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PHARMACOKINETIC STUDY OF CLARITHROMYCIN IN HUMAN FEMALE OF PAKISTANI POPULATION

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ABSTRACT

The study was designed to assess the various pharmacokinetic parameters of a commercially available clarithromycin Tablet (Klaricid® 250 mg Abbot, Pakistan) in plasma sample of healthy adult female volunteers by applying a rapid, sensitive and accurate HPLC-UV analytical method. The human plasma samples were evaluated by using an isocratic High-Performance Liquid Chromatography (HPLC) system of Sykam consisted of a pump with a column C18 column (250×4.6mn, 5µm) UV-detector. The mobile phase comprises of potassium dihydrogen phosphate (50 mM, pH 6.8, contained 0.7% triethylamine), methanol and acetonitrile (30:25:45, v/v/v) was delivered with injection volume of 20µL at flow rate of 1 mL/min. The detection was performed at λ_{max} 275 nm. By applying this method, important pharmacokinetic parameters C_{max}, T_{max}, Area under curve (AUC), half-life ($t_{1/2}$), Volume of distribution (Vd) and Clearance (Cl) were measured. The parameters of pharmacokinetics of clarithromycin were calculated by software (APO) pharmacological analysis. Maximum plasma concentrations C_{max} 2.78 ±0.33 µg/ml, time to reach maximum co ncentration t_{max} 2.82 ± 0.11 h and Area under curve AUC was 20.14h.µg/ml. The mean ± SD values obtained for the pharmacokinetic parameters showed a significant difference in pharmacokinetic parameters observed in previous literature which emphasizes the need for dose adjustment of clarithromycin in Pakistani population.

1. INTRODUCTION

Antibacteria Clarithromycin is semi-synthetic broad-spectrum macrolide antibiotic having both bactericidal and bacteriostatic activity [1].It is an acid stable drug showing better oral absorption, lower frequency of gastrointestinal intolerance and longer half-life [2].It is primarily metabolized to its biologically active 14-hydroxy-6-O-methyl erythromycin metabolite in both human and animal [3]. Clarithromycin kill bacteria by interrupting with their protein synthesis and bind reversibly to 50S ribosomal sub unit inhibiting translation and translocation of peptides [4].

The Asian countries have diverse environmental, topological and nutritional condition from west which ultimately affects the genetic makeup of man. As most of the drug literature is acquired from western countries so, Pakistan being an importer of raw and finished drugs must investigate the pharmacokinetic parameters by conducting different clinical and pre-clinical investigation [5]. Studies conducted on it shown the varied results of pharmacokinetics parameters under different indigenous condition as specified in literature. Pharmacokinetic studies provide essential data for the calculating dosage regimen of the drug. In an order to individualize the dose and to know the kinetics of drug in a specified environment, pharmacokinetics studies must be carried out. In most cases the genetic makeup of indigenous animals and environmental conditions are different from their foreign counterparts and this affects the biodisposition of drugs. So, evaluation of kinetic parameters in indigenous animal species and human is necessary [6].

The analytical methods stated earlier for the quantification of clarithromycin in biological fluids were microbiological bioassay and high-performance liquid chromatography (HPLC). Different HPLC methods have been developed for analysis of clarithromycin in human serum using Electrochemical, Mass Spectroscopy, fluorescent detection and UV [7]. Among all these UV detector is the most inexpensive commonly distributed and requiring only a small volume of biological sample [8]. The present study was designed to evaluate the pharmacokinetic variation and dose determination of clarithromycin in Pakistani population.

2.MATERIALS AND METHODS

2.1 Chemicals

Clarithromycin (Klaricid® 250 mg) was from Abbott Pharmaceutical Company (Pakistan). Certified reference materials (CRMs) of clarithromycin USP28 (984µg/mg) was supplied by Zhejiang Better Pharmaceuticals Co., Ltd., China. HPLC grade acetonitrile (ACN) and methanol (MeOH) were obtained from Fisher Scientific limited (New Jersey, USA). Analytical grade triethylamine (TEA) was kindly provided by Danas Pharmaceutical Islamabad. Potassium dihydrogen phosphate, ethyl ether, sodium hydroxide (NaOH), concentrated phosphate acid and dichloromethane (CH_2Cl_2) were obtained from Department of Physiology and Pharmacology, UAF, Faisalabad. Water was glass-double distilled and further purified for HPLC.

