Pharmacokinetics and Bioequivalence Evaluation of Two Brands of Ciprofloxacin 500 mg Tablets in Iranian Healthy Volunteers

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Key words
- bioequivalence
- ciprofloxacin
- pharmacokinetics
- HPLC

Abstract

Background: In the present study pharmacokinetics and bioequivalence of 2 brands of ciprofloxacin 500 mg were evaluated in 24 healthy male volunteers after a single dose oral administration in an open, randomized, 2-way crossover study.

Methods: Blood samples were taken before and within 12 h after the administration of the drug. Plasma concentrations of ciprofloxacin were determined by a simple HPLC method with ultraviolet detection. The used method was validated for specificity, accuracy, precision and sensitivity. The mobile phase consisted of 0.025 M phosphoric acid, acetonitrile, and triethylamine. Analytical column was 5 µm Eurosphere C8 with a Eurosphere C8 guard column. The detector wavelength was set at 278 nm and the retention time was 10 min. The pharmacokinetic parameters, including peak plasma concentrations and time needed to reach the peak were obtained directly from plasma concentration–time profiles. The area under the curve was calculated using non-compartmental methods.

Results: The C_{max} of 1476.8 ± 319.9 ng/mL and 1 423.0 ± 278.4 ng/mL were attained in about 1.67 and 1.58 h for test and reference formulations, respectively. The mean ± SD values for $AUC_{0-\infty}$ were 9 665.3 ± 2 880.2 and 9 716.1 ± 2 572.1 ng.hr/mL for test and reference formulations, respectively. The pharmacokinetics parameters AUC0-t, $AUC_{0-\infty}$ and Cmax were calculated for bioequivalence after log-transformation of data. The 90% confidence intervals of test/reference for AUC0-t, $AUC_{0-\infty}$ and C_{max} were (95.6–109.9%), (91.8–106.3%) and (95.2–112.8%), respectively and all were within the bioequivalence acceptance range of 80–125%.

Conclusion: These results indicate that 2 tested formulations are bioequivalent and thus could be prescribed interchangeably.

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Introduction

Fluoroquinolones are broad spectrum antibacterial agents and are effective against most Gram-negative and Gram-positive aerobic bacteria. They also have some activity against mycobacteria, mycoplasmas, rickettsias and the protozoa plasmodium falciparom. Ciprofloxacin (CPR, CAS Number: 85721-33-1) is a potent fluoroquinolone of the second-generation group of nalidixic acid derivatives, first commercially introduced in the 1980 s. CPR is one of the 4-quinolone antimicrobial agents highly active against a broad spectrum of microbial pathogens and is used in a wide range of gastrointestinal, urinary and respiratory infections [1–7]. Following oral dosing in human, CPR is rapidly absorbed from gastrointestinal tract into the systemic circulation and reaches the maximal concentration in 1–2 h. The bioavailability of ciprofloxacin is between 70% and 80%. Concomitant administration of the oral tablet with food has been reported to result in a prolonged time to reach peak serum levels, although overall bioavailability does not appear to be significantly altered [8–12]. About 65% of unchanged ciprofloxacin and 10–15% of metabolites are excreted in the urine and about 15% in feces [13, 14]. Absorption and disposition kinetics studies are important to compare the rate and extent of systemic absorption of a drug manufactured by different manufacturers. Variations in excipients and manufacturing process can affect the disintegration and dissolution rate of tablets given through the oral route. The bioequivalence of 2 formulations of the same drug is concluded based on the lack of difference in the rate and extent of absorption (AUC) in drug formulations. In the present study the bioequiva-

lence of 2 CPR tablets was evaluated by comparing the pharmacokinetic parameters derived from serum CPR concentration–time profiles.

Materials and Methods

Materials

All chemicals (phosphoric acid, trichloroacetic acid, acetonitrile, and triethylamine) were HPLC grade and were purchased from Merck Company (Darmstadt, Germany). The test formulation was ciprofloxacin HCL 500 mg tablet, Ciproxir®, manufactured by EXIR, Boroujerd, Iran with a batch number of 0030409 (Expiration Date: 04-2011), and the reference formulation, Ciproxin® 500 mg was from Bayer Schering Pharma with a batch number of BXF6×41 (Expiration Date: 02-2013).

