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Comparison of High-Pressure Liquid Chromatography and Microbiological Assay for the Determination of Biliary Elimination of Ciprofloxacin in Humans

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Serum kinetics and biliary, urinary, and fecal elimination of ciprofloxacin, a new quinolone derivative, were studied in 12 recently cholecystectomized patients provided with T-tube drainage during 24 h after oral administration of a single 500-mg dose of this substance. Drug concentrations were measured by both high-pressure liquid chromatography (HPLC) and microbiological assay. The results were comparable for the concentrations in serum (average of peaks, 2.0 ± 0.2 μg/ml by HPLC and 2.3 ± 0.3 μg/ml by the microbiological method) and urine (0 to 6 h, 267 ± 74 and 241 ± 58 μg/ml, respectively). This was not the case for biliary values, for which the microbiological assay yielded significantly higher concentrations than did HPLC (average of peak concentrations, 21.2 ± 2.6 and 16.0 ± 2.5 μg/ml, respectively [$P < 0.02$]), nor for total 24-h biliary output (2,167 ± 288 and 1,587 ± 222 μg, respectively [$P < 0.01$]). This suggests hepatic biotransformation of ciprofloxacin into microbiologically active metabolites. The apparent broad antibacterial spectrum of ciprofloxacin and its higher biliary levels than simultaneously determined serum concentrations suggest that this derivative is suitable for the treatment of biliary tract infections.

During recent years, the derivatives of the quinolone group have been extended (2, 10) by a new class of fluorine compounds substituted in position 7 by a piperazinyl group. These second-generation quinolones, like ciprofloxacin, norfloxacin, ofloxacin, and pefloxacin, have interesting pharmacologic and antibacterial properties in comparison with the first-generation group—better gastrointestinal absorption, absence of cross resistance with other antibacterial agents, and a broader in vitro antibacterial spectrum against clinically relevant gram-positive and gram-negative rods (particularly *Pseudomonas aeruginosa* [5, 10, 12]). Therefore, the treatment of systemic or localized infections by an orally administered quinolone derivative may be an interesting alternative to parenterally administered beta-lactam or aminoglycoside antibiotics. In this respect, it would also be of interest to assess whether the oral administration of quinolone derivatives produces sufficient biliary concentrations to deal with biliary tract infections. The purpose of the present study was to investigate the biliary elimination of one of these new derivatives, ciprofloxacin (formerly named Bay o 9867), in cholecystectomized patients provided with a T tube.

MATERIALS AND METHODS

Patient selection. Patients (five women and seven men) recently cholecystectomized and provided with T-tube drainage were included in this study. There was no evidence of liver or kidney impairment. Their ages ranged from 28 to 58 years (average, 47.5 ± a standard error of the mean of 2.7 years), and they weighed from 64 to 78 kg (average, 71.3 ± 1.5 kg). Written informed consent was obtained from the patients participating in this study. No other antimicrobial agents were given concurrently with ciprofloxacin.

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Investigational procedure. The protocol applied was the same as that used to study the biliary elimination of other antibiotics (1, 3). The fasting patients were given a single oral dose of 500 mg of ciprofloxacin (supplied by Bayer-Pharma, Paris-Puteaux, France). Blood samples were drawn before drug administration and 0.25, 0.50, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 24 h after drug administration. Bile was collected at hourly intervals for the first 4 h after drug administration, every 2 h for the next 8 h, and again after another 12 h. Urine was collected after each of the first two 6-h periods after drug administration and again after another 12 h. Total feces were collected during the 24 h after drug administration. These investigations were performed on postoperative day 8, a time considered sufficiently remote from the operation but still with an adequate bile flow through the T drain (11). All samples were immediately refrigerated and stored at −70°C until assay.

