Comparative bioavailability and pharmacokinetic study of Cefadroxil capsules in male healthy volunteers of Pakistan

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Abstract: The current study was aimed to judge bioequivalence between two formulations of cefadroxil capsules as guided by FDA guidelines. Another objective was to conduct pharmacokinetic evaluation in Pakistani population. A single-dose, randomized, cross-over pharmacokinetic study was conducted during the month of May’2013 to August’2013. Washout period was one week. Fourteen healthy male adult volunteers were enrolled in the study, however twelve completed the study. Cefadroxil plasma concentration was analyzed by using validated HPLC method. Protein precipitation was achieved by the addition of 6% tri chloro acetic acid in 1:1 ratio and detection was done at 260 nm. Retention time was 7.792 min and correlation coefficient (R²) was 0.9953 showing linearity of the method. Blood sampling was carried out at different time intervals after administration of either test (TEST 500 mg) or reference (REF® 500mg) formulation. Pharmacokinetic parameters (AUC 0 →∞, AUC 0 →t, C max, T max, t 1/2 and k el) were calculated using Kinetica® PK/PD software. The geometric mean ratios and 90 % confidence interval (CI) of these pharmacokinetic parameters for cefadroxil (test and reference) formulations were 0.986 (90.83-106.98%) for AUC 0-t; 0.967 (89.13-104.92%) for AUC0-∞ and 0.999 (91.06-109.69%) for C max. The differences between T max of both formulations were not found to be statistically significant (p-value was more than 0.05). The 90% CI of the test/reference AUC and C max ratio of cefadroxil were within the FDA recommended range for bioequivalence. Maximum plasma concentration C max was 12.5 µg/ml for test and 12.47µg/ml for reference formulations. Average time to reach C max for test and reference formulation was 1.54 and 1.5 hrs. The two formulations of cefadroxil studied during the above study were verified bioequivalent. Maximum plasma concentration of cefadroxil was lower than those mentioned in some previous studies, while Tmax and half-life were near to values reported in literature

Keywords: FDA; cefadroxil; pharmacokinetic study; comparative bioavailability.

INTRODUCTION

Cefadroxil is semi synthetic, bactericidal antibiotics prescribed for skin, soft tissues, up per respiratory and urinary tract infections especially in children. Adult usual dose is 500-1000 mg/day in single or divided doses (Shetty et al., 1999). It is well tolerated and readily absorbed after oral administration. It is mainly excreted as intact molecule in urine. Protein binding is 20% and half-life is 1.5 to 2 hrs. Its activity is similar to cephalexin, whereas its half life is 1.1 hrs after 500mg oral dose as compared to 0.7 hrs after similar dose of cephalexin (Welling et al., 1985). In contrast with other cephalosporins like cephalexin, cefadroxil produces considerable higher concentration in physiological fluids and tissues like lung, skin, sputum, pleura after oral administration. The drug concentration attained is high enough to defeat bacterial growth (Bernhardt 1980; Nightingale 1986; Tanrisever and Santella 1986; Akimoto et al., 1994).

FDA defines bioequivalence as “the absence of any significant change in rate and extent of absorption of any active pharmaceutical ingredient from its pharmaceutical products at similar molar dose administered under identical conditions”. The basic aim of bioequivalence study is that if two products of same active pharmaceutical ingredient are revealed to be bioequivalent at similar molar dose and dosage form, but different formulation ingredients; they are therapeutically equivalent regarding efficacy and safety; such products can be used substitutable (Meredith 1996). FDA guidance for industry endorses the requirement of bioequivalence studies whenever they apply for the approval of a new dosage form of established active pharmaceutical ingredient (FDA 2003). So, manufacturers have to submit applications to the regulatory authorities to prove the safety and efficacy of their product in contrast with innovator’s brand. They have to document proof of similar rate and extent of absorption which should be within recommended limits (FDA 2003) and (EMA-CHMP 2010). To prove bioequivalency, the plasma drug concentration verses time curve and different Pharmacokinetic parameters including Cmax, Tmax and
AUC of test formulation should be 80-125% that of reference formulation.

