Bioequivalence evaluation of two different formulations of ciprofloxacin tablets in healthy volunteers

Hassan Y, Alfadly S O, Azmin M N, Peh K K, Tan T F Y, Noorizan A A, Ismail O

ABSTRACT

Introduction: A bioequivalence study of two oral formulations of 500 mg tablets of ciprofloxacin (RAZA Pharmaniaga, Malaysia) as test and Ciprobay (Bayer AG, Germany) as reference, was carried out in 24 healthy human volunteers. Each volunteer received a single dose of ciprofloxacin.

Methods: The study method used was a double-blind, two-period, two-treatment, two-sequence, and crossover randomised design. Blood samples were taken before, and within 24 hours after drug administration. Plasma concentrations of ciprofloxacin were determined by a high-performance liquid chromatographic method with ultraviolet detection. The pharmacokinetic parameters, C_{max} and T_{max}, were obtained directly from plasma data, k_e was estimated by log-linear regression, and the area under the curve (AUC) was calculated by the linear trapezoidal rule. The parameters, $AUC_{0-\infty}$ and C_{max}, were tested for bioequivalence after log-transformation of data, while the differences of T_{max} were evaluated nonparametrically.

Results: When $AUC_{0-\infty}$ and C_{max} were analysed using analysis of variance, no statistically significant difference was observed between the two different formulations. The 90 percent confidence intervals of the mean values for the test/reference ratios were 0.95-1.07 for $AUC_{0-\infty}$ and 0.90-1.07 for C_{max}, respectively. Both of these values were within the bioequivalence acceptance range of 0.80-1.25.

Conclusion: We found that both formulations are bioequivalent and, therefore, interchangeable.

Keywords: bioequivalence, ciprofloxacin, healthy volunteers, high-performance liquid chromatographic analysis, pharmacokinetics

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Centre for Bioequivalence Study, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia

Hassan Y, BPharm, PharmD
Associate Professor in Clinical Pharmacy

Clinical Pharmacy Programme

Alfadly S O, MPharm
PhD Candidate

Noorizan AA, MSc, PharmD
Associate Professor

Department of Pharmaceutical Technology

Azmin MN, BPharm, PhD
Associate Professor

Peh KK, BPharm, PhD
Associate Professor

Tan TFY, BPharm, MSc, PhD
Associate Professor

Department of Cardiology, Penang Hospital, Jalan Utama 10450, Penang, Malaysia

Ismail O, MD, FACC
Consultant and Head

Correspondence to:
Dr Yahaya Hassan
Tel: (60) 1 9551 5611
Fax: (60) 657 0017
Email: yahaya@usm.my

INTRODUCTION

Bioequivalence of different preparations containing the same active ingredient has gained considerable importance over the last few years because of increasing generic substitution.[1] Ciprofloxacin is a relatively new broad-spectrum fluoroquinolone antibacterial agent active against a wide range of aerobic gram-positive and gram-negative bacteria. The primary mechanism of ciprofloxacin, like other fluoroquinolones, is inhibition of bacterial DNA gyrase (a type 11 topoisomerase), which disrupts DNA replication.[2] Its chemical name is 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid.[3] Ciprofloxacin is a relatively small molecular weight Zwitterion molecule with pKa of 6.0 and 8.8; the isoelectric point is 7.4.[4] Ciprofloxacin is very active in vitro against most gram-negative bacteria, including *Escherichia coli*, *Salmonella* species (spp.), *Neisseria* spp., *Moraxella catarallis* and *Haemophillus* spp., and gram-positive bacteria, such as *Staphylococcus aureus*, *Staphylococcus epidermis* and most strains of the Streptococcus spp. However, as with other flouroquinolones, ciprofloxacin has little activity against anaerobes. Ciprofloxacin is a good alternative drug in the treatment of lower respiratory tract infections, complicated urinary tract infections, skin, bone and gastrointestinal infections, as well as sexually transmitted diseases.[5]