Methods

Study Design

24 Iranian healthy male volunteers aged between 20 and 34 years (22.7±3.6 years) and weighed from 51 to 93 kg (70.7±10.3 kg) were enrolled in this study after providing written informed consent. The volunteers were examined and assessed for their eligibility to participate in the present study. The examinations and tests included medical history, physical examination, and measurement of weight, height and vital signs (heart rate and blood pressure). This study was a single-dose, randomized, open label, crossover. The 2 phases of study were separated by a 1 week washout period. The washout period was determined based on 5–7 times of the elimination half life ($T_{1/2}$) of CPR. After an overnight fast for 12 h, the volunteers received 500 mg of either formulation of CPR, taken with 200 mL of water. 5 mL of blood samples were obtained just before drug administration and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hr after that. The plasma was separated by centrifugation at 5000 g for 5 min at room temperature (20 °C), followed by direct transfer into polypropylene tubes and storage at −20 °C until analysis. This study was conducted in accordance with the ethical standards for studies in humans of the Declaration of Helsinki and its amendments and approved by the local ethical committee of Tabriz University of Medical Sciences (5/4/4202, July 2010).

HPLC method

In the present study plasma concentrations of ciprofloxacin were analyzed using a sensitive and selective HPLC method with ultraviolet detector. The HPLC instrumentation was manufactured by Knauer, Germany. 1 mL of sample was first deproteinized with an aqueous solution of trichloroacetic acid (10% v/v). The mixture was vortexed for 10 s and centrifuged for 15 min. 20 µL of clear supernatant was injected onto the HPLC column. Short-term stability studies showed that CPR is stable in acidic media at least for 12 h at room temperature. The mobile phase consisted of 0.025 M phosphoric acid (pH=3), acetonitrile, and triethylamine (88:12:0.1, v/v). Analytical column used for chromatographic separations was 5 µm Eurosphere C8 (150×4.5 mm) with a Eurosphere C8 (5 µm, 4.6×10 mm) guard column. The flow rate was 1 ml/min and the detector wavelength was set at 278 nm. Under these conditions the retention time for ciprofloxacin was 10 min. The method used was validated for specificity, accuracy, precision and sensitivity. Validation parameters were according to the recommendation of the guidance CDER and ICH guidelines [15–18]. The accuracy was determined as a relative error (%) calculated from the following equation: accuracy (%)=100 ($C_{added} - C_{measured}$)/C_{added} which was calculated from the calibration curve equation. The range of the usability of HPLC method was limited within the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ) [3,15,17–19]. The lower limit of quantification was determined as the lowest concentration on the standard calibration curve which was measured with a precision of 20% and accuracy of ±20%. The linearity of the peak height against standard concentration was verified using linear regression. The specificity of the method was verified using different blank plasma samples obtained from healthy human volunteers before drug administration. The precision, as the measure of intra-day repeatability, was expressed as the coefficient of variance (CV %) of 6 identically prepared and measured calibration samples during 1 day measurement series. Inter-day precision (reproducibility) was performed as a CV (%) of 6 consecutive days' measurements of plasma samples [15,20–22]. All plasma samples of a given volunteer collected in the 2 treatment periods were measured in the same chromatographic run (analytical own control). Each run had a separate daily calibration. Runs contained quality control samples (QC) at 6 concentration levels. There was no reason for re-analysis of study samples in the present research work. However, since the use of calibration standards and QC samples during validation may not mimic the actual study samples, accuracy of incurred samples by reanalysis of study samples in separate runs at different days were measured. Almost 10% of samples (preferably samples around C_{max} and in the elimination phase) were reanalyzed. For more than 80% of repeats the concentration obtained for the initial analysis and the concentration obtained by re-analysis were within 20% of their mean. The pharmacokinetic parameters for test and reference formulations were evaluated. The C_{max} and the corresponding time of peak plasma concentration (T_{max}) were taken directly from the individual plasma data. The elimination rate constant (k_e) was estimated as the slope of the semi logarithmic plot of the 3–4 last points of the plasma concentration vs. time curve. The area under the plasma concentration vs. time curve, AUC_{0-t}, was calculated by linear trapezoidal method. $T_{1/2}$ was calculated as ln2/k_e. For the purpose of bioequivalence analysis AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were considered as the primary variables. The values of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were analyzed statistically using an analysis of variance (ANOVA), which distinguishes effects due to product, group and periods. SPSS version 17.0 (SPSS Inc., Ehningen, Germany) was used for statistical data analysis. Parametric 90% confidence intervals (CI) based on the ANOVA of the mean test/reference (T/R) ratios of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were computed [1,13,16–18,23].

Results and Discussion

Both formulations were readily absorbed from the gastrointestinal tract and ciprofloxacin was measurable at the first sampling time (0.5 h) in almost all the volunteers. Plots of the mean of CPR serum concentration–time for the 2 formulations over the 12 h sampling period are shown in **Fig. 1**.

