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Enantioselective Disposition of Ofloxacin in Humans

OSAMU OKAZAKI,[1]* CHIEKO KOJIMA,[1] HIDEO HAKUSUI,[1] AND MITSUYOSHI NAKASHIMA[2]

Drug Metabolism and Analytical Chemistry Research Center, Developmental Research Laboratories, Daiichi Pharmaceutical Co., Ltd., 16-13, Kita-kasai 1-Chome, Edogawa-ku, Tokyo 134,[1] and Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu 431-31,[2] Japan

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The enantioselective disposition of ofloxacin (OFLX) was studied in healthy subjects after oral administration of (±)-OFLX at a dose of 200 mg. *S*-(−)-OFLX and *R*-(+)-OFLX concentrations in serum and urine were measured separately by high-performance liquid chromatography, and various pharmacokinetic parameters were calculated from the data. The ratio of *S*-(−) to *R*-(+) enantiomer concentrations in serum showed a increase with time, with *S/R* ratios of 1.01 at 2 h and 1.31 at 24 h. The terminal elimination half-life of *S*-(−)-OFLX was 6.9 h, which was significantly greater ($P < 0.05$) than that of the *R*-(+) enantiomer (6.3 h). *S*-(−)-OFLX also revealed a significantly greater area under the concentration-time curve in serum, mean residence time, and total body clearance than the *R*-(+) enantiomer did. The renal clearance of *S*-(−)-OFLX (7.14 liters/h/1.73 m^2) was significantly lower than that of the *R*-(+) enantiomer (7.53 liters/h/1.73 m^2). Although the difference in the pharmacokinetic parameters of the enantiomers was small, their disposition in humans was found to be stereoselective. The difference between the enantiomers may be explained by the difference in their renal excretion.

Ofloxacin [(±)-OFLX] is a fluorinated quinolone exhibiting a marked bactericidal activity mediated by inhibition of DNA gyrase (6, 16). (±)-OFLX has a methyl group at the C-3 position in the oxazine ring, resulting in an asymmetric carbon. Recently, OFLX enantiomers have been successfully prepared by asymmetric synthesis (15). It has been shown that the antibacterial activity of *S*-(−)-OFLX against gram-positive and gram-negative bacteria is 8 to 128 times more potent than that of the *R*-(+) enantiomer and twice as potent as that of (±)-OFLX (3, 5).

Numerous investigators have described differences in the metabolism and disposition of the enantiomers of many chiral drugs (2, 7, 8, 18). In a previous paper, we reported the stereoselective disposition of the enantiomers of OFLX in rats (13), showing that there are marked pharmacokinetic differences between the enantiomers of OFLX caused by stereoselective glucuronidation. The major metabolic pathway of OFLX in rats is glucuronidation of the carboxyl group at the C-3 position of the quinolone ring (20). However, there exist species differences in OFLX glucuronidation, as the small amount of glucuronide excreted in urine by dogs and monkeys indicates (19). In humans, (±)-OFLX has been shown to undergo only limited metabolism with urinary excretion of small amounts of metabolites (desmethyl OFLX and OFLX *N*-oxide) and most of the administered drug is excreted unchanged in the urine (11, 24, 25).

OFLX is used clinically as a racemic mixture of *S*-(−) and *R*-(+) enantiomers, and its therapeutic efficacy against various infectious diseases is mainly derived from the *S*-(−) enantiomer. Therefore, it is of great importance in seeking a more thorough understanding of the clinical efficacy of the enantiomers to know how they are handled by the body. The objective of the study presented here was to clarify the pharmacokinetics of OFLX enantiomers after oral administration of racemic OFLX in healthy subjects, using a sensitive and stereoselective high-performance liquid chromatography (HPLC) assay (9).

* Corresponding author.

MATERIALS AND METHODS

Study design and drug administration. Five adult male subjects, with an average age of 33 years (27 to 36 years) and an average weight of 71 kg (60 to 97 kg), participated in this study. Subjects were judged to be in good health on the basis of their medical history, physical examination, and laboratory profiles, which were performed within 2 weeks before the study. All subjects signed an informed consent agreement that had been approved by the Local Ethics Committee of the Hamamatsu University School of Medicine. The subjects were given an oral dose of 200 mg of racemic OFLX in two tablets with water 2 h after receiving regularly scheduled meals. During the investigation period, the intake of other drugs and of alcohol was not allowed. Blood samples were withdrawn from the forearm vein before drug administration and at 0.25, 0.5, 1, 2, 3, 4, 6, 10, and 24 h following drug administration. Serum samples were prepared by centrifuging the blood samples at 2,500 × *g* for 10 min and were stored at −20°C until analysis. Urine samples were collected in plastic bags before dosing and at 2, 4, 6, 8, 12, 24, and 48 h after it. The volumes of the samples were measured immediately after collection, and the urine samples were stored at −20°C until analysis.