In Pakistan, there are more than 400 registered pharmaceutical companies, out of these 370 are local companies contributing the market share of 46.7% (Drug Control Organization 2005). The pharmaceutical products marketed by local and multinational companies registered in local index of pharmaceuticals have large variation in the cost. In developing countries like Pakistan, health related expenses have to be born by the patient himself. In this scenario, use of generics should be encouraged instead of brands (Karim et al., 1996; Simoens and De Coster 2006; Jamshed et al., 2009). As 77% of medical expenditure in Pakistan is out of pocket, the introduction and use of generic medicines should be encouraged to solve the issues of access and affordability being a cost containment approach used worldwide (WHO 2004). The substitution should be supported by bioequivalence studies conducted in local population.

Approximately ten brands of cefadroxil are available in Karachi mostly produced by local companies. However, literature does not contain any published report about their pharmacokinetic and bioequivalence study conducted on Pakistani population. Physician and clinical pharmacist are prescribing such products according to the pharmacokinetic data available from developed countries. It is also observed that some significant variations in pharmacokinetic are found in different products of same active pharmaceutical ingredient as these products are manufactured by different pharmaceutical companies. Therefore, the present study was planned to investigate comparative bioavailability of a local formulation of cefadroxil (TEST capsule 500mg) with its reference brand (REF® capsule 500mg). Bioequivalence study was performed using randomized, two treatment, two period, cross over design as guided by FDA-CDER 2003 (FDA 2003). Pharmacokinetic variation in local population was also investigated.

**MATERIALS AND METHODS**

**Formulations**
The reference (REF® 500mg) and test formulation (TEST 500mg) were selected from available brands of cefadroxil in Karachi. The reference formulation was the innovator’s brand of multinational origin, while the formulation with lowest price was selected as test. These formulations were purchased from local market. Information written on labels was noted including price, manufacturing date, expiry date and batch number.

**Chemical assay and dissolution profile comparison**
These formulations were assayed by assay method as given in official monograph of cefadroxil (USP, 31). RP-HPLC method was adopted with UV detection at 230 nm. Mobile phase comprised of buffer pH 5.0 and acetonitrile in the ratio of 960:40 and flow rate was 2 ml/min. Results were calculated as percent drug content of manufacturers claim. Dissolution profile of the test product (n=12) was compared with that of reference (n=12) in four different dissolution media (HCl pH 1.2, buffer pH 4.5, buffer pH 6.8 and distilled water). Dissolution apparatus (Pharmatest DT70, Germany) and spectrophotometer (Spekol 2000 series, Analytikjena) were used. Similarity (f2) and dissimilarity (f1) factor was calculated using DDSolver® excel based Add in program by Zhang (Zhang et al., 2010).

**Study subjects**
Fourteen participants were enrolled in the clinical part of the study. Their selection was based on inclusion/exclusion criteria. The clinical examinations like medical history, height, weight, body temperature, body-mass index, heart rate, systolic and diastolic blood pressure were completed. They were communicated about the side effects that may be encountered after administration. Such side effects are nausea, vomiting, diarrhea, abdominal discomfort and hardly ever allergic reactions including skin rash or urticaria. During the study period, volunteers were not permitted to ingest alcohol or cold drinks and foods having caffeine from 48 hrs earlier to administration of products till the termination of clinical part of study. The volunteers were instructed to refrain from taking medicines during 4 weeks before and during the study.

**Criteria for inclusion and exclusion of volunteers**
As the study was conducted on healthy subjects, so that inclusion and exclusion criteria was just for healthy volunteers as follows:

**Inclusion criteria**
Volunteers were Pakistani, male, age ranges from 18 to 38 years. Body weight can deviate from average body weight by 10%. They should be healthy deprived of sign/symptoms of any clinical condition and medical history of chronic diseases like diabetes, hypertension. They should not be allergic to beta lactam antibiotics including penicillins and cephalosporins.

