Inti. Chem. Pharm. Med. J. Vol. 2. pp.189-196 (2005)

ASSESSMENT OF BIOEQUIV ALENCE AND PHARMACOKINETICS OF CIPROFLOXACIN IN HEAL THY MALE SUBJECTS

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ABSTRACT: Pharmacokinetics and bioequivalence studies of two ciprofloxacin brands (Ciprofloxacin & Ciproxin) were conducted in 14 healthy male volunteers after oral administration. Each brand (test and reference) consisted of 500 mg of Ciprofloxacin. The drug was analyzed in plasma samples with a microbiological assay using *Streptococcusfaecalis* as test organism. The elimination half-life of 3.92 :!: 0.45 and 3.72 :!: 0.29 h was calculated for both brands. The peak plasma concentrations of (3.95 :!: 0.33 *ug/mL)* and (3.89 :!: 0.22 */lg/mL)* was attained in about I hour for both Test and Reference Ciprofloxacin respectively. The mean :t SE values for total area under the curve (AUC 0-00) were 22.11 :!:1.94, and 19.33 :!: 0.75 h.mglI for both test and reference tablets respectively. The mean:!: SE values of clearance were 29.02 :!: 1.70 and 31.63 :!: 1.39 l/h for both formulations respectively. The mean:!: SE volume of distribution was 147.95 :!: 17.25 and 165.56 :!: 9.44 L respectively for both test and reference tablets of Ciprofloxacin. This study indicated that all pharmacokinetic and bioequivalence parameters for both ciprofloxacin formulations are statistically non-significant, hence both formulations are bioequivalent.

KEYWORDS: Pharmacokinetics, Bioequivalence, Microbiological assay Ciprofloxacin & Ciproxin

INTRODUCTION

Infectious diseases remain a constant threat to human and animal's health throughout the world. The problems are more prevalent in developing countries because of poor hygienic conditions and lack of education. Prevention of infectious diseases is a consistent endeavor to enhance the quality of health and life. Antibiotics playa significant role to check infectious diseases and are one of the extensively used drugs throughout the world but more so in the developing countries. Development of antibiotic resistance in bacteria continuously incites the scientists to modify the existing drugs or to develop newer remedies, which has resulted in a constant flow of the products in the market.

Quinolones are composed of a bicyclic aromatic core, a dual-ring chemical structure. During the 1980s, fluorinated derivatives of quinolones were introduced (Ciprofloxacin, 1987) and are now freely available in Pakistan. The addition of the fluorine atom improves potency; enhances antimicrobial activity; and alters pharmacokinetic properties, which provide tremendous therapeutic advantages. These fluoroquinolones are useful for treating infections because pharmacokinetic characteristics render them suitable for treating systemic infections (Thomas and File, 2001).

Ciprofloxacin (a zwitterions) has good penetration and accumulation in tissues with a wide distribution throughout the body. One of the most impressive properties of Ciprofloxacin is its ability to exert a very rapid bactericidal effect on bacteria. Within 5-10 minutes of Ciprofloxacin being added to the culture medium, the number of organisms falls dramatically. Unlike Penicillin and Cephalosporin, Ciprofloxacin shows equal bactericidal activity during the resting and reproductive phase of bacteria. This would explain the rapid onset of action of Ciprofloxacin and its potency (Zeiler and Grohe, 1984).

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 excepients and manufacturing process can affect the disintegration and dissolution rate of tablets given through the oral route. Since, local population shows distinct nutritional habits and thrives in particular environments; therefore, there is a likelihood of differences in biodisposiiion of Ciprofloxacin. Seth *et at.* (1995) recorded disposition kinetics of Ciprofloxacin and suggested the need to be cautious while treating patients with renal problems and proposed to use lower doses in Indian patients to achieve desirable results. Therefore, it is always advisable to perform disposition kinetics and renal handling studies in the target population and environments.

The term "bioavailability" refers to the rate and extent to which a drug/nutrient reaches its site of action or a biological fluid such as blood that has access to its site of action. The term "bioequivalence" refers to pharmaceutically equivalent drug products where the rates/extents of bioavailability of the active ingredients are not significantly different under suitable test conditions. In other words, this is a comparison of two or more products with respect to their bioavailability.

