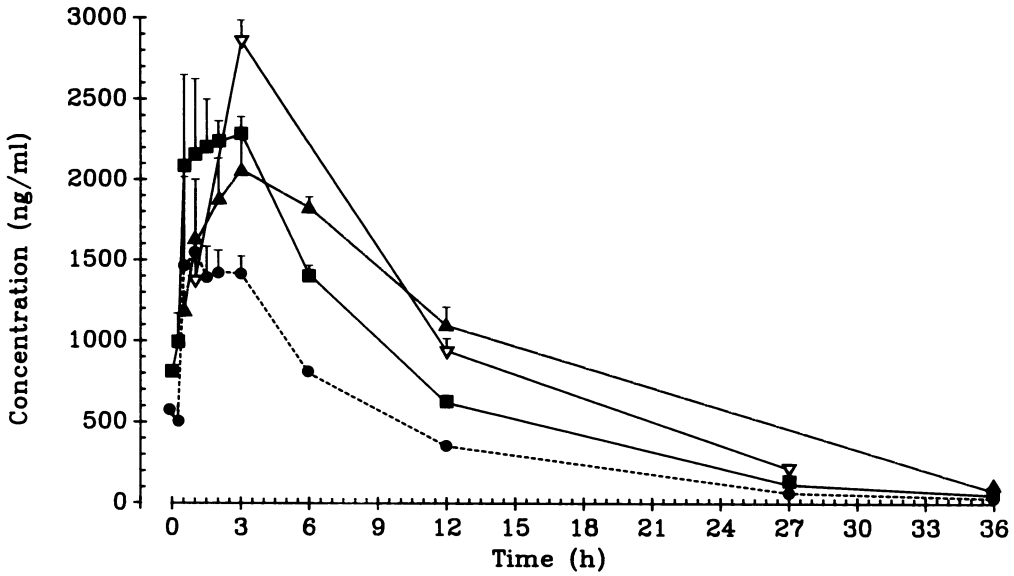


FIG. 1. Ofloxacin concentrations in serum (■), saliva (●), CBF (▲), and SBF (▽) after steady-state administration of twice-daily oral doses of 200 mg. Mean values (+ standard error of the mean [bars]) in six healthy volunteers.

high-performance liquid chromatographic method requiring only small sample volumes (minimum, 30 to 50 μl) (R. Warlich and E. Mutschler, Abstr. 7th Danube Symp. Chromatogr., Leipzig, German Democratic Republic, abstr. no. MO-138, 1989).

BF, serum, and saliva were deproteinized by adding 0.7 M perchloric acid. After centrifugation, a 10-μl portion of the supernatant was directly chromatographed by using a reversed-phase high-performance liquid chromatography system. The stationary phase was NovaPak $C_{18}$ (Waters Millipore, Eschborn, Federal Republic of Germany). The mobile phase consisted of water, acetonitrile, and triethylamine (850:150:1.4 [vol/vol/vol]) adjusted to pH 2.6 by addition of concentrated phosphoric acid and delivered isocratically at 1.0 ml/min at ambient temperature. Eluents were monitored fluorimetrically (excitation and emission wavelengths, 295 and 505 nm).

For all body fluids, linear results were obtained in the range of $10^{-3}$ to 5.0 μg/ml. The coefficients of variation for the within-day performance (between-day performance) ranged from 0.6 to 2.5% (1.5 to 3.1%) for serum, from 1.5 to 2.6% (1.8 to 3.0%) for saliva, and from 0.5 to 2.5% (0.9 to 3.6%) for BF. Recovery varied from 64 to 72% for serum and BF; in the case of saliva, a value of 100% was determined.

Serum standards for the calibration graphs were prepared by using blank human serum; BF standards were prepared by using diluted serum (2:1 [vol/vol]). Saliva standards were made in phosphate buffer, pH 6.

**Pharmacokinetic calculations.** Maximum ofloxacin concentrations ($C_{max}$) in serum, saliva, and skin BF, as well as times to maximum concentrations ($T_{max}$), were obtained from the measured data. The elimination rate constant ($k_{el}$) was calculated from the terminal log-linear decline of concentrations in the measured fluids. The areas under the ofloxacin concentration-time curve ($AUC_{0-12}$) were calculated by the trapezoidal rule. With respect to CBF and SBF, it was assumed that the concentration at $t = 0$ was identical to the drug level at 12 h. Drug penetration into saliva and skin BF was determined by the area ratios for saliva/serum and BF/serum. Oral clearance was calculated by $D/AUC_{0-12}$, and the volume of distribution ($V_\beta$) was calculated by $D/(AUC_{0-12} \cdot k_{el})$.

**Statistical evaluation.** All data are arithmetic mean values; variation is expressed as standard error of the mean. The data were statistically evaluated by using the Wilcoxon test for tied pairs. A $P \leq 0.05$ was considered as significant.

## RESULTS

The mean concentration-time curves for serum, saliva, and skin BF are depicted in Fig. 1; individual pharmacokinetic data are listed in Tables 1 and 2.

