Bioequivalence evaluation of two brands of cefaclor 500 mg capsules: quantification of cefaclor using solid phase extraction technique

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SUMMARY

Objective: To assess the bioequivalence of two cefaclor 500 mg capsule formulations, and to develop a new high performance liquid chromatographic (HPLC) method using solid phase extraction technique for the quantification of cefaclor in human plasma.

Method: An open, randomized, two-way, crossover trial with a one-week washout period in 25 healthy volunteers. The two commercial brands used were Recocef® (Julphar, United Arab Emirates) as test and Ceclor® (Eli Lilly, UK) as reference product. The drug was administered with 240 mL of water after a 10-h overnight fast. After dosing, serial blood samples were collected for a period of 8 h. Plasma harvested from blood was analysed for cefaclor by a new HPLC method using a solid phase extraction technique. The limit of detection of cefaclor was 17.6 ng/mL; average recovery was 96.5%; the intraday CV was less than 8% and interday CV was less than 13%. Various pharmacokinetic parameters, including AUC0-t, AUC0-∞, Cmax, Tmax, T1/2, and Kel were determined from plasma concentrations for both formulations. Statistical analysis (ANOVA and 90% confidence intervals) were applied to AUC0-t, AUC0-∞ and Cmax for bioequivalence evaluation of two brands. The new HPLC method with solid phase extraction circumvented the problem of mixed polarity of cefaclor and facilitated its extraction from the complex plasma matrix while keeping the background free from interference due to endogenous plasma compounds.

Results: No significant difference was observed between the two brands of cefaclor capsules.

Conclusion: Recocef® was judged bioequivalent to Ceclor®, and the two products can therefore be considered to be interchangeable in medical practice.

Keywords: bioequivalence, cefaclor, HPLC, pharmacokinetics

INTRODUCTION

Bioequivalence of two formulations of the same drug is equivalence with respect to the rate and extent of the drug’s absorption. Whereas the area under the concentration time curve (AUC) generally reflects the extent of absorption, the peak concentration (Cmax) and the time of its occurrence (Tmax) reflect the rate of absorption, especially with rapid release drug formulations (1,2).

The present study was conducted to evaluate the bioequivalence of two brands of cefaclor 500 mg capsules in fasting, healthy human volunteers. Although several studies have been published on cefaclor pharmacokinetics, very few of them have focused on the issue of bioequivalence between two brands.

Cefaclor is a second generation semi-synthetic cephalosporin antibiotic with a broad spectrum of activity against Gram positive and Gram negative bacteria (3,4). It is used in the treatment of otitis media caused by Streptococcus pneumoniae, upper respiratory tract infections caused by susceptible group A β-haemolytic streptococci, lower respiratory tract infections caused by susceptible S. pneumoniae, H. influenza, or group A β-haemolytic streptococci, urinary tract infections caused by E. coli, Proteus or Klebsiella, and skin or skin structure infections caused by Staphylococcus aureus (5–7).
Cefaclor is well absorbed after oral administration to fasting subjects; average peak serum level of 13 µg/mL was obtained within 30–60 min after 500 mg oral dose (5). Total absorption was similar regardless of whether the drug was given with or without food. However, if the drug was administered with food, the peak concentration was 50–75% of that found in fasting subjects and was generally achieved about one hour later (5). Cefaclor is widely distributed throughout the body and reaches therapeutic concentrations in most tissues and body fluids (4). It does not undergo either hepatic or renal biotransformation (4) and 60–85% of the drug is excreted unchanged in the urine within 8 h (5). The reported elimination half-life is 0.6–0.9 h, increasing to 2.3–2.8 h in patients with reduced renal function (5).

The aim of this study was to assess the bioequivalence of two commercial 500 mg capsule formulations of cefaclor in healthy human volunteers and to develop a new high performance liquid chromatographic (HPLC) method using solid phase extraction technique for the quantification of cefaclor in human plasma.

MATERIALS AND METHODS

Drug preparations

The two commercial formulations used in the study were Recocef® 500 mg capsules (Gulf Pharmaceutical Industries, Julphar, United Arab Emirates), which were used as the test product, and Ceclor® 500 mg capsules (Eli Lilly, UK), which were used as the reference product.

Subjects

The subjects were 25 healthy adult male volunteers recruited at Jordan University Hospital, Amman, Jordan. Their mean age was 33 ± 6.7 years (range 18–43 years) and mean body weight was 77 ± 6.4 kg (range 67–85 kg). On the basis of medical history, clinical examination and laboratory investigation (haematology, blood biochemistry, and urine analysis), no subject had a history or evidence of hepatic, renal, gastrointestinal or haematological deviations or any acute or chronic diseases or drug allergy. The subjects were instructed to abstain from taking any medication for at least one week prior to and during the study period. Informed consent was obtained from the subjects after explaining the nature and purpose of the study. The study protocols were approved by the Institutional Review Board (IRB) of Jordan University Hospital.

