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Pharmacokinetic Analysis of Levofloxacin in Healthy Korean Volunteers

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ABSTRACT – A sensitive and simple method of determining the plasma levofloxacin (LFX, CAS 100986-85-4) concentrations in human volunteers by liquid-liquid extraction were developed and validated by using a high-performance liquid chromatography/diode array detector. The method was also applied to pharmacokinetic study of LFX. LFX was orally administered to 8 healthy male Korean volunteers at single lowest dose of 200 mg, compared to the published reports in which more than 500 mg of LFX was orally administered. LFX in human plasma was determined. The detection limit of LFX was 0.05 μg/mL. C_{max} value was 2.48 ± 0.67 μg/mL. $AUC_{0\rightarrow 24\ hr}$ and $AUC_{0\rightarrow\infty}$ were 14.52 ± 3.35 μg/mL and 16.00 ± 3.66 μg·hr/mL, respectively. The terminal half-life was 6.87 ± 0.46 hr. Our pharmacokinetic parameters were very consistent with that previously reported, showing good correlation between LFX doses and AUC (r^2 = 0.995). This method can be useful for the pharmacokinetics and bioequivalence study with relatively low dose for reducing the main side effects of LFX.

Key words – Levofloxacin, Antibiotics, Pharmacokinetics, Human volunteers, High-performance liquid chromatography

Levofloxacin (L-ofloxacin; S-(-)-9-Fluoro-2,3-di-hydro-3-methyl-10-(4-methyl-l-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid; CAS 100986-85-4; LFX) is the bacteriologically active L-isomer of the racemic fluoroquinolone ofloxacin.[1,2] The stereoselective antibacterial activity of ofloxacin is known to be resided almost entirely within the L-isomer, even if the D- and L-isomers contribute equally to the toxicological profile. LFX has broad spectrum of *in vitro* activity against both Gram-positive and Gram-negative organisms.[3]

Many attempts were made to determine ofloxacin by using reversed-phase HPLC with various mobile phases. Most of the method for extracting LFX in biological samples was conducted by using solid-phase procedure[4-7] rather than the liquid-liquid extraction,[8] because the latter has the disadvantage of a poor limit of detection and lack of selectivity. However, the solid-phase extraction may be ineffective in consideration of cost, since bioequivalence data are obtained from the determination of several hundreds of samples, even if the method is simple and gives high recovery.

The pharmacokinetic data of LFX have been reported after high doses of LFX such as 500 to 1000 mg in human,[9-11] and 500 mg in patients with lower respiratory tract infections,[12] partly in order to make the detection of LFX in samples favorable. Such doses of LFX have been reported to result in the main side effects as headache and gastrointestinal disturbance.[10]

In this work, a sensitive and simple method of determining the plasma LFX concentrations in human volunteers by liquid-liquid extraction were developed and validated. The method was applied to pharmacokinetic analysis of relatively low dose of LFX (200 mg) in 8 healthy male Korean human. The comparison of our results to the reported several pharmacokinetic data of LFX showed the good correlation (r^2 = 0.995) between LFX doses and AUC.

Materials and Methods

Chemicals

Levofloxacin (>99%) and its 100 mg tablet formulation were kindly supplied from Jeil Pharmaceutical Co. (Seoul, Korea). Enoxacin was purchased from Sigma (St.Louis, MO, USA). HPLC grade of methanol, acetonitrile and methylene chloride were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals and reagents were of analytical grade unless specified otherwise.

Oral administration of LFX tablets to healthy volunteers

A 21-gauge scalp-vein set was established in the arm vein of each volunteers and 8 mL blood was collected as blank. According to the prescription directed by a doctor, two tablets

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(total 200 mg LFX) were orally given to each volunteer. Blood was collected into heparin-treated tubes (Vacutainer®, Becton Dickinnson, Rutherford, NJ, USA) at 0.33, 0.67, 1, 1.5, 2, 2.5, 4, 6, 8, 12 and 24 hr after the oral administration. The time interval of blood sampling between volunteers was 2 min to consider blood collection time. Blood was centrifuged to obtain plasma. The plasma was stored at −70°C until analyzed.

