Pharmacokinetics and bioequivalence of levofloxacin in healthy Chinese subjects

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Abstract

Aim A randomized, two-way crossover study was conducted to determine the pharmacokinetics and bioequivalence of levofloxacin in 20 healthy Chinese male subjects under fasting conditions. Methods Levofloxacin test and reference tablets were administered as a single dose on two treatment days separated by a one-week washout period. After dosing, serial blood samples were collected for a period of 24 hr, and serum levofloxacin concentrations were determined by a validated reversed-phase high performance liquid chromatography (HPLC) assay and pharmacokinetic parameters were analyzed by the Drug and Statistics (DAS) Software. Results The serum concentration-time course followed a two-compartment open model. The main pharmacokinetic parameters of levofloxacin test and reference formulations were as follow: $t_{1/2}$ were $(7.37 \pm 0.88)$ h and $(7.39 \pm 0.96)$ h, $t_{max}$ were $(1.13 \pm 0.30)$ h and $(1.06 \pm 0.24)$ h; $C_{max}$ were $(2.08 \pm 0.42)$ mg·L$^{-1}$ and $(2.03 \pm 0.35)$ mg·L$^{-1}$, $AUC_{0-24}$ were $(13.17 \pm 2.32)$ mg·h·L$^{-1}$ and $(13.73 \pm 2.89)$ mg·h·L$^{-1}$, respectively. The relative bioavailability of test tablets was $(97.7 \pm 16.7)$%. No significant differences between the two formulations were found, and the parametric confidence intervals (90%) of the mean values of the pharmacokinetic characteristics for test/reference ratio were within the bioequivalence acceptable ranges of 0.8-1.25 and 0.70-1.43 respectively for $AUC$ and $C_{max}$. Conclusion The results indicate that the two tablet formulations of levofloxacin are equivalent in the rate and extent of absorption.

Key words bioequivalence; high performance liquid chromatography; levofloxacin; pharmacokinetics

Introduction

Levofloxacin, an oral fluoroquinolone antibacterial agent, is the optical $S$(-) isomer of the racemic drug substance ofloxacin. Levofloxacin is significantly more active than ofloxacin. In vitro it is generally twice as potent as ofloxacin. In vitro it has a broad spectrum of activity against Gram-positive...
and Gram-negative bacteria, as well as certain other pathogens such as Mycoplasma, Chlamydia, Legionella and Mycobacteria spp\textsuperscript{[1,2]}. Hence, it provides clinical and bacteriological efficacy in a range of infections, including those caused by both penicillin-susceptible and -resistant strains of \textit{S. pneumoniae}. It has a favorable pharmacokinetic profile that is compatible with once-daily administration and allows for sequential intravenous to oral therapy\textsuperscript{[3,4]}. 

The purpose of the present work was to determine the pharmacokinetics and bioequivalence between the two tablet formulations of levofloxacin and to ascertain equal effect and safety in medical practice in our population.

Materials and methods

Drugs and reagents

The test formulation was levofloxacin 100 mg tablet provided by Hubei Heng’an Pharmaceutical Co., Ltd. (Hubei, China; Batch No. 030603); the reference product was levofloxacin 100 mg tablet manufactured by Guangdong Bituo Pharmaceutical Co., Ltd. (Guangdong, China; Batch No. 20030402). The standard substance of levofloxacin (purity: 99.9\%) was supported from Hubei Heng’an Pharmaceutical Co., Ltd. Acetonitrile and methanol were of HPLC grade. Others reagents were of analytical grade. Distilled, deionized water was used in the preparation of all reagents and the mobile phase throughout the study.

Chromatographic systems and conditions

The Waters HPLC system consisted of two model 510 pumps and a model 484 UV-Vis absorbance detector operated at 294 nm, and an EChrom98 chromatographic workstation (Dalian Elite Instrument Company, China). The analytical column was hypersil-ODS (150 mm×4.6 mm, 5 µm, United States) protected with a guard column packed with the same material. Acetonitrile: 0.04 mol·L\textsuperscript{-1} phosphoric acid: triethylamine (21:79:0.4) was used as mobile phase at a flow rate of 1.0 mL·min\textsuperscript{-1}. The column temperature was maintained at 30\textordmasculine C.

Study subjects

Twenty healthy adult male volunteers completed this study at Drug Clinical Research Organization of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). Their mean age was (23.2 ± 1.5) years with a range of 20 to 25 years. Mean height was (168.7 ± 5.8) cm with a range of 160 to 176 cm and mean body weight was (60.9 ±8.6) kg with a range of 50 to 72 kg. None of the subjects smoked. The subjects were selected after completing a thorough medical history and physical examination, and after a normal laboratory examination (hematology, blood biochemistry, and urine analysis). The volunteers had no evidence of hepatic, renal, pulmonary, cardiac, gastrointestinal, neurologic, or hematologic disorders or any acute or chronic disease. Subjects confirmed that they had abstained from taking alcohol, cigarette, or caffeine-containing beverages or food for 48 hr prior to the study and from the time of drug administration until the last blood sample was collected. Subjects were instructed to abstain from taking any drug, including over-the-counter (OTC) products, for at least 2 weeks prior to and during the study period.

