Bioequivalence of Two Oral Extended Release Formulations of Ciprofloxacin Tablets in Healthy Male Volunteers under Fed and Fasting Conditions

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Abstract

The bioavailability of a single dose of ciprofloxacin 1000 mg Extended release (XR) tablets manufactured by a Jordanian manufacturer (Hikma PLC), was compared with a reference ciprofloxacin 1000 mg XR tablets (Ciprol® XR, Bayer-health care, Germany) in two different studies (under fasting and fed conditions). In each study, 28 healthy, male, Jordanian volunteers were enrolled. However, only 25 subjects in fasting study and 23 subjects in fed study completed the crossover. Each study was designed as single-center, open-label, single-dose, two-way crossover study. Nineteen blood samples were taken during 24hrs. Samples were frozen and kept until time of analysis. Ciprofloxacin concentrations in subjects’ plasma were determined by using a validated HPLC fluorescence technique. Confidence intervals (90%) for the peak plasma concentration (Cmax) and area under the concentration-time curve (AUC0-t) were determined by calculating log-transformed Test/Reference ratio using standard non-compartmental method and ANOVA statistics. The 90% CI result in fasting study for Cmax was 88.87 (82.17 - 96.10)% and for AUC0-t was 87.60 (80.38-95.46)%. In fed study the results were 102.09 (92.77-112.34)% and 104.06 (100.01-108.27)% for Cmax and AUC0-t, respectively. In conclusion, it is evident that the 90% CI for the primary pharmacokinetics parameters was within the bioequivalence acceptable boundaries of 80-125%, while for AUC0-t, and 75-133% for Cmax. Therefore, it was concluded that both products were bioequivalent.

Keywords: Ciprofloxacin; Extended Release; Pharmacokinetics; Bioavailability; Bioequivalence

Introduction

Ciprofloxacin is a quinoline carboxylic acid derivative with broad antibacterial activity against both gram-positive and gram-negative bacteria. Chemically it is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolin-carboxylic acid [3, 5, 11]. It was found to be more active against enterobacteriaceae than the older drugs of this class, such as nalidixic acid, with minimum inhibitory concentrations ranging from 0.008 to 2.0 mg/l [3, 4]. The adverse effects are also less likely [7]. Ciprofloxacin extended release is indicated only for the treatment of urinary tract infections, including acute uncomplicated pyelonephritis, caused by susceptible strains. Ciprofloxacin XR and ciprofloxacin immediate-release tablets are not interchangeable [6].

Once-daily ciprofloxacin XR was safe, effective, and noninferior to twice-daily ciprofloxacin immediate-release (IR) in the treatment of acute UTI. Additionally, ciprofloxacin XR was associated with significantly reduced frequencies of nausea and diarrhea. [8, 12, 15, 19, 14]. [18] showed that ciprofloxacin XR possessed larger AUCs than levofloxacin. Patients’ compliance improvement using ciprofloxacin XR and ciprofloxacin immediate-release tablets are not interchangeable [6].

Studies to establish bioequivalence (BE) between two products are important for certain changes before approval regulatory submissions. In BE studies, an applicant compares the systemic exposure profile of a test drug product to that of a reference drug product. For two orally administered drug products to be bioequivalent, the active drug ingredient or active moiety in the test product must exhibit equivalent rate and extent of absorption to that of the reference drug product. Product quality BE frequently rely on pharmacokinetics measures such as AUC and Cmax that are reflective of systemic exposure [9]. For modified-release products, the following studies are recommended: (1) a single-dose, nonreplicate, fasting study comparing the highest strength of the test and reference listed drug product and (2) a food-effect, nonreplicate study comparing the highest strength of the test and reference product. For immediate release products, a single-dose, fasting study is recommended. In addition, in vivo BE studies are to be accompanied by in vitro dissolution profiles on all strengths of each product.

Literature showed many bioequivalence studies for ciprofloxacin immediate release tablets [2, 1], while no bioequivalence studies were published on the 1000 mg XR formulation. The aim of this study was to determine bioequivalence of two XR tablet formulations: Ciprofloxacin...
Subjects and Methods

Subjects

Table 1 shows number and demographic data for subjects enrolled in the two studies, fast and fed. In either study, 28 subjects were enrolled, however, 3 in fasting and 5 in fed withdrew due to personal reasons or medical conditions before study drug administration. Consequently, a total of 25 and 23 subjects completed the crossover in fast and fed, respectively. All subjects were healthy, adults, male Jordanian volunteers. Clinical results of the screened laboratory examinations (biochemistry, serology, hematology and urine analysis) were within normal ranges. All subjects were informed about the objectives, drugs, potential risks, dates and activities during the clinical part of the study. A written consent form was signed by each subject. Any subject having drug allergy, alcohol abuse, GIT conditions that may have significantly affected drug absorption, etc., was excluded. The use of either prescription or OTC drugs was abstained 2 weeks before study time.

Both Fasting and fed studies were approved by The Institutional Review Board (IRB) of IPRC, Amman, Jordan, which operates in accordance with the principles and requirements described in Guidelines on Research Involving Human Subjects. The study protocols were reviewed by IRB of IPRC, approved and given the code Nos.CIP-T015 and CIP-T016 for fasting and fed studies respectively.