2.2 Instrumentation and chromatography

Chromatography was performed with a High-Performance Liquid Chromatograph (Sykam, S-1122) and analyses were determined using UV detector (Sykam, S-3210). A stainless-steel column packed with YMC pack A-312 (BDS-C18 with 250 x 4.6mm dimensions and 5µm particle size) was used. The output of the detector was monitored with computer software (Peak Simple Chromatography Data System, Buck Scientific Inc., East Norwalk). Analytical Balance (Sartorius, Germany). Centrifugation Machine (MSE Micro Centaur, Sanyo UK). Sonication apparatus (Oqawa seiki Co, Japan).

2.3 Study design

Eight healthy female volunteers were recruited to participate in this study. The average age was 22years (range18-26) and the average weight was 57kg range (45-70 kg). The study protocol was approved by the ethical committee at University of Agriculture Faisalabad. The nature of the study was explained to the volunteers and a written consent was obtained from each volunteer. All the volunteers had normal kidney and liver functions and were free from any chronic disease such as hypertension, diabetes, hypotension or liver

abnormalities. Blank plasma was prepared from heparinized whole-blood samples collected from healthy volunteers. Then, the blood samples were centrifuged at 4000 rpm for 30 min. Each volunteer received clarithromycin Tablet (250 mg) as a single oral dose after overnight fasting. Blood samples (5 ml) were taken at 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours post medication then frozen immediately at – 20^{0}C until assayed.

2.4 Mobile phase preparation

Mobile phase consisted of 50Mm potassium dihydrogen phosphate (contained 0.7% Triethylamine v/v, adjust with concentrated phosphate acid to pH 6.8) acetonitrile–methanol at a ratio of 30:45:25 v/v/v. Analyses were run at a flow rate of 1ml/min. The mobile phase was passed through the filtration assembly, having the Whatman filter paper. Then finally, the filtered mobile phase was sonicated to remove air bubbles for 15minutes. The detection was carried out at 275nm.

2.5 Standard solutions

Standard stock solutions of clarithromycin were prepared in methanol to a concentration of 50mg/50 ml. Working solution of different concentration ranging from 0.1-10 µg/ml was prepared by diluting the stock solution with water as needed to construct the calibration curve. The working solutions were freshly prepared for daily analysis. These solutions were added to drug free plasma in volumes not exceeding 8% of the plasma volume. The solutions were filtered through a phenomenex membrane of 0.45 µm pore size and 20µl was injected into HPLC for analysis. Calibration graph was prepared by using peak area versus concentration of working solutions.

2.6 Sample preparation

To150µl of plasma sample add 20µl of 0.25M NaOH. The solution was vortexed briefly and added1.0 ml of ethyl ether again vortexed for 5 min and centrifuged for another 5 min at 4000 rpm. The organic layer was separated and transferred into another clean Eppendorf tube and dried under a stream of N_2 at room temperature. The residue was resolved with 600µl of frappe CH_2Cl_2. Then 200µl of water was added and vortexed 1 min to terminate the reaction. The solution was centrifuged for 5 min and the water layer was discarded, and the obtained organic layer was dried under a stream of N_2 at room temperature. Then 150 µl mobile phase was added in the residue and samples were passed through filter paper having size 0.45µm. Finally, 20µl of the reconstituted solution was injected onto the HPLC column.

2.7 Standard curve

Working standard having clarithromycin concentration 10, 5, 1, 0.5 and 0.1 µg/ml were prepared. The working standards were analysed by using HPLC. Concentration versus peak area data was plotted on a graph to construct the calibration curve. The assay was fully validated for linearity, selectivity, precision, accuracy and stability.

2.8 Determination of clarithromycin in plasma

The concentration of clarithromycin in the plasma sample was calculated by comparison with peak area obtained from standard solutions.

2.9 Pharmacokinetics Analysis

The plasma verses concentration data was plotted on graph by computer software MW-PHARM version 3.2 (Holland). Pharmacokinetic calculations were done with APO software program. Based on the goodness of fit statistics, the compartment model was selected. Thus, one compartment open model was selected to explain and compare the pharmacokinetics parameters of clarithromycin. Peak plasma concentration (C_{max}) and time to peak concentration (t_{max}) were derived from the individual subject concentration- time curves. Half-life ($t_{1/2}$) was calculated as 0.693 divided by K. The area under the plasma concentration time curve from time zero to the last measurable concentration at time t (AUC_{0-t}) was calculated using the trapezoidal rule. The mean value and standard error of means ±(SE) for each concentration and parameters was calculated [9].