Equipment

The HPLC system was consisted of HPLC1090 series (Hewlett-Packard, CA, USA), and a diode array detector (294 nm) was used with an auto liquid sampler. LFX was separated by using a Symmetry Shield RP 18 column (3.9 mm × 150 mm, particle size 5 μm, pore size 100 Å; Waters, Milford, MA, USA). The mobile phase was consisted of 0.3% triethylamine and acetonitrile (90 : 10, v/v) and the flow-rate of the mobile phase was 1 mL/min. Data integration and manipulations were performed using the HP ChemStation software.

Blood sampling from volunteers

At first, after approval of the proposal by the Korea Food and Drug Administration (KFDA), male volunteers who submitted the informed consent to participate in this project were medically examined. Eight volunteers were selected by a medical doctor at the Bestian Hospital (Seoul, Korea), based on clinical examination including seropathological (hemoglobin, hematocrit, WBC, platelet), serochemical (blood urea nitrogen, creatinine, total protein, albumin, SGOT, SGPT, total bilirubin, cholesterol, glucose fasting, alkaline phosphatase), and urological (specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC) data. The physical data of healthy volunteers were summarized in Table I. The mean body weight and height of 8 male volunteers were 69.0 ± 6.6 kg and 173.4 ± 6.1 cm, respectively. Age of these volunteers was ranged from 24 to 28 years with the mean value of 26.1 ± 1.3. Seropathological, serochemical, and urological data for volunteers were obtained and all of these values were located within normal ranges (data not shown). No clinically significant adverse effects were observed during the study.

The subjects were instructed not to take any medicine for at least 1 week prior to and during the study period. They were accommodated to the same place one day before blood collection. They were fasted overnight before administration of the tablets. Lunch and dinner were allowed 4 and 12 hr, respectively, after drug intake. A physical and biological examination was carried out before and after completion of the study.

Table I–*Physical Parameters of Healthy Volunteers Involved in the Pharmacokinetic Study of Levofloxacin*

Subjects	Sex	Age (years)	Weight (kg)	Height (cm)
1	M	26	65	180
2	M	27	70	169
3	M	26	65	174
4	M	28	78	171
5	M	24	67	170
6	M	26	58	165
7	M	27	74	174
8	M	25	75	184
Mean		26.1	69.0	173.4
S.D.		1.3	6.6	6.1

Preparation of calibration curve of LFX in human plasma

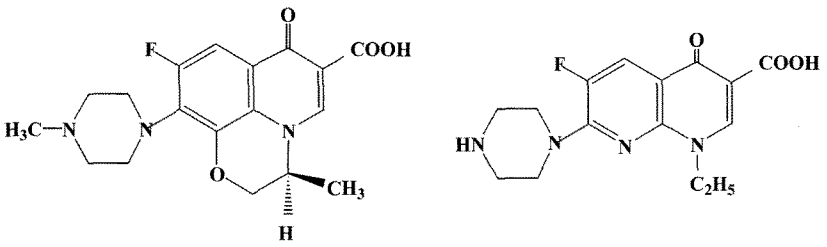
To 1 mL of the LFX-free blank plasma, 0.05, 0.1, 0.2, 0.5, 1, 1.5, 2 and 4 μg of LFX prepared in methanol and the internal standard of enoxacin (100 μg/mL, 20 μL) were added. The clean- up procedure was the same as described below.

