Phase I Study of Levofloxacin, (*S*)-(−)-Ofloxacin

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Phase I study of levofloxacin, an optically active isomer of ofloxacin, was conducted in 21 normal healthy male volunteers.

In the single-dose studies, levofloxacin was orally administered at doses of 50, 100, and 200 mg after breakfast. The serum concentration of levofloxacin peaked at 0.92 to 2.41 hr and reached 0.57, 1.22, and 2.04 μg/ml, respectively, in a dose-dependent manner. The $t_{1/2}$ of serum levels were about 4 to 6 hr. Approximately 85 to 92% of the dose was excreted unchanged into urine within 48 hr and 3.9% into feces within 72 hr. The absorption of levofloxacin tended to be more rapid under fasting condition than after meal, whereas the urinary recovery was not altered. The pharmacokinetic profiles of levofloxacin and ofloxacin, determined at a single dose of 200 mg, were quite comparable, except for the significant difference in V_d and mean residence time. In the multiple-dose study, levofloxacin was not accumulated on the basis of serum concentrations and urinary recoveries. The salivary to serum concentration ratio of levofloxacin was about 0.7 until 10 hr after administration. There was no obvious observation of chiral conversion of (*S*)-(−)-isomer, levofloxacin, to (*R*)-(+)-isomer in the body. Levofloxacin was well tolerated.

Key words: levofloxacin, enantiomer of ofloxacin, phase I study

Introduction

Levofloxacin (DR-3355) is a new quinolone antibacterial which is an optically active isomer [(*S*)-(−)-isomer] of ofloxacin (Fig. 1). Like ofloxacin, levofloxacin has a broad antibacterial spectrum against gram-positive and gram-negative organisms.[1,2] It demonstrates approximately twice higher antibacterial activity than ofloxacin, and is considered as the active entity of ofloxacin.[1-4]

From the preclinical studies of levofloxacin on toxicity, general pharmacology, and pharmacokinetics, it was suggested that levofloxacin had comparable safety and pharmacokinetic profiles to those of ofloxacin.

In this study, phase I study of levofloxacin was conducted in normal healthy male volunteers to determine its safety and pharmacokinetics.

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Materials and Methods

Drugs

The drugs used were levofloxacin, 50 mg tablets (Lot No. 1603-PLC), and ofloxacin, 100 mg tablets (Lot No. 246), which served as the reference drug.

Subjects

Twenty-one healthy male subjects, with ages ranging from 23 to 48 yr, body weights from 49 to

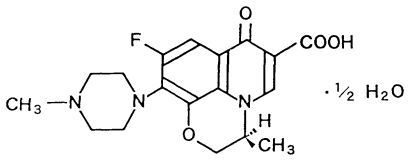

Fig. 1 Chemical structure of levofloxacin. (−)-(*S*)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido [1, 2, 3-*de*] [1, 4] benzoxazine-6-carboxylic acid hemihydrate.

97 kg, and height from 161 to 183 cm, were enrolled in this study. The subjects signed an informed consent after being informed of the purpose and contents of the study in detail. All were ascertained to be in good health by interview and the screening tests of hematology, blood biochemistry, serum immunology, urinalysis, and physical examinations conducted just prior to the study. The subjects were hospitalized from the afternoon of the day before administration to the next morning following the final administration. They took scheduled meals simultaneously during the experimental period. For breakfast, a light meal with a calorific value of about 500 to 600 cal was taken. The subjects avoided the use of other drugs for 1 wk before the study initiation and drinking alcohol was prohibited from the day before and throughout the study. The experimental protocol was approved by the local ethics committee.

Dosage and administration

The first dose of levofloxacin was set at 50 mg, which is half the lowest therapeutic dose of ofloxacin. A single dose of levofloxacin was administered orally to 5 subjects per each step, by doubling the dosage after confirming the safety of the previous dose: step 1, a dose of 50 mg; step 2 a, 100 mg; step 3 a, 200 mg. The doses were given 30 min after breakfast. To determine the effect of meal on the absorption of levofloxacin, the 5 subjects of step 2 a were given 100 mg dose after a 12-hr fasting (step 2 b). Moreover, the 5 subjects of step 3 a were given 200 mg of ofloxacin (step 3 b) to compare the pharmacokinetics of levofloxacin and ofloxacin. These comparative experiments were made with an interval of 1 wk as washout. In the final experiment (step 4), 6 subjects were given 200 mg of levofloxacin 3 times daily after meals at 8 : 00, 14 : 00, and 20 : 00 for 7 consecutive days (a total of 19 doses). In all steps, the drugs were taken with 100 ml of tap water.