Protein binding study. The protein binding of the ^{14}C-*S*-(−) and ^{14}C-*R*-(+) enantiomers of OFLX in serum was determined by the ultracentrifugation method (4). Each enantiomer was added separately to the serum to yield final concentrations of 1 and 10 μg/ml. After incubation at 37°C for 1 h, 0.25 ml of serum samples were transferred to a centrifuge tube (Hitachi, Tokyo, Japan) and were centrifuged at 40,000 × *g* for 16 h at 4°C. The protein binding rate was determined from drug concentrations in the supernatant fraction of serum samples (50 μl) and in the original serum samples analyzed by radiochemical assay.

Determination of enantiomers of OFLX in serum and urine. Concentrations of *S*-(−)- and *R*-(+)-OFLX in serum and urine were measured separately by an HPLC method described elsewhere (9) by using DS-4632 {*R*-(+)-9-fluoro-3-methyl-10-(4-ethyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-py-

rido-[1,2,3-de] [1,4]benzoxazine-6-carboxylic acid}, as an internal standard. Briefly, serum (0.2 ml) and urine (1 ml) were mixed with 1 ml of phosphate buffer (pH 7.0) containing internal standard and shaken with 2 ml of dichloromethane for 20 min. One milliliter of the dichloromethane extract was transferred to another tube, and 20 μl of diphenylphosphinyl chloride (0.105 nmol) and 20 μl of triethylamine (0.144 nmol) were added to the extract. The mixture was stirred for 10 s. After further addition of 500 μl of the L-leucinamide solution (ca. 0.15 mmol), the mixture was shaken for 10 min and extracted with 200 μl of 1 N HCl. Approximately 10 to 100 μl of the extracted solution was injected onto a reverse-phase column (Nucleosil $5C_{18}$; particle size, 5 μm; inside diameter, 15 cm by 4.6 mm; Macherey Nagel, Germany) by using an automatic injector (Sample Injector Model 231; Gilson, France). The mobile phase contained 0.2 M tetraethylammonium phosphate buffer (pH 1.85) and acetonitrile (8/2 [vol/vol]). The effluent was monitored with a fluorescence detector (F-1000; Hitachi), using 298 nm for excitation and 458 nm for emission.

Pharmacokinetic calculation. The peak concentration in serum (C_{max}) and the time to peak concentration (T_{max}) were obtained from the observed data. The elimination rate constant (k_{el}) was obtained by linear regression analysis without weighting factor. The terminal log linear portion of the serum concentration-time curve was defined by the last n ($n \geqq 3$) datum points, where n was selected to minimize the mean square error. The elimination half-life in serum was calculated by dividing 0.693 by k_{el}. The area under the concentration-time curve in serum ($AUC_{0-\infty}$) was calculated by the trapezoidal rule and extrapolated to infinity by dividing the last observed concentration by k_{el}. Total body clearance (Cl_{tot}) was calculated as the dose divided by AUC and was corrected by the body surface area (1.73 m^2). The apparent volume of distribution (V) was calculated as Cl_{tot} divided by k_{el}. Calculation of Cl_{tot} and V was based on the assumption that bioavailability from oral dosing was 100% (11). Student's paired t test was used to distinguish the statistical significance of differences in the pharmacokinetic parameters obtained for the enantiomers.

