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PHARMACOKINETICS AND PK/PD MODELING OF AMOXICILLIN IN HUMAN MALE AND FEMALE VOLUNTEERS

Naima Batool[1], Neelam Iqbal[1], Mehak Gull[1] and Zahid Manzoor[2]*

[1]Nishtar Medical University, Multan, Pakistan.

[2]Department of Biomedical Sciences, King Edward Medical University, Lahore, Pakistan.

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***Corresponding Author**
Dr. Zahid Manzoor
Department of Biomedical Sciences, King Edward Medical University, Lahore, Pakistan.

ABSTRACT

The present study aimed to investigate the pharmacokinetic behavior in terms of Pharmacokinetic(PK)/Pharmacodynamic (PD) Modeling of amoxicillin in human male and female volunteers. For this purpose, ten healthy, adult male and ten healthy adult females of 20-25 years of age were randomly selected. The detailed study procedure was told to the each human volunteer and consent was taken from each individual prior to the study. Amoxicillin (500 mg capsule) was administered orally to each volunteer. Blood samples were collected at specific time intervals. Plasma drug concentrations and time data was subjected to WinNonlin® for the estimation of pharmacokinetic parameters. Based upon the pharmacokinetic and pharmacodynamic data, PK/PD indices were estimated in order to optimize the dosage regimen of amoxicillin in humans. Student's T-test was applied to observe the significant difference of the pharmacokinetic parameters between male and female human volunteer. $P \leq 0.05$ was considered as significant. Amoxicillin was well distributed in the body after its absorption as indicated by their respective AUC values in both male and female human volunteers. However, the drug was slowly eliminated ($P < 0.05$) in human female volunteers and stayed in the body for longer period of time as compared to human male volunteers. The values of PK/PD indices fall below the recommended values suggesting the individual dose optimization of amoxicillin in humans.

KEYWORDS: Amoxicillin, Human volunteers, Pharmacokinetics, PK/PD Modeling.

INTRODUCTION

The impetus to search for a novel antibiotic always remains a challenge for human being. Antimicrobial agents play a vital role to combat the bacterial infections. Amoxicillin is an

antimicrobial agent belongs to the beta-lactam antibiotics and is commonly prescribed in respiratory diseases, skin infections, and urinary tract infections.[1-2] It has good affinity to penicillin-sensitive gram-positive and gram-negative bacteria.[3] Amoxicillin has very good tissue absorption and has very little toxicity, that's why the use of this drug is very common in clinics.[4] It shows its antibacterial action by inhibiting an enzyme (DD-transpeptidases), which is mainly responsible to maintain the integrity of bacterial cell wall.[5] Due to the inhibition of this enzyme, the transpeptidation reaction may not occur and bacterial cell wall becomes fragile ultimately leading to the death of bacterial cell. Hence, the mode of action of amoxicillin is bactericidal in nature.

Amoxicillin has very little affinity to bind with plasma proteins. Hence, the major fraction of the drug is widely distributed in the body and penetrates to the different organs and tissues. But it fails to cross the blood brain barrier except in case of meningitis.[6] Amoxicillin is mainly metabolized in the liver where its two metabolites (amoxicilloic acid and diketopiperazine) are formed. These metabolites have no any significant role in the antibacterial activity of the drug and its bactericidal action is mainly due to the parent compound of the drug.[7]

The resistance to antibiotics is a major concern in humans. It appears due to extensive use of antibiotics and in inaccurate doses. Therefore, it is essential to use antibiotics only when it is required and it should be used in proper dosage regimen. In addition, the pharmacokinetic profile of a drug varies with a change in the environment and topography as the environment has direct effect on the genetics of human population. Asian people have different environment as compared to Western countries so the pharmacokinetic profile of the same drug will be different when investigated in European and Asian people. Such environmental and genetical variations are explained in animals.[8-9-10] Therefore, the current study has been planned in order to investigate the variation in the pharmacokinetic profile among Asian and Western people and also to observe the sex-based variation in the disposition of amoxicillin in human volunteers. Moreover, modern PK-PD approach in order to optimize the dosage regimen of an antibiotic is also estimated in the current study.