Drug administration and sample collection

The study was a single dose, randomized, two-way treatment crossover design. On the morning of phase I, after an overnight fast (10 h), volunteers were given a single dose of either formulation (reference or test) of cefaclor with 240 mL of water. No food was allowed until 4 h after dose administration. Water was allowed 2 h after the dose; water, brunch and lunch were given to all volunteers according to a pre-planned schedule. Volunteers were ambulatory during the study but prohibited from strenuous activity. Approximately 10 mL of blood was drawn from each subject into heparinized tubes through an indwelling cannula before (0 h) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6 and 8 h after dosing. Blood samples were centrifuged at 4000 r.p.m. for 3 min (at −4 °C), and plasma was separated and kept frozen at −70 °C in coded polypropylene tubes containing glacial acetic acid. After a period of 7 days the study was repeated in the same manner to complete the crossover design.

Chromatographic conditions

An HPLC method using solid phase extraction was developed and validated for cefaclor analysis in plasma samples at PRU Laboratory. All solvents used were of HPLC grade and were purchased from Merck (LiChrosolv-Darmstadt, Germany); the cefaclor reference standard was provided by Gulf Pharmaceutical Industries.

The HPLC system was an isocratic system consisting of a solvent delivery pump (Varian Star model 9002, Varian Analytical Instruments, Sunnyvale, CA, USA), UV/Vis detector (Varian Star Model 9050), autosampler (Varian Star Model 9100) and a Varian Star chromatography workstation for data collection. The extraction of cefaclor from plasma was performed by solid phase extraction technique using C18 solid phase extraction columns (Supelco, Bellefonte, PA, 100 mg silica bonded
phase packed as 1 cm³ in a 0·5 × 6·5 cm column). The separation was performed by using a stainless steel Hypersil BDS C₁₈, Shandon (250 × 4·6 mm; Shandon Life Sciences International (Europe) Ltd, Runcorn, UK) column with a particle size of 5 μm. Hypersil BDS C₁₈ (10 × 4·0 mm) with 5 μm particle size was used as a guard column. The mobile phase consisted of acetonitrile and water in 12 : 88 (v:v) ratio; the pH was adjusted to 3·83 with acetic acid. The mobile phase was pumped at a flow rate of 1·2 mL/min; effluent was monitored at wavelength of 264 nm and corresponding peak areas were recorded.

**Extraction of cefaclor from plasma using solid phase extraction technique**

The solid phase extraction columns were initially wetted with methanol (1 mL) and conditioned with phosphate buffer (pH 3·0, 1 mL). Internal standard (hydrochlorothiazide, 1·98 μg/mL) was added to plasma samples (0·5 mL). The plasma samples were then acidified with 25 μL glacial acetic acid (pH 3·0) and diluted with a buffer solution (pH 3·0) before being aspirated into the wetted preconditioned C₁₈ columns. After equilibration, the plasma components were washed with an aliquot of the wash solvent (1 mL phosphate buffer, pH 3·0). Cefaclor molecules were subsequently eluted from the dried columns using 1 mL of an eluting solution (acetonitrile and water: 15 : 85). A 100-μL elute was then injected into the HPLC column and peak areas were recorded.

**Pharmacokinetic analysis**

Pharmacokinetic analysis was performed by means of a model-independent method. The maximum cefaclor concentration (Cₘₐₓ) and the corresponding peak times (Tₘₐₓ) were determined by the inspection of the individual drug plasma concentration–time profiles. The elimination rate constant (Kₑ) was obtained from the least square fitted terminal log–linear portion of the plasma concentration–time profile. The elimination half-life (T₁/₂) was calculated as 0·693/Kₑ. The AUC to the last measurable concentration (AUC₀₋₉) was calculated by the linear trapezoidal rule. The AUC extrapolated to infinity (AUC₀₋∞) was calculated as AUC₀₋₉ + Cₙ/Kₑ, where Cₙ is the last measurable concentration.

**Statistical analysis**

For the purpose of bioequivalence analysis AUC₀₋₉, AUC₀₋∞ and Cₘₐₓ were considered as the primary variables. Bioequivalence was assessed by means of an analysis of variance (ANOVA GLM model) (8) for crossover design and by calculating standard 90% confidence intervals (9–13) of the ratio test/reference (T/R) using log-transformed data. The products were considered bioequivalent when the difference between two compared parameters was found statistically insignificant (P ≥ 0·05) and 90% confidence intervals for these parameters fell within 80–125% (9).

**RESULTS**

The mean concentration–time profiles for the two brands of cefaclor 500 mg capsules are shown in Fig. 1. The pharmacokinetic parameters of both formulations are shown in Table 1. For bioequivalence evaluation, various statistical modules were applied to AUC₀₋₉, AUC₀₋∞ and Cₘₐₓ in accordance with current US Food and Drug Administration (FDA) guidelines (9). The difference between two compared parameters was considered statistically significant with P ≤ 0·05 with a 95% confidence level. Table 2 shows the 90% confidence interval for AUC₀₋₉, AUC₀₋∞ and Cₘₐₓ for log-transformed data.

According to the mean plasma levels of the 25 subjects completing the study, the relative bioavailability was found to be 102·1%, 102·9% and 103·4% on the basis of mean AUC₀₋₉, AUC₀₋∞ and Cₘₐₓ, respectively.

