Pak. J. Sci. Ind. Res. 2004 ***47****(3) 214-217*

SINGLE-DOSE PHARMACOKINETIC STUDY OF CIPROFLOXACIN AFTER ORAL ADMINISTRATION TO THE HEALTHY FEMALE VOLUNTEERS

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(Received October 15, 2002; accepted April 21, 2004)

The disposition kinetic of ciprofloxacin was evaluated in 10 adult healthy female volunteers. Appropriate mathematical model was applied for the estimation of the basic pharmacokinetic parameters because the statistical tests and profiles formed the basis for accepting or rejecting a proposed model. After single oral dose of 500 mg ,the blood samples were collected and ciprofloxacin concentrations were determined in serum by High Performance Liquid Chromatography. The mean value of plasma elimination half life (t ½) was estimated as 4.08 ± 1.3 h, maximum plasma concentration (C max) was 1.56 ± 0.49 mg/l obtained at mean value of (Tmax) 2.11 ± 1.9 h. The average value of area under the curve was 8.96 ± 7.70 h-mg/l and is calculated from t_o to t_α, while the average absorption rate constant was 1.56 ± 1.84 l/h. The average value for clearance was 78.08 ± 41.36 l/h. Volume of distribution and mean resident time showed an average value of 197.0 ± 98.88 litre and 4.82 ± 4.22 h, respectively. There was a significant deviation from the literature trends in respect to Vd and clearance.So this study supports the need for comprehensive evaluation of drug under indigenous conditions to obtain pharmacokinetic parameters on which the rational dosage regimens of drug could be based.

Key words: Ciprofloxacin,Pharmacokinetics ,HPLC.

Introduction

Ciprofloxacin is introduced in 1980, as broad spectrum antibiotic. It belongs to the fluoroquinolone class of antimicrobial agents. The quinolones uniquely inhibit the replication of DNA by interfering with the action of DNA gyrase. This enzyme is responsible for coiling and supercoiling of the DNA within the cell. When this enzyme is inhibited, DNA transcription, which results in protein synthesis, and DNA replication which results in cell division are inhibited (Kidwai *et al* 1998).

Most gram negative bacteria are highly susceptible to this agent *in vitro* whereas gram positive bacteria are generally susceptible or moderately susceptible. The quinolones are bactericidal but are not effective against anaerobes. Ciprofloxacin is particularly useful in treating infections caused by multiple resistant bacteria. It is an alternative to more toxic drugs, such as the aminoglycosides, or drug that require parentral administration. (Harvey and Pamela 1995).

Ciprofloxacin is a good alternative in a wide variety of situations such as lower respiratory tract infections, complicated urinary tract infections, gastrointestinal, skin and bone infection and sexually transmitted diseases. (Davis *et al* 1985). It has good bioavailability and pharmacokinetic profile, large apparent volume of distribution showing concentration in several compartments. Renal unmetabolized excretion is the primary route of elimination.

Ciprofloxacin is currently enjoying wide acceptance and usage in Pakistan, however it is observed that investigation in all aspects of the pharmacokinetics of all clinically relevant quinolones have been carried out in drug exporting countries. In these studies considerable variation of the pharmacokinetic of parameters in men have been observed (Nawaz and Shah 1985; Nawaz 1994) because the genetic make up of men and animals, nutritional and environmental conditions are entirely different among drug importing and exporting countries. Pharmacogenetic variations may effect the biodisposition and fate of drugs that are extensively metabolized. Several studies have shown that pharmacokinetic behavior, renal clearance and urinary excretion of the investigated drugs were different under indigenous conditions when compared with the values given in literature. (Nawaz and Shah 1985). Thus these variations warrant depiction of therapeutic standards and dosage regimen on the basis of indigenous investigation.

Microbiological assay and HPLC (Joos *et al* 1985; Catchpole *et al* 1994) has been used to quantify ciprofloxacin in biological fluids. Both methods are reproducible and accurate but HPLC is recommended due to the presence of microbiologically active metabolites. It gives exact concentration of drug in sample so a precise method is evaluated to work in the conditions of individual labs.

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Materials and Methods

A total of 10 healthy Pakistani female subjects, participated in this study after their written consent. All subjects were determined to be in good health prior to study on the basis of physical examination and medical history. Subjects with a history of allergy or hypersensitivity to quinolone drugs, those being treated by a long-term administration of other drugs, those who have gastrointestinal tract problems and those who were found to have abnormalities during pretreatment were excluded from this study. No other medication were permitted one week prior to and during the study.