3. RESULTS

The results for calibration curve of metronidazole are given in Table I and Figure 1. The curve was linear over the range of 0.1 to 10µg/ml with regression equation (R^2=0.9863;y=31.941x +33.964). Retention time of drug was found to be 15 min.

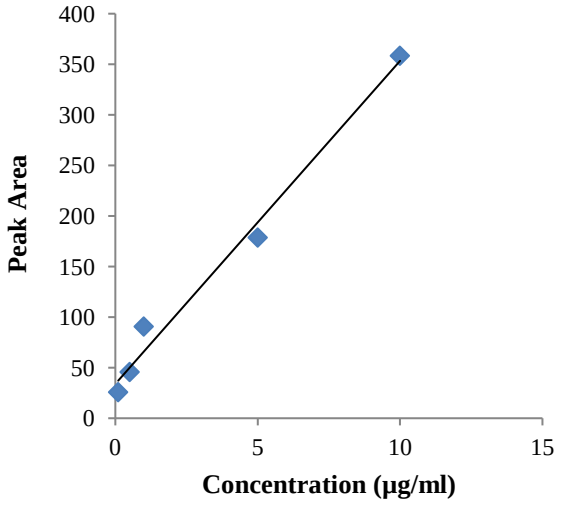

Figure 1: Calibration curve for clarithromycin

3.1 Compartment model

The plasma concentration-time data was analyzed by one compartmental open model and the values of different pharmacokinetic parameters were determined in healthy female volunteers.

3.2 Plasma concentration C_{max} (µg/ml)

The Table II and Figure 2 show the clarithromycin concentration in healthy female volunteers. Following oral administration of clarithromycin, the plasma concentration in healthy volunteers at 0.5 hr was 0.43µg/ml means that drug absorption in blood started immediately after drug administration. The concentration of clarithromycin in plasma after 1 hour of drug administration was 1.24 µg/ml showing that very little absorption takes place after 1 hr which reaches at a concentration 4.58µg/ml after 3 hours and then decline to 0.43µg/ml after 12 hrs showing the decline was progressive. The concentration increases with passage of timing showing an average C_{max} 2.7 ±0.33 µg/ml SD.

Table 1: Plasma concentration (µg/ml) of clarithromycin at different time intervals following oral administration of 250 mg to patients (Mean+SD, n=8)

Time (hour)	Concentration(µg/ml)
0.5	0.43±0.12
1	1.24±0.34
2	2.41±0.26
3	4.58±0.40
4	2.78±0.21
6	1.42±0.22
8	1.03±0.37
10	0.88±0.09
12	0.43±0.09

3.3 Time to peak concentration T_{max}(hour)

It is the time at which maximum concentration of drug is attained. The T_{max} of clarithromycin after 250mg of oral dose was found to be 2.82 ±0.11 hours in healthy females.

3.4 Half-Life $t_{1/2}$ (hour)

The time needed for the concentration or amount of drug in the body to be decrease to exactly one-half of a given concentration or amount. The mean

half- life of clarithromycin recorded in healthy volunteers after 250mg of single clarithromycin dose was 2.55±0.34hours.

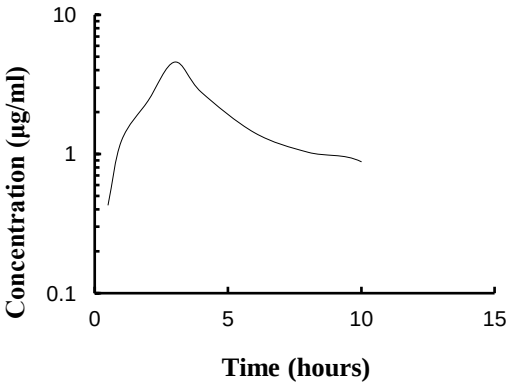

Figure 2: Plasma Concentration of clarithromycin in 8 healthy volunteers

3.5 Total body clearance Cl_B (L.h/kg)

The total body clearance of a drug is the amount of blood that is cleared of the drug in a unit of time. The total body clearance of clarithromycin was 0.23±0.05L/hr/kg in 8 healthy female volunteers.

3.6 Area under the concentration curve AUC (h.µg/ml)

$AUC_{0-\infty}$ is the total area under plasma concentration curve from t_0 to t_∞ $AUC_{0-\infty}$ of clarithromycin after 250 mg of oral dose was found to be was 20.14 h.µg/ml. The average values of pharmacokinetic parameters in healthy volunteers is shown in Table 2.