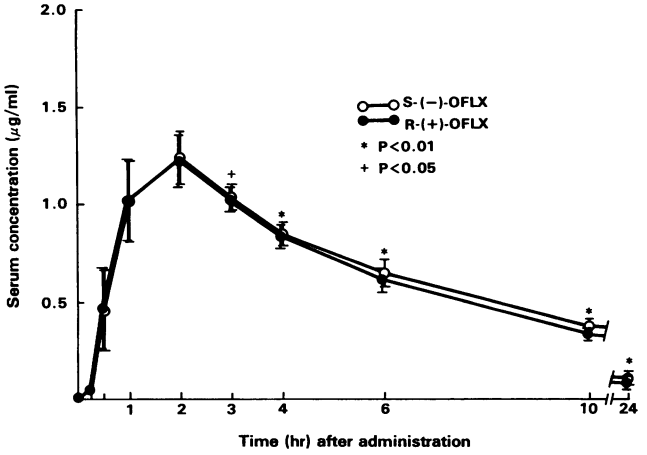

FIG. 1. Concentrations of S-(−)-OFLX and R-(+)-OFLX in serum from healthy male subjects after a single oral administration of 200 mg of (±)-OFLX. Each value represents the mean ± standard error of five determinations.

TABLE 1. Enantiomer concentration ratios (S/R) in serum after an oral administration of (±)-OFLX (200 mg) to healthy volunteers

Time (h)	S/R[a] in serum
0.5	0.97 ± 0.04
1	1.00 ± 0.01
2	1.01 ± 0.01
3	1.01 ± 0.01
4	1.02 ± 0.01
6	1.06 ± 0.01
10	1.13 ± 0.02
24	1.31 ± 0.07

[a] Each value represents the mean ± standard deviation of five determinations.

RESULTS

Concentration of S-(−)- and R-(+)-OFLX in serum. S-(−)- and R-(+)-OFLX concentrations in serum after an oral administration of 200 mg of racemic OFLX were measured separately by HPLC. As shown in Fig. 1, each enantiomer reached a peak at 2 h after administration and the C_{max} values of S-(−)- and R-(+)-OFLX were 1.24 and 1.22 μg/ml, respectively, which were not significantly different. However, the concentrations of S-(−)-OFLX in serum at 3 h and thereafter were significantly higher than those of the R-(+) form. Table 1 presents the S/R concentration ratios in serum. The concentration ratios of S-(−) to R-(+) forms increased with time, resulting in a value 1.3 times higher at 24 h after administration.

Urinary excretion of S-(−)- and R-(+)-OFLX. Table 2 shows the urinary excretions of S-(−)- and R-(+)-OFLX after oral administration of (±)-OFLX. Urinary concentrations of S-(−)-OFLX in the earlier period after dosing were lower than those of the R-(+) form. However, S-(−)-OFLX was predominant in the urine at 6 h after medication and thereafter. The S-(−)/R-(+) concentration ratios in urine from the 0- to 2-, 6- to 8-, and 24- to 48-h periods were 0.93, 1.02, and 1.47, respectively (Table 3). The cumulative urinary excretions of S-(−)- and R-(+)-OFLX 48 h after dosing were 82.9 and 79.7% of the dose, respectively, so there was

TABLE 2. Urinary concentrations of OFLX enantiomers after an oral administration of (±)-OFLX (200 mg) to healthy volunteers

Time (h)	Concn (μg/ml) in urine[a]	
	S-(−)-OFLX	R-(+)-OFLX
0–2	111.2 ± 34.3	120.9 ± 40.2
	(10.8 ± 3.1)	(11.6 ± 3.3)
2–4	138.2 ± 61.1	147.6 ± 65.3
	(27.9 ± 3.9)	(29.0 ± 3.5)
4–6	121.7 ± 16.7	126.2 ± 14.6
	(39.4 ± 5.2)	(41.7 ± 4.5)
6–8	73.7 ± 11.5	72.7 ± 11.2
	(50.2 ± 6.4)	(52.4 ± 5.5)
8–12	42.5 ± 17.4	40.4 ± 19.2
	(62.3 ± 6.4)	(63.7 ± 4.8)
12–24	21.9 ± 4.4	18.1 ± 3.4
	(75.9 ± 7.7)	(74.9 ± 5.3)
24–48	5.5 ± 1.3	3.8 ± 1.0
	(82.9 ± 5.7)	(79.7 ± 3.9)

[a] Each value represents the mean ± standard deviation of five determinations. Values in parentheses are cumulative urinary excretions of OFLX enantiomers.