Study drugs

Two products of Ciprofloxacin 1000mg XR tablets were studied. The test product was Ciprofloxacin 1000mg XR tablets (Hikma-Pharma, Jordan, B# JES-P9, Exp date: 04/10). The reference was [6] tablets (Bayer Health care-Germany, after oral administration to healthy volunteers under both fed and fasting conditions.

Pharmacokinetic calculations

Pharmacokinetic parameters of ciprofloxacin were estimated using standard non-compartmental methods. The maximal plasma concentration (C_max) and the time to the peak plasma concentration (t_max) of ciprofloxacin were taken directly from the measured data. The area under the plasma concentration-time curve (AUC) was calculated from measured data points from time of administration to time of last quantifiable concentration (C_last) by the linear trapezoidal rule. The area under the plasma concentration-time curve extrapolated to infinity (AUC_{0-∞}) was calculated according to the following formula:

\[ AUC_{0-∞} = AUC_{0-t} + \frac{C_{last}}{\ln 2/t_{1/2}} \]

where C_last is the last quantifiable concentration and t_{1/2} is the elimination half-life of ciprofloxacin.

The method was validated according to the FDA current bioanalytical guidance. Accordingly the method validation was evaluated in terms of specificity, selectivity, linearity, sensitivity, inter- and intra-day accuracy and precision, recovery and stability under different conditions. Samples from 25 subjects in fasting study and 23 subjects in fed study were analyzed.

Methods

The method was designed as single dose, two-treatment, two-period, two-sequence crossover with a 7-days washout period for each study. Subjects were admitted the night before the study drug administration, supervised for at least 10 hours overnight fasting, and confined until collecting the 24 hour sample. On day 1 of each study period, each subject was given either one tablet of Ciprofloxacin 100mg XR tablet (test product) or Cipro XR 1000mg (Reference product) according to a randomization plan, along with 240ml of water. Diet consumption before and after drug administration are shown in Table 2.

The consumption of alcohols, methylxanthin-containing beverages (coffee, etc.) or grapefruits were prohibited enough time prior to drug administration. Physical activities were controlled throughout the study period. For blood samples collection, a cannula was inserted to each subject’s forearm vein and remain there until the 24-hour sample. Eight milliliters blood was collected each sample as follows: immediately before at 0.00 (predose) and at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 20.00 and 24.00 hours. Blood samples were collected into tubes containing heparin as an anticoagulant (Dispo™, AFMA, Jordan), slightly shaken and centrifuged (at approximately 3500 rpm) for 10 minutes, transferred immediately to plastic tube (Dispo™, AFMA, Jordan) and stored at -20°C. The total amount of blood loss during the whole study did not exceed 317.5ml.

Ciprofloxacin concentrations in human plasma were determined by a specific high performance liquid chromatography (HPLC) with fluorescence detection method. The technique was developed at IPRC laboratories. After spiking 200 µl of plasma samples with levofloxacin as internal standard, a protein precipitation technique using 1 ml methanol was employed to extract both analytes. Supernatants then were centrifuged, evaporated to dryness under nitrogen gentle stream and reconstituted with 800 µl mobile phase. Only 20 µl were injected into a precapped C18, 5 µm (3.9x150 mm) reversed phase analytical column. Mobile phase was composed of 7% glacial acetic acid, 5% acetonitrile and 88% (0.025 M sodium acetate trihydrate), with flow rate at 1.50 ml/min. Separations were monitored at 280 and 440 nm as excitation and emission wavelengths, respectively. Analysis was accomplished at column oven temperature 25°C. Chromatography separation and drug determination was accomplished by using Shimadzu (Japan) HPLC composed of LC-10AD vp pump, RF-10A XL fluorescence detector and SCL-10A vp system controller.

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\[ AUC_{0-∞} = AUC_{0-t} + \frac{C_{last}}{\ln 2/t_{1/2}} \]

where C_last is the last quantifiable concentration. The ratio AUC_{0-∞} / AUC_{cmax} as a percent was determined as an indicator for the adequacy of sampling time. The elimination
half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/(-b)$, where $b$ was obtained as the slope of the linear regression of the ln-transformed plasma concentrations versus time in the terminal period of the plasma curve. The pharmacokinetic calculations were performed on a Pentium MMX MHz Computer using the computer program Kinetica™ 2000.

**Statistical analysis**

Statistical analysis was performed by using the Kinentica™ 2000 program, with the aid of Microsoft® Excel (2002). The extent of absorption is determined by $AUC_{0-t}$ and $AUC_{0-\infty}$. The rate of absorption is determined by $C_{peak}$. For the parametric analysis of bioequivalence for ln-transformed data, the acceptance boundaries were set at 80.00-125.00% for both $AUC_{0-t}$ and $C_{peak}$. A multiplicative model with respect to the untransformed bioequivalence parameters was selected. A logarithmic transformation of the original data was used. Under the assumption of a logarithmic normal distribution, a parametric approach was used by [16] based on the inclusion of the shortest 90% confidence interval in the bioequivalence range. An analysis of variance (ANOVA) was performed on $AUC_{0-t}$, $AUC_{0-\infty}$, $t_{max}$, $t_{1/2}$, $K_{e}$, $Ln\ AUC_{0-t}$, $Ln\ AUC_{0-\infty}$, and $Ln\ C_{max}$.