Schedules of sampling and laboratory examination

Blood samples were withdrawn from the forearm vein before (0 hr) and 0. 25, 0. 5, 1, 2, 3, 4, 6, 10, and 24 hr after the administration in the single-dose studies. In the multiple-dose study, samples were taken at 0, 0. 25, 0. 5, 1, 2, 3, 4 hr after the first dose, 0 and 2 hr after the second and third doses of day 1, before the morning dose (0 hr) on days 2, 3, 5, 6, and 0 and 2 hr after every dose on day 4, and 0, 0. 25, 0. 5, 1, 2, 3, 4, 6, 10, and 24 hr after the final dose on day 7. Serum samples were prepared by centrifuging the blood samples at $2,500 \times g$ for 10 min. Urine samples were collected in plastic vessels at 0, 2, 4, 6, 8, 12, and 24 hr after dosing in the single-dose study, and every 24 hr in the multiple-dose study. The volume of each urine sample was measured immediately, and an aliquot (5 to 6 ml) was stored for analysis. Salivary samples were collected at 0, 0. 5, 1, 2, 3, 4, 6, 10, and 24 hr after dosing in steps 1 and 2 a by dipping paper discs, 8 mm in diameter, Toyo Filter Papers, Tokyo, in saliva of each volunteer. Fecal samples were collected in step 3 a, at 24, 48, and 72 hr after the dose. All the above samples were stored at −80℃ or lower until analyzed. Laboratory tests were carried out at intervals according to the previously fixed schedule; the test parameters consisted of hematology, blood biochemistry, serum immunology, urinalysis, electrocardiography, blood pressure, pulse rate, electroencephalography, and body temperature. Interview was done periodically to detect subjective and objective symptoms. They were followed up for 1 wk after the final administration, subjected to the above examinations except for electroencephalogram.

Measurement of drug concentration

Serum, urinary and fecal concentrations of levofloxacin [(*S*)-(−)-ofloxacin] and DR-3354 [(*R*)-(+)-ofloxacin] were measured by high performance liquid chromatography (HPLC) as described by Okazaki et al.[5] The concentrations of levofloxacin were also measured by bioassay using *Bacillus subtilis* ATCC 6051 and *Escherichia coli* Kp as indicator organisms. The concentrations of levofloxacin determined by HPLC and bioassay correlated well with ratio of approximately 1 : 1; therefore only the data by HPLC were shown in this paper. The detection limit of the drug concentrations in serum, urine, and feces by HPLC were

0.01, 0.3, and 0.3 μg/ml, respectively. In the ofloxacin dosing group, the concentrations of ofloxacin were calculated as a total of those of levofloxacin and DR-3354. Salivary concentration of levofloxacin was measured by bioassay ; the detection limit being 0.1 μg/ml.

Pharmacokinetic analysis

Pharmacokinetic analysis of serum concentrations after single-dose administration was based on a one-compartment open model with first-order absorption (program APAS 98).

Statistical analysis

Differences between the pharmacokinetic parameters after meal and under fasting condition, and between those of levofloxacin and ofloxacin were analyzed by Student's *t*-test.

Results

Pharmacokinetics

Single-dose study

Serum concentrations after single oral doses of levofloxacin at 50 mg (step 1), 100 mg (step 2 a), 200 mg (step 3 a) after meal and at 100 mg after over-night fasting (step 2 b) are shown in Fig. 2. Table 1 summarizes the pharmacokinetic parameters obtained in the steps. After the administration of levofloxacin after breakfast, the serum concentrations increased rapidly and peaked at 0.92 to 2.41 hr (t_{max}). The mean maximum serum concentrations (C_{max}) were 0.57, 1.22, and 2.04 μg/ml for the doses of 50, 100, and 200 mg, respectively, in a dose-dependent manner. The apparent $t_{1/2}$ was about 4 to 6 hr regardless of doses. The mean area under the serum concentration-time curve (AUC) was 4.70, 7.46, and 19.88 μg·hr/ml for doses of 50, 100, and 200 mg, respectively. The mean residence time (MRT) was about 6 to 9 hr. Urinary concentration reached a peak at 0 to 2 or 2 to 4 hr after the administration. Even in the 12 to 24-hr urine samples, concentrations of about 17 to 45 μg/ml were retained. Within the first 48 hr, 85.3 to 91.9% of the given dose was recovered unchanged into urine, regardless of the doses.