Determination of LFX in human plasma

To an aliquot of human plasma (1 mL), was added 20 μL (100 μg/mL) of enoxacin as internal standard. After the addition of 0.5 mL acetonitrile, each sample was vortex-mixed for 30 s and centrifuged at 2000 g for 5 min. The organic layer was transferred into clean test tubes (16 × 125 mm, Pyrex, Corning, NY, USA). The samples were extracted with 3 mL of methylene chloride by a vortex-mixing type of shaker (IKA - Labortechink, Janke & Kunkel Co., Germany) at 1800 rpm for 10 min, and centrifuged at 850 g for 10 min (Sorvall RT 6000B, Du Pont Co., Newtown, CT, USA). The organic layer (lower phase; about 2.7 mL) was transferred into a fresh test tube. After evaporation under nitrogen flow, the sample was reconstituted in 100 μL of acetonitrile. The sample was transferred to a filtering tube (Ultrafree-MC centrifugal filter units, 0.22 μm; Millipore, Canton, MA, USA) and centrifuged at 300 g for 5 min (Hm-150IV, Hanil Co., Seoul, Korea). The filtrate was transferred to a vial for HPLC analysis and 10 μL of the solution was injected to the HPLC by the auto liquid sampler. The plasma concentrations of LFX were evaluated by the calibration curve from the ratio of the peak area of LFX to that of the internal standard.

Pharmacokinetic calculations

The highest concentration (C_{max}) and the time to reach the highest concentration (T_{max}) were read directly from the time-



Levofloxacin **Enoxacin**

Figure 1—Chemical structures of enoxacin and levofloxacin. Enoxacin was used as internal standard.

plasma concentration curves of LFX. The area under the curve of time-plasma concentrations until the last sampling time ($AUC_{0\rightarrow last}$) was calculated by the trapezoidal rule, whereas the area was extrapolated to the infinity to determine $AUC_{0\rightarrow\infty}$ by the equation of $AUC_{0\rightarrow\infty}=AUC_{0\rightarrow last}+C_{last}/\beta$, where β is the slope of the terminal phase of the time-plasma concentration curve using log-transformed concentrations and C_{last} is the concentration obtained at the last sampling time.[13]

Data are presented as mean ± standard deviation. Pharmacokinetic parameters were determined from the time-plasma concentrations of LFX by non-compartmental analysis by using WinNonlin software (Scientific Consulting Inc., Cary, NC, USA).

Results

Determination of LFX in human plasma

Typical chromatograms obtained from human plasma samples were showed in Figure. 2. There were no peaks of interfering with LFX and enoxacin (shown the chemical structures in Figure.1) at their retention times from the blank plasma. Each identical peak spectrum was confirmed with the diode array detector (data not shown). By using the flow rate of 1 mL/min, the retention times of the peaks were 9.0~9.2 min for LFX, and 10.4~10.6 min for enoxacin.

The calibration curve prepared from 1 mL of plasma spiked 0.05-4.0 μg/mL of LFX gave good linearity ($y=1.5091x-0.0477$, $r^2=0.9990$), as showed in Figure 3. The detection limit of LFX was less than 0.05 μg/mL, at which the precision was satisfied the criteria of less than 20% but the accuracy was not. The limit of quantitation was 0.1 μg/mL at which the criteria of precision and accuracy are satisfied.

Intra-day precision was less than 10.33% and accuracy was less than 12.09%. Inter-day precision was less than 10.47% and accuracy was less than 13.30%(Table. II).