Sample collection for drug determination. After an overnight fasting, control blood samples were collected from all volunteers. Each volunteer received single 500 mg tablet of ciprofloxacin with 250 ml of water. The volunteers took a breakfast before/after two hours following drug administration. 5 ml venous blood was drawn in heparinized centrifuge tubes at 30, 60, 90, 120, 150, 180, 240, 300, 360, 480 and 720 min time intervals. The pH of fresh blood samples was recorded, then the sample was centrifuged at 4000 rpm for 10 min and plasma was separated and stored at -20°C till further analysis.

*Analysis of plasma.*The HPLC method described by Kamberi *et al* (1998) was used for the detection and quantification of unchanged ciprofloxacin in plasma. The plasma samples were deproteinized with acetonitrile and after evaporation and reconstitution of the supernatant, samples were analyzed by injecting 20 µl manually into HPLC System. The HPLC system consisted of a pump (Jasco - PU.980) and U.V. spectrophotometer (Jasco- U.V. 975). The output of the detector was monitored with an integrator (JASCO - 807 - IT). A stainless steel column packed with ODS was used.

Working standards. Standard samples of plasma having concentration 0.01, 0.5, 1.0, 2.5 µg/ml of ciprofloxacin were prepared in drug free control plasma. Area versus concentration of standards were plotted and a linear curve was obtained (Fig 1).

Chromatographic conditions. Separation of ciprofloxacin was achieved at 50°C, using an isocratic mode. The mobile phase consisted of mixture of 5% acetic acid, acetonitrile and methanol having ratio 18:1:1. In order to de gas the mobile phase it was first filtered under vacuum and then sonicated (EYELA Sonicator) for 30 min. The U.V. detector was set at 280 nm and sensitivity was set at 0.02 absorbance. The flow rate was 1.5 ml/min and retention time was approximately 5.9 min.

Table1
Demographic data

n = 10	Age (y)	Weight (kg)	Height (cm)
Mean	24.00	57.00	155.80
SD	5.53	7.24	4.28
Min	18.00	47.00	150.00
Max	29.00	69.00	162.00

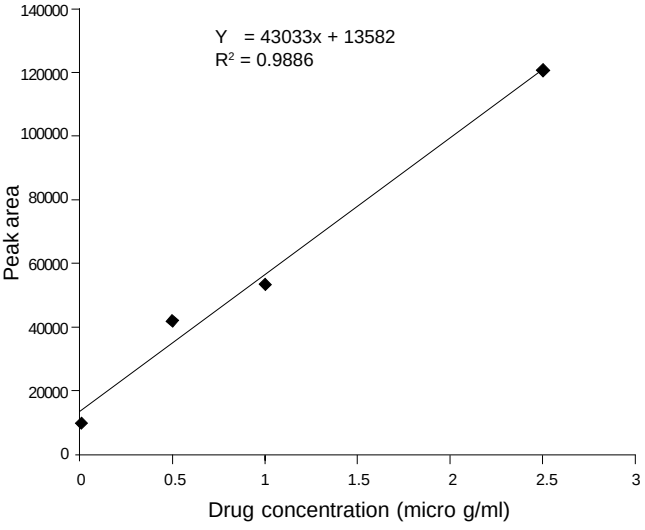

Fig 1. Ciprofloxacin Standard Curve in Plasma.

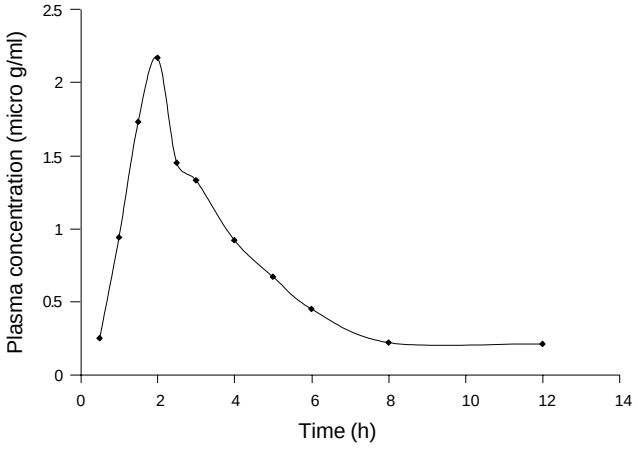

Fig 2. Average representative plot of plasma concentration (micro g/ml) versus time (h) after the oral administration of 500 mg ciprofloxacin.

Pharmacokinetic parameters and statistical analysis. The pharmacokinetic profile of ciprofloxacin was evaluated by using computer programme MW, PHARM version 3.02 (Rombout 1987). The data on plasma concentration at different time intervals were analyzed by single compartment model.

The statistical calculations were done according to the standards and the results were given as an average ± SD.