MATERIALS AND METHODS

Human Subjects

To determine the pharmacokinetic profile of amoxicillin, ten healthy male volunteers were selected randomly from local population of Multan District. The protocol of the study was according to FDA guidelines.

Ethical consideration

The detailed procedure was demonstrated to all the human subjects. Complete information regarding drug, its safety profile, toxicity, interaction of drug with food and other drugs, experimental procedure was provided to all the human subjects. A consent form was filled from all the human subjects prior to the study.

Place of study

The study was conducted in the Department of Pharmacology, Nishtar Medical University, Multan.

Selection criteria

The pharmacokinetic profile of a drug may be influenced by certain factors like age, genetic makeup, diet, disease status of subjects, gender, drug interactions, environmental changes etc. Therefore, ten males and ten female healthy human subjects from same age group 21-25 years were selected for the study. History of any disease and clinical investigations of all human volunteers were taken to declare healthy status of these subjects. Same kind of food was provided to all human volunteers on the day of sampling. No medication was taken by any volunteer at least 7 days before initiation of the study.

Drug administration

After overnight fasting, the selected human volunteers were given 500 mg amoxicillin capsule (Glaxosmithkline®, GSK, Pakistan).

Collection of blood samples

For the collection of blood, one of the brachial veins was cannulated under aseptic conditions with plastic cannula. The blood samples were collected in EDTA (Ethylene diamine tetra acetic acid) tube. A blank blood sample (3 mL) was collected before drug administration. Then blood samples were collected at specific intervals of time i.e. 1, 2, 3, 4, 6, 8, 10, 12, 24,

36, and 48 h post drug administration. Blood samples were centrifuged at 4000 rpm for 10 min and plasma was separated and preserved at -20°C until analysis.

Analytical procedure

The concentration of amoxicillin in samples were determined by High Performance Liquid Chromatography (HPLC) according to the method described by Mascher and Kikuta.[11]

MATERIALS

All the reagents and chemicals were HPLC grade. Amoxicillin was purchased from (Glaxosmithkline®, GSK, Pakistan). Fluram was purchased from Sigma-Aldrich. All other chemicals and reagents were procured from Merk Globe Chemicals, Private Limited, Pakistan.

Instrumentation of HPLC

The HPLC system consists of Agilent 1200 Series binary LC gradient system. The system was connect with quaternary pump having flow range of 0.001-10 mL/min. A C18 column (ZORBAX SB RRHT C18) with dimensions 2.1mm × 50 mm was packed with the system. The pore size of the analytical column was 1.8µm. The column was thermostatted at 30ºC. A standard auto-sampler injection was calibrated with HPLC system. A vacuum degasser with internal volume of 12 mL having capacity of 10mL/min was integrated with system. A fluorescence detector for multisignal detection and online fluorescence spectra was connected to the HPLC system. The output of the system was controlled by Agilent ChemStation software.

Composition of mobile phase

It consists of a mixture of 0.02M methanesulfonic acid and acetonitrile (92:8, v/v). The flow-rate of the mobile phase was 1.1 ml/min. The post-column reaction was made by using two different pumps: first for delivering the 0.2M buffer (pH 8; 27.2 g of KH_2PO_4) and 7.45 g of NaOH per 500 ml of water) and second for the reagent (0.01% Fluram in acetonitrile).

Sample preparation

Serum samples were thawn at 20ºC for 10 minutes in a water bath. First of all, 0.3 mL of sample was taken and mixed tieh 50 mL of 20% perchloric acid for 15 seconds (pH: 1.0). This mixture was then subjected for centrifugation at 2000 g for 2 minutes. After centrifugation, 150 mL of the clear supernatant were transferred into an autosampler glass

vial and mixed with 150 mL of 1M sodium acetate solution whose pH was 5.0 within 30 minutes. 20µL of this solution was subjected to HPLC system.

