SINGLE DOSE PHARMACOKINETICS OF MEFLOQUINE IN HEALTHY NIGERIAN MALE SUBJECTS.

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ABSTRACT

Single dose pharmacokinetics of mefloquine was determined in six healthy Nigerian male subjects. Mefloquine 500mg single dose was administered and blood samples were collected at intervals. Plasma concentrations were determined by RP-HPLC method after sample pre-treated step by solid phase extraction technique. Analytical characteristics of the HPLC system showed a plasma recovery of between 82.3 and 93% with an intra-day precision of 9.83% and 6.1% inter-day precision. Absorption was rapid with a $t_{1/2a}$ of 4.2h and a T_{max} of 10h. The corresponding Cmax was 1.77ug/ml and an AUCo-x of 19.05mg/L. day. The volume of distribution Vd/f was small, 9.43L/kg with a corresponding total plasma clearance of 0.453L/day/kg. The elimination was slow with a $t_{1/2B}$ of 18. 60 days.

INTRODUCTION

Mefloquine is a quinolinemethanol antimalarial found to be effective as single dose therapy for all species of malaria infecting man, including multi-drug-resistant *Plasmodium falciparum*[1]. It is used in both prophylaxis and treatment of malaria. There is no parenteral formulation [2].

Mefloquine, (R*S*)-(±)-a-2-piperidinyl-2-8-bis(trifluromethyl)-4-quinoline-methanol) hydrochloride was recently introduced to Nigeria. Mefloquine has been assayed in biological fluids by different chromatographic methods such as, TLC[3] with a limit of detection of 50mg/L, GC[4,5] and HPLC[6,78,9]. Bergqvist et al[8] reported analysis of the enantiomers of mefloquine in 100ul plasma adsorbed on chromatographic paper. All the methods described however used the conventional liquid-liquid extraction at the sample pre-treatment stage. This paper reports a solid phase extraction technique before HPLC analysis of mefloquine.

Desjardins *et al.*,[9] studied the pharmacokinetics of mefloquine in healthy male subjects using oral suspension and tablets. They reported an absorption half-life ($t_{1/2a}$) of 1.1h for oral suspension and 3.8h for tablets, with AUC of 35% higher than the oral suspension. Similarly many other workers studied the pharmacokinetics of mefloquine in humans (Kenyan, Brazilian, Malaysians, Caucasians and Thai) [1,10,11,12]. The time to peak concentration ranges from 7 to 20.7h with corresponding concentration of between 250 and 1872ug/L with an elimination half-life range of 6.5 to 22.7 days. These parameters are so varied and wide apart for a general statements for all races. Mefloquine like the other quilines is concentrated in the red cells by high affinity binding to the erythrocyte with a whole blood concentration of up to double those in the corresponding plasma samples of healthy volunteers[11]. However, some other workers reported to the contrary, that whole blood and plasma concentrations are similar[2,12].

All the reports stated that mefloquine is extensively bound to tissues and cleared slowly from the body with great inter-subject and ethnic variations. Based on the aforementioned we therefore find it worthwhile to study the pharmacokinetics of mefloquine in healthy Nigerian subjects. The sample pre-treatment stage involved solid phase extraction technique which has not been reported for mefloquine.

MATERIALS AND METHOD

Acetonitrile and methanol were obtained from Rathburn Chemicals (Walkerburn, UK), sodium lauryl sulphate (SLS) by Fison (Loughborough U.K), ammonium hydroxide and orthophosphoric acid by BDH chemical (England, UK). Sodium dihydrogen phosphate was obtained from Merck (UK). Water was distilled and further purified by using a Millipore Milli-Q system (Millipore-Waters, Milford, USA). Aquasil was obtained from Pierce (Illinois USA). All other reagents were of AnalaR or equivalent grade. The liquid chromatograph used was a modular system consisting of a jasco 980 pump and jasco 975 variable wavelength ultraviolet detector (jasco, Tokyo, Japan) and incorporated with Rheodyne 7125 injection valve (Cotati, CA, USA) fitted with a 20ul loop. The wavelength of detection used was 285nm. The chromatographic column was 100 x 2 mm I.D., slurry packed in the laboratory with 3 um particle size ODS Hypersil (HETP, Macclesfield, UK) at a pressure of 55 Mpa in a solvent of propan-2-01/hexane/methanol.

