PHARMACOKINETIC PROFILE OF CHLOROQUINE IN HEALTHY PAKISTANI SUBJECTS

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ABSTRACT

Aim of Study: To study pharmacokinetics of chloroquine in healthy adult Pakistani subjects and evaluate the role of pharmacokinetic factor in the dynamics of drug resistance in Plasmodium falciparum.

Design: This was an experimental study.

Place and Duration of Study: The study was conducted at National Institute of Health Islamabad during the year 2001.

Materials and Methods: Eleven healthy male volunteers, who met the defined inclusion criteria, were recruited into the study after obtaining informed consent. Blood samples were drawn at specified time intervals before and after administration of chloroquine tablets equivalent to 600 mg base. Plasma was separated and extracted according to the methods described in literature. Chloroquine estimation was carried out using High Performance Liquid Chromatography.

Results: Data of plasma concentration of chloroquine in 10 healthy volunteers is reported in this study. Mean pharmacokinetic parameters worked out from the data are Area under the Curve = 10.827, Peak Concentration = 201 ng/ml, Time to Peak = 6.1 hours, Half Life Phase-1 = 2.62 hours, Half Life Phase-2 = 41 hours, Volume of Distribution = 149.24 Litres, Clearance = 55.95 litres/hour, Mean Residence Time = 58.53 hours.

Conclusion: The pharmacokinetic profile of chloroquine in healthy adult male Pakistani subjects is comparable to that described in other ethnic populations of the world. The pharmacokinetic factor may play a role in selection of partially chloroquine-resistant parasites when the drug is used for prophylaxis of malaria on weekly basis.

Keywords: Pharmacokinetics, chloroquine, chloroquine-resistance.

INTRODUCTION

The safe and effective use of drugs requires an understanding of their pharmacokinetic profile. The optimal size and frequency of individual doses administered by various routes, the appropriate size of loading dose, when required, and the time course of accumulation in the body during continued administration are all functions of pharmacokinetic fate of a drug. [1]. The use of mathematical models to describe the Pharmacokinetic processes allows predicting about drug concentrations in various parts of body at a specified time as a function of dose and route of administration. The knowledge of pharmacokinetic behavior enables the clinician to choose dosage schedules that will rapidly produce and constantly maintain the desired concentration of the drug at its site of action. There has been increasing application of pharmacokinetics to clinical medicine, particularly with a view to individualizing of dosing [2].

Basic pharmacokinetics of chloroquine were investigated and described during 1940s soon after its introduction in clinical use. However, the techniques available at that

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time for estimation of chloroquine concentrations in biological fluids were primitive and less sensitive. With the development of more specific and highly sensitive assays there has been a renewed interest in exploring the pharmacokinetics of chloroquine. Particularly, in the wake of development of drug-resistance in Plasmodium falciparum, there is a need to explore the role of pharmacokinetic factor in this phenomenon [3].

Pharmacokinetics of chloroquine has been described in Caucasians [4] and Africans [5]. However, pharmacokinetic data of the drug has not been published in Pakistani population. In view of the fact that pharmacokinetic profile of a drug may vary in different populations due to environmental and genetic factors [6], we planned to study the pharmacokinetics of chloroquine in healthy Pakistani subjects and evaluate the role of pharmacokinetic factor in the dynamics of drug-resistance.

SUBJECTS, MATERIALS AND METHODS

The study was carried out at the Drugs Control & Traditional Medicines Division of the National Institute of Health Islamabad during the year 2001. Fifteen healthy male volunteers were recruited into the study but sampling could be completed in 11 subjects due to non-compliance of the remaining to report at specified intervals of time. Their ages ranged from 23 to 48 years and weights from 58 to 74 kilograms. The nature and purpose of the study was carefully explained to the volunteers and only those who willfully consented to participate were admitted to the study. The following exclusion criteria [7] were followed.

