Bioequivalence Evaluation of Fleroxacin Tablets in healthy Korean

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Fleroxacin is an orally absorbed antimicrobial agent used to treat urinary tract infections.\(^1\) The mechanism of action is the antimicrobial effect, which inhibits the DNA gyrase and topoisomerase IV of bacteria.\(^2\)-\(^4\) Quinolones, such as nalidixic acid, are bactericidal to most common gram negative bacteria that cause urinary tract infections, and their intrinsic activity is limited. In contrast, fluoroquinolones, e.g. fleroxacin, are rapid bactericidal agents in vitro, and are considerably more potent against E. coli and various species of Salmonella, Shigella, Enterobacter, Cmylobacter, and Neisseria.\(^5\) The therapeutic uses include urinary tract infections, prostatitis, sexually transmitted diseases, gastrointestinal and abdominal infections, other infections including bone, joint, and soft tissue infections.\(^6\)

There are relatively few side effects resulting from the use of fleroxacin, and microbial resistance to their action does not develop rapidly.\(^7\) Fluoroquinolones are generally well tolerated: the most common adverse reactions being nausea, abdominal discomfort, headache, and dizziness. In rare cases, hallucinations, delirium, and seizures have occurred but mainly in patients receiving theophylline or non-steroidal anti-inflammatory drugs. Fluoroquinolones have decreased the incidence of gram-negative rod bacteremias when used as prophylaxis in neutropenic patients. However, these drugs are generally not recommended for prepubertal children or pregnant women.\(^8\)

The minimum inhibitory concentrations of fluoroquinolones for 90% of these strains (MIC90) are usually < 0.2 µg/ml.\(^9\) Fleroxacin has MIC90 values from 0.5 to 3 µg/ml for M. fortuitum, M. kansassi and M. tuberculosis, and is active in an animal models of leprosy.\(^10\) However, there is limited clinical experience with these pathogens. Most anaerobic microorganisms are resistant to fluoroquinolones, with the exception of sparflaxacin. The peak serum levels of fluoroquinolones are obtained within 1 to 3 hours of an oral dose of 400 mg.

This study evaluated the bioequivalence of the test and reference fleroxacin tablets, Fleroxacin® 100 mg and Megarocin® 100 mg, respectively, according to the guidelines and standard protocols to the bioequivalence test of the Korea Food and Drug Administration.\(^11\)-\(^12\)

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Therefore, the typical bioavailability including AUC_t, and C_max parameters were compared. Twenty-four healthy male Korean volunteers were divided into two groups using a randomized 2x2 cross-over design. The pharmacokinetic parameters (AUC_{72hr} and C_max) of the test and reference fleroxacin tablets were determined. Statistical analysis of the PK parameters was carried out to determine if the test and reference tablets were comparable.

**MATERIALS AND METHODS**

**Materials**

Enoxacin, potassium dihydrogen phosphate, methanol, and other reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). The test and reference fleroxacin tablets were Fleroxacin® 100 mg and Megarocin® 100 mg, respectively.

**Sample analysis**

**Preparation of calibration standards**

A stock solution of fleroxacin was prepared at a concentration of 1 mg/mL, and used to prepare the calibration standards. “Fleroxacin-free” human serum was obtained after administering fleroxacin orally (100 mg, 3 tablets) to the healthy Korean subjects. The collected serum with fleroxacin administration was used as the blank serum. The blank serum was spiked with the diluted fleroxacin stock solution, and the calibration standards were prepared over the concentration ranges of 0.1, 0.2, 0.5, 1, 2, 5, 10 µg/mL.

**Sample extraction**

The experimental serum and calibration standard samples were combined with 100 µL of an enoxacin solution (internal standard, 50 µg/mL). The sample mixture was deprotonized with 10µL of a 6% aqueous TCA solution, vortex-mixed for approximately 1 min and centrifuged for 10 min at 13,000 rpm. Only a 10 µL aliquot of the supernatant was injected into the HPLC/UV system.

**Chromatographic condition**

Fleroxacin were analyzed using a HPLC/UV detector system with a C18 column (SI-II, Shiseido Co., Tokyo, JAPAN). Chromatographic separation was achieved on a Gemini C18 (5 m, 250 mm x 4.6 mm, Phenomenex, USA) with a C18 guard column (4.0 mm x 3.0 mm, Phenomenex, USA). Fleroxacin was retained in the pre-column with mobile phase 1 (50 mM KH_2PO_4: methanol = 76:24 v/v % ) at a flow rate of 1.5 ml/min. It was then eluted through the analytical column with mobile phase 2 (water: acetonitrile (57:43, v/v %)) at a flow rate of 0.7 ml/min. The column temperature was maintained at 40°C, and fleroxacin was detected at 280 nm. The injection volume used was 10 µL.