**Exclusion criteria**
The volunteers who failed in their physical examination and did not fit in the inclusion criteria were not enrolled in the study. Smokers and volunteers who are taking any type of medication were not included. Those who participated in clinical trial three months before the study and intended to hospitalize within three months after the study were also excluded.

**Study protocol**
A single-dose, randomized, cross-over pharmacokinetic study was executed on adult male volunteers proved healthy. Volunteers were randomly divided in two groups and each group received the one drug treatments at one
time. Table 1 displays the details of two periods of the study. The capsules were administered with water (240 ml) in the morning of the study day after overnight fasting. Breakfast was served at 4 hrs and lunch at 8 hrs after administration. A non-blind approach was adopted. Both volunteer and researchers were aware of the formulations under test. Blood samples (5ml) were withdrawn using an indwelling cannula inserted on the forearm, into Vacutainer™ tubes (containing EDTA) at time intervals of 0 (predose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 10 hrs after drug administration. The blood samples were centrifuged at 6000 rpm for 5 min; plasma samples were stored in cryo vials and kept frozen at -20°C until analyzed. Volunteers were inquired about the incidence of any side effects/adverse event through the treatments periods. Radial pulse and blood pressure were examined as vital signs before study and through the study period.

**Ethical issues**
The study protocol was submitted and got approval from Ethical & Scientific Review Committee, Karachi Medical and Dental College, Abbasi Shaheed Hospital, Karachi. Participation of the subjects in the study was voluntarily and anonymous. They were briefed about the rationale, length and risks of the study. They were also informed about the opportunity of withdrawal whenever they want. Participants were asked to read carefully and signed the informed consent approved by above-mentioned ethical committee before their enrolment in the study. Helsinki declaration and good clinical practices were followed (ICH 1996; WMA 2000).

**HPLC analysis of plasma samples**
Validated high performance liquid chromatographic method was used for plasma sample analysis (Rahim et al., 2014). Schimadzu HPLC (20A) with LC solution software was used with Waters Spherisorb ODS2, C18 (5µm, 150mm×4.5 mm) column. Protein extraction was achieved by using 6% trichloroacetic acid in the ratio of 1:1. Sodium dihydrogen phosphate buffer pH 4.0 and methanol (96:4) was used as mobile phase. 1.5 ml/min was flow rate and 100µl was injection volume. UV detection was recorded at 260 nm. Method specificity was achieved as the blank plasma presented no peak near the retention time of cefadroxil. Linearity was found over the range of 0.5-30µg/ml (R²=0.9953). LOD and LLOQ were 0.03 and 0.06µg/ml, respectively. The precision was in the acceptable range of COV% i.e. 0.35-4.01%, 1.88-7.9% for inter-day and intra-day precision, respectively. Calibration curve is mentioned in fig. 1.

**Calculation of pharmacokinetic parameters**
The plasma concentration of cefadroxil verses time curve was plotted. The values of \( C_{\text{max}} \), \( T_{\text{max}} \), \( \text{AUC}_{0-t} \) and \( \text{AUC}_{0-\infty} \) were estimated from Kinetica® PK/PD software as guided in FDA documents, approved for two period, two treatment, cross-over bioequivalence studies. In addition, other parameters including \( k_a \), absorption half-life, \( k_{el} \), terminal half-life and total clearance were also determined from the above-mentioned software.

**STATISTICAL ANALYSIS**
The pharmacokinetic parameters after logarithmic transformation (multiplicative model) were subjected to an analysis of variance (ANOVA) with the factors sequence; subject nested within sequence; period and drug formulation using a general linear model procedure. For evaluation of bioequivalence 90% confidence intervals for the formulations geometric mean ratio in the parameters \( \text{AUC}_{0-t} \), \( \text{AUC}_{0-\infty} \) and \( C_{\text{max}} \) were determined using the log-transformed data. If the calculated 90% confidence intervals were within 0.80 to 1.25, bioequivalence was proved.