- Persons with any hepatic or renal disease.
- Persons showing any abnormality of routine blood or urine analysis.
- Persons having used chloroquine during previous three months.
- Persons taking any other drugs.
- Smokers

Four tablets of chloroquine sulfate (Nivaquin P, Rhone Poulenc Rorer, Pakistan) each containing 200 milligram of the salt, equivalent to 150 mg of chloroquine base, were administered to each study subject orally with water after an overnight fast. The subjects were allowed a light breakfast three hours after the drug administration. Venous blood samples were drawn from antecubital vein at 0, 0.5, 1, 2, 4, 8, 12 and 24 hours after administration of the drug. Subsequently, blood was taken at 24 hours intervals for a period of 7 days. The samples were drawn through an indwelling canula during the first 24 hours and through direct venepuncture subsequently. Three ml of blood was withdrawn on each occasion into a lithium-heparin bottle. Plasma was separated by immediate centrifugation at 1000 G, transferred to screw-capped plastic bottles and stored at 20^{O}C until analyzed [8].

Urine was collected before administration of chloroquine and for 24 hours on first, second and 7th day after drug administration. Aliquots of 20 ml were stored at 20^{O}C in plastic tubes.

Extraction of Samples:

One ml of plasma was mixed with an equal volume of 1N sodium hydroxide and 30 ml of n heptane and was shaken for 30 minutes. After extraction, 25 ml of the mixture containing chloroquine was transferred to another tube and dried in a water bath at 30^{O}C. The residue from the tube was redissolved in 1:1 mixture of methanol with 0.1 M phosphoric acid and allowed to dry for further concentration. The dried samples were reconstituted in methanol / phosphoric acid solution to a volume of 100µL.

In case of urine samples, 100 µL were diluted to 1 ml with water and then extracted as described for plasma samples.

Chloroquine Estimation:

Concentrations of chloroquine in plasma and urine samples were measured by high performance liquid chromatography (HPLC), model LC-9A, Schimadzu Corporation Japan, using ultraviolet (UV) detector (SPD-6AV-VIS, Schimadzu Corporation Japan). The method described by Brown et al [9], 1982 was modified and standardized to use the UV detector instead of the fluorescence detector, which was used by the original authors but was not available at our centre. UV absorbance of chloroquine was scanned and optimum wavelength was determined (fig. 1). The calibration curve of chloroquine was prepared (fig. 2). The method is sensitive enough to detect as low as 5 nanogram of chloroquine on the column. The analysis was carried out using a 33 mm x 3.9 mm I.D, 10µm Bondapak C 18 column. The internal standard was (Amino-4 methyl 1 butyl amino)-4-chloro-7 quinoline (provided by Rhone Poulenc Rorer, France). The mobile phase consisted of acetonitrile (HPLC grade, E Merck) with 0.02M 1-heptanesulfonic acid (to adjust the pH at 3.4) at a pumping ratio of 66:34 with a flow rate of 1 ml/ minute. The column pressure ranged from 62 to 76 bars and the oven temperature was maintained at 37^{O}C. Ten µL of the sample was introduced into the column through the injector and absorbance was read at 344 nm. All samples were analyzed in duplicate and the results have been expressed as means. Peak heights/ areas were measured and concentration of chloroquine was computed with the help of an on-line integrator.

DATA ANALYSIS

The plasma concentration versus time data of chloroquine were analyzed on computer program APO, MWPHARM 3.02, Mediwall Products, Holland. Mean, standard deviation (SD) and graphs were used to describe the data.

RESULTS

Eleven healthy male volunteers were included in the study. Average age of subjects was 33.5 years and average weight was 66 kilograms.

Data of plasma concentration of chloroquine in 10 healthy volunteer subjects has been analyzed and reported in this study. Subject number 4 was an outlier from the remaining group and therefore, has been excluded from the final analysis.

The plasma concentration profile of chloroquine, after oral administration of a single dose of 600 mg base as chloroquine sulfate tablets (table-1). The plasma

Fig. 1: UV absorbance of chloroquine.