In comparison with nonfluorinated quinolones, ciprofloxacin has a good oral absorption and a better bioavailability. It has an approximate bioavailability of 70% after oral administration.[4] Maximum plasma concentrations between 0.8 and 3.9 μg/ml are achieved at 1–2 hours after oral administration of single 250–750 mg doses. The drug has a large apparent volume of distribution (2.1–5 L/kg) and is concentrated in many body tissues and fluids, including bile, kidney, liver, gallbladder, prostate and lung tissue.[2,5,6] Ciprofloxacin is largely excreted unmetabolised in the urine and faeces, although small amounts of metabolites have

been detected. Intestinal excretion appears to be the predominant route of gastrointestinal elimination, but bile excretion also occurs.[7] The elimination half-life is approximately 3–5 hours.[8] The aim of this study was to compare the rate and extent of absorption of a generic product, the Ciprofloxacin tablet (Ciprofloxacin HCL 500 mg, Pharmaniaga Manufacturing Bhd, Kuala Lampur, Malaysia) with that of a reference product (Ciprobay®, Bayer AG, Germany), in a single-dose, two-treatment, two-sequence, crossover randomised study in 24 healthy male volunteers.

METHODS

The test product used was Ciprofloxacin HCL 500 mg tablet manufactured by Pharmaniaga, Malaysia with a batch number of FT008, and the reference product was Ciprobay® 500 mg tablet manufactured by Bayer AG, Germany with a batch number of CDBFU1. 24 healthy adult male volunteers with a mean age of 22.8 (± 3.7) years , a mean body weight of 63.8 (± 9.5) kg and a mean height of 169.9 (± 5.8) cm, participated in a single-dose, randomised, fasting, two-period, two-treatment, two-sequence crossover, double-blind study after providing written informed consent. Prior to the study, the volunteers were briefed on the nature, purpose, duration and risk of the study. They were informed that they could withdraw from the study at any time. No consumption of alcohol was permitted for the subjects 48 hours prior to the drug administration and until the end of the 24-hour sample period. Similarly, beverages and food containing caffeine were not permitted throughout the study. The volunteers were instructed to abstain from taking any medication two weeks before, and over the entire course of the study period. The study protocol was approved by the Ethics Committee of the Joint Penang Hospital/School of Pharmaceutical Sciences, Universiti Sains Malaysia Committee on Bioavailability Studies.

The volunteers were hospitalised at 7.00 pm and had standard dinner fare in the hospital. On the morning of phase one, each volunteer of group one received one 500 mg tablet of Ciprobay® and those of group two received one 500 mg tablet of Ciprofloxacin. After a washout period of two weeks, the two groups received the alternate product. The products were administered at 10.00 am after an overnight fast with 150 ml of water. Food was withheld until four hours after the dose administration. Lunch and dinner were served at four and ten hours, respectively, after dosing. Venous blood samples of 5 ml each were collected via an indwelling cannula placed on the forearm into Vacutainer™ tubes (containing sodium heparin) at preset time intervals of 0 (predose), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.5, 6.0, 8.0, 12.0, 18.0, and 24.0 hours after dosing. The blood samples were centrifuged at 3,500 rpm for 15 minutes and the plasma samples were transferred to Vacutainer™ tubes (no additive) and stored frozen at –20° C until further analysis. The volunteers were examined and assessed for their ability to participate thereafter. The examinations and tests performed included medical history, physical examination, measurement of height, weight and vital signs (heart rate, systolic and diastolic blood pressure, and body temperature), renal and liver function tests and electrocardiogram. The volunteers were informed to report any abnormalities during and after the study. The results were documented in the individual case report forms. No drug-related adverse drug reaction was found in the study.

Table I. Demographical data.

n = 24	Age (years)	Weight (kg)	Height (cm)
Mean	22.88	63.83	169.88
SD	3.66	9.50	5.78
Min	20	50	157
Max	39	79	178