**Analytical method validation**

The linearity of the analytical method was examined over the standard concentration range, 0.1~10 mg/mL. A calibration equation was constructed by fitting the normalized peak area (ratio of the fleroxacin peak area to the internal standard peak area) as a function of the fleroxacin concentration in each calibration standard, and was obtained from least-square linear regression. The intra-day and inter-day precision and accuracy were calculated for all calibration standard concentrations. The intra-day precision was assessed from 5 consecutive determinations within a single day run, and the inter-day precision was determined from 5 consecutive day determinations. The precision results are presented as the coefficient of variation (CV %). The intra-day and inter-day accuracy was examined by calculating the agreement between the measured and nominal concentrations, and the accuracy is presented as the mean±S.D.

**Pharmacokinetics of fleroxacin in Koreans**

**Measurement of the fleroxacin concentrations**

Healthy male Korean volunteers were enrolled in this study to measure the fleroxacin concentration. Physical and biological examinations were performed at Chung Ang University Yongsan Hospital (Seoul, Korea). Those volunteers not meeting the selection criteria were
rejected. Prior to initiating the study, the selected subjects (n=12) signed a written informed consent document detailing the purpose, procedure, and risks of the study. The average age of the subjects was 21.3 (±2.1) years, and their average weight was 67.3 (±7.5) kg.

All subjects were asked to refrain from taking all medications including antibiotics and analgesics. In addition, they were asked not to smoke or consume beverages containing xanthines for a 10 day period before the study. On Day 1 of the study, blood samples (5 mL) were withdrawn from the upper arm vein of the subjects at 7:30 am, and thereafter from 8:00 am(0 hour) to day 3(72 hour). During the day, all subjects were provided with a standard meal for lunch and dinner. The withdrawn blood samples were centrifuged at 3,000 rpm for 10 minutes. The serum was separated and stored at -70°C until analyzed.

Bioequivalence study of fleroxacin in Korean

The pharmacokinetic parameters of the test and reference fleroxacin tablets in 24 healthy Korean male subjects were determined. The physical and biological examinations were performed at Chung Ang University Hospital Yongsan (Seoul, Korea). The subjects were divided into two groups using a randomized 2×2 crossover design. The night before the study, the subjects were hospitalized at 6:30 pm and fasted until noon the next day. During the study, the subjects were asked to refrain from physical activity, smoking, and beverages containing xanthines. The blank blood samples were withdrawn at 8:00 am the next day morning (0 hr), and 3 tablets of fleroxacin (30 mg) were administered orally to the first subject at 8:00 am with a two-minute interval between subjects. Blood samples (5 mL) were withdrawn 0.33, 0.66, 1, 1.5, 2, 4, 8, 12, 24, 48 and 72 hrs after administering the drug. The blood samples were centrifuged at 3,000 rpm for 10 minutes, and serum was separated and stored at -70°C until analysis.

The pharmacokinetic parameters of the test and reference tablets, such as the maximum serum concentration (C\text{max}), time to reach the C\text{max} (T\text{max}), and area under the serum concentration-time curve (AUC\text{72hr}) were determined. The AUC\text{72hr} and C\text{max} were transformed logarithmically to stabilize the variance and obtain a symmetric data distribution. The bioequivalence between the test and reference tablets were assessed using statistical analysis (ANOVA) of ln (AUC\text{72hr}), ln (C\text{max}) and T\text{max}.

RESULTS AND DISCUSSION

Chromatography of fleroxacin

A blank serum was obtained after administering fleroxacin and its chromatogram is shown in Figure 1 (A). Figure 1 (B), (C) and (D) show chromatograms of the serum spiked with fleroxacin and enoxacin. Fleroxacin and enoxacin were eluted at 9.8 and 11.9 minutes,
Sensitivity and linearity

The peak areas of fleroxacin and the internal standard in the calibration standards were determined by automated integration. The lower limit of quantification (LOQ) was 0.1 ng/mL with a signal-to-noise ratio of 10. The calibration equation of this method was $y = 0.00491x + 0.18477$ ($R^2 = 0.99961$), where $y$ is the normalized peak area of fleroxacin and $x$ is the standard concentration.