Pharmacokinetic analysis of LFX

From the time-plasma concentration curves of LFX (Figure

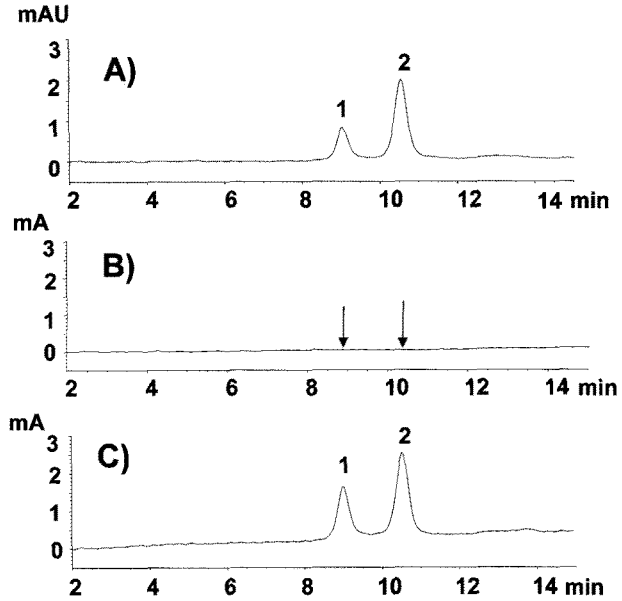


Figure 2—Chromatograms obtained by a high-performance liquid chromatography/diode array detector from authentic standard of levofloxacin (A), the plasma blank not spiked internal standard (B), and human plasma sample obtained after oral administration of a single 200 mg of levofloxacin (C). Peak 1, internal standard enoxacin; peak 2, levofloxacin.

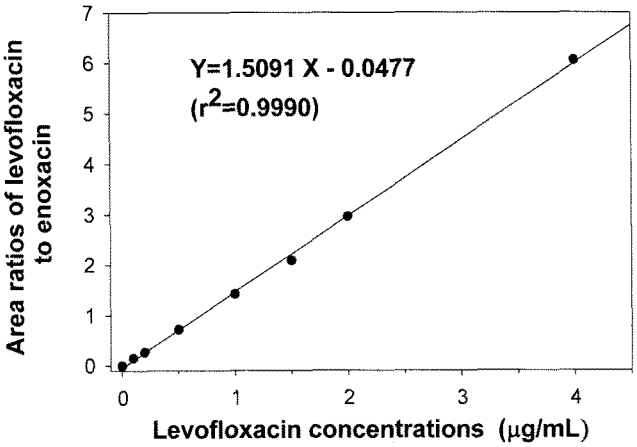


Figure 3—The calibration curve of levofloxacin in the plasma. The curve showed high linearity ($r^2>0.999$) at concentrations ranged from 0.1 to 4.0 μg/mL.

Table II–*Intra- and Inter-day Precision and Accuracy for the Determination of Levofloxacin in the Plasma of Human Volunteers*

Concentrations of levofloxacin (μg/mL)	Precision (C.V.%)		Accuracy (bias%)	
	Intra-day	Inter-day	Intra-day	Inter-day
0.05	2.25	4.71	31.64	23.06
0.1	6.49	10.47	12.09	13.30
0.2	10.33	8.89	6.95	3.89
0.5	3.22	5.62	4.09	−0.11
1.0	1.31	7.46	−3.67	−2.49
1.5	2.40	5.00	−4.79	−6.44
2.0	3.94	4.78	−3.97	2.47
4.0	3.64	2.96	1.79	0.42

Each value for within (intra-) and between (inter-) days precision and accuracy was obtained from 5 repeated experiments; C.V.% is determined from (standard deviation/mean × 100); Accuracy bias % is determined from [100-{(observed concentration/theoretical concentration) × 100}].

4) obtained from 8 healthy male Korean volunteers orally administered 200 mg of LFX that is the lowest dose, compared to the published reports, the principal pharmacokinetic parameters were calculated. The parameters for individual subjects are presented in Table III. The mean C_{max} was 2.48±0.67 μg/mL. T_{max} was 1.13±0.62 h. $AUC_{0\rightarrow 24\ hr}$ and $AUC_{0\rightarrow\infty}$ were 14.52 ±3.35 and 16.00±3.66 μg·h/mL, and the ratio of $AUC_{0\rightarrow 24\ hr}$ to $AUC_{0\rightarrow\infty}$ was 90.7%. This indicates that the time intervals after the administration of LFX are appropriate. K_e and $t_{1/2}$ were decided to be 0.101±0.007 hr^{-1} and 6.87±0.46 hr, respectively.