Analytical procedures. (i) HPLC. Serum, bile, and urine were assayed by the previously described method of high-pressure liquid chromatography (HPLC) (9). Separations were performed on a C18 reversed-phase analytical column (Ultrasphere ODS). The mobile phase consisted of a mixture of 0.005 M tetrabutylammonium bromide-acetonitrile (90:10 [vol/vol]), and the pH was adjusted to 2.0 with phosphoric acid. Biological fluids (500 μl; serum and urine diluted to 1/20, bile diluted to 1/10) were added to 3.5 ml of methylene chloride in a 6-ml screw-capped glass tube.

After mixing for a few seconds on a Vortex mixer, the tubes were gently shaken for 10 min by rotation (20 rpm) and then centrifuged for 10 min at 1,000 × *g*. The upper aqueous layer was aspirated and discarded, and 3 ml of the lower organic phase was transferred into a second screw-capped glass tube. Ciprofloxacin was then extracted into 200 μl of phosphoric acid (pH 2.0) by rotation for 30 min. Centrifugation at 1,000 × *g* for 10 min produced two phases. Part (20 μl) of the upper aqueous phase was then injected into the

TABLE 1. Mean ciprofloxacin concentrations comparatively determined

Measurement method	Mean concn (μg/ml) ± SEM at Serum									
	0.25	0.50	1	2	3	4	5	6	7	8
HPLC	0.14 ± 0.11	0.38 ± 0.26	0.69 ± 0.28	0.74 ± 0.18	0.81 ± 0,14	0.95 ± 0.26	0.97 ± 0.17	0.88 ± 0.17	0.85 ± 0.17	0.83 ± 0.17
Microbiological assay	0.13 ± 0.11	0.42 ± 0.30	0.72 ± 0.30	0.89 ± 0.27	0.86 ± 0.18	1.06 ± 0.25	1.08 ± 0.19	1.04 ± 0.20	0.97 ± 0.20	0.91 ± 0.19
Statistical significance[a]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]NS, Not significant.

liquid chromatograph, and quantitation was determined by absorbance at 254 nm.

(ii) Microbiological assay. The quantitative determination of microbiologically active drug in plasma, bile, and urine was made by an agar diffusion assay. The test organism used was *Escherichia coli* ICB4004 (Bayer AG, Wuppertal, Federal Republic of Germany). Isotonic Sensitest agar (Oxoid Ltd., London, England) medium was prepared as directed by the manufacturer and poured onto assay plates (20 by 20 cm) (about 80 ml per plate). The final agar thickness was 0.2 cm on the plates. After cooling of the agar, 2-mm-diameter wells were punched. The arrangement of the punched holes and the positioning of the different samples were randomized. An exactly measured volume of 5 μl of sample was dispensed into each well. Standard serum concentrations (5, 2.5, 1.2, 0.6, and 0.3 μg/ml) were obtained by diluting the stock solution with antibiotic-free serum. For urine and bile, standard concentrations of 10, 5, 2.5, 1.2, and 0.6 μg/ml were prepared with buffered saline (pH 7.2). Each sample was run in triplicate. Results were read after overnight incubation at 37°C. Inhibition zones were measured with the aid of a light projector.

(iii) Determination of fecal ciprofloxacin. Feces triturated with a phosphate buffer (pH 4.0) were paper filtered, and microfiltration of this filtrate was carried out on a Millipore (0.45-μm pore size) membrane filter (Millipore Corp., Bedford, Mass.). This last filtrate was used for HPLC and the microbiological assay.