Method

The subjects were six healthy young adults aged from 30 to 37 years (mean $\pm$ SD = 32.2 $\pm$ 2.6 years) and weighing from 58 to 73kg (mean $\pm$ SD = 64.8 $\pm$ 6.0KG). Ethical approval was obtained from the Jos University Teaching Hospital. All subjects were confirmed fit to undertake the study and their written consent was obtained. All subjects wee non-smokers and none of the subjects took alcohol or any other medication a week before and during the study.

After an overnight fast the subjects were administered with single oral 500mg dose mefloquine tablet (Roche) with approximately 150ml of water. Venous blood (3ml) was then drawn through an indwelling catheter just before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 6, 9, 12, 24, 48 hours, 7, 15, 30 and 45 days after dosing. Blood was collected in EDTA-pretreated plastic blood sample bottles. The whole blood sample was centrifuged at 50 for 5min and 0.1ml plasma was pipetted out with a micropipette and adsorbed on filter paper. The filter paper was then air dried at room temperature (30°C). Subjects were requested not to eat or drink within the first 3 hours of the study.

Sample Pre-treatment

The blood-spotted parts of the filter paper were cut into pices (2-3mm diameter a piece) and placed into a plastic centrifuge tube (pre-treated with 0.2% aquasil to minimise drug adsorption) and 2M ammonium hydroxide (3ml) was added. A volume (0.1ml) of the internal standard, 3,4-dichlorophenybiguanide (1ug/ml), was immediately added. The centrifuge tube was stoppered and vortexed for 1 minute and allowed to sand at ambient temperature for 2 hours before subjecting to ultrasonication treatment for 1 hour at room temperature (26°C). The Bond Elut C18 cartridge was activated by adding methanol (2ml) to the cartridge and allowing it slowly pass through. The Bond Elut was subsequently conditioned by passing 2M ammonium hydroxide (0.5ml) through it.

The supernatant from the filter paper extract was transferred to the C18 Bond Elut cartridge which had previously been activated with methanol (2ml) and subsequently conditioned with ammonium hydroxide (1ml) and the washing also applied to the cartridge. A stream of air was allowed to through the cartridge. The cartridge was washed with ammonium hydroxide (1ml), followed by water (1ml) and finally with 65% methanol in water. The compounds, mefloquine and 3,4-dichlorophenylbiguanide were eluted 0.1% w/v perchloric acid in methanol (1ml). The extracts were collected in small conical plastic centrifuge tubes, evaporated under the stream of nitrogen at ambient temperature and the residue reconstituted in mobile phase (100ul) just before injection of 20ul into the chromatograph.

Chromatographic Separation

The ion-pair system of Taylor *et al.,*[7] for multidrug assay of antimalaria was adopted, modified and optimised. The mobile phase was Acetonitrile: 20mM phosphate buffer (42:58) containing 12mM TBA and 120mM SLS at pH 2.4 (with ortho-phophoric acid). The mobile phase was pumped with system pressure of 180kg/cm^2 at a flow rate of 0.4ml/min. The eluent was monitored with ultraviolet detector at wavelength 285nm and sensitivity set at 0.01 AUFS and chart speed of 0.2cm/min.

Recovery

The recoveries obtained by the above treatment of spiked samples were determined by plasma with Mefloquine at 0.5, 1.0 and 2.0ug/ml. Samples (plasma, 0.1ml) of these were adsorbed on filter paper, dried and subjected to the sample pre-treatment and calibration steps. The peak heights obtained during the subsequent chromatography were compared with those obtained by direct injection of aqueous standards of mefloquine containing the theoretical mass corresponding to complete recovery of each of the mefloquine concentrations.

Determination Limit

Determination limit for mefloquine was determined by preparing serial dilutions of mefloquine in aqueous solution and subsequently in plasma and applying 0.1ml of these to the filter papers. Extraction and chromatography was carried out as described above and the resulting peak height measured. Detection limit was taken as the concentration that have signal to noise ratio of 3 to 1. While determination limit was taken as two times the detection limit.

Accuracy and Precision

The within day (intra-day) accuracy and precision of the method was determined by assaying ten samples of filter paper piked with treated plasma using a single calibration procedure. Calibration equations were obtained by linear regression of peak height ratio (compound/internal standard) on concentration.