Different methods for the determination of CPR have been proposed such as spectrophotometry, fluorophotometry, capillary electrophoresis, solid phase spectrofluorometry, differential electrolytic potentiometric, enzymatic rotating biosensor, high performance capillary electrophoresis, reversed phase ion pair-

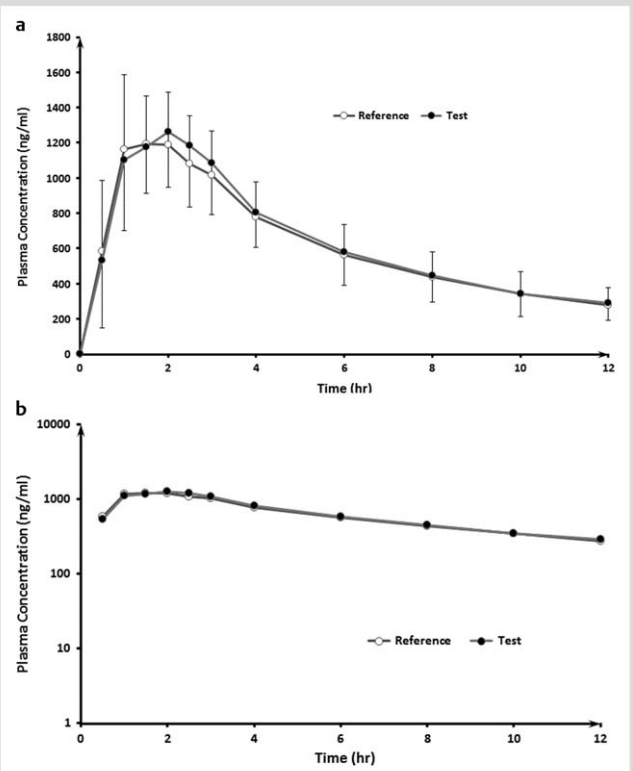

Fig1 Mean Plasma concentration-time curves of ciprofloxacin after oral administration of reference and test formulations in 24 healthy volunteers on the linear scale (top) and log-linear scale (down).

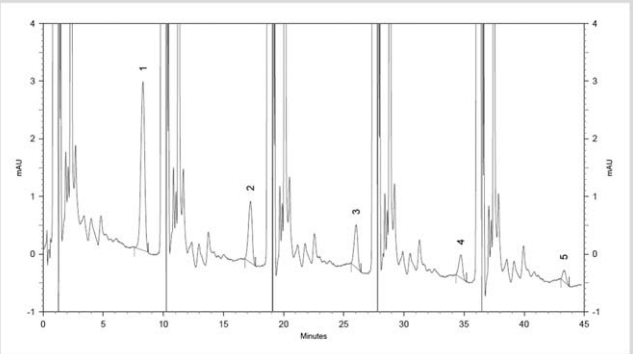

Fig. 2 A typical chromatogram obtained from standard ciprofloxacin samples.

high performance liquid chromatography, differential pulse polarography, cathodic stripping voltammetry, High performance reversed phase chromatography (HPLC) with fluorescence detector and more recently tandem mass spectrometric [1,3,4,11,23–26]. Plasma CPR concentrations were determined using a validated HPLC procedure with UV detection. Peak heights were used for sample quantitation. Linear regression of the peak height ratios vs. the drug concentration was performed on the standard curve to determine the slope, intercept, and strength of the correlation. The used method was linear in the concentration range of 100–2 000 ng/ml, with a correlation coefficient (r^2) of 0.9963 (n = 5). **Fig. 2** shows a typical chromatogram obtained from the standard ciprofloxacin samples.

Lower Limit of quantification was 50 ng/ml with inter-day and intra-day precision and accuracy of 10.78 and 12.33 ng/ml respectively. Limit of detection was found to be 15 ng/ml. The respective accuracy was 96.3 and 97.7 %. Intra-day and inter-day precision and accuracy of this method for 6 quality control samples (100, 200, 250, 400, 500 and 1 000 ng/ml) were shown in **Table 1**. The quality control samples were prepared by adding the known amounts of drug in blank plasma to make concentrations in standard curve range and were used to accept or reject the run. Sample analysis was performed 4 times for each intra-day and inter-day precision and accuracy. These results show that an adequate accuracy and precision were obtained for the determination of CPR plasma concentration by this method.