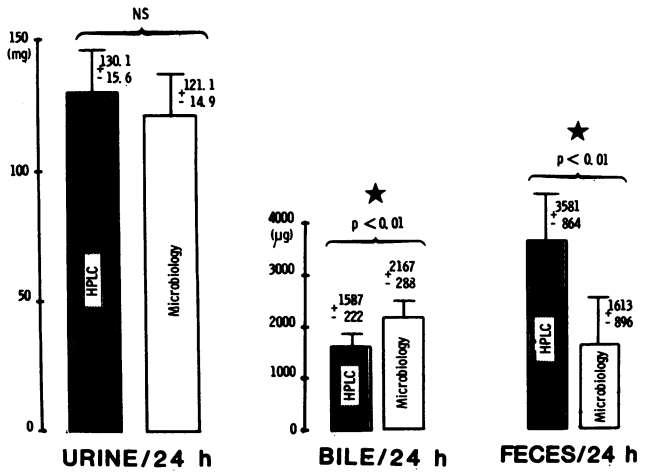

FIG. 1. Elimination of ciprofloxacin in urine, bile, and feces 24 h after oral administration of 500 mg of the antibiotic. The determinations were performed by HPLC and microbiological assay. Vertical bars indicate the standard error of the mean. Stars indicate statistically significant differences.

Calculations. Serum concentration-time curves were analyzed by linear regression analysis of the time points between 0 and 12 h (6). The following parameters were studied: serum elimination half-life, serum overall elimination rate constant, absorption half-time, and absorption rate constant. Pharmacokinetics were expressed as the means of the data on individual patients. Areas under the serum and biliary concentration curves were established by using the Simpson trapezoidal rule. The statistical significance of differences of means was tested by the Wilcoxon signed rank test.

RESULTS

The average serum concentration-time curves of ciprofloxacin found by HPLC did not significantly differ from those found by microbiological assay (Table 1). With both methods, respective mean values of individual peak concentrations of 2.0 ± 0.2 and 2.3 ± 0.2 μg/ml were reached about 3.5 h after administration of the antibiotic.

The absorption half-times were 0.8 ± 0.3 and 0.9 ± 0.3 h, the serum half-lives were 3.4 ± 0.5 and 3.2 ± 0.6 h, the absorption rate constants were 0.91 ± 0.32 and 0.78 ± 0.29 h^{-1}, and the overall elimination rate constants were 0.210 ± 0.027 and 0.220 ± 0.034 h^{-1} with HPLC and the microbiological assay, respectively.

The mean concentrations of ciprofloxacin found in urine by HPLC and the microbiological assay did not differ significantly. For 0 to 6 h, they were 267 ± 74 versus 241 ± 58 μg/ml, respectively. For 0 to 12 h, the means were 213 ± 53 and 210 ± 54 μg/ml, and for 12 to 24 h they were 89 ± 20 and 79 ± 20 μg/ml. The total 24-h elimination of the antibiotic was 26.0 ± 3.1% of the dose given by HPLC and 24.2 ± 3.0% by the microbiological assay (Fig. 1).

The average 24-h values for the elimination of ciprofloxacin in feces were 0.72 ± 0.17 and 0.32 ± 0.18% for HPLC and the microbiological assay, respectively ($P < 0.01$) (Fig. 1).

The mean concentrations of the antibiotic found in bile by the microbiological assay were significantly higher (average of individual peak values, 21.2 ± 2.6 μg/ml; 4.3 ± 0.8 h after administration) than those found by HPLC (average of individual peak values, 16.0 ± 2.5 μg/ml; 3.9 ± 0.7 h after administration; $P < 0.02$) (Table 1). The ratios of peak drug concentrations in bile and serum were 8.0 by HPLC and 9.2 by the microbiological method. Also, the total 24-h mean biliary output of ciprofloxacin was higher by the microbiological assay than by HPLC (Fig. 1)—0.43 ± 0.06 versus 0.32 ± 0.04%, respectively, of the dose given ($P < 0.01$). The