The day to day (inter-day) precision was taken as the variation in the slope of the calibration lines obtained on different days over a period of five days.

Pharmaokinetic Analysis

The standard pharmacokinetics equations for one and two compartment model are shown below as Equation (a) and Equation (b) respectively.

$C = B.\exp^{B1} - Co.\exp^{-kat}$ (a)

$C = B.\exp^{B1} + A.\exp^{x1} - Cpo.\exp.^{-kat}$ (b)

C = drug concentration at time t [ngml^{-1}, ugml^{-1}]
B = elimination rate constant (h^{-1})
ka = absorption rate constant (h^{-1})
x = distribution rate (h^{-1})
Cpo = hypothetical drug concentration at time 0, obtained from A+B = Cpo [ngml^{-1}]
B = intercept of back-extrapolated monexponential elimination slope b with ordinate [ngml^{-1}]
A = intercept of monoexponential distribution slope a with ordinate [gml^{-1}]

Preliminary pharmacokinetics values were obtained from concentration-time profile. Data were then fitted to these relationship above equation (a) and (b) using the Dixon, (1985)[12], non-linear regression curve fitting program, to obtain the least squares fit to the individual concentration-time curves. Standard pharmacokinetics parameters were then calculated. Elimination and absorption half lives were calculated from the ration 0.693/b and 0.693/ka respectively. AUCO-x was calculated by the trapezoidal rule from the beginning of the drug administration to the last data point and with extrapolation to infinity. The area from the last data point Ct to infinity was obtained as Ct/B. This was done with the non-compartment model. AUC is the area under the curve [ng/ml.h, or mg/L.day], obtained from the integral of drug blood level over the time from zero to infinity. It is a measure of the amount of drug absorbed and is in the body.

Total body clearance ClT (or Plasma clearance Cl_P) was calculated from the ratio of dose to area under the plasma drug concentration - time curve, assuming bioavailability was equal to one (F=1). Apparent Volume of distribution V_D/f[L or L/kg] was calculated from the ratio of plasma clearance to rate of elimination.

Student's two t-test was used to assess difference between paired data. Minitab software package for two group analysis was employed. Group data are presented as mean $\pm$ SD.

RESULT AND DISCUSSION

The system is selective for mefloquine, there was no interference by its metabolites, or other antimalarial drug. Taylor *et al.*,[7] in their analysis involving other commonly used antimalarial using a similar system, reported non interference of system with other antimalarial. The metabolites of mefloquine or any other polar metabolites were not retained on this ion-pair mobile phase system in the RP-HPLC.

Extraction from Filter Paper and Solid Extraction

Ammonium hydroxide 2M was found to be efficient and adequate for the extraction of mefloquine from the filter papers at the experimental temperature. A similar extraction was reported[8] for plasma dried on chromatographic paper but with mefloquine recovery of 70%. The extraction of mefloquine from the plasma/filter mixture in ammonium hydroxide, was carried out by the use of methanol activated C18 Bond Elut cartridge conditioned with ammonium hydroxide. The SPE method used avoided the problems of phase emulsion, large solvent volumes, impure and wet extracts associated with liquid-liquid extraction.

Recovery, Precision and Accuracy

Table I and II show the recovery, precision and accuracy of the assay method. The recovery in plasma ranged from 82.3 to 93%. These recoveries compared well with the reported recoveries[6,8,15]. The level of recovery is large enough for quantitative work. The intra-day (within-day) assay precision of method average 9.83% in plasma. This low coefficient of variation shows the analytical method to be good.

The calibration of the drug in plasma required the use of internal standard from the extraction stage. Dichlorophenylbiguanide [3,4-CPB] a metabolite of chloproguanil, an antimalarial agent not commonly used in Nigeria was used as internal standard. The retentation time of mefloquine was 10min and that of 3,4-CPB was 6.7min in this system. The calibration curve showed good lenity over the concentrations considered. The mean correlation coefficient was 0.990. The inter-day (day-to-day) assay precision of method was found to be

6.1% (C.V) and intra-day (within day) assay precision averaged 9.8% plasma.