Table 2: Pharmacokinetic parameters of clarithromycin in healthy volunteers

Parameters	Mean Value±SD
AUC (h.µg/ml)	20.14±3.03
$T_{1/2}$(h)	2.55±0.34
Cl (L.h/kg)	0.23±0.05
T_{max} (h)	2.82±0.11
C_{max} (µg/ml)	2.78±0.33
B(µg/ml)	5.51±0.78

4. DISCUSSION

The major emphasize of present study was to evaluate the pharmacokinetic parameters of clarithromycin and then determine the dose accordingly. There is a linear relationship between therapeutic effect and pharmacological activity of the drug and it shows that pharmacological response is directly proportional to the plasma concentration. C_{max} depends on both the rate of drug absorption and extent of drug absorption in plasma. So, it is a strong indicator of rate of absorption of drugs i.e. higher the peak concentration, faster the rate of absorption. In addition, C_{max} also give warning of possible toxic levels of drugs (Shargel and Yu, 1999). The finding in the present study when compared to the previous literature observed in healthy volunteers showed C_{max} 3.19±0.50 µg/ml [7]4.2±0.45 µg/ml [8]. This statistically significant difference is attributed probably due to the decreased absorption of clarithromycin from GIT. According to Zuckerman 2004, clarithromycin is metabolized by cytochrome P-450 particularly the CYP3A enzyme which is polymorphically expressed which results in pharmacokinetic variations in individual. But the variation in C_{max} was observed with in the therapeutic safety index of drug.

T_{max} is the time at which maximum concentration of drug is achieved and the rate of drug absorption exactly equals to the rate of drug elimination [10]. The T_{max} value in the current study was different from 3.0 ±1.1h SD [7] and 5.7 ±2.8 hrs [11]. It shows that in our population the drug took slightly less time to reach to its peak because of rapid absorption from the walls of intestine. There are the two main reasons for this difference:

i) Previous study has employed male volunteers
ii) Different brand of clarithromycin was used

The Food and Drug Administration (FDA) studied various new drug applications and observed 40% pharmacokinetic variability due gender difference in some drugs. So, this fact reveals that there decrease in T_{max} is probably due to the gender difference.

The AUC from time zero to 24 h (AUC0-24) was calculated using the log-linear trapezoidal rule [12]. It is stated that AUC has a direct relation with the dose of drug administered. AUC is used to estimate the extent of drug absorption that actually appears in the blood stream [13]. AUC is the total amount of active drug that reaches the systemic circulation [10]. The present $AUC_{0-\infty}$ value in healthy volunteers was different to literature cited values that is 27.49 ±6.03 h.µg /ml SD [2] and as 39.6 h µg/ml [8].

Previously reported half-life of a clarithromycin was 4.31±0.87 hours [14] which was significantly different from observed half-life. If clarithromycin concentration in serum increases greater than 1mg/ml it indicates saturation to the process of binding of plasma protein and major availability for distribution to the infected tissue [11]. The absorption of drug is related to four major functions

1) Volume of tissue in which the drug distributes.
2) Partition co efficient of drug between tissue and circulatory blood.
3) The blood flow to tissue
4) Binding of drug to plasma or tissue protein.

All these factors are linked to genetics. So, apparent volume of distribution may vary according to genetic polymorphism. Volume of distribution is known as a "primary pharmacokinetic parameter" means that this parameter was determined by the physiochemical properties of the drug and physiologic properties of the body. It is extensively bind to plasma protein which indicates less volume of distribution. It is clinically important for determining the loading dose essential for a desired blood concentration of a drug, and is also used for measuring the blood concentration in the treatment of overdose.

The clearance value found in the previous literature of clarithromycin after oral drug administration was 19.5±5.5 L/h [15]. Dose modification do not seem to be necessary in hepatic impairment patient with normal kidney function because there is less conversion of clarithromycin to 14-hydroxy metabolite resulting in decreased plasma concentration of metabolite and also high excretion of unchanged drug [4]. The low clearance value is also a function of plasma protein binding.