**Ofloxacin levels in serum.** Ofloxacin levels prior to the final dose, i.e., at zero hour ($C_0$), amounted to 0.81 μg/ml. Ofloxacin was rapidly absorbed from the gastrointestinal tract. Peak time was 1.9 h, and peak concentration was 2.96 μg/ml. Drug levels declined to 0.62 μg/ml at 12 h and to 0.05 μg/ml at 36 h after the final dose. Terminal elimination half-life amounted to 6.6 h; $AUC_{0-12}$ was 17.8 μg · h/ml. Mean volume of distribution was 1.51 ± 0.05 liters/kg, and oral clearance was 188 ± 5.5 ml/min.

TABLE 1. Pharmacokinetic data derived from ofloxacin levels in serum after 200 mg twice a day for 3.5 days to six healthy volunteers[a]

| Subject no. | $C_0$ (μg/ml) | $C_{12}$ (μg/ml) | $C_{max}$ (μg/ml) | $T_{max}$ (h) | $t_{1/2}$ (h) | $AUC_{0-12}$ (μg · h/ml) |
|---|---|---|---|---|---|---|
| 1 | 0.91 | 0.67 | 2.68 | 3.0 | 6.6 | 18.5 |
| 2 | 0.74 | 0.57 | 4.01 | 0.5 | 7.0 | 18.5 |
| 3 | 0.83 | 0.71 | 2.66 | 1.5 | 6.6 | 18.2 |
| 4 | 0.86 | 0.58 | 3.69 | 0.5 | 6.5 | 18.9 |
| 5 | 0.78 | 0.62 | 2.17 | 3.0 | 6.6 | 15.7 |
| 6 | 0.75 | 0.59 | 2.53 | 3.0 | 6.6 | 17.0 |
| Mean | 0.81 | 0.62 | 2.96 | 1.9 | 6.6 | 17.8 |
| SEM | 0.03 | 0.02 | 0.30 | 0.5 | 0.1 | 0.5 |

[a] $C_0$, Concentration prior to the final dose; $C_{12}$, concentration 12 h later; $C_{max}$, maximum concentration; $T_{max}$, time to maximum concentration; $t_{1/2}$, half-life.

TABLE 2. Pharmacokinetic data derived from ofloxacin levels in CBF, SBF, and saliva after 200 mg twice daily for 3.5 days to six healthy volunteers[a]

| Source of serum specimen and subject no. | $C_{max}$ (μg/ml) | $T_{max}$ (h) | $t_{1/2}$ (h) | $AUC_{0-12}$ (μg · h/ml) |
|---|---|---|---|---|
| CBF | | | | |
| 1 | 2.41 | 3.0 | 6.4 | 19.4 |
| 2 | 2.78 | 2.0 | 6.5 | 20.3 |
| 3 | 1.95 | 3.0 | 6.1 | 20.4 |
| 4 | 2.73 | 1.0 | 6.3 | 21.8 |
| 5 | 1.63 | 6.0 | 5.6 | 16.5 |
| 6 | 1.99 | 6.0 | 6.8 | 18.9 |
| Mean | 2.25 | 3.5 | 6.3 | 19.6 |
| SEM | 0.19 | 0.8 | 0.2 | 0.7 |
| SBF | | | | |
| 1 | 3.39 | 3.0 | 6.3 | 24.9 |
| 2 | 2.95 | 3.0 | 6.4 | 21.7 |
| 3 | 2.74 | 3.0 | 7.1 | 23.6 |
| 4 | 2.84 | 3.0 | 7.5 | 23.3 |
| 5 | 2.84 | 3.0 | 7.3 | 21.8 |
| 6 | 2.41 | 3.0 | 7.3 | 19.9 |
| Mean | 2.86 | 3.0 | 7.0 | 22.5 |
| SEM | 0.13 | 0 | 0.2 | 0.7 |
| Saliva | | | | |
| 1 | 1.33 | 3.0 | 7.0 | 9.4 |
| 2 | 2.95 | 0.5 | 7.5 | 12.1 |
| 3 | 1.63 | 1.0 | 7.4 | 9.5 |
| 4 | 3.50 | 0.5 | 6.2 | 14.5 |
| 5 | 1.40 | 3.0 | 8.4 | 9.5 |
| 6 | 1.59 | 3.0 | 6.4 | 9.8 |
| Mean | 2.07 | 1.8 | 7.2 | 10.8 |
| SEM | 0.38 | 0.5 | 0.3 | 0.8 |

[a] $C_{max}$, Maximum concentration; $T_{max}$, time to maximum concentration; $t_{1/2}$, half-life.

**Ofloxacin levels in saliva.** In saliva, a peak concentration of 2.07 μg/ml was obtained at 1.8 h. Ofloxacin levels in saliva declined in parallel with levels in serum. After 12 and 36 h, mean concentrations were 0.35 μg/ml (range, 0.30 to 0.45 μg/ml) and 0.03 μg/ml (range, 0.02 to 0.04 μg/ml). The terminal half-life of ofloxacin in saliva was 7.2 h. The mean saliva AUC was 10.8 μg · h/ml (60.6% of the serum AUC).