Plasma concentrations of ciprofloxacin were analysed using a sensitive and selective high-performance liquid chromatographic (HPLC) method. The HPLC system consisted of a pump (Gilson 307, USA) with model 10 WSC washable pump head equipped with sample injector port (Gilson 7725i) fitted with 100 μL sample loop and a programmable UV/Vis detector (model 151) with wavelength range of 190–700 nm. The detector was operated at a wavelength of 278 nm. The HPLC system control and data analysis were done by using Gilson 712 HPLC Controller Software and Gilson 506C system Interface Module. The chromatographic separation was performed on a reversed phase C18 analytical column; 150 × 4.6 i.d; 5μm particle size (Phenomenex, CA, USA). The mobile phase consisted of a mixture of 0.05 M potassium dihydrogen phosphate, water, acetonitrile, and tetra-n-butyl ammonium bromide in the proportion of 60, 27, 8, and 5 (v/v). The pH of phosphate buffer and tetra-n-butyl ammonium bromide was adjusted to 3.00 using 1M phosphoric acid. The mobile phase was eluted at a flow rate of 1.2 ml/min at room temperature. The method was validated by following international guidelines.[9] The samples were quantified using peak area ratio of ciprofloxacin over the internal standard norfloxacin. Each analysis run required a maximum of 12.5 minutes. The relationship between concentration and peak area ratio was found to be linear within the range of 0.05 to 5.5 μg/ml, with a limit of quantitation of 0.05 μg/ml.The precision was expressed as the percentage of coefficient of variation. The intraday precision of the method was less than 6% and the accuracy (% error) value was below 7.7%. The interday precision and accuracy values were less than 7.5% and 7.3%, respectively. The absolute

recovery for ciprofloxacin was about 93% for the three concentrations of 0.05, 0.5 and 2.5 μg/ml. The stability study showed that ciprofloxacin was stable in plasma for six weeks when stored at –20°C. 0.5 ml of plasma samples in screw-capped glass tubes were spiked with 15 μL of internal standard norfloxacin solution, (57.5 μg/ml) and vortexed for 15 seconds. To each tube, 50 μL acetonitrile and 25 μL perchloric acid (60%) were added and vortexed for one minute to precipitate the protein. 500 μL chloroform was added to each tube and vortexed for another one minute to extract less polar materials from the aqueous phase. The tubes were centrifuged at 4,000 rpm for ten minutes. The supernatants were transferred into clean 1.5 ml Eppendorf tubes. 100 μL of each supernatant was injected directly onto the HPLC column.

Three pharmacokinetic parameters, namely, maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}) and total area under the plasma concentration-time curve ($AUC_{0-\infty}$), were obtained from plasma concentration versus time profiles. The values of C_{max} and T_{max} were obtained directly from the plasma levels. The elimination rate constant k_e was obtained as the slope of the linear regression of the log-transformed plasma concentration values versus time data in the terminal phase.[10] The terminal half-life ($t_{½}$) was estimated as $0.693/k_e$. The $AUC_{0-\infty}$ was the summation of AUC_{0-t} (the area from time zero to the last sampling time t) and $AUC_{t-\infty}$ (the area from time t to infinity). AUC_{0-t} was calculated using the linear trapezoidal rule and $AUC_{t-\infty}$ was determined by dividing the last measurable plasma drug concentration with elimination rate constant k_e. In all cases, the $AUC_{t-\infty}$ was less than 20% of the $AUC_{0-\infty}$. For the purpose of bioequivalence analysis, $AUC_{0-\infty}$ and C_{max} were considered as the primary variables. The values of $AUC_{0-\infty}$, C_{max}, k_e and $t_{½}$ were analysed statistically using an analysis

Table II. Pharmacokinetic parameters of ciprofloxacin 500 mg tablets.