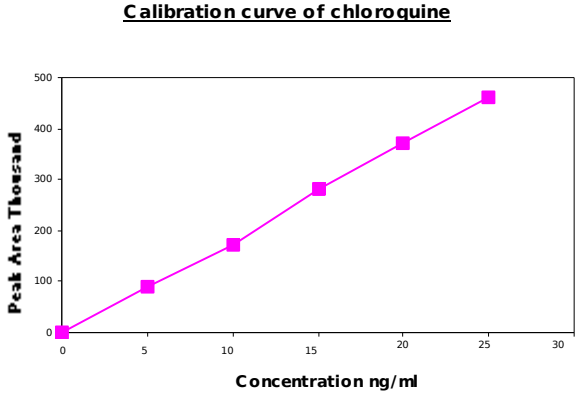

Fig. 2: Calibration curve of chloroquine.

concentration-time curves of chloroquine prepared on arithmetic and logarithmic scales (fig. 3&4 respectively) have been used to work out various pharmacokinetic parameters of the drug (table-2).

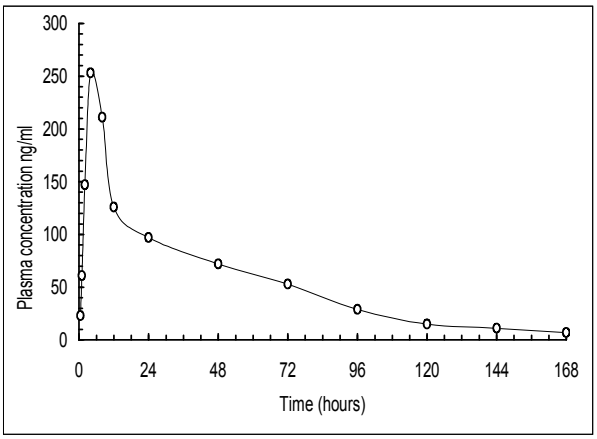

Fig. 3: Plasma concentration-time curve of chloroquine in healthy male volunteers (Mean ± SD, Arithmetic scale).

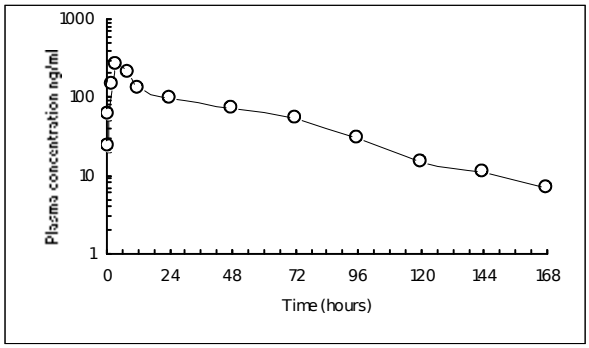

Fig. 4: Plasma concentration-time curve of chloroquine in healthy male volunteers (Mean ± SD, Log scale).

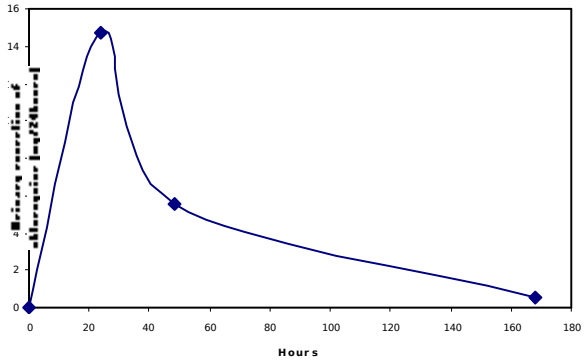

Fig. 5: Graph showing excretion of chloroquine in urine after single dose (600 mg base) oral administration in healthy volunteers.

The pattern of urinary excretion of chloroquine in the study subjects (table-3) and the same is depicted in (fig. 5).

DISCUSSION

Pharmacokinetic profile of chloroquine has been studied in healthy volunteers for the convenience of exploring the single-dose

Table-1: Plasma concentration profile of chloroquine following an oral dose of 600 mg base in healthy male volunteers.

Time (hour)	Mean	SD	Min	Max
0.5	37	8.4	23	50
1	65	9.7	53	81
2	124	14	107	147
4	230	23	187	261
8	224	23	173	251
12	138	12	123	157
24	110	9.5	95	123
48	85	8.4	72	98
72	65	7.8	53	79
96	37	6.9	27	51
120	22	5.2	15	33
144	15	3.9	10	22
168	11	4.5	5	18

Table-2: Pharmacokinetic parameters of chloroquine in healthy male subjects after oral administration of 600 mg base of the drug.