**RESULTS**
The percent cefadroxil content in test and reference formulation were 101.75% and 98.9%, respectively. Results of dissolution profile study are given in fig. 2 and table 2. Similarity \( (f_2) \) and dissimilarity \( (f_1) \) was within the recommended range i.e. \( f_2 \) should be 50-100% and \( f_1 = 1-15 \). A total of fourteen male healthy Pakistani volunteers
comply with the selection criteria were enrolled. Twelve volunteers were completed the study. Their ages were in between 18 and 38 years, with average body weight and body mass index of 74.33kg and 24.31kg/m² (table 3). Blood samples of them were collected after the administration of test and reference formulations and analyzed through validated HPLC method (Rahim et al., 2014). Detection was done at 260nm and mean retention time was 7.792min. Inter-day and intra-day analysis showed adequate precision.

Fig. 3: Linear and (B) semi-log plot of mean cefadroxil plasma concentration verses time in human volunteers.

Pharmacokinetic evaluation of both cefadroxil formulations was done by Kinetica® PK/PD software. The linear and semi log plot of mean plasma concentrations versus time of volunteers (n=12) for the two formulations are mentioned in fig. 3. Pharmacokinetic parameters are depicted in table 4 including Cmax, Tmax, AUC0-t, AUC0-∞, t1/2 and k-el as estimated by Kinetica® PK/PD. ANOVA was applied on the log transformed data of such pharmacokinetic parameters to prove the assumption that period/carry over effect on these parameters was statistically insignificant. For the bioequivalence of two drugs, the 90% confidence interval should be fall in the range given by FDA i.e. 0.8-1.25. We did not found any significant effect of sequence, subject, period and drug formulation for all PK parameters. The 90% confidence interval for geometric mean ratios of test and reference formulations of AUC 0-t, AUC0-∞, and C max were observed as 90.83-106.98%, 89.13-104.92% and 91.06-109.69%, respectively. All these 90% confidence intervals were found within the FDA recommended range for bioequivalence i.e. 80%-125% (table 5). In vitro dissolution profile comparison already revealed that the two products were similar in releasing drug from two products under test and can produce similar rate and extent of absorption in vivo (table 2).

The two formulations of cefadroxil investigated during the study were proved to be bioequivalent. The test formulation is a generic product, which can substitute the reference formulation of innovator’s brand to reduce the cost of treatment in the patient interest. Analogous studies of comparative bioavailability of cefadroxil oral dosage forms (tablets, capsules, suspensions) have been executed formerly in different populations (Oliveira et al., 2000; Otoom et al., 2004; Kano et al., 2008; Mansour 2012). But, such studies outwardly did not assess the same formulation that was tried in the current study. The product under test had its particular formulation unlike from those studied in previous studies. Therefore, the findings of this bioequivalence study were categorically distinctive only to the tested formulation used in the study and cannot be indiscriminated to other cefadroxil generics.