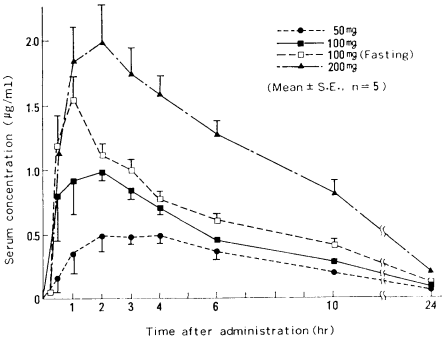

Fig. 2 Serum concentrations of levofloxacin after a single oral administration.

Influence of meal on absorption

When 100 mg of levofloxacin was given under the fasting condition, a mean t_{max} of 0.82 hr and a mean C_{max} of 1.36 μg/ml were obtained (Tab. 1). Although t_{max} tended to be shorter and C_{max} tended to be larger for the fasting state than for the postmeal state, no significant difference was seen. The absorption rate constant (K_a) was larger

Tab. 1 Pharmacokinetic Parameters for Levofloxacin after a Single Oral Administration[a)]

Step	Dose	No. of volunteers	K_a (hr^{-1})	K_e (hr^{-1})	V_d (*l*/kg)	$t_{1/2}$ (hr)	MRT (hr)	t_{max} (hr)	C_{max} (μg/ml)	AUC_{0-inf} (μg·hr/ml)
1	50 mg, after meal	5	8.43 ±4.86	0.16 ±0.00	1.09 ±0.02	4.34 ±0.09	7.82 ±0.66	2.41 ±0.84	0.57 ±0.05	4.70 ±0.32
2 a	100 mg, after meal	5	11.67 ±4.74	0.18 ±0.01	1.19 ±0.07	3.96 ±0.26	6.19 ±0.61	0.92 ±0.31	1.22 ±0.08	7.46[b)] ±0.36
2 b	100 mg, fasting	5	15.75 ±6.73	0.14 ±0.02	1.10 ±0.09	5.12 ±0.48	7.77 ±0.73	0.82 ±0.18	1.36 ±0.16	10.42[b)] ±0.43
3 a	200 mg, after meal	5	4.23 ±1.55	0.12 ±0.01	1.25 ±0.06	5.97 ±0.38	9.27 ±0.47	1.48 ±0.31	2.04 ±0.21	19.88 ±1.15

The data shown are mean±S.E. [a)] Calculated by one-compartment open model. [b)] $P<0.01$ between groups.

Tab. 2 Cumulative Urinary Excretion of Levofloxacin after a Single Oral Administration

Step	Dose	No. of volunteers	Cumulative urinary excretion (% of dose)						
			2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	48 hr
1	50 mg, after meal	5	11.5 ±4.3	32.2 ±3.4	48.4 ±1.4	59.0 ±0.8	71.6 ±1.1	85.5 ±1.7	91.9 ±1.5
2a	100 mg, after meal	5	16.6 ±4.0	33.8 ±2.6	45.4 ±2.0	54.9 ±1.7	67.4 ±1.4	79.6 ±1.2	85.3 ±1.6
2b	100 mg, fasting	5	19.7 ±1.9	36.3 ±2.3	47.8 ±2.6	58.0 ±2.5	68.0 ±2.8	81.6 ±2.1	88.3 ±1.9
3a	200 mg, after meal	5	11.4 ±2.1	28.8 ±2.4	40.8 ±3.1	52.0 ±3.5	65.7 ±3.5	80.3 ±3.0	86.7 ±2.8

The data shown are mean±S. E.

in the fasting state (15.75 hr^{-1}) than in the postmeal state (11.67 hr^{-1}), though no significant difference was observed. AUC was significantly greater ($P<0.01$) for the fasting (10.42 $\mu g \cdot hr/ml$) than for the postmeal group (7.48 $\mu g \cdot hr/ml$). Table 2 shows the urinary recovery of levofloxacin after administration of 100 mg under fasting or postmeal condition. In the fasting group, about 88% of the given dose was excreted in the urine within 48 hr after administration, which was not significantly different with that in the postmeal group.