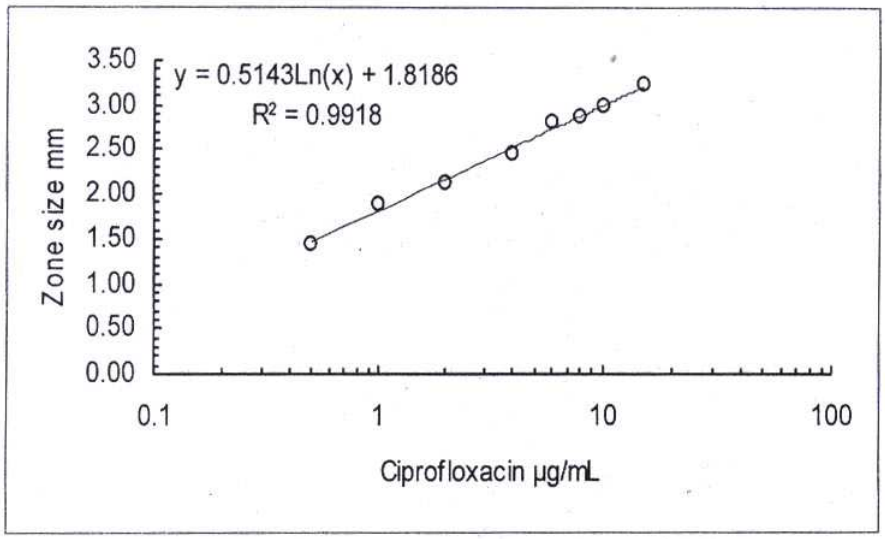
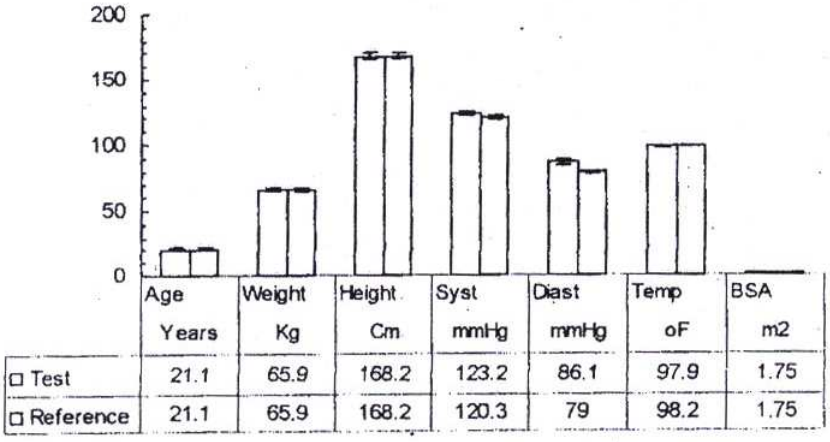


Figure 1: Standard curve of Ciprofloxacin in plasma



	Age Years	Weight Kg	Height Cm	Syst mmHg	Diast mmHg	Temp oF	BSA m2
□ Test	21.1	65.9	168.2	123.2	86.1	97.9	1.75
□ Reference	21.1	65.9	168.2	120.3	79	98.2	1.75

Figure 2: Mean age, body weight, height, blood pressure, body temperature and body surface area of 14 healthy male volunteers who participated in study of bioequivalence of Reference and Test Tablets of Ciprofloxacin 500 mg.

Bio-equivalent simply means that one brand or dosage form of a drug or supplement is equivalent to a reference brand or dosage form of the same drug or supplement in terms of various bioavailability parameters (21 CFR, 1991).

The interchange ability of pharmaceutically equivalent drug products is a matter of concern to health of peoples and health authorities in Pakistan, particularly in view of increasing international drug trade facilitated import and export in many countries. The availability of different formulations of the same drug given at the same strength and in the same dosage form possesses a special challenge to health care professionals making these issues relevant to pharmacists in all practice settings.