Pharmacokinetic parameters

The obtained data of plasma concentrations at different time intervals were subjected to a computer program WinNonlin® (Pharsight, version 4.6, USA). Data were analyzed by non-compartmental and compartmental analysis. Minimum Akaike Information Criteria was compared to determine the best fit model among these human subjects.

Pharmacokinetic-Pharmacodynamic Modeling

Amoxicillin has very good potential against Staphylococcus species of bacterial pathogen. To estimate the PK-PD surrogate markers, the MIC_{90} of 2.0 µg/mL of amoxicillin against *Staphylococcus aureas* has been used in this study.[12] From the *in vivo* PK parameters and MIC_{90} values, the surrogate marker (AUC_{24}/MIC_{90}, C_{max}/MIC_{90}) was determined after oral administration of amoxicillin in human subjects.

Statistical analysis

Mean with standard deviation values for each parameter was calculated by Microsoft Excel version 2007. Two tailed Student's T test was used to compare the pharmacokinetic parameters in human male and female volunteers. $P \leq 0.05$ was considered as significant.

RESULTS

Pharmacokinetic

Data were best fitted by one compartmental model in each human subject. The (mean±SD) plasma values of amoxicillin at different time intervals in human male and female volunteers are presented in Figure 1.

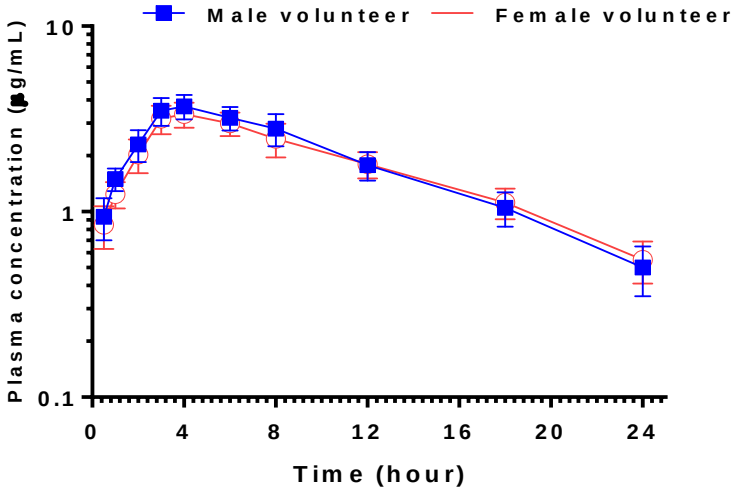

Figure 1: (Mean±SD) plasma concentration of amoxicillin (500 mg) after oral administration in human male and female volunteers on logarithmic scale at different time intervals.

The pharmacokinetic parameters of amoxicillin following oral administration (500 mg) in human male and female volunteers depicted in Table 1.

Table 1: Pharmacokinetic parameters of amoxicillin after oral administration of 500mg capsule in human male and female volunteers (n = 10).

Parameters	Unit	Male volunteers	Female volunteers
C_{max}	(µg/mL)	3.70±0.14	$3.35±0.12^{NS}$
T_{max}	(h)	$4.05±0.10^{NS}$	$4.15±0.53^{NS}$
K_{abs}	(h^{-1})	$0.37±0.03^{NS}$	$0.41±0.06^{NS}$
$T_{1/2abs}$	(h)	$1.08±0.02^{NS}$	$1.16±0.04^{NS}$
V_c	(L/kg)	$9.48±0.21^{NS}$	$10.24±0.14^{NS}$
K_{el}	(h^{-1})	$0.14±0.04^{NS}$	$0.10±0.06^{NS}$
$T_{1/2\ el}$	(h)	4.73±0.93*	4.96±1.24*
Cl_B	(L/h/kg)	$0.37±0.12^{NS}$	$0.29±0.14^{NS}$
AUC_{0-24}	(µg.h/mL)	$21.4±1.47^{NS}$	$19.9±1.97^{NS}$
MRT	(h)	11.47±0.68*	12.58±0.47*

*Means are significant ($P < 0.05$) to each other in a row, ^{NS}Non-significant difference, C_{max} = Maximum plasma concentration, T_{max} = Time to reach maximum concentration, K_{abs} = Absorption rate constant, $T_{1/2abs}$ = Absorption half-life, V_c = Volume of central compartment, K_{el} = Elimination rate constant, $T_{1/2el}$ = Elimination half-life, Cl_B = Total body clearance, AUC = Area under plasma concentration-time curve, MRT = Mean Residence Time.