Comparative pharmacokinetic study with ofloxacin

A comparative study was made on the pharmacokinetics of levofloxacin with that of ofloxacin. Two hundred mg of each drug was given after meal to the same 5 subjects. A 7-day washout period was provided between the administration of the two drugs. As shown in Tab. 3, there were no differences in t_{max}, C_{max}, or AUC between the two drugs. The $t_{1/2}$ of levofloxacin (5.97 hr) tended to be longer than that of ofloxacin (4.13 hr), but the difference was not significant. On the contrary, V_d was 1.25 l/kg for levofloxacin and 0.93 l/kg for ofloxacin, and MRT was 9.27 and 7.24 hr for levofloxacin and ofloxacin, respectively; both parameters of levofloxacin were significantly larger than those of ofloxacin ($P<0.05$). As shown in Tab. 4, cumulative urinary excretion of levofloxacin and ofloxacin were not significantly different, showing 86.7% and 81.3% of recoveries within 48 hr after dosing, respectively.

Multiple-dose study

Figure 3 shows the serum concentrations of levofloxacin in repeated administration of 200 mg of levofloxacin 3 times daily after meals for 7 consecutive days. The mean serum concentration of levofloxacin was 1.51 μg/ml at 2 hr after the first dosing and 3.15 μg/ml at 2 hr after the third dosing on the first day of treatment, reaching a near steady state thereafer. Furthermore, a steady level ranging from 1.05 to 1.39 μg/ml immediately before the first dosing was maintained after day 2. The

Tab. 3 Comparison of Pharmacokinetic Parameters for Levofloxacin and Ofloxacin after a Single Oral Administration[a)]

Drug	No. of volunteers	K_a (hr^{-1})	K_e (hr^{-1})	V_d (l/kg)	$t_{1/2}$ (hr)	MRT (hr)	t_{max} (hr)	C_{max} (μg/ml)	$AUC_{0\text{-}inf}$ ($\mu g \cdot hr/ml$)
levofloxacin, 200 mg	5	4.23 ±1.55	0.12 ±0.01	1.25[b)] ±0.06	5.97 ±0.38	9.27[c)] ±0.47	1.48 ±0.31	2.04 ±0.21	19.88 ±1.15
ofloxacin, 200 mg	5	2.16 ±0.93	0.18 ±0.02	0.93[b)] ±0.06	4.13 ±0.36	7.24[c)] ±0.29	2.17 ±0.48	2.23 ±0.22	18.66 ±1.83

The data shown are mean±S. E. [a)] Calculated by one-compartment open model. [b,c)] $P<0.05$ between groups.

Tab. 4 Comparison of Urinary Excretion of Levofloxacin and Ofloxacin after a Single Oral Administration

Drug	No. of volunteers	Cumulative urinary excretion (% of dose)						
		2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	48 hr
levofloxacin 200 mg	5	11.4 ±2.1	28.8 ±2.4	40.8 ±2.1	52.0 ±3.5	65.7 ±3.5	80.3 ±3.0	86.7 ±2.8
ofloxacin 200 mg	5	11.2 ±1.4	28.1 ±1.7	40.6 ±2.2	51.3 ±2.7	63.0 ±2.5	75.4 ±2.9	81.3 ±2.1

The data shown are mean±S.E.

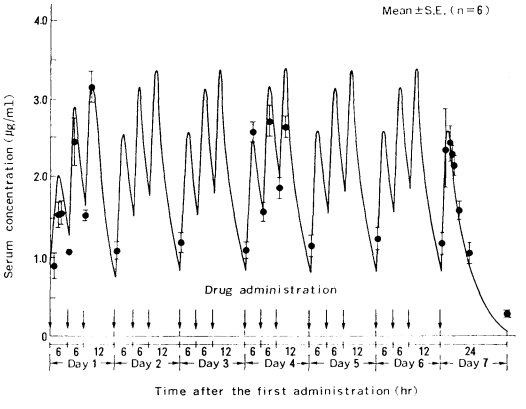

Fig. 3 Serum concentrations of levofloxacin during multiple oral administration. Simulation curve was obtained from the mean serum concentrations after a single oral administration of 200 mg.

actual values obtained during the treatment period roughly coincided with the simulated values constructed on the basis of the pharmacokinetic parameters of serum concentration after a single 200 mg dose. Cumulative urinary excretion reached a plateau at day 3, and in total 84.8% of the given dose was excreted into urine until 48 hr after the final dosing (data not shown). These findings suggested that levofloxacin was not accumulated.

Penetration into saliva

The penetration of levofloxacin into saliva was examined in the 50 and 100 mg dose groups (steps 1 and 2 a). Salivary concentrations of levofloxacin paralleled with that of the serum concentrations, reaching a peak at 2 hr. At a dose of 100 mg, observed peak salivary and serum concentrations were 0.72 and 0.98 μg/ml, respectively. The saliva to serum concentration ratio was about 0.7 until 10 hr (data not shown).