Table 2: Statistical comparison of bioavailability (Bioequivalence) parameters for Test and Reference Tablets containing 500 mg of ciprofloxacin

Bioequivalence Parameters	Units	Test		Reference		P(T <=t) One-tail	P(T<=t) Two-tail
		Mean	:!::SE	Mean	:!::SE		
AUC	[h.mg/l]	22.11	1.94	19.33	0.75	0.0507	0.1014
Ln(AUC)	h.mg/l	2.87	0.06	2.77	0.04	0.0580	0.1160
Tmax	[h]	0.91	0.06	0.97	0.06	0.2138	0.4277
Cmax	[mg/L]	3.95	0.33	3.89	0.22	0.2845	0.5690
Ln(Cmax)	[mg/L]	1.37	0.09	1.34	0.05	0.3904	0.7808

t Critical one tail =1.709, t Critical two tail =(P.0.05)

Table 3: Critical Bioequivalence metrics comparison of bioavailability (Bioequivalence) for the Test and Reference Tablets containing 500 mg of Ciprofloxacin

Bioequivalence Parameters	Units	Test	Reference	Test% of Reference	90%CI Lower	Limits Upper
AUC*	[h.mg/I]	22.11	19.33	111.43	99.6	122.41
Ln(AUC)*	h.mg/l	2.87	2.77	103.48	99.59	106.62
Ton,,,'	[h]	0.91	0.97	93.77	81.94	85.15
Cm".	[mg/L]	3.95	3.89	105.03	88.4	120.12
Ln(Cm,,)	[mg/Li	1.37	1.34	101.97	87.26	114.6

*Critical Bioequivalence metrics that should not differ beyond 80 125 percent.

the study. Each volunteer signed the "Informed Consent Form" at the time of registration.

Drug information

Test drug: Ciprofloxacin 500 mg Tablets B.
No Test
Mfg. 01-2002
Exp. 12-2005
Biyon Pharmaceuticals (Pvt.) Ltd
48- A Industrial Estate, Hayatabad, Peshawar, Pakistan

Reference drug: Ciproxin9 500 mg Tablets
Ciprofloxacin 500 mg
Batch No. 132-B
Mfg. Date 05-2001
Exp. Date 04-2006
Manufactured by:
Bayer Pakistan (Pvt.) Ltd.,
C 21, S.I.T.E., Karachi, Pakistan.

After an overnight fast of at least 8-12 hours, subjects were randomized to receive a single dose of 500-mg Ciprofloxacin standard or test tablet with 240 to 250 ml of water. The volunteers were randomly divided into 2 groups of 14 subjects in each group. A replicated-crossover design for the bioequivalence studies with two formulations was used. A seven days washout period was provided between dosing of test and reference tablets.

Sample collection and handling

Before drug administration, a control/blank venous blood sample was collected from each volunteer through a sterile venous Branula 18G (J Vasocan@ Braniile@, B. Braun Melsungen AG). Following drug administration, serial blood samples were drawn at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, and 12 hours in heparinized centrifuge tubes specially prepared for this purpose. These tubes were chilled and centrifuged under refrigeration for 15 minutes at approximately 2000 rpm. The plasma was separated and stored at <-20 DC until analysis.

Demographic and clinical data

The age, weight, height, blood pressure (Systolic/ Diastolic), temperature and body surface area (BSA)

of each volunteer was recorded. The Body surface area was calculated with the following formula:

$$BSA = (W\ 0.425 \times H\ 0.725) \times 0.007184$$

Where, 'W stands for weight and 'H' stands for height

The clinical data including Glucose, Blood urea, Serum Creatinine, Cholesterol, Bilirubin total, SGPT, SCOT, and CPK of all the volunteers was also determined to check the health status of individuals under study.

Microbiological Agar Diffusion Method Ciprofloxacin concentration in the plasma samples was measured with microbiological assay.
This method has the advantage to detect the microbiologically active moieties, hence, considered a valid method for assay of most of antibiotics in biological samples.

For assay of Ciprofloxacin, the Disc Agar Diffusion Method was standardized and validated for accuracy and precision by using Streptococcus faecalis as test organism according to the method of Arrct *et at.* 1971. The samples were run at least in duplicate. The zones of inhibition were measured with Zone Reader and the concentrations of Ciprofloxacin in the plasma samples were calculated by sample zones with standard curve regression equation (Figure I). The standards were run with each analysis. The curve shows the value of regression coefficient ($R2 = 0.991\ 8$).