Pharmacokinetics

Figure I show the mean (n_{-6}) concentration-time profile of mefloquine in plasma following 500mg mefloquine tablet. The mean pharmacokinetics parameters of mefloquine in plasma of healthy subjects were presented in Table III. The rate of absorption k_a was found to be $5.27h^{-1}$ with the corresponding $t_{1/2a}$ being 4.2±3.2h. The $t_{1/2a}$ of 4.2h in plasma is in close agreement with the reported [9] $t_{1/2a}$ 3.8h but longer than the 2h reported by Schwartz *et al.*,[3]. This absorption phase is fairly rapid. The time to peak concentration T_{max} was 10±7h in plasma. This agree with the reported[9] T_{max} 10.5h but shorter than the reported cases of about 14 to 20h by other researchers[1,2,3]. The Cmax was 1.77±0.2ug/ml obtained is higher than the ones reported earlier[1,9,14] but lower than that of Franseen *et al.*,[15]. The AUCo-x was found to be 19.05± 7.10mg/L.day. The volume of distribution V/day/kg was 9.43±3.77l/kg, with the corresponding total clearance of $Cl_{T/f}$ 0.453±0.15l/day/kg in plasma. The terminal elimination half-life $t_{1/2B}$ of mefloquine in this study was found to be 18.60±9.8 days.

The pharmacokinetics properties of mefloquine after a single oral dose of 500mg mefloquine hydrochloride tablet were adequately described by the triexponential function. In this study, the plasma concentration in healthy Nigerian adults after 500mg single dose oral melfoquine are generally similar to earlier reports[1,2,14,15]. The peak plasma concentration (1.77ug/mL) is relatively high when compared with some early reports[14,15] (1.1ug/mL) using similar dose treatment. The sample collection and treatment in plastic containers, [to which mefloquine get adsorbed], used by earlier workers might explain the low recoveries reported. In this study aquasil was applied to all glassware to prevent adsorption of mefloquine to glass. Also inter-subject and ethnic variability which has been reported by most of the authors cited above might have contributed to the differences in the plasma concentrations. However these values are still within the general range of C_{max} in the literature. Franseen *et al.*,[15] reported peak plasma concentration of 1.872ug/mL after 500mg dose followed by a further 500mg 8h later. The volume of distribution Vd/f of 9.43 L/kg in plasma is smaller than the Vd/f of 13.3kg reported by Schwartz *et al.*,[3] after 1000mg mefloquine dose. The volume of distribution is generally low as compared to earlier report[1,2,3], which probably explain the low clearance obtained in the report. The terminal elimination half-life $t_{1/2B}$ in this report was found to be 18.6h in plasma. This agreed with the reported half-life elimination[1,14].

CONCLUSION

The paper has reported an efficient, method of sample collection, storage and transportation. The solid phase extraction technique developed here has made mefloquine one of the chemical compounds being extracted with this versatile method. Pharmacokinetics parameters are herein reported for Nigeria subjects. The results showed inter-subject variations and when compared with earlier reports racial variation. The reported pharmacokinetics will be of value in calculating therapeutic doses in clinical practice in Nigeria.

Table 1: The analytical characteristics of method (inter-day precision of method in quantification of mefloquine).

Biological fluid	Mean Calibration slope, (n=5)	C.V. (Day-to-day Precision)	Mean correlation (n=5)	Determination limit ug/ml
Plasma on filter paper	0.472	6.10	0.990	0.04

Table 2: Analytical characteristics of method showing recoveries, intra-day (within-day) precision and accuracy of method

Biological fluid	Conc. spiked ($ugml^{-1}$)	n	Recovery (%)	C.V.	Conc. Obtained ($ugml^{-1}$)	C.V.
Plasma	0.5	10	83.3	12.5	0.460	13.0
"	1.0	10	93.3	10.0	1.060	12.2
"	2.0	10	82.3	7.0	2.12	16.5

Table 3: Pharmacokinetics parameter of mefloquine in six healthy subjects following oral 500mg

Parameter	Mefloquine	± SD
Ka(h^{-1})	5.27	2.38
B(day^{-1})	0.0445	0.018
$t_{1/2a}$(h)	4.20	3.15
$t_{1/2b}$(day)	18.56	9.79
T_{max} (h)	8.00	3.10
C_{max} (ug/ml)	1.77	0.23
AUCO-x(mg/L. day)	19.05	7.01
V_D(Lkg-1)	9.43	3.77
C_{LT}/f(L/day/kg)	0.453	0.151