Table 2
Disposition kinetic parameters of ciprofloxacin following oral administration of 500 mg tablets to each of the 10 healthy female volunteers

	AUC [h,mg/l]	Clearance (Cl) [l/h]	Vd [l]	t1/2 [l]	MRT [h]	Tmax [h]	Cmax [mg/l]
n	10	10	10	10	10	10	10
Geom.mean	7.37	67.59	177.7	3.82	3.92	1.7	1.474
Median	7.62	65.56	150.5	3.6	3.15	1.583	1.553
Mean	8.96	78.08	197.0	4.08	4.82	2.11	1.56
SD	7.70	41.36	98.88	1.38	4.2	1.9	0.49
Min	2.87	16.5	107.3	1.3	2.6	0.9	0.75
Max	30.3	174.1	400.2	5.6	16.5	7.57	2.4

Table 3
pH of blood

Volunteer	A	B	C	D	E	F	G	H	I	J	Mean	SD
pH	7.70	7.70	7.73	7.65	7.78	7.67	7.78	7.67	7.53	7.70	7.68	0.14

Results and Discussion

The demographic data of the study population are presented in Table 1.

The linearity of calibration curve was verified from 0.01 to 2.5 µg/ml for ciprofloxacin in plasma. The plasma concentration of ciprofloxacin versus time data was plotted on semilogarithmic scale. Biexponential equation on data was not found to be best fitted and showed a poor correlation coefficient. Thus two compartments model was found unsuitable. However, the monoexponential equation showed its goodness to fit our data, so the "one compartment open model" was selected to explain disposition kinetics of ciprofloxacin, which is similar to earlier observations in dogs, sheep and horse (Baggot 1977).

Following oral administration of 500 mg ciprofloxacin tablet to 10 healthy female volunteers, the plasma concentration at 0.5 h was 0.25 ± 0.28 µg/ml which shows very little absorption after 30 min. The concentration increases with passage of time showing average Cmax ± SD of 1.56 ± 0.49 mg/l at 2.11± 1.97 h (Tmax). In the present study the area under curve was 8.96 ± 7.70 h.mg/l. These values are comparable with previous data given in literature. (Ledergerber *et al* 1985; Hoffken *et al* 1985; Graffor *et al* 1989; Shah *et al* 1999). In our study the value of t½ is 4.08 ± 1.38 comparable to t½ of 3 to 5 h given in literature (Davis *et al* 1985; Tartaglione *et al* 1986).

The summary of results following pharmacokinetic evaluation of blood level data is presented in Table 2. Mean plasma profile versus time are presented in Fig 2.

Clearance is the most important concept to be considered when rational regimen for drug administration is to be designed. Clearance is altered by three factors (i) blood flow to the organ (ii) fraction of bound drug in blood (iii) maximal ability of that organ to remove the drug. In our study the clearance is 78.08 ± 41.36 l/h which is much higher than given in literature 46.3±2.6 l/h and 42.0l l/h (Bergan *et al* 1987; Sudo *et al* 1990). With respect to volume of distribution, in this study there is variation from literature trend. In our study the value of volume of distribution is 197 ± 98 l (3.4l/kg) while in literature the value is 2.7 l/kg (Bergan *et al* 1987). Factors that effect the value of the extent of distribution include plasma protein binding and tissue binding and partition coefficient of the drug between tissue and circulatory blood. It is found that ciprofloxacin has a large apparent Vd showing concentration in several tissues.

The present study revealed considerable variation in the behavior of ciprofloxacin in healthy female volunteers with the similar studies conducted under different environmental conditions as seen in literature. These differences attributed to the specific biochemical and physiological characters, which have been evidenced by Nawaz and Shah (1985) and Nawaz (1994). In man and most species of animals usually accepted value of pH is about 7.4 while under indigenous conditions the value of blood pH is higher in different species. Most of the drugs are either weak acids or bases therefore, the unionic diffusion depends upon the pKa value of the drug and pH of the surrounding media. Blood protein synthesis is under genetic control, such geonetic variations are reflected in the electrophoresis pattern of normal serum proteins. Most of the drug bound to albumen (circulatory protein of the blood) so any change in the albumen will change the protein binding of

the drug. Under nutrition and various deficiencies are common in drugs importing countries .In starvation, the decreases in protein synthesis, loss of muscle and hypoalbumenaemia are well documented, the low level of albumen (32 g/l) in undernourished than in the normal human subjects (42g/l), has exhibited poor protein binding of tetracyclines in the blood of former, thus causing rapid clearance of drug from the body. In view the specificity of disposition kinetic of ciprofloxacin under indigenous condition, it is obvious that dosage regimen of this chemotherapeutic agent be defined in the condition in which it is to be used in the clinics.

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