Statistical Calculations:

a) Pharmacokinelics Pammeters:
For computation and analysis of Ciprofloxacin in plasma, the computer software programme "Microsoft Excel 7.0" was used. The plasma concentration of Ciprofloxacin from each volunteer was plotted on a semi logarithmic scale against time. The plasma concentration versus time data was used to calculate pharmacokinetics and bioavailability parameters with the help of a PC-Computer Program, APO, MWPHARM version 3.02 a MEDI WARE product Holland. Calculations also included area under curve (AUC) from time t to $_{00}$ (infinity) calculated with polyexponential and trapezoidal methods.

b) Bioequivalence/Bioavailability parameters:
(Bioavailability parameters such as Cma" T max and AVC were determined) Bioequivalence comparisons were performed' using Student t-test: paired two .samples for means. For the ratios of the mean bioavailability parameters, models were used to construct 90% confidence intervals for test versus reference tablet.

RESULTS

The demographic data of individuals who participated in the study of phannacokineticlbioequivalence of Ciprofloxacin Test and Reference formulations is represented in Figure 2. This is evident from the data that volunteers in both study groups are homogenous in terms of mean :t SE age (21.1 :t 0.42 years), weight (65.9 :t 1.42 kg), height (168.2 :t1.89 cm), and body surface area (BSA) (1.73 :t 0.03 m\ Composite plasma drug concentration-versus-time profiles collected from study individuals following oral administration of Ciprofloxacin are presented in Figure 3. The peak plasma concentrations of (3.95 :t 0.33 ug/mL) and (3.89 :t 0.22 ug/mL) was attained in about 1 hour for both Test and Reference Ciprofloxacin respectively. Mean pharmacokinetic data for both Ciprofloxacin preparations are presented in Table 1. The mean + SE values for total area under the curve (AUC 0-(0) were 22.11 :f: 1.94, and 19.33 :f: 0.75 h.mg/l for both test and reference tablets respectively. The mean :t SE values of clearance measured in *1/h* were 29.02 :t 1.70 and 31.63 :t 1.39 for both formulations respectively. This is evident from table 1 that the mean :t SE volume of distribution was 147.95 :t 17.25 and 165.56 :t 9,44 L respectively
for both test and reference tablets of Ciprofloxacin.
The elimination half-life of 3.92 :t 0.45 h was calculated for test Ciprofloxacin and 3.72 :t 0.29 h was observed for reference formulation. (Table 1) This is evident from Table 1 that all the pharmacokinetic parameters for both Ciprofloxacin formulations are statistically non-significant.

The comparison of mean :t SE "bioequivalence" parameters of Ciprofloxacin Test and Reference formulations have been presented in Table 2, while Table 3 presents critical bioequivalence metrics comparison for both Test and Reference Ciprofloxacin. Statistical appraisal of the bio-equivalence between the Unavailability parameters of two formulations did not reveal any significant differences.

DISCUSSION

Ciprofloxacin has become an extremely popular antimicrobial agent for use in human (Owens *et ai,* 1997), dogs, cats, pigs, cattle and poultry (Brown 1996). The availability of this important drug in various brands in Pakistan raises the need to conduct pharmacokinetic and bioequivalence studies for various formulations in target population. The present project was undertaken to investigate the disposition and bioequivalence of two orally administered formulations of Ciprofloxacin. The systemic absorption of an orally administered drug in a solid dosage form is comprised of three distinct steps that can significantly affect the pharmacokinetic and bioequivalence parameters:

1. Disintegration of the drug product.
2. Dissolution of the drug in the fluids at the absorption site.
3. Transfer of drug molecule across the membrane lining the gastrointestinal tract into the systemic circulation.

There is controversy in literature regarding selection of suitable compartmental model to best describe the disposition of ciprofloxacin. The kinetics of ciprofloxacin in domestic animals was mainly described with two compartment open model, as reported in chickens (Anadon *et ai.,* 1995), horses (Garcia Ovando *et ai;* 1996), pigs & bovines (Nouws *et ai.,* 1988) and ponies (Dowling *et ai.,* 1995). However, Pharmacokinetics behavior of orally administered Ciprofloxacin has been described in terms of three compartmental models in volunteers (Hoftken *et ai.,* 1985), in patients subjected to lung surgery for bronchial epithelioma (Breilh *et ai.,* 2001) and by two compartmental models in healthy male volunteers (Abdallah *et ai.,* 2002). Pharmacokinetics of Ciprofloxacin has also been studied by using non-compartmental model in healthy volunteers (Maya *et ai.,* 2003). In the present study, pharmacokinetics parameters determined by two compartmental open models and non-compartment analysis did not reveal any significant differences. However, a decision about two or three compartmental model seems to depend on the frequency of blood sampling during the initial phase of experiments. In case of three compartmental models, frequent sampling within first hour makes it possible to distinguish between two distribution phases (Xi a *et ai.,* 1983).