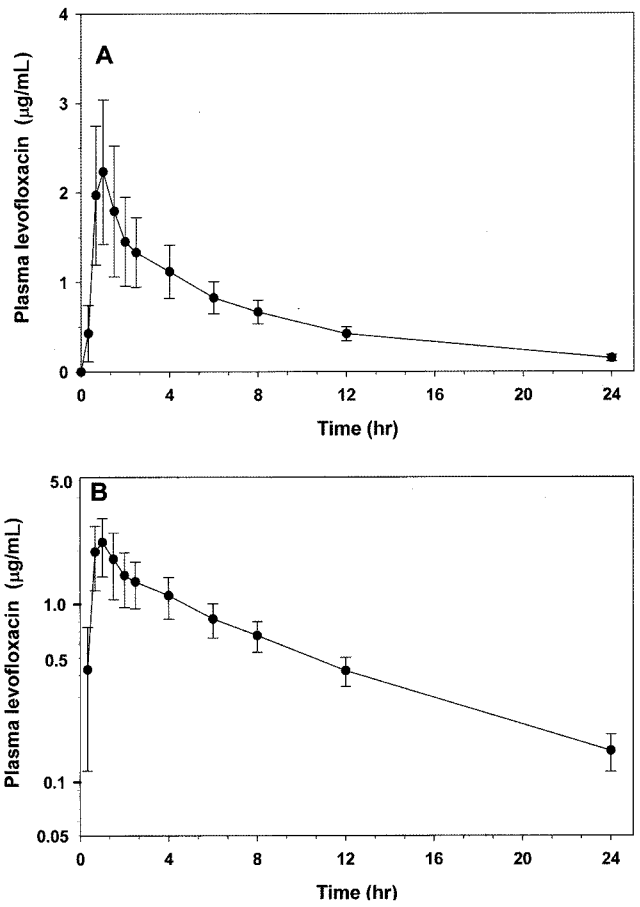


Figure 4–The time-plasma concentration curves after oral administration of two 100 mg tablets of levofloxacin (200 mg) to 8 healthy volunteers either in regular (A) or semi-logarithmic (B) scales. Each point was represented mean ± S.D. of 8 human volunteers.

Table III–*Pharmacokinetic Parameters Obtained from the Time-plasma Concentrations of Levofloxacin after Oral Administration of 200 mg Levofloxacin in 8 Male Volunteers*

Subjects	Pharmacokinetic parameters					
	AUC (μg·hr/mL)		C_{max} (μg/mL)	T_{max} (hr)	K_e (1/hr)	$t_{1/2}$ (hr)
	$AUC_{t\rightarrow 24\ hr}$	$AUC_{0\rightarrow\infty}$				
1	16.719	18.362	3.037	0.667	0.1000	6.933
2	14.873	16.478	2.245	1.500	0.0991	6.996
3	21.536	23.693	3.729	1.000	0.1057	6.555
4	10.445	11.750	2.166	1.000	0.0940	7.372
5	12.957	14.414	2.123	0.667	0.0959	7.226
6	13.634	15.024	2.238	1.000	0.0994	6.975
7	13.115	14.464	2.774	0.667	0.0993	6.980
8	12.857	113.824	1.544	2.500	0.1175	5.899
Mean	14.52	16.00	2.48	1.13	0.101	6.87
SD	3.35	3.66	0.67	0.62	0.007	0.46

Pharmacokinetic parameters were obtained by non-compartmental analysis by using WinNonlin software.