**Ofloxacin levels in skin BF.** Maximum concentrations of 2.25 μg/ml in CBF and 2.86 μg/ml in SBF were reached after 3.5 and 3.0 h, respectively. Mean concentrations declined to 1.10 μg/ml (range, 0.74 to 1.43 μg/ml) and 0.94 μg/ml (range, 0.73 to 1.27 μg/ml) after 12 h in CBF and SBF. At 27 h, levels in SBF were 0.21 μg/ml (range, 0.14 to 0.29 μg/ml). Concentrations in CBF ranged from 0.06 to 0.10 μg/ml (mean, 0.08 μg/ml) 36 h after ofloxacin intake.

$AUC_{0-12}$s were 22.5 μg · h/ml for SBF and 19.6 μg · h/ml for CBF. AUC ratios were 1.3 for SBF and 1.1 for CBF. Mean terminal half-lives were 6.3 h for CBF and 7.0 h for SBF.

Drug-related side effects were not observed.

## DISCUSSION

Ofloxacin and other antibiotics are predominantly administered for several days or even weeks. Pharmacokinetic experiments, however, in general are performed after a single dose. This holds true especially with respect to studies in which skin blistering techniques (14) are used for the evaluation of tissue penetration. The present study was performed to get detailed information on ofloxacin penetration into various human body fluids after repeated drug intake.

Although ofloxacin was administered under steady-state conditions, concentrations in serum and saliva before the final dose (0.81 and 0.54 μg/ml) exceeded the concentrations 12 h thereafter (0.62 and 0.35 μg/ml); $P \leq 0.05$. This results from volunteers fasting for 12 h after the evening dose and receiving the drug with food in the morning. Food delays ofloxacin absorption from the gastrointestinal tract (21) and thus can account for the differences in the $C_0$ and $C_{12}$ values.

Ofloxacin penetrated excellently into CBF and SBF, with mean peak levels of 2.25 and 2.86 μg/ml (corresponding serum value, 2.96 μg/ml). Peak concentrations in CBF and SBF were reached after 3.5 and 3.0 h, respectively. The relatively short $T_{max}$s can be explained by the low protein binding of ofloxacin (25% [9]), since drugs extensively bound to plasma proteins show delayed penetration into skin BF (14, 18). From 3 h after the final dose to the end of the investigation period, drug levels in BF exceeded concentrations in serum. Drug penetration into the tissue compartment is best calculated by the area ratios ($AUC_{tissue}/AUC_{serum}$) (2). AUC ratios for CBF (1.1) and SBF (1.3) and the course of the concentration-time curves in skin blisters and serum both demonstrate the particular ability of ofloxacin to penetrate into the extravascular compartment. The ratios are in good correspondence with data reported in previous single-dose studies. Kalager and co-workers (6) determined a ratio of 1.0 for SBF, and Lockley et al. (8) determined a ratio of 1.3 for CBF.

The pharmacokinetic behaviors of ofloxacin in SBF and CBF were similar. Elimination half-lives, $T_{max}$, and AUC values (Table 2) were very similar. Higher peak levels, however, were reached in SBF (2.86 μg/ml) than in CBF (2.25 μg/ml; $P \leq 0.05$). This finding results from differences between the two blister types. The composition of SBF is identical to that of interstitial fluid (20), whereas CBF is an inflammatory exudate (19). Thus, the penetration into and out of CBF may be influenced by the inflammatory reaction. Kalager et al. (6) postulated that the inflammatory process may accelerate drug elimination from blisters, yet identical half-lives for SBF and CBF were observed here. Moreover, BF half-lives were identical to serum half-lives. After the single-dose experiments, half-lives in SBF and CBF exceeded the respective serum half-lives (10.6 h [6] and 8 h [8] versus 6.3 and 7.0 h). This may result from the fact that the periods of blister fluid sampling (10 and 12 h [6, 8]) were too short to obtain a sufficient decrease in ofloxacin concentrations.

In addition to levels in BF, ofloxacin concentrations were determined in saliva. Peak concentrations in saliva and serum were reached simultaneously, and saliva half-lives were close to serum half-lives. Saliva area was 60.6% of serum area. There is a rather close relation of levels in saliva and serum. With respect to drug concentrations during the elimination phase, there is an even closer relation (correlation coefficient, 0.989). Thus, ofloxacin determination in saliva seems to be suitable for a noninvasive evaluation if noncompliance is suspected. Furthermore, drug monitoring in patients suffering from severe anemia (e.g., hemodialysis patients) seems to be practicable by saliva sampling (12).

Levels in CBF and SBF close to 1 μg/ml suggest that infections due to gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* (5) may be successfully treated with 200 mg of ofloxacin twice daily. With respect to gram-negative organisms, this dosage is

sufficient to exceed the MIC for 90% of strains tested for, e.g., members of the family *Enterobacteriaceae* (10) and *Neisseria gonorrhoeae* (3). In the treatment of infections caused by less susceptible strains of *P. aeruginosa* (10), however, a higher dosage, which is also well tolerated (8, 21), has to be recommended.

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