TABLE 3. Enantiomer concentration ratios (*S*/*R*) in urine after an oral administration of (±)-OFLX (200 mg) to healthy volunteers

Time (h)	*S*/*R*[a] in urine
0–2	0.93 ± 0.04
2–4	0.94 ± 0.04
4–6	0.96 ± 0.04
6–8	1.02 ± 0.04
8–12	1.08 ± 0.07
12–24	1.21 ± 0.08
24–48	1.47 ± 0.08

[a] Each value represents the mean ± standard deviation of five determinations.

no difference between amounts of urinary excretion with the two enantiomers.

Protein binding of *S*-(−)- and *R*-(+)-OFLX in vitro. The protein binding of *S*-(−)- and *R*-(+)-OFLX to human serum was studied in vitro. The protein binding of OFLX enantiomers after 1 μg of each enantiomer per ml was added to a separate sample of serum ranged from approximately 47 to 52%. In addition, the protein binding of OFLX in serum did not change between drug concentrations (1 and 10 μg/ml). Moreover, there was no stereoselectivity in the protein binding in serum between the enantiomers.

Comparison of pharmacokinetic parameters between the enantiomers. The pharmacokinetic parameters calculated from all subjects are presented in Table 4. There were no significant differences in the C_{max}, T_{max}, or V of the two enantiomers; however, the AUC values of the *S*-(−) and *R*-(+) forms were 11.66 and 10.62 μg · h/ml, respectively, and these values did show a significant difference between the enantiomers. The half-life of *S*-(−)-OFLX (6.93 h) was significantly longer than that of *R*-(+)-OFLX (6.26 h). Moreover, mean residence time and the total body clearance also revealed enantioselectivity. The renal clearance of *R*-(+)-OFLX was 7.53 liters/h/1.73 m^2 and was significantly greater ($P < 0.01$) than that of the *S*-(−) enantiomer (7.14 liters/h/1.73 m^2). The creatinine clearance of the subjects was 4.46 liters/h/1.73 m^2 and was lower than the renal clearance of the enantiomers.

DISCUSSION

There are a number of chiral drugs that are used therapeutically as racemic mixtures. Recently, progress in the development of analytical methods for the efficient resolution of enantiomers has facilitated studies of the differences in the pharmacological properties of the enantiomers of many chiral agents, and it has been shown that such differences can originate from stereoselectivity in their absorption (22), distribution (1, 26), receptor binding (23), metabolism (17, 18) and/or excretion (10). Among the quinolone antibacterial agents there are many drugs, such as lomefloxacin, sparfloxacin, and OFLX, which possess an asymmetric carbon in their chemical structures. At the present time, however, there are no reports on the stereoselective pharmacokinetic and pharmacodynamic properties of quinolones other than OFLX. We have demonstrated stereoselective disposition of OFLX in rats that is caused by stereoselective glucuronidation in rat hepatic microsomes (12). Racemic OFLX, however, is metabolized to a very small degree compared with other quinolones in humans (11, 14). Approximately 4 to 5% of the administered drug is excreted in the urine as either the desmethyl or the *N*-oxide metabolite, and most of the drug given is recovered in the urine in its unchanged form. Consequently, it is supposed that there is little possibility of stereoselectivity in the metabolism of OFLX enantiomers in humans. The study reported here examined whether the pharmacokinetics of OFLX enantiomers in healthy subjects is stereoselective.

Many pharmacokinetic parameters of OFLX have been established in healthy (11) and renal failure (25) subjects. The present data show that concentration profiles of *S*-(−)-OFLX and *R*-(+)-OFLX in serum after oral (±)-OFLX administration in humans were almost equal to those of the racemic drug reported in healthy subjects. However, there is a significant difference in the pharmacokinetic parameters of the OFLX enantiomers. This difference can be detected by the separate quantitation of OFLX enantiomers by a highly

TABLE 4. Comparison of pharmacokinetic parameters of *S*-(−)-OFLX and *R*-(+)-OFLX after an oral administration of (±)-OFLX (200 mg) to healthy volunteers[a]