Bioequivalence is a comparison of the bioavailability of 2 or more drug products. The 2 products or formulations containing the same active ingredient are bioequivalent if their rates and extents of absorption do not show a significant difference. The mean plasma profiles of Ciproxir® and the reference formulation were visually very similar in shape and pattern (**Fig. 1**). The parameters $AUC_{0-\infty}$ and T_{max} were related to the extent and rate of drug absorption respectively, while C_{max} was related to both of these processes (**Table 2**) [1, 13, 15, 17, 22, 23, 25]. After oral administration of CPR, the mean C_{max} of 1 476.8 ± 319.9 ng/mL and 1 423.0 ± 278.4 ng/mL were attained in about 1.67and 1.58 h for both test and reference formulations respectively. In the present study the mean values of AUC_{0-t} were 7 616.8 ± 1 688.7 and 7 438.5 ± 1 636.6 ng.hr/mL and $AUC_{0-\infty}$ values were 9 665.3 ± 2 880.2 and 9 716.1 ± 2 572.1 ng.hr/mL for test and reference CPR formulations respectively.

The 90 % confidence intervals for the log transformed data were also calculated and the results are shown in **Table 3**. It is generally accepted that for basic pharmacokinetic characteristics, such as AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}, the standard 90 % Confident Interval (CI) range is 0.8–1.25.The CI obtained in this study were 95.6–109.9, 91.8–106.3 %, and 95.2–112.8 % for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} respectively. After log-transformation of the data, the result of the ANOVA test for these parameters showed no statistically significant difference between the 2 formulations either in periods, group, or product. Therefore, the CI for the main parameters reflecting the extent and rate of absorption are within the acceptance range, confirming the bioequivalence of both brands of CPR.

Conclusion

The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When 2 formulations of the same drug are equivalent in the rate and extent to which the active drug ingredient is absorbed, and becomes equally available at the site of drug action, they are bioequivalent and thus are assumed to be therapeutically equivalent. Statistical comparison of the critical bioequivalence parameters included AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} clearly indicated no significant difference between test and reference, 500 mg formulations. The 90 % CI for the ratios of mean AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} indicated that these values are entirely within the bioequivalence acceptance range of 80–125 %. Consequently both test and reference formulations are therapeutically bioequivalent and interchangeable and therefore can be considered equally effective in medical practice.

Table 1 Intra-day and Inter-day precision and accuracy obtained from 6 levels of QC samples.

Added concentration (ng/ml)	Intra-day				Inter-day			
	Mean (ng/ml)	SD (ng/ml)	CV (%)	Accuracy (%)	Mean (ng/ml)	SD (ng/ml)	CV (%)	Accuracy (%)
100	100.24	8.13	8.11	100.2	96.70	8.31	8.59	96.7
200	199.75	4.27	2.14	99.9	198.75	3.30	1.66	99.4
250	243.97	8.12	3.33	97.6	249.68	18.68	7.48	99.9
400	398.75	7.13	1.79	99.68	401.25	5.56	1.39	100.3
500	514.56	22.76	4.42	102.9	502.91	28.82	5.73	100.6
1000	1010.02	56.13	5.56	101.0	1006.97	73.43	7.29	100.7

Table 2 Pharmacokinetic parameters for the test and reference preparations (500 mg of ciprofloxacin) after oral administration in 24 healthy volunteers.

Parameter	Test			Reference		
	Arithmetic Mean	SD	Interindividual CV (%)	Arithmetic Mean	SD	Interindividual CV (%)
$AUC_{0-12\ (ng.hr/mL)}$	7616.8	1688.7	22.2	7438.5	1636.6	22.0
$AUC_{0-\infty\ (ng.hr/mL)}$	9665.3	2880.2	29.8	9716.1	2572.1	26.5
C_{max} (ng/mL)	1476.8	319.9	21.7	1423.0	278.4	19.6
T_{max} (hr)	1.67	0.62	37.18	1.58	0.52	33.14
$T_{½}$ (hr)	5.04	1.19	23.62	5.46	1.57	28.75

Table 3 The 90 % CI for the ratios test/reference of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values.

Pharmacokinetic parameter	ANOVA (P-value) Variation source			C.I. 90 % for the ratios
	Product	Group	Period	
C_{max}	0.4814	0.2887	0.3794	95.2–112.8
AUC_{0-t}	0.5493	0.0755	0.2452	95.6–109.9
$AUC_{0-\infty}$	0.7823	0.2727	0.06056	91.8–106.3

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Conflicts of Interest

The authors indicate that they have no conflicts of interest in this report.

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