Volunteer	Ciprobay®				Ciprofloxacin			
	$AUC_{0-\infty}$ (h.µg/ml)	C_{max} (µg/m)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0-\infty}$ (h.µg/ml)	C_{max} (µg/ml)	T_{max} (h)	$t_{½}$ (h)
1	3.44	0.93	1	6.42	4.28	0.88	1	5.68
2	6.03	1.34	1	4.3	4.98	0.91	1	5.25
3	4.91	1.05	0.5	4.08	5	1.41	0.5	3.26
4	7.85	1.72	1	2.42	7.89	1.82	1	3.15
5	5.83	1.62	1	5.7	5.52	1.07	2	3.15
6	6.62	0.87	3.5	5.04	7.15	1.32	1.5	6.25
7	3.75	1.3	1	1.86	3.52	1.12	0.5	2.01
8	3.38	0.91	0.75	2.16	4.41	1.38	0.5	2.26
9	8.19	1.62	1.5	6.72	7.21	1.49	0.75	7.42
10	9.15	1.41	1.5	4.54	7.57	1.95	1	5.3
11	8.91	2.01	0.75	3.28	7.9	1.73	0.75	5.03
12	3.46	1.14	1	1.69	4.16	0.98	1.5	1.35
13	5.8	2.08	1	1.68	5.73	1.47	1.5	1.16
14	4.76	1.35	0.75	2.52	6.52	1.47	0.75	3.09
15	4.5	2.37	0.5	1.94	4.42	1.84	1	2.02
16	6.95	2.21	1	1.99	7.97	2.04	1	0.93
17	6.11	1.82	0.75	3.7	5.9	1.98	1	1.94
18	4.8	1.78	1	1.71	4.7	1.72	0.75	2.38
19	6.38	1.24	1.5	3.47	8.5	1.35	1.5	4.46
20	4.69	0.73	1	2.52	3.21	0.76	2.5	2.32
21	3.9	1	1.5	3.12	3.51	0.99	1.5	3.03
22	5.45	1.19	0.5	6.18	5.67	1.56	0.75	5.11
23	3.99	0.95	1.5	3.42	4.31	1.04	1.5	1.88
24	4.92	1.81	0.5	2.28	4.29	1.09	1	2.3
Mean	5.57	1.44	1.08	3.45	5.59	1.39	1.11	3.36
SD	1.70	0.46	0.61	1.60	1.62	0.38	0.49	1.77

$AUC_{0-\infty}$: total area under the plasma concentration-time curve; C_{max}: maximum plasma concentration; $t_{½}$: elimination half-life; t_{max}: time to reach C_{max}.

Table III. 90% confidence interval for the ratios test/reference of AUC and C_{max} values (log-transformed).

Parameter	90% confidence interval	Point estimate
$AUC_{0-\infty}$	0.95–1.07	1.02
C_{max}	0.90–1.07	1.05

of variance (ANOVA), which distinguishes effects due to subjects, sequence, periods and treatment.[11] The C_{max} and $AUC_{0-\infty}$ values were logarithmically transformed before analysis. Conversely, the T_{max} values were analysed nonparametrically using the Wilcoxon signed rank test for paired samples. Probability of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

The two studied products (Ciprofloxacin and Ciprobay® 500 mg tablets) were well tolerated by all subjects; undesirable effects that could have influenced the outcome of the study did not occur. There was no dropout; all volunteers who participated in the study stayed on till the end and were discharged in good health. Both formulations were readily absorbed from the gastrointestinal tract and ciprofloxacin was measurable at the first sampling time (0.25 hour). Plots of the mean (± SD) plasma ciprofloxacin concentration versus time profiles of volunteers for the two formulations over the 24-hour sampling period are shown in Fig.1. The mean plasma profiles of Ciprobay® and ciprofloxacin were very similar. The peak plasma concentrations of both formulations were attained at about one hour and thereafter, declined gradually over a period of 23 hours. Table I shows the mean demographical data of the subjects. Table II presents the individual volunteer numerical values of $AUC_{0-\infty}$, C_{max}, T_{max} and $t_{½}$. 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 processes. The mean $AUC_{0-\infty}$ value of ciprofloxacin was 5.59 hr.μg/ml (SD 1.62 hr.μg/ml) slightly higher than that of Ciprobay® 5.57 hr.μg/ml (SD 1.70 hr.μg/ml). All the percentages of extrapolated AUC for both formulations were less than 20%. The mean C_{max} value for ciprofloxacin was 1.39 μg/ml (SD 0.38 μg/ml), while that of Ciprobay® was 1.43 μg/ml (SD 0.46 μg/ml).