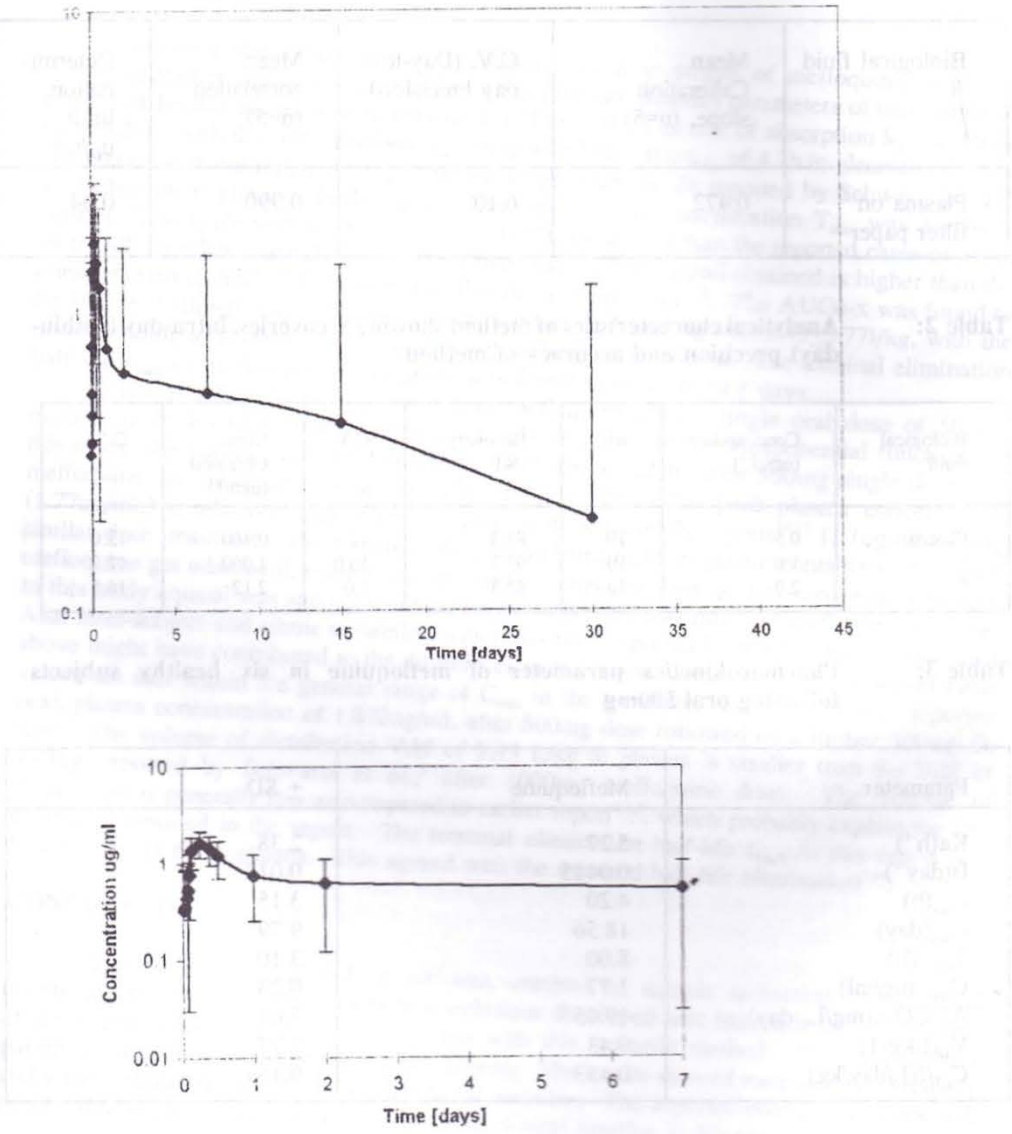

Figure 1: The mean concentration-time profile of mefloquine in plasma of six healthy subjects following oral 500mg mefloquine hydrochloride. (time scale; Upper 45 days, lower scale, first 8 days)

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