5. CONCLUSION

The objective of this study to assess the pharmacokinetic parameters of clarithromycin in human females at an oral dose of 250 mg. The value of different pharmacokinetics varies when compared to literature leads to the conclusion that there is a need of dose adjustment in Pakistani population in an order to achieve the desired therapeutic effect. Difference with the literature could be due to decrease volume of tissue to which the drug distributes, decrease blood flow, the plasma protein binding, environmental condition, dietary habits, fast metabolism, genetic morphology etc. However, more studies are required to evaluate the genetic effect on pharmacokinetic of clarithromycin in human on a large scale to verify the factors that are cause of difference. Also, studies of clarithromycin are required in established pain model for further modification in dose.

REFERENCES

[1] Bekele, L.K. and Gebeyehu, G.G. 2012. Application of different analytical techniques and microbiological assays for the analysis of macrolide antibiotics from pharmaceutical dosage forms and biological matrices. ISRN Analytical chemistry, 1, 1-17.

[2] Farshchi, A., Ghaisi, G., and Bahrami, G. 2009. A sensitive liquid chromatographic method for the analysis of clarithromycin with pre-column derivatization: Application to a bioequivalence Study. Iranian journal of basic medical sciences, 12, 25-32.

[3] Wibawa, J.I.D., Shaw, P.N., and Barret, D.A. 2003. Quantification of clarithromycin, its 14- hydroxy and decladinose metabolites in rat plasma, gastric juice and gastric tissue using high-performance liquid

chromatography with electrochemical detection. Journal of chromatography, 783, 359-363.

[4] Zuckerman, J.M. 2004. Macrolides and ketolides: azithromycin, clarithromycin, telithromycin. Infectious disease of clinical north America, 18, 621-649.

[5] Javed, I., Rahman, Z.U., Khan, F.H., Muhammad, F., Iqbal, Z., and Aslam, B. 2006. Renal clearance and urinary excretion of kanamycin in domestic ruminants species. Pakistan Veterinary Journal, 26, 1-8.

[6] Ali, A., Afzal, S., Ashraf, M., Amin, S., Usman, M.J.A. 2012. Pharmacokinetic study of ketoprofen in healthy sheep under local conditions of Pakistan. The Journal of Animal and Plant Sciences, 22, 588-592.

[7] Bahrami, G., and Mohammadi, B. 2007. Determination of clarithromycin in human serum by high-performance liquid chromatography after pre-column derivatization with 9-fluorenylmethyl chloroformate: Application to a bioequivalence study. Journal of Chromatography, 850, 417–422.

[8] Forouten, S.M., Zarghi, A., Shafaati, A., Madadian, B., and Abolfathi, F. 2013. Rapid high-performance liquid chromatography method for clarithromycin in human plasma amperometry detection: Application in pharmacokinetic and bioequivalence studies. Iranian journal of pharmaceutical research, 12, 65-69.

[9] Steel, R.G.D., Torrie, J.H., and Dicke, D.A. 1997. Principles and procedures of statistics: A biometrical approach, 3rd Edition, McGraw Hill Book Co, 284-231.

[10] Shargel, L., and Yu, A. 1999. Applied Biopharmaceutics and Pharmacokinetics, 4th ed., Appleton & Lange, Stamford, 154-163.

[11] Alkhalidi, B.A., Tamimi, J.J., Salem, I.I., Ibrahim, H., and Sallam, A.A. 2008. Assessment of the bioequivalence of two formulations of clarithromycin extended-release 500-mg Tablets under fasting and fed conditions: a single- dose, randomized, open-label, two-period, two-way crossover study in healthy Jordanian male volunteers. Clinical therapeutics, 30, 1831-1843.

[12] Alffenar, J.W.C., Nienhuis, De Velde, F., Zuur, A.T., Wessels, A.M.A., Almeida, D., Grosset, J., Adjei, O., Uges, D.R.A., and Werf, T.S.V.D. 2007. Pharmacokinetics of rifampin and clarithromycin in patients treated for Mycobacterium ulcerans infection. Journal of antimicrobial chemotherapy, 54, 3878-3883.

[13] Madan, P.L. 2000. Biopharmaceutics and Pharmacokinetics, 1st ed., Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, 406-494.

[14] Amini, H., and Ahmadiani, A. 2005. Sensitive determination of clarithromycin in human plasma by high-performance liquid chromatography with spectrophotometric detection. Journal of chromatography, 817, 193-197.

[15] Shin, J., Pauly, D.F., Johnson, J.A., and Frye, R.F. 2008. Simplified method for determination of clarithromycin in human plasma using protein precipitation in a 96-well format and liquid chromatography–tandem mass spectrometry. Journal of chromatography, 87, 130–134.