Enantiomer	Subject no.	C_{max} (μg/ml)	T_{max} (h)	k_{el} (h^{-1})	$t_{1/2}$ (h)	V_{area} (liter/kg)	$AUC_{0-\infty}$ (μg · h/ml)	MRT (h)	Cl_{tot} (liter/h/1.73 m^2)	Cl_{ren} (liter/h/1.73 m^2)	Cl_{cre} (liter/h/1.73 m^2)
S-(−)-OFLX	1	1.57	1.0	0.080	8.63	1.45	14.27	7.41	7.26	5.41	4.20
	2	1.45	1.0	0.099	6.99	1.20	11.71	6.70	8.30	7.04	4.67
	3	1.64	2.0	0.096	7.23	1.37	14.24	7.42	7.69	6.26	4.74
	4	0.89	2.0	0.114	6.06	1.16	7.76	6.79	10.13	8.76	4.24
	5	1.17	3.0	0.121	5.71	1.13	10.34	6.93	9.19	8.21	4.46
	Mean	1.35	1.8	0.102	6.93	1.26	11.66	7.05	8.51	7.14	4.46
	SD	0.31	0.8	0.016	1.14	0.14	2.76	0.34	1.16	1.37	0.24
R-(+)-OFLX	1	1.59	1.0	0.093	7.44	1.39	12.81	6.95	8.09	5.95	4.20
	2	1.45	1.0	0.110	6.31	1.17	10.80	6.33	9.00	7.31	4.67
	3	1.62	2.0	0.100	6.93	1.43	13.04	7.06	8.40	6.78	4.74
	4	0.88	2.0	0.124	5.57	1.16	7.16	6.44	11.00	9.14	4.24
	5	1.15	3.0	0.138	5.04	1.10	9.31	6.54	10.20	8.45	4.46
	Mean	1.34	1.8	0.113	6.26	1.25	10.62	6.67	9.34	7.53	4.46
	SD	0.32	0.8	0.018	0.98	0.15	2.47	0.32	1.23	1.28	0.24
	P	NS	NS	<0.01	<0.05	NS	<0.01	<0.001	<0.001	<0.01	

[a] Abbreviations: C_{max}, peak concentration in serum; T_{max}, time to peak concentration; k_{el}, elimination rate constant; $t_{1/2}$, elimination half-life; V_{area}, volume of distribution; $AUC_{0-\infty}$, area under the concentration-time curve in serum; MRT, mean residence time; Cl_{tot}, total body clearance; Cl_{ren}, renal clearance; Cl_{cre}, creatinine clearance; NS, not significant.

sensitive and stereoselective HPLC assay that allowed this quantitation in biological fluids after administration of racemic OFLX despite the very small stereoselective difference.

Not only the peak concentration in serum but the time to peak concentration in serum show no difference between the enantiomers, suggesting that the absorption of OFLX is not stereoselective in humans. There are no drugs showing stereoselective absorption except for L-dopa (22) and the β-lactam antibiotics ceftibuten (27) and cephalexin (21), which are transported by a carrier system across the intestinal mucosa. There is no difference between the two OFLX enantiomers in their aqueous or lipid solubility. If OFLX absorption in fact occurs through passive transport, it is probable that no stereoselectivity of drug absorption exists. One of the factors that play an important role in the stereoselectivity of enantiomers is protein binding (26). In OFLX, there is no difference in protein binding in serum in humans in vitro between the enantiomers. Consequently, protein binding makes no contribution to the stereoselectivity in the disposition of OFLX enantiomers in humans.

The renal clearance of the enantiomers was significantly different. Stereoselective renal excretion of enantiomers often results from differences in their secretion and/or metabolism in the kidney (10). OFLX, however (as mentioned above), undergoes limited metabolism in humans, resulting in only a small amount of metabolite excretion in the urine (11). In OFLX enantiomers, renal clearance (7 to 8 liters/h/1.73 m^2) exceeds the normal glomerular filtration rate calculated by the creatinine clearance (approximately 4 liters/h/1.73 m^2). These data indicate that net renal secretion of OFLX enantiomer may have occurred. Most of the total body clearance of OFLX takes place in the kidney, and other clearance, such as metabolic clearance, does not contribute greatly to the total body clearance. Consequently, only a small difference in renal clearance may affect stereoselectivity in concentration profiles of the OFLX enantiomers in serum. The mechanism of stereoselective renal excretion of OFLX enantiomers has not been elucidated, but it is considered that the binding affinities of the enantiomers to the carrier protein concerned with renal secretion are different, or that competition for the cation transport in renal excretion is greater in the *R*-(+) than in the *S*-(−) form of OFLX. Further experiments with administration of each enantiomer alone and with administration of racemic OFLX are needed to indicate whether the enantiomers influence each other's pharmacokinetics and, in particular, their renal excretion.

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