by HPLC and microbiological assay of serum and bile of 12 patients

time (h) after administration in:											
			Bile								
9	10	24	0–1	1–2	2–3	3–4	4–6	6–8	8–10	10–12	12–24
0.67 ± 0.14	0.61 ± 0.13	0.15 ± 0.07	1.1 ± 0.5	7.5 ± 2.8	6.1 ± 1.8	5.4 ± 2.3	6.1 ± 1.5	5.2 ± 1.5	4.7 ± 1.8	2.6 ± 0.9	1.1 ± 0.4
0.71 ± 0.16	0.58 ± 0.15	0.03 ± 0.02	0.9 ± 0.5	10.0 ± 3.4	8.2 ± 2.1	8.5 ± 2.5	9.0 ± 2.2	8.2 ± 2.5	6.9 ± 2.5	3.7 ± 1.6	0.9 ± 0.4
NS	NS	$P < 0.01$	NS	$P < 0.02$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.02$	$P < 0.02$	NS	NS

same held true for the ratios of the 24-h areas under the curves for drug concentrations in serum and bile, which were 6.6 and 9.2 ($P < 0.01$) by the microbiological and HPLC methods, respectively.

DISCUSSION

The present data on the biliary excretion of ciprofloxacin cannot be compared with other, so far unavailable, reports. The lower serum concentrations found in this study compared with those in healthy volunteers receiving the same dose (4, 8) cannot be explained. But the more prolonged time-serum concentration curve and larger areas under the curves in our investigation (12.95 ± 1.99 and 12.82 ± 1.68 μg · h/ml by HPLC and the microbiological method, respectively) than those found previously (9.9 ± 2.4 [4] and 7.383 ± 1.601 μg · h/ml [8], respectively) are compatible with still impaired renal and hepatic excretory functions on postoperative day 8.

According to our data, biliary elimination of ciprofloxacin seems to be relatively low but greatly exceeds the concentrations of the drug in serum in the 12 h after drug administration. No significant difference was observed in our study between the total biliary elimination of ciprofloxacin in five women (1,721 ± 181 and 2,190 ± 318 μg by HPLC and the microbiological method, respectively) and seven men (1,492 ± 666 and 2,193 ± 544 μg, respectively).

In contrast to serum concentration-time curves and urinary values, which did not significantly differ by HPLC and the microbiological assay, fecal elimination of the antibiotic assessed by HPLC was significantly higher than that found by the microbiological assay (Fig. 1). The cause of this discrepancy is not clear. The possibility that the antibacterial activity of ciprofloxacin might have been inhibited by a factor present in feces was excluded by identical findings with the two methods after a 36-h in vitro incubation of ciprofloxacin with human feces. As for the biliary values, they were higher by the microbiological assay than by HPLC (Fig. 1 and Table 1). This suggests hepatic metabolism of ciprofloxacin to one or more microbiologically active metabolites (7, 9, 14; H.-J. Zeiler, V. Petersen, and W. Gau, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 983, 1984) with high biliary elimination and poor excretion into urine.

On the other hand, we demonstrated that the addition of ciprofloxacin to bile and buffer produced no difference in the inhibitory effect against test organisms. Standard curves of ciprofloxacin added to serum and bile were similar when the microbiological assay or HPLC was used. This argues against a different degradation of ciprofloxacin in the two biological fluids.

The average 24-h volume of bile collected by the T tube in our patients on postcholecystectomy day 8 amounted to 553 ± 108 ml. This agrees with the volume of 500 to 600 ml indicated by Rundle et al. (11) for the same period.

The organisms commonly responsible for biliary infections (*E. coli*, enterococci, streptococci, *P. aeruginosa*, *Salmonella* spp., *Enterobacter* spp., and *Proteus* spp.) are, with the exception of anaerobes, included in the antibacterial spectrum of ciprofloxacin. The minimal concentration necessary for their inhibition is reported (5, 10, 12) to be no higher than 4.0 μg of ciprofloxacin per ml. As is apparent from the present work, this concentration is reached or exceeded in the bile between 2 and 10 h after oral administration of 500 mg of the antibiotic. These data are, therefore, consistent with the view that ciprofloxacin may favorably affect biliary infections, provided that no hindrance (choledochal obstruction or impairment of liver function) to its biliary elimination is present. However, these considerations call for further clinical trials.

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