After oral administration, drug was absorbed from the stomach and intestine to the systemic circulation with $T_{1/2abs}$ values of 1.08 and 1.16 hours in human male and female volunteers respectively. The difference between the absorption half-life was insignificant among both genders. Drug reached its maximum concentration as 3.70±0.14 and 3.35±0.12 µg/mL at T_{max} of 4.05±0.10 and 4.15±0.53 hours in human male and female volunteers respectively. There was an insignificant difference for these values in both genders. After achieving the maximum concentration in plasma, amoxicillin was decreased gradually with a constant rate of K_{el} = 0.14±0.04 hour in human male volunteer and K_{el} = 0.10±0.06 hours in female volunteers. The elimination half-life was observed to be 4.73±0.93 hour in human male and 4.96±1.24 hour in human female volunteers. Amoxicillin was slowly eliminated ($P < 0.05$) in female volunteers and remained in the body for longer period of time as indicated by their respective MRT values when compared to human male volunteers. The total body clearance was 0.37±0.12 L/h/kg in human male and 0.29±0.14 L/h/kg in human female volunteers.

PK/PD Modeling

To suggest the proper dosage regimen of an antibiotic three surrogate markers are used for antibiotics. These surrogate markers (AUC_{24}/MIC_{90}, C_{max}/MIC_{90}, $T > MIC_{90}$) depends on the pharmacokinetic and pharmacodynamic data in order to generate the required levels of these PK/PD indices. The pattern of amoxicillin is a time-dependent bacterial killing,[13] hence, the PK/PD indices of $T > MIC_{90}$ is mainly concerned in order to optimization of dosage regimen of amoxicillin in humans as shown in Figure 2.

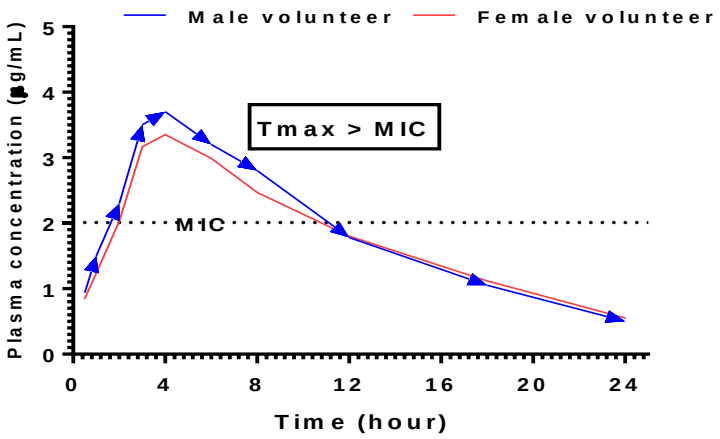

Figure 2: Mean plasma concentration of amoxicillin after its oral administration (500 mg) in human male and female volunteers at different time intervals indicating the MIC value of *Staphylococcus aureas* against amoxicillin in humans.

The values of PK/PD indices are depicted in Table 2. The value of AUC_{24}/MIC_{90} of 10.7 and 9.95 hour was suggested in male and female volunteers respectively. The ratio of C_{max}/MIC_{90} were 1.85 and 1.67, whereas $T > MIC_{90}$ values were 9.86 and 8.94 suggested for amoxicillin in human male and female volunteers.

Table 2: PK-PD indices of amoxicillin after oral administration (500 mg) in human male and female volunteers (n = 10).