The study was conducted in accordance with good clinical practice (GCP) guidelines of State Food and Drug Administration regulation in P. R. China, and the Declaration of Helsinki (as revised in Edinburgh 2000). Approval for the study was gained from the independent Ethical Committee of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China) and written informed consent was obtained from all subjects after explaining the aim and risks of the study prior to participation.

Study design

The study was of a single dose, randomized, two
treatments, two-period crossover design. 20 subjects were randomly divided into 2 groups (test and reference). After an overnight fasting, all subjects were given a single oral dose of a 200 mg levofloxacin test or reference tablets with 250 mL water. Regular standardized low-fat meals were provided until 4 h after dose administration; water intake was allowed after 2 h. Water, lunch, and dinner were given to all volunteers according to a time schedule. During the whole test period, all subjects remained under closely medical supervision at the study site. A crossover study was followed by a one-week washout period.

Safety was monitored by routine clinical laboratory tests conducted before and 24 h after administration of levofloxacin, by recording reported adverse events, and by conducting physical examinations before and after the study.

Blood samples collection
Blood samples (5 ml each) were drawn before (0 hr) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 hr after drug administration. An intravenous cannula was placed into the volunteers’ forearm vein before drug administration and left in place until the 12-hr blood sample was collected. The blood samples were immediately subjected to centrifugation at 4000 r·min⁻¹ for 10 min. The serum was decanted in coded polypropylene tubes and stored at -80°C until analysis.

Sample preparations
Ethyl acetate (4 mL) was added to 1 mL aliquot of serum. The mixture was vortex mixed for 5 min, then centrifuged at 3000 r·min⁻¹ for 10 min at 4°C. The supernatant was decanted into a clean centrifuging tube and evaporated to dryness under a gentle nitrogen stream at 40°C. The residue was reconstituted in 100 μL of mobile phase, and then 20 μL of the solution was injected into the HPLC system for analysis.

Pharmacokinetic analysis
Values for peak serum concentration (C_max) and time to C_max (t_max) were taken directly from the observed concentration-time profiles. The terminal-phase half-life (t_1/2) was calculated as (ln2)/K_e, where K_e is the slope of the log-linear regression of the terminal concentration data points. The area under the serum concentration versus time curve from 0 to the last measurable concentration (AUC_{0-24}) was calculated with the linear trapezoidal rule. The area under the serum concentration versus time curve from 0 to infinity (AUC_{0-∞}) was calculated as AUC_{0-∞}+C_t/K_e, where C_t is the last measurable concentration, and the bioavailability (F) was calculated according to the equation:

\[ F = \frac{AUC_{0-24}(test)}{AUC_{0-24}(reference)} \times 100\% \]

The pharmacokinetic parameters were calculated by Drug and Statistics Software (DAS) (Mathematical Pharmacology Professional Committee of China).

Statistical analysis
For the aim of bioequivalence analysis between two formulations, AUC_{0-24}, AUC_{0-∞}, and C_max were considered as primary variables. The bioequivalence of the two products was assessed by means of an analysis of variance (ANOVA) for crossover design and calculating standard 90% confidence intervals (CI) of the ratio test/reference (T/R) using log-transformed data. The parameter t_max was analyzed with Wilcoxon’s rank sum test. In addition, bioequivalence between the two formulations was evaluated by paired two-one-sided t-test. The products were considered bioequivalent if the difference between the two compared parameters was statistically insignificant (\( P >0.05 \)) and 90% confidence intervals for these parameters fell within 0.8-1.25 and 0.70-1.43 respectively for AUC and C_max, which is the range accepted by the US and China State Food and Drug Administration[5-7]. All data were expressed as mean ± SD. A two-tailed \( P <0.05 \) was considered statistically significant.

Results
Method validation

The described analytical method used for measurement of levofloxacin in serum was proved to be accurate and sensitive. The regression equation was described as: \( C = 0.0042A - 0.0544 \) \((r=0.9999, n=5)\) (A: Peak area of levofloxacin; C: Concentration of levofloxacin), and the lower limit of quantitation (LOQ) in serum was 0.1 mg·L\(^{-1}\) (defined as 10 times background noise). The calibration curve was in good linearity over the range of 0.1~5 mg·L\(^{-1}\), and the intra-day and inter-day coefficient of variation was <4.8% and 5.2%, respectively. The absolute recovery was 81.0% to 81.3% while the relative recovery ranged from 99.1% to 102.0%. A stability study showed that levofloxacin was stable in serum at room temperature for at least 12 hr, as well as for 30 days at -80\(^\circ\)C and after three freeze-thaw cycles. The retention time of levofloxacin was 3.82 min. The HPLC chromatograms of levofloxacin in serum were showed in Fig 1.

![HPLC chromatograms of levofloxacin](image)

Pharmacokinetics and bioavailability

The mean serum concentration-time curves after oral administration of levofloxacin tablets in 20 healthy volunteers were shown in Figure 2, and corresponding main pharmacokinetic parameters were listed in Table 1. A two-compartment open model with first-order absorption was fitted to the concentration-time data using the DAS program.