Pharmacokinetic parameters	Mean	SD	Min	Max
Area Under the Curve (AUC) [h.mg/l]	10.827	1.340	9.131	13.19
AUC polyexponential (t= 168)	10.213	1.073	8.825	12.04
AUC trapezoidal rule (t= 168)	10.384	1.071	9.00	12.21
Clearance (CL) [l/h]	55.95	6.844	45.49	65.72
Volume of distribution comp.1 [l]	69.29	10.58	59.60	95.36
Volume of distr. steady state [l]	137.67	5.40	129.77	148.05
Volume of distribution [l]	149.24	6.42	139.91	162.77
Half-life phase 1 [h]	2.62	0.34	2.15	3.30
Half-life phase 2 [h]	41.00	5.03	34.45	49.14
Rate constant k10 [1/h]	0.037	0.005	0.031	0.048
Rate constant k12 [1/h]	0.122	0.018	0.093	0.151
Rate constant k21 [1/h]	0.127	0.034	0.089	0.208
Mean Residence Time (MRT) [h]	58.53	6.49	51.26	69.92
Absorption rate constant (ka) [1/h]	0.269	0.032	0.213	0.323
Absorption half-life [h]	2.61	0.33	2.15	3.25
Lag-time [h]	0.14	0.09	0.02	0.32
Time to peak Tmax				

kinetics of the loading dose of the drug, which is administered clinically to saturate the binding sites in the body and to produce the therapeutic plasma levels. The data of this study can be extrapolated to malaria patients, because previous researchers have shown that pharmacokinetics of chloroquine is not significantly altered by the disease [10].

Table-3: Urinary excretion of chloroquine in healthy male volunteers after oral administration of 600 mg base of the drug.

Time	Mean ± SD (mg/24 Hrs)
Before Drug	0
24 Hrs	13.95±3.50
48 Hrs	5.01 ± 2.16
168 Hrs	0.32 ± 0.46

Absorption of chloroquine in our subjects started early with a lag time of 8.4 minutes. The samples drawn at 30 minutes after administration of the drug showed mean levels of 37 ng / ml. This level is slightly higher than the effective concentration of the drug against sensitive strains of Plasmodium falciparum i.e. 30 ng/ml and it is more than double than the effective concentration against Plasmodium vivax i.e. 15 ng/ml [11]. Detection of chloroquine levels above the minimum therapeutic levels in plasma, 30 minutes after oral administration, has also been reported by other workers [10,12].

In our study, absorption of chloroquine from gut progressed gradually and resulted in a mean peak concentration of 201 ng/ml. The mean time to reach the peak (Tmax) was 6.10 hours. In majority of the study subjects (7 out of 10), the peak was observed in the 4th hour plasma samples. The absorption half-life of 2.61 hours found in our study indicates prolonged absorption of chloroquine from gut. In the studies conducted by previous workers, dose- and route dependence of the peak plasma concentration of chloroquine has been described. Peak levels as low as 73 ng/ ml were found by Gustafsson et al, after oral administration of 300 mg of chloroquine to healthy male Caucasians [4]. On the other hand, the highest peak plasma concentration of chloroquine, after administration of an initial dose of 10mg/KG body weight followed by 3 doses of 5 mg/KG body weight each at 6,24 and 48 hours through naso-gastric tube, was 897 ng/ml that was observed in Gambian children [13]. Administration of a similar dosage regimen by oral route to Nigerian children suffering from acute falciparum malaria produced a peak concentration of 250 ng/ml [10]. In another study in the same country on similar subjects, the same dosage regimen produced a peak concentration of 143.9 mg/ ml [12]. Thus, there can be a wide variation in the peak plasma concentration of chloroquine under different and even similar clinical conditions. The peak concentration observed in our study is within the range described in the literature and is sufficient to exert anti-plasmodial action of the drug on the sensitive strains of the parasite.