Fecal recovery

Fecal excretion of levofloxacin was examined in the single 200 mg dose group (step 3 a). About 2.1% of the dose was excreted in feces within 24 hr after administration, and was 3.9% within 72 hr.

Chiral conversion in the body

To determine whether levofloxacin, the (*S*)-(−)-isomer of ofloxacin, was converted to the (*R*)-(+)-isomer, the serum concentration of the (*R*)-(+)-isomer was measured in the subjects given levofloxacin. (*R*)-(+)-isomer was hardly detected in any serum samples tested, indicating that chiral conversion might not occur in the body (data not shown).

Tolerance and safety

In 21 subjects enrolled in this study, levofloxacin produced no remarkable subjective and objective symptoms, nor any abnormal changes in laboratory tests such as hematology, blood biochemistry, serum immunology, and urinalysis, and physical examinations such as electrocardiography, blood pressure, pulse rate, and electroencephalography.

Discussion

Levofloxacin is an optical isomer [(*S*)-(−)-isomer] of the racemic mixture, ofloxacin. A phase I study was conducted to determine the safety and pharmacokinetics in healthy male subjects.

Levofloxacin is approximately twice as potent as ofloxacin[1-4] and is similar to ofloxacin with regard to oral absorption, tissue distribution, and elimination.[6] Toxicity of levofloxacin in animals is also comparable to that of ofloxacin [unpublished data]. In consideration of these preclinical study results, levofloxacin would be clinically used at a half dose of ofloxacin. On this basis, the first dose in the present trial was set at 50 mg, a half of the lowest therapeutic single-dose of ofloxacin. Dosage was

successively doubled thereafter with confirmation of safety of the previous step.

Regarding safety, levofloxacin was well tolerated both subjectively and objectively. There were no significant changes attributable to the drug in any laboratory tests and physical examinations. From the above data, levofloxacin was considered to be as safe as ofloxacin.

After single 50, 100, and 200 mg doses of levofloxacin, serum concentration increased rapidly, with t_{max} of 0.92 to 2.4 hr. C_{max} and AUC increased in a dose-dependent manner. V_d, as determined by one-compartment open model, was 1 l/kg or more regardless of doses. These results indicated that oral absorption and tissue distribution of levofloxacin was excellent. Levofloxacin was excreted efficiently via urinary route, at the rate of 85 to 90% of the dose. Fecal excretion was marginal, with the rate of 3.9% of the dose. Chiral conversion was hardly observed in vivo.

Levofloxacin was absorbed more rapidly when given to fasting rather than non-fasting subjects. But, total amount of levofloxacin excreted into urine was almost the same between the two conditions, indicating the total amount absorbed was hardly affected by meal.

When levofloxacin was administered at 200 mg 3 times daily for 7 consecutive days, neither daily increase in serum C_{max} nor increase in the trough serum level was noted. Observed values of serum concentration were well fitted with the simulation curve calculated based on the pharmacokinetic parameters of a single 200 mg oral dose. This observation was also substantiated by the fact that the cumulative percentage urinary excretion reached plateau on day 3 of treatment and that the 48-hr value was about 85%, which was comparable to that in the single-dose study, even after the 19 th dosing. From these results, it could be concluded that no accumulation occurred after the multiple-dose.

The concentration of levofloxacin in saliva paralleled with that in serum. The saliva to serum concentration ratio was about 0.7 until 10 hr after dosing. These findings indicated that the penetration of levofloxacin into the saliva was satisfactory.

The above pharmacokinetic profiles including dose effect and meal effect, urinary excretion, fecal excretion, penetration into the saliva equated to those obtained in the phase I trial of ofloxacin.[7] In this study, pharmacokinetics of the two drugs revealed to be comparable with each other, except for the difference in V_d and MRT. Cumulative recovery in urine was almost equal between the two drugs.

In conclusion, levofloxacin had excellent pharmacokinetic profiles in humans like ofloxacin, such as high oral absorption, high urinary excretion, and good tissue distribution. Levofloxacin was well tolerated by healthy subjects. Based on the twice higher antibacterial activity of levofloxacin than ofloxacin, levofloxacin is expected to produce similar clinical effects at the half dose of ofloxacin. This may contribute to reducing possible side-effects of ofloxacin.

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