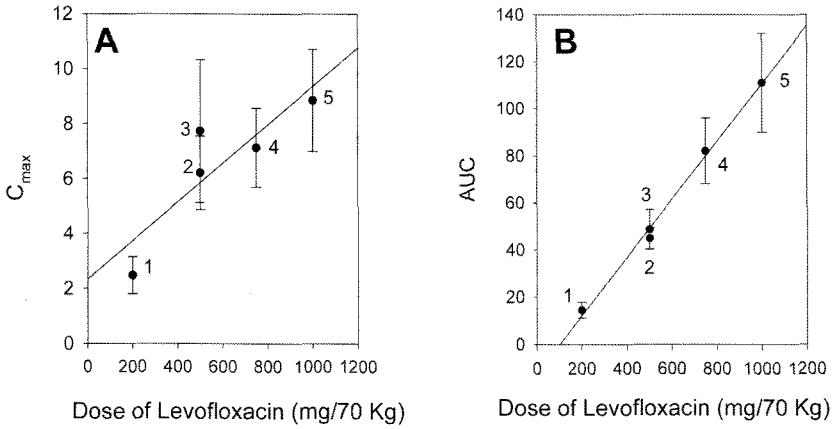


Figure 5—Relationships between administered doses of levofloxacin and C_{max} (A) or AUC (B). Numbers around the closed circles, 1-5, presented the pharmacokinetic parameters obtained from our results, Grant et al.,[9] Lubasch et al.,[10] and Chien et al.,[11] respectively.

LFX doses were plotted with C_{max} and AUC that was obtained from published reports including our results[9-11] as shown in Figure 5. Correlations between LFX doses and C_{max} and between LFX doses and AUC were good with 0.757 and 0.995 of relative coefficient (r^2), respectively. The high correlations of these parameters between different researchers suggest that our results are very similar to the results previously published. For instance, our half-life of 6.87 hr was very consistent to the published results that were ranged from 6.6 to 7.9 hr.[9-11] These pharmacokinetic data showed that LFX follows a linear pharmacokinetic behavior in human.

Discussion

Several pharmacokinetic data of LFX have been reported in healthy human volunteers or in patients with lower respiratory tract infections. Lubasch et al. has conducted pharmacokinetic study in healthy Caucasians with 6 male and 6 female after oral administration of 500 mg LFX, and its half-life was decided to be 6.95 ± 0.81 hr that was very similar to our value (6.87 ± 0.46 h).[10] Chien et al. investigated pharmacokinetics of once-daily oral LFX in healthy male volunteers who received orally 750 mg and 1000 mg LFX continuously with an appropriate washout period.[11] Grant et al. studied pharmacokinetics about the interaction between LFX (500 mg) and oxycodone.[9] In all these works, administered doses of LFX were 2.5 to 4 times higher than the one we used. This may be explained by feasibility that high dose of LFX was more easily detected in human plasma.

The half-life of LFX in our work (6.87 ± 0.46 hr) was very similar to that reported in Lubasch et al. (6.95 ± 0.81 hr) and Grant et al. (6.6 ± 1.1 hr).[9,10] The normalized C_{max} of our work (12.4 μg/mL if the dose is converted to 1000 mg LFX) was very similar to that reported in Lubasch et al. (12.24 ± 12.4 μg/mL).[10] In most pharmacokinetic parameters compared, our study was the closest to that of Lubasch et al., indicating that pharmacokinetic parameters of LFX have very similar property between Caucasians and Koreans. However, in the results obtained by Chien et al.[11] and Grant et al.,[9] the normalized C_{max} values were lower than that of our study and AUC values were about 34 to 53% higher that our results. Only based on these two parameters, this data suggest that absorption and excretion may be slower, compared to Koreans. In general, difference in AUC between research groups may be made from the difference of drug formulation, variation due to the method of determining drug concentrations, and individual variation such as gastrointestinal motility. Therefore, we could not find the significant difference in pharmacokinetic parameters of LFX between races by the comparison of these parameters.

By administering relatively a single low dose of LFX (200 mg) to human volunteers, this method can be useful for determining the plasma concentrations of LFX at least until 24 hr. A single dose of 200 mg LFX has not been reported except for this study. Usually high dose of LFX have been reported to cause side effects such as headache and gastrointestinal disturbance.[10]

In summary, a simple and sensitive method was applied to study pharmacokinetics of LFX after oral administration of a single 200 mg LFX in healthy male Korean volunteers. Correlations between LFX doses and C_{max} and between LFX doses and AUC were generally good with 0.757 and 0.995 of relative coefficient (r^2), respectively. Our pharmacokinetic parameters were very similar to that previously reported in human volunteers administered high doses of LFX. This method may be useful for the pharmacokinetics and bioequivalence study with relatively low dose for reducing the main

side effects of LFX.

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