The statistic analysis showed that there were no significant differences for pharmacokinetic parameters AUC\(_{0-24}\), AUC\(_{0-\infty}\), C\(_{max}\), T\(_{max}\) and T\(_{1/2}\) between the two formulations \((P>0.05)\). The mean ratio of AUC\(_{0-24}\)/AUC\(_{0-\infty}\) for test and reference formulation of 90.0% and 89.7%, respectively, indicated that the sampling time was adequate. The relative bioavailability for test formulation was (97.7 ±16.7)% The 90% confidential interval of AUC\(_{0-24}\), AUC\(_{0-\infty}\) and C\(_{max}\), of test formulations were 90.2%-103.0%, 90.0%-102.6% and 96.1%-108.1%, respectively. According to the bioequivalence criteria, the two formulations were bioequivalent.
Fig 2  Mean serum concentration - time curves of levofloxacin after a single oral administration of 200 mg in healthy volunteers (Mean ± SD, n =20)

Table 1. Main pharmacokinetic parameters of levofloxacin after a single oral dose of 200 mg in healthy volunteers (Mean ±SD, n = 20)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test formulations</th>
<th>Reference formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;/mg·L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>2.08 ±0.42</td>
<td>2.03 ±0.35</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt;/h</td>
<td>1.13 ±0.30</td>
<td>1.06 ±0.24</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;/h</td>
<td>7.37 ±0.88</td>
<td>7.39 ±0.96</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt;/mg·h·L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>13.17 ±2.32</td>
<td>13.73 ±2.89</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt;/mg·h·L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>14.64 ±2.56</td>
<td>15.30 ±3.20</td>
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<tr>
<td>F&lt;sub&gt;0-24&lt;/sub&gt;/%</td>
<td>97.7 ±16.7</td>
<td></td>
</tr>
<tr>
<td>F&lt;sub&gt;0-∞&lt;/sub&gt;/%</td>
<td>97.4 ±16.5</td>
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</tbody>
</table>

**Safety evaluation**

Both formulations used in this study were well tolerated at the dose administered by all the volunteers. Unexpected adverse events that could have influenced the outcome of the study did not occur. None of the changes in laboratory test values and vital signs during the study were considered clinically important. There were no drop-outs and all volunteers who started the study continued to the end and the biochemical parameters remained unchanged and within the reference range.

**Discussion**

A single oral dose of 200 mg levofloxacin test and reference tablets were given to 20 healthy male Chinese volunteers in a single dose, randomized, two-treatment, two-period crossover study, and serum levofloxacin concentrations were determined with the validated RP-HPLC method developed in our study. It was a specific, simple, sensitive and reproducible procedure and, therefore, a suitable and valuable tool in the investigation of the clinical pharmacokinetics and bioavailability of levofloxacin.

Pharmacokinetics of levofloxacin was well described by a two-compartment open model with first-order absorption. Both tablet formulations were readily absorbed from the gastrointestinal tract, which was measurable at the first sampling time (0.25 h) in the majority of the volunteers. The mean concentration-time profiles of two formulations were closely similar and superimposable (Figure 2). The peak concentration of the test and reference products was 2.08 mg·L<sup>-1</sup> and 2.03 mg·L<sup>-1</sup> for levofloxacin at 1.13 h and 1.06 h after administration, respectively. Concentration then declined but remained detectable...
up to 24 h after administration. There is a wide inter-subject variability in both formulations. The pharmacokinetic results were consistent with other reports\(^8,9\). Throughout the whole study period, there were no adverse events reported.

The most important objective of any bioequivalence study is to assure the safety and efficacy of the test and reference products. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent\(^10\). The mean and standard deviation of $AUC_{0-24}$, $AUC_{0-\infty}$, and $C_{max}$ of the two formulations did not differ significantly, suggesting that the serum profiles generated by the test formulation were comparable to those of the reference formulation. ANOVA, after log-transformation of the data, showed no statistically significant difference between the two formulations ($P>0.05$). Furthermore, the parametric confidence intervals (90%) of the mean values of the pharmacokinetic characteristics for T/R ratio were in each case well within the bioequivalence acceptable ranges of 0.8-1.25 and 0.70-1.43 respectively for AUC and $C_{max}$. These results were confirmed by the Schuirmann's two one-sided t tests, which indicated that the lower and upper limits of the calculated t value were greater than the critical t value for the three parameters. Therefore, the two tablet formulations can be considered bioequivalent with regard to the extent and rate of absorption.

In conclusion, statistical analysis (ANOVA and 90% CI) for $AUC_{0-24}$, $AUC_{0-\infty}$, and $C_{max}$ clearly indicated no significant difference in the two levofloxacin tablets. Based on the pharmacokinetic and statistical results of this study, it is concluded that two levofloxacin 100 mg tablet formulations are bioequivalent, and that the two formulations can be considered equally effect and safe in medical practice.

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References