The plasma concentrations of chloroquine remained above the therapeutic level for 96 hours after the single dose administration in our study. This shows inadequacy of single dose administration of chloroquine on weekly basis for prophylaxis of malaria. Such prophylaxis has been incriminated in promoting drug-resistance by selection of less sensitive strains of the parasite. Our results confirm that sub-therapeutic levels of chloroquine persist in plasma during the last three days of the week. In face of the decreasing sensitivity of P. falciparum to chloroquine and the changing epidemiological pattern of falciparum malaria in Pakistan, prophylactic use of the drug is not recommended.

Pharmacokinetics of chloroquine is characterized by high tissue binding which is evident from a large volume of distribution (Vd). The mean Vd found in our study is 149.24±6.42 Litres/Kg, which is within the range of 116 to 285 L/Kg described in the literature [3]. Based on the data reported in different studies, White et al [14] have calculated the mean Vd for chloroquine at

204L/Kg which is greater than that found in our subjects.

The rates of exchange of chloroquine from plasma to tissue compartment and then back to plasma are depicted by Rate Constants K12 and K21 respectively. Redistribution of chloroquine from tissues to plasma seems to continue over a long period of time. The variable affinity of chloroquine for various tissues makes the pharmacokinetics of the drug extremely complex and poses particular difficulties in the calculation of its half -life.

Half-life phase-1 of chloroquine was estimated to be 2.7 hours in the present study. This indicates half time of distribution phase of chloroquine in the body. This parameter has been reported in very few published studies and most of the researchers have focused their attention on the half-life in elimination phase of the drug. Adelusi et al, [10] have reported half-life of chloroquine for initial 24 hours after administration of the first dose of 10 mg/Kg body weight. They found it to be 8.06 hours, which is significantly longer that that found in the present study. However, in their study, a second dose of 10 mg/kg body weight, 12 hours after the first dose, had also been administered, which may have had altered the distribution phase of the drug by increasing the plasma levels.

Terminal half-life of chloroquine during the elimination phase was calculated to be 41.18 hours in our analysis. An extremely wide variation of this parameter is evident from the data reported in the literature. Frisk-Holmberg et al [15] calculated the terminal half life of chloroquine to be 42.9 hours based on sampling carried out for 50 hours. In our study the sampling was carried out for 168 hours but the terminal half-life is very close to that calculated by these workers. Tissues behave as a separate compartment for chloroquine with slow elimination rate constants. The drug therefore, continues to enter the circulation from tissues at a very slow rate. The calculation of terminal half- life therefore, depends on the duration of sampling. In the study carried out by Frisk-Holmberg et al [15] two different half-lives i.e. 42.9 and 312 hours were calculated after sampling for 50 and 1176 hours respectively. Aderounmu et al [7] have reported a terminal half-life of 216.9 hours based on sampling for 672 hours. The terminal half-life in our study is within the range described in literature, though longer periods of sampling may give extended value of this pharmacokinetic parameter. However, Titus [16] is of the view that terminal phases are of minor importance in determining effective half-life of chloroquine. This view is corroborated by the Mean Residence Time of chloroquine, which is calculated at 58.53 hours with our data.

Total Plasma Clearance of chloroquine was found to be 55.95 liters/hour or 932.47 ml/minute. A wide variation of this parameter has been reported by different researchers. A scientific group of World Health Organization [3], after reviewing the available data, has described the limits of clearance from 750 to 1050 ml/minute. The plasma clearance ascertained in our subjects is within this range.

The urinary excretory pattern of chloroquine in our study subjects is depicted in (table-3) and (fig. 3). It closely resembles that observed in healthy adult African subjects [7].

CONCLUSION

We conclude that the pharmacokinetic profile of chloroquine in healthy adult Pakistani subjects is comparable to that reported in other ethnic groups of African and South East Asian countries. It produces sufficient drug levels in plasma to exert antiplasmodial activity against sensitive strains of parasite. However, it is not suitable to administer the drug on weekly basis for prophylaxis, which can lead to selection of less sensitive strains of plasmodia.

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