Gender	Surrogate Marker	Value	Unit
Male Volunteers	AUC_{24}/MIC_{90}	10.7	hour
	C_{max}/MIC_{90}	1.85	hour
	$T_{max} > MIC_{90}$	9.86	Hour
Female Volunteers	AUC_{24}/MIC_{90}	9.95	Hour
	C_{max}/MIC_{90}	1.67	Hour
	$T_{max} > MIC_{90}$	8.94	Hour

DISCUSSION

The various pharmacokinetic parameters found in our study are in good agreement with those reported previously.[14][15] However, there is a great variation regarding the C_{max} value of the drug[14]. Spyker and his co-workers[14] administered 500 mg of amoxicillin orally, and Welling and his co-workers[16] administered two 250 mg capsules, reported peak levels of 11.8 and 10.0 µg/ml respectively. In addition, Arancibia & his colleagues[17] reported the C_{max} value of amoxicillin after intravenous administration as 10.4 µg/mL. Kirby[18] reported this value as 7.6 µg/mL. Since there are many factors which influence the absorption of drugs from the gastrointestinal tract, hence, this difference is not surprising. It has been reported that the volume of water taken with the capsules may significantly influence the amount of amoxicillin absorbed. Reducing the water volume from 250 to 25 ml caused a significant reduction in the serum amoxicillin level in fasted subjects.[15]

Dose optimization of antibiotics is a very serious concern as the emergence of bacterial resistance arises when an inappropriate dosing of antibiotic is used in humans. To minimize the bacterial resistance, it is necessary to use an accurate dose of an antibiotic which is sufficient to kill the mutant resistant bacteria in order to achieve successful outcome of the therapy. Hence, antibiotic dose should be optimized in human beings. Amoxicillin has a great potential against a variety of microorganisms *in vitro*. Various pharmacokinetic/pharmacodynamic (PK/PD) indices have been proposed to predict the effectiveness of an antibiotic.[19] Three PK/PD indices are commonly used. These include T > MIC when the antibiotic is time dependent, AUIC (AUC/MIC) and C_{max}/MIC when antibiotic

is concentration dependent. Recently, Nielsen and Friberg[20] propesed that the surrogate marker (AUC/MIC) is the better predictor of antibacterial activity of β-lactams antibiotics. White[21] determined the MIC_{50} of amoxicillin of 0.125 µg/mL for the majority of the bacterial pathogens. It is reported that the value of AUC/MIC ≥125 hour provide optimal efficacy against most pathogens.[22] To calculate AUIC in the current study, the MIC_{50} of 0.125 µg/mL has been used for amoxicillin against most of the pathogenic bacteria. The obtained AUIC values were 171 and 159 hour in human male and female volunteers respectively. So, it is speculated that the current dose (500 mg) of amoxicillin is sufficient to kill most of the bacteria in humans. But the optimal dosage is that amount of drug which rapidly kills the microorganisms and prevents the re-growth of bacteria without any support of the defensive system of the body.[23] So, MIC_{90} should be used in order to calculate the optimal dosage regimen of an antibiotic. Veloo[24] suggested the MIC_{90} of amoxicillin to be 2.0 µg/mL for majority of the bacterial pathogens. By incorporating (MIC_{90} = 2.0 µg/mL) of amoxicillin, the determined values of AUIC were 10.7 and 9.95 hours in human male and female volunteers respectively. The values of the surrogate marker obtained by PK-PD integration in the present study fall below of suggestive values (AUIC = 125 hours). Hence, it is supposed that the current dose of 500 mg will promote the resistant mutants in humans. However, further detailed pharmacodynamic studies of amoxicillin in humans are required to identify an actual as opposed to an assumed MIC_{90} for confirming the dose by generating sufficient data from field isolates in future studies before issuing final recommendations.

CONCLUSION

Based on the findings of the present study it was concluded that the current administered dose of 500 mg by oral administration seemed to be effective against most of the respiratory pathogens in human male and female volunteers. However, in the light of PK/PD modeling, the optimal doses should be optimized in order to achieve successful therapeutic outcome and prevention of mutant selection pressure in humans. It is prudent to conduct further detailed pharmacodynamic studies of amoxicillin against enteric disease causing organisms in humans in order to establish PK-PD inter-relationships.

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