A multiplicative linear model was used for the two-way crossover design: $Y_{ijk} = \log (X_{ijk}) = \mu + G_k + S_{ik} + P_j + F(j, k) + e_{ijk}$, Where, $Y_{ijk}$ is a pharmacokinetic parameter of the $i$th subject ($i = 1, 2, \ldots, n_k$) in the $k$th sequence, which is administered at the $j$th period; $G_k$ is the fixed effect of the $k$th sequence; $S_{ik}$ is the random effect of the $i$th subject in the $k$th sequence; $P_j$ is the fixed effect of the $j$th period; $F(j, k)$ is the fixed effect of the formulation in the $j$th period (and, $e_{ijk}$ is the (within subject) random error in observing $Y_{ijk}$). It was assumed that $S_{ik}$ and $e_{ijk}$ are mutually independent and normally distributed with mean zero and variances $\sigma_s^2$ and $\sigma_e^2$.

**Results**

The described analytical method was proved selective and specific. Retention times were 7.4 and 8.8 min for the internal standard and drug, respectively. No interferences were observed. Concomitant drugs do not interfere with ciprofloxacin or internal standard analysis. The method was proved sensitive and accurate for the determination of ciprofloxacin in human plasma. Under the described conditions, the limit of quantitation for ciprofloxacin was 50 ng/ml with 99.60% accuracy and 15.06% CV. The method was found linear within the range 50 - 5000 ng/ml, with accuracy ranging 99.15 - 100.70% and precision 0.98 - 15.06%. Correlations coefficients were 0.99. Intraday accuracy of ciprofloxacin method ranged from 94.93 to 97.80%, while precision ranged from 1.24 to 5.73%. Interday accuracy ranged from 95.00 to 99.31%, while the interday precision ranged from 4.07 to 7.58%. Mean recovery was proved 86.57% with 7.74% CV. Ciprofloxacin long-term stability was tested and was found stable for 90 days at -20°C (99.59% with 1.09% CV).

Drug plasma levels were designated as surrogate parameters to indicate clinical activity. Primary pharmacokinetic parameters were set to be $C_{peak}$ and $AUC_{0-t}$ were also considered to be the bioequivalence determinants. Finally, $K_{e}$, $AUC_{0-\infty}$, $t_{max}$, $AUC_{0-t}/AUC_{0-\infty}$ and $t_{1/2}$ were set as the secondary pharmacokinetic parameters. The detailed results for fasting and fed studies are shown in Tables 3-6, respectively. Bioequivalence could be demonstrated for Ciprofloxacin within the prescribed 90% confidence interval of 80.00-125.00% for $AUC_{0-t}$ and $C_{peak}$ with respect to the parametric method on log-transformed data. The results are shown in Figure 2 and 3 for fed and fasting studies, respectively.

**Discussion**

Assessment of bioequivalence of generic product to reference product is required to exclude any clinically important differences in the rate or extent at which the active entity of the drugs becomes available at the site of action. Two drug products are considered to...
be bioequivalent if they are pharmaceutically equivalent and their bioavailability is so similar that they are unlikely to produce clinically relevant differences in regard to safety and efficacy [9]. Food has been shown to alter the bioavailability of some drugs which can have negative impact on the interpretation of bioequivalence results between test and reference products [13].

In this study, the effect of food on the bioavailability of ciprofloxacin is somewhat more noticeable for reference product (Cipro® XR) than test product. This might be due to the difference in the formulation composition. That is, the test product formula contains Ciprofloxacin HCl only, while that of the reference product contains a combination of ciprofloxacin base and HCl. Thus test product will be less affected by change effect due to food. The AUC values in Table 3 and 4 shown to be higher under fasting than those under fed conditions. This effect can also be noticed if Figures 1 and 2 are compared. Furthermore, the values of Cmax is shown to be higher in fasting than in fed conditions which reflects effect of food on the rate of drug absorption.

The results of this bioequivalence study showed the equivalence of the two studied products in terms of the rate of absorption as indicated by Cmax and in terms of the extent of absorption as indicated by AUC0-t and AUC0-∞. The parametric 90% confidence intervals of the mean values for the Test/Reference ratio were in each case well within the bioequivalence acceptable boundaries of 80.00-125.00% for AUC0-t and Cmax. ANOVA analysis on the log-transformed data, C max, AUC0-t, AUC0-∞, t1/2e, and tmax showed that sequence effect for all these parameters did not significantly influence the outcome of the study. The mean plasma curves of both products are almost superimposable suggesting that not only Cmax and AUC0-t, but also the time course of plasma levels over the whole sampling period are identical.

Conclusions

In conclusion, pharmacokinetic parameters, namely, Cmax, AUC0-t, and AUC0-∞...
and $AUC_{0-\infty}$ of the two ciprofloxacin 1000mg extended release products showed comparable values indicating that they are bioequivalent.

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