Accuracy and Precision

Table 1 shows the intra-day and inter-day precision and accuracy. The coefficient of the variations in the intra-day and inter-day precision were < 15% except for the lower limit of quantification, which were 8.54 and 10.00%, respectively. The intra-day and inter-day accuracy ranged from 94.5% to 100%. (Table 1)

Pharmacokinetics of fleroxacin

The above described HPLC method was applied to a bioequivalence study of the two fleroxacin tablet formulations. Figure 2 shows the mean (±S.D.) plasma concentration-time profiles of fleroxacin after administering an oral dose of 300mg of both formulations in tablet form. No significant sequence effect was observed for any of the bioavailability parameters, indicating that the cross-over design was appropriate. Table 2 shows the geometric means of the parameters for the test and reference formulations, both separately for each period and as combined estimates. The parametric point estimates for the mean of the test medication/mean of the reference medication for the AUC$_{72}$ and C$_{max}$ were 1.005% and 0.942%, respectively, and the parametric 90% confidence intervals for AUC$_{72}$ and C$_{max}$ were 0.957$\delta$1.057 and 0.837$\delta$1.063, respectively, which were within the accepted range of 0.80-1.25% by the Korean Food and Drug Administration. Detailed bioequivalence analysis for the T$_{max}$ value under the assumption of the non-parametric model is also given. The pharmacokinetic parameters, such as the AUC$_{72}$, AUC$_{\infty}$, t$_{1/2}$, C$_{max}$ and T$_{max}$ of the test drug were similar to those of the reference drug, as shown in Table 2. This demonstrates no significant difference between the bioavailability of Fleroxacin® (test drug) and Megarocin® (reference drug). The 90% confidence intervals for the ratios of the test drug to the reference drug in terms of the AUC$_{72}$, AUC$_{\infty}$ and C$_{max}$ were within 80-125%.

CONCLUSION

A convenient HPLC method was developed to determine the fleroxacin level in human plasma. The bioequivalence of two different 100mg fleroxacin tablet formulations was examined based on the oral administration of 300 mg of the drug (consisting of three tablets) to 24 healthy, normal male volunteers. Statistical analysis based on the compositions of the three pivotal parameters (AUC$_{72}$, and C$_{max}$) suggested that these two tablet formations of fleroxacin are bioequivalent, and

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Intra (n=5)</th>
<th>Inter (n=5)</th>
<th>Intra (n=5)</th>
<th>Inter (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>8.54</td>
<td>10.00</td>
<td>98.00</td>
<td>100.00</td>
</tr>
<tr>
<td>0.3</td>
<td>2.68</td>
<td>7.42</td>
<td>104.00</td>
<td>97.33</td>
</tr>
<tr>
<td>4</td>
<td>1.95</td>
<td>2.63</td>
<td>98.40</td>
<td>97.75</td>
</tr>
<tr>
<td>8</td>
<td>2.53</td>
<td>1.60</td>
<td>94.68</td>
<td>95.28</td>
</tr>
</tbody>
</table>
may be prescribed interchangeably

ACKNOWLEDGEMENTS

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REFERENCE


Table 2. Pharmacokinetic parameters of the reference and test formulations of fleroxacin based on the plasma concentration

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Megarocin® (mean±SD)</th>
<th>Fleroxacin® (mean±SD)</th>
<th>Confidence limit 90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax (h)</td>
<td>1.20±0.96</td>
<td>1.65±0.96</td>
<td>-</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>4.11±1.63</td>
<td>3.78±1.01</td>
<td>83.69-106.26</td>
</tr>
<tr>
<td>AUC72 (µg/mL)</td>
<td>44.26±7.12</td>
<td>45.16±10.04</td>
<td>95.70-105.65</td>
</tr>
<tr>
<td>AUC∞ (µg/mL)</td>
<td>46.96±6.85</td>
<td>48.69±9.30</td>
<td>98.93-106.58</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>10.41±1.68</td>
<td>10.33±1.72</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. Statistical results of the bioequivalence test between the test and reference fleroxacin tablets

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Criteria for bioequivalence</th>
<th>AUC0~10 hr</th>
<th>Cmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent difference of averages</td>
<td>≤ ±20%</td>
<td>+ 0.55 %</td>
<td>- 5.68 %</td>
</tr>
<tr>
<td>Point estimate of the average ratio</td>
<td>NA</td>
<td>1.005</td>
<td>0.942</td>
</tr>
<tr>
<td>Confidence interval at a = 0.05</td>
<td>0.8 = δ = 1.25</td>
<td>0.9575 = δ = 1.0565</td>
<td>0.8369 = δ = 1.0626</td>
</tr>
</tbody>
</table>

The AUCt and Cmax were calculated based on the log-transformed data. NA: None available.