DISCUSSION

The present study was conducted with two drop out due to non-compliance of volunteer. The cross-over design as recommended by FDA for a bioequivalence study was adopted since it can eradicate inter-subject variations. The carry-over effect may hinder in a cross-over design study. It was excluded by providing washout period (one week) large enough to eliminate drug from the body. A total of twelve male healthy Pakistani volunteers were participated till the end of the study. Blood samples of subjects were withdrawn after the administration of both formulations. A validated high performance liquid chromatographic method was adopted. The concentration at first sampling point i.e. 0.5 hr after dosing was above the limit of quantification of the bioanalytical method adopted (i.e. 0.06µg/ml) (Rahim et al., 2014). The plasma drug concentration verses time plots of test as well as reference formulation were comparable (fig. 3). Pharmacokinetic parameters as mean ±SD are depicted in table 4 including Cmax, Tmax, AUC0-t, AUC0-∞, t1/2 and k-el as estimated by Kinetica® PK/PD. ANOVA was applied on the log transformed data of such pharmacokinetic parameters to prove the assumption that period/carry over effect on these parameters was statistically insignificant. For the bioequivalence of two drugs, the 90% confidence interval should be fall in the range given by FDA i.e. 0.8-1.25. We did not found any significant effect of sequence, subject, period and drug formulation for all PK parameters. The 90% confidence interval for geometric mean ratios of test and reference formulations of AUC 0-t, AUC0-∞, and C max were observed as 90.83-106.98%, 89.13-104.92% and 91.06-109.69%, respectively. All these 90% confidence intervals were found within the FDA recommended range for bioequivalence i.e. 80%-125% (table 5). In vitro dissolution profile comparison already revealed that the two products were similar in releasing drug from two products under test and can produce similar rate and extent of absorption in vivo (table 2).

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Table 1: Study design for comparative bioavailability study of two formulations of cefadroxil.

<table>
<thead>
<tr>
<th>Study Period</th>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period one</td>
<td>1</td>
<td>One capsule (500 mg dose) of cefadroxil test formulation*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>One capsule (500 mg dose) of cefadroxil reference formulation®</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One week washout period</td>
</tr>
<tr>
<td>Period two</td>
<td>1</td>
<td>One capsule (500 mg dose) of cefadroxil reference formulation®</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>One capsule (500 mg dose) of cefadroxil test formulation*</td>
</tr>
</tbody>
</table>

*Test = TEST 500 mg capsule (manufactured by local company of Pakistan), Batch number 12647, manufacturing date 6/2012, expiration 5/2014.

®Reference = REF® 500 mg capsule (manufactured by multinational company), Batch number 2G552, manufacturing date 7/2012, expiration 7/2015.

Table 2: Dissolution profile comparison of cefadroxil test and reference products (n=12 units)

<table>
<thead>
<tr>
<th>Dissolution media</th>
<th>Similarity factor ($f_2$)</th>
<th>Dissimilarity factor ($f_1$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl pH 1.2</td>
<td>73.49</td>
<td>3.14</td>
</tr>
<tr>
<td>Buffer pH 4.5</td>
<td>67.19</td>
<td>4.33</td>
</tr>
<tr>
<td>Buffer pH 6.8</td>
<td>50.24</td>
<td>14.04</td>
</tr>
<tr>
<td>Distilled water</td>
<td>51.14</td>
<td>8.29</td>
</tr>
</tbody>
</table>

Recommended ranges of $f_2$ = 50-100 and $f_1$ = 1-15.

Table 3: Demographics of the volunteers participated in the study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21</td>
<td>5.48</td>
<td>18-38</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>74.33</td>
<td>7.27</td>
<td>60-80</td>
</tr>
<tr>
<td>Body height (Inches)</td>
<td>68.08</td>
<td>2.84</td>
<td>62-73</td>
</tr>
<tr>
<td>BMI* (Kg/m²)</td>
<td>24.31</td>
<td>1.66</td>
<td>19.8-27</td>
</tr>
</tbody>
</table>