Fig. 1. Plasma concentration–time profile of Cefaclor 500 mg capsules.
Table 1. Pharmacokinetic parameters of cefaclor 500 mg capsules

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Recocef® (Test)</th>
<th>Celer® (Reference)</th>
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<tbody>
<tr>
<td>AUC₀₋₄ (µg/ml·h)</td>
<td>23.53 ± 4.92</td>
<td>24.02 ± 4.37</td>
</tr>
<tr>
<td>AUC₀₋∞ (µg/ml)</td>
<td>24.12 ± 5.34</td>
<td>24.51 ± 4.77</td>
</tr>
<tr>
<td>C_máx (µg/mL)</td>
<td>18.16 ± 5.38</td>
<td>19.10 ± 6.29</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>0.85 ± 0.28</td>
<td>0.89 ± 0.32</td>
</tr>
<tr>
<td>T₁/₂ (h)</td>
<td>0.80 ± 0.43</td>
<td>0.70 ± 0.23</td>
</tr>
<tr>
<td>Ke (/h)</td>
<td>0.94 ± 0.32</td>
<td>0.97 ± 0.23</td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation.

Table 2. 90% Confidence interval (CI) analysis for bioequivalence evaluation

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>90% CI</th>
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<tbody>
<tr>
<td>AUC₀₋₄</td>
<td>90.3–103.6</td>
</tr>
<tr>
<td>AUC₀₋∞</td>
<td>90.7–103.9</td>
</tr>
<tr>
<td>C_máx</td>
<td>85.8–105.2</td>
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The mean and standard deviation of both parameters for the two brands were found to be very close, indicating that the plasma profiles generated by Recocef® are comparable to those produced by Celer®. Analysis of variance (ANOVA) for these parameters, after log-transformation of the data, showed no statistically significant difference (P > 0.05) between the two brands. In addition, 90% confidence intervals demonstrated that the ratio of the AUC₀₋₄ or AUC₀₋∞ of the two brands lie within the FDA accepted range of 80–125%.

For bioequivalence evaluation ANOVA was performed on C_máx values of both products. ANOVA showed that the two formulations were not statistically different (P > 0.05) from each other. Moreover, 90% confidence intervals also demonstrated that the ratio of the C_máx of both formulations lay within the FDA accepted range of 80–125%.

DISCUSSION

All calculated pharmacokinetic parameter values were in good agreement with previously reported values (3–7). Cefaclor was well tolerated by the volunteers in both phases of the study. All volunteers who started the study continued to the end and were discharged in good health; unexpected incidents that could have influenced the outcome of the study did not occur. Both formulations were readily absorbed from the gastrointestinal tract and cefaclor was measurable at the first sampling time (0.25 h) in almost all volunteers.

In the present work a solid phase extraction technique was employed to overcome the problem of mixed polarity of cefaclor, which makes the solvent extraction procedure insufficient (14). Reported cephalosporin analytical methods for biological samples have predominantly relied on protein precipitation with cold methanol and 0.1 M sodium acetate or with methanol alone prior to HPLC separation using an RP C₁₈ column (14–17) and spectrophotometric determination (18–20).

In this study the solid phase extraction facilitated the extraction of cefaclor from the complex plasma matrix while keeping a background free from interference due to endogenous plasma compounds (21). All the experimental variables including polarity, stability in plasma, storage temperature effect, pH effect, etc. were investigated during method development and validation. The method was validated (22) using a linearity range of 0.56–14.7 µg/mL with a limit of detection of 1.76 ng/mL and average recovery of 96.5%. The intraday CV was 4.5% (1.7–7.1%) and interday CV was 4.16% (1.7–12.5%).

A non-compartmental approach was used to determine the pharmacokinetic parameters of cefaclor. The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. 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, the two formulations are bioequivalent and thus considered therapeutically equivalent (23). To demonstrate bioequivalence, certain limits should be set depending on the nature of drug, patient population, and clinical end points. It is generally accepted that for basic pharmacokinetic characteristics, such as AUC and C_máx the standard equivalence range is 0.8–1.25 (9).

CONCLUSION

The HPLC method with solid phase extraction described above for the quantification of cefaclor
overcame the problem of mixed polarity of cefaclor, which usually makes the solvent extraction procedure inefficient. The statistical comparison of $\text{AUC}_{0-t}$, $\text{AUC}_{0-\infty}$ and $C_{\text{max}}$ clearly indicated no significant difference between the two brands of cefaclor 500 mg capsules. In addition, 90% confidence intervals for the mean ratios ($T/R$) of $\text{AUC}_{0-t}$, $\text{AUC}_{0-\infty}$ and $C_{\text{max}}$ indicated that the reported values were entirely within the bioequivalence acceptance range of 80–125% (using log-transformed data). Based on the above pharmacokinetic and statistical results of this study, we can conclude that Recocet® 500 mg capsules are bioequivalent to Ceclor® 500 mg capsules and that the two products can be considered interchangeable in medical practice.

REFERENCES