When analysed using ANOVA, no statistically significant difference was observed between both formulations in the logarithmically transformed $AUC_{0-\infty}$ ($p > 0.05$) as well as logarithmically transformed C_{max} ($p > 0.05$) values. Moreover, the sequence (or group) effect was not statistically significant in the analysis of both parameters, indicating that there was no carryover effect. The within subject variation, denoted by coefficient of variation CV%, was calculated using the mean square error obtained from the logarithmically transformed $AUC_{0-\infty}$ and C_{max} values, were 12.22% and 18.04%, respectively. These results were less than 20%. Additionally, the 90% confidence interval for the ratio of the logarithmically transformed $AUC_{0-\infty}$ values of ciprofloxacin over those of Ciprobay® lay between 0.95 and 1.07, while C_{max} values lay between 0.90 and 1.07. These are within the acceptable bioequivalence limit of 0.80–1.25 as shown in Table III. Based on the plasma levels of the 24 volunteers completed this study, the mean relative bioavailability of ciprofloxacin (test) was 100.36% for $AUC_{0-\infty}$ compared with Ciprobay®. The mean T_{max} values of ciprofloxacin and Ciprobay® were 1.11 hr and 1.08 hr, respectively. When analysed using the Wilcoxon signed rank test for paired samples, no statistical significance difference ($p > 0.05$) was observed between the two formulations. The $t_{½}$ values for Ciprobay® and ciprofloxacin were 3.45 hr and 3.36 hr, respectively, within the normal range of 3–5 hr stated in the literature.[5]

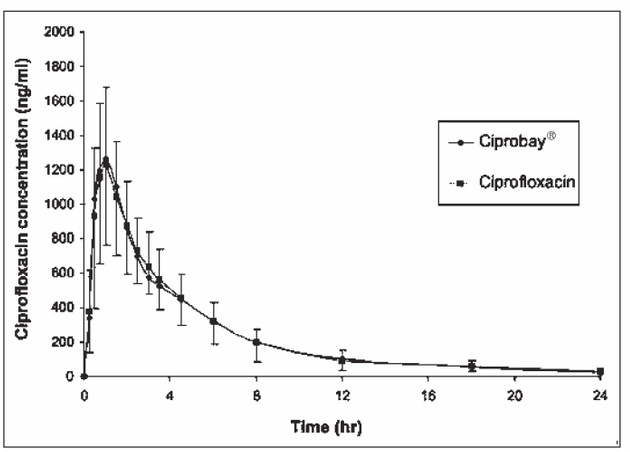

Fig. 1 Graph shows mean (± SD) plasma concentration of ciprofloxacin 500 mg tablets after oral administration of single dose of Ciprobay® and ciprofloxacin to 24 healthy human volunteers.

DISCUSSION

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 ingredient is absorbed, and becomes equally available at the site of drug action, they are bioequivalent and thus are assumed to be therapeutically equivalent.[12] To demonstrate bioequivalence, certain limits should be set, depending on the nature of the drug, patient population and clinical end-points.[13] It is generally accepted that the 90% confidence interval for the ratio of averages of logarithmically transformed AUC and C_{max} should lie within the range of 0.80–1.25.[14,15] Our study data show that both ciprofloxacin formulations are bioequivalent for the rate and extent of absorption. The mean and standard deviation of $AUC_{0-\infty}$ of the two formulations did not differ

significantly, suggesting that the plasma profiles generated by ciprofloxacin (test) are comparable to those produced by Ciprobay®.

In addition, the power of the test (1-β) in detecting a difference of 20% between ciprofloxacin and Ciprobay® based on a type 1 error (α) significance level of 0.05, using 24 healthy volunteers, was estimated to be greater than 90% for both ln-transformed $AUC_{0-\infty}$ and ln-transformed C_{max} values.[16] In conclusion, statistical comparison of the $AUC_{0-\infty}$ and C_{max} clearly indicates that no significant difference was observed between ciprofloxacin (test) and Ciprobay®. The 90% confidence intervals for the mean ratio of $AUC_{0-\infty}$ and C_{max} indicate that these values are within the acceptable bioequivalence limit of 0.80–1.25. Based on the above results, ciprofloxacin is bioequivalent to Ciprobay® in both the rate and extent of absorption. Thus, it can be assumed that the two formulations are therapeutically equivalent and interchangeable in clinical practice.

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