*body mass index

Table 4: Pharmacokinetic parameters of cefadroxil (test and reference) formulations determinet by Kinetica® from plasma-drug concentration data.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Test (TEST)</th>
<th>Reference (REF®)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC₀-t (µg/ml).hr</td>
<td>35.28±2.24</td>
<td>36.31±7.31</td>
</tr>
<tr>
<td>AUC₀-∞ (µg/ml).hr</td>
<td>36.08±2.59</td>
<td>37.85±7.74</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>12.5±1.72</td>
<td>12.47±1.314</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>1.54±0.144</td>
<td>1.5±0.001</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>1.72±0.41</td>
<td>1.99±0.538</td>
</tr>
<tr>
<td>$k_d$ (1/hr)</td>
<td>0.82±0.41</td>
<td>0.7±0.22</td>
</tr>
<tr>
<td>$t_{1/2k_d}$ (hr)</td>
<td>0.32±0.11</td>
<td>0.232±0.133</td>
</tr>
<tr>
<td>$k_d$ (1/hr)</td>
<td>2.6±1.51</td>
<td>4.2±2.8</td>
</tr>
<tr>
<td>Clearance (L/hr)</td>
<td>14.6±2.04</td>
<td>14.86±3.06</td>
</tr>
<tr>
<td>MRT (mean residence time (hr))</td>
<td>1.47±0.7</td>
<td>1.64±0.61</td>
</tr>
</tbody>
</table>

Values in table 4 represent mean ±SD.

Table 5: Results of statistical analysis of log transformed data of pharmacokinetic parameters of cefadroxil test and reference formulations.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Geometric mean ratio (test/reference)</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC₀-t</td>
<td>0.9858</td>
<td>90.83-106.98</td>
</tr>
<tr>
<td>AUC₀-∞</td>
<td>0.967</td>
<td>89.13-104.92</td>
</tr>
<tr>
<td>Cmax</td>
<td>0.999</td>
<td>91.06-109.69</td>
</tr>
</tbody>
</table>
During the study, different pharmacokinetic parameters were studied in local Pakistani population, as regional variation may cause amendments in drug absorption, distribution, metabolism and excretion (Zhou et al., 1989; Shoaib et al., 2008). The plasma concentration data was the best fitted in two compartmental model, demonstrating linear pharmacokinetics, whereas elimination was carried out from the central compartment. Such type of information was also documented by other investigators (Marino et al., 1982; Shen et al., 2007). Previously some researchers also suggested that cefadroxil monohydrate followed one compartmental model after oral administration (Pfeffer et al., 1977; Lode et al., 1979). Maximum plasma concentrations achieved were 12.5µg/ml for test and 12.47µg/ml for reference. These values were slightly lower than the values of Cmax reported by other researches (Kano et al., 2008; Mansour 2012). Kano et al. in 2008 reported Cmax as 16.04 µg/ml for test and 16.01 µg/ml for reference formulation (Kano et al., 2008). The mean time for the attainment of Cmax as reported in the present study was 1.5 hr and 1.54 hr for reference and test formulations. The values of Tmax observed were in close agreement of previous results reports by (Prenna and Ripa 1980) and shorter than another report (Otoom et al., 2004). The t1/2 (mean±SD) of cefadroxil was 1.72±0.3 hr and 1.99±0.538 hr for test and reference formulations. These values were near to the reports of (Barbhaiya 1996) and (Oliveira et al., 2000), whereas higher than the t1/2 reported by Prenna and Ripa (Prenna and Ripa 1980) and Otoom, et al. (Otoom et al., 2004). Otoom et al., in 2004 reported the t1/2 as 1.4 hrs for reference and 1.36 hrs for test (Otoom et al., 2004), while Olivera et al. in 2000 documented 1.8 hr for test and 1.9 hr for reference formulation analyzed (Olivera et al., 2000). All though most of the pharmacokinetic parameters of cefadroxil were similar to published data from other parts of the world, however some variations were observed that might be due to inter-subject and inter-racial variability.

CONCLUSION

In the current single-dose, two period, cross-over study in healthy fasted volunteers, the two formulations of cefadroxil capsules i.e. REF® 500mg and TEST 500mg were verified bioequivalent and can substitute each other. Maximum plasma concentration of cefadroxil observed in Pakistani population was lower than reported in some previous studies, while Tmax and half-life were near to values reported in literature

ACKNOWLEDGMENTS

The authors are very much grateful to the volunteers joined in the study.

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