In present study the mean values of volume of distribution were 147.9 :t 17.25 and 165.5 :t 9.44L for both test and reference formulations. These parameters are comparable to 145.86 :t 97.51 L (Breilh *et ai.,* 2001) and 129 :t 32 L (Garrelts *et ai.,* 1996) after oral administration of 500' mg Ciprofloxacin in patients. Such higher values for volumes of distribution suggested effective diffusion in the extravascular space. The half-life of a drug is a derived parameter that changes as a function of both clearance and volume of distribution (Booth and McDonald, 1998). In present study the mean values of *tIn* B of test and reference drugs was 3.92 and 3.72 h respectively (Table 1). These values are comparable to 4.02 :t 0.89 h (Breilh *et ai.,* 2001), 4.2 h (Catchpole *et ai.,* 1994), and 5.37:t0.82 h (Lubasch *et ai.,* 2000) in healthy volunteers. The mean :t SE values of clearance measured in 1/h were 29.02 :t 1.70 and 31.63 :t 1.39 for both brands of Ciprofloxacin respectively (Table 1). These values are also similar to 29.1 :t 17.5 1/h in patients after oral administration of Ciprofloxacin (Garrelts *et ai.,* 1996).

Bioequivalence is a comparison of the bioavailability of two or more drug products. The two products or formulations containing the same active ingredient are bioequivalent if there rates and extents of absorption are same. For bioequivalence studies Cmax. T roax and AVC are commonly used parameters (Table 2). After oral administration of Ciprofloxacin, the mean peak plasma concentrations (Croax) of 3.95 ::!: 0.33 Ilg/mL and 3.89 ::!: 0.22 Ilg/mL were attained in about 1 hour (Troax) for both Test and Reference tablets respectively (Figure 2). These values are comparable to the literature values of 3.9 :t 1.7 mg/L (Catchpole *et ai.,* 1994) and 2.9 Ilg/mL (Lebel, 1998) after a single 500 mg oral dose. In present study the mean values of area under the curve (AVC) were 22.11 :t 1.94 and 19.33 :t 0.75 h mg/L for test and reference Ciprofloxaein formulations. This parameter is similar to the reported values of 20.7 ::!: 16.6 h Ilg/ml (Garrelts *et ai.,* 1996) and greater than 12.11 h mg/I (Escobar and Hoyo, 2003).

It has been reported that the time at which plasma or biological fluid concentrations of antibiotic exceed minimum inhibitory concentration (MIC) is highly correlated with success of therapy for antibacterial agents exhibiting time-dependent activity (Rao *et ai.,* 2002). Previous studies suggest that fIuoroquinolones kill bacteria in a concentration-dependent manner and

area under inhibitory curve (AVC) calculated by AVCIMIC is highly correlated with the outcome of successful treatment (Drusano *et al.*, 1993, Aliabadi and Lees, 1997). For effective eradication of bacteria and good clinical therapy, it has been suggested that an AUIC>lOO is required *for* gram-negative bacteria and > 30 is needed *for* gram-positive organisms (Nightingale *et al.*, 2000; Walker, 2000). Although MIC values of ciprofloxacin *for* many pathogens of genus *Pasturella, Escherichia, Haemophilus, Moraexella,* and *Salmonella* are reported to be in the range of *0.01-0.06* Jlg/mL (Prescott and Yielding, 1990; Bottner *et al.*, 1995). On the basis of MIC reported *for* highly sensitive pathogens (0.0 1-0.06 Jlg/ml) and AVC (22.11 :!:: 1.94, and 19.33 :!:: *0.75* h mg/L) determined in the present study, AVIC would be much greater than 100.

In present study the critical bioequivalence parameters included AUC, Tmax and Cmax of both test and reference Ciprofloxacin are within the range of 80 to 125 % (Table 3). This study concludes that the bioequivalence metrics between the Bioavailability parameters of both Ciprofloxacin formulation did not show significant differences, hence both test and formulations are bioequlvalent.

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Manuscript received 05 - 10 - 2004
Accepted for publication 04 - 12 - 2004