PHARMACOKINETICS OF LUMEFANTRINE IN HEALTHY PAKISTANI VOLUNTEERS

Nusrat Jafery, Muzammil Hassan Najmi

Army Medical College Rawalpindi

ABSTRACT

Objective: To study the pharmacokinetics of lumefantrine in healthy Pakistani volunteers so as to see the adequacy of the regimen in vogue for the treatment of malaria and prevention of recrudescence.

Place and duration of study: Department of Pharmacology and Therapeutics, Army Medical College Rawalpindi, from December 2006 to December 2007.

Study design: Quasi experimental study.

Subjects and Methods: Twelve healthy Pakistani male adult volunteers after informed consent participated in the study. Plasma concentration time profiles were measured after a single oral dose administration of 480mg of lumefantrine equal to four tablets of artemether –lumefantrine combination (Exafal). After extraction of lumefantrine with hexane-diethyl ether (70:30v/v) from plasma, it was analysed by HPLC (High performance liquid chromatography) using a C18 reverse phase ODS stainless steel column and a mobile phase of acetonitrile-0.1 M ammonium acetate (90:10, v/v) adjusted to pH 4.9 with ultra violet detection at 335 nm.

Results: The median absorption half-life of lumefantrine was 4.5 hours, with Time to reach peak plasma concentration (Tmax) 8.5 hours, plasma clearance 2.44 l/h and terminal elimination half-life of 89.5 hours. The Mean residence time (MRT) calculated ranged from 62.5 to125.6 (mean 98.17+17.18) hours. The day seven plasma concentrations in all the subjects, less one, were more than the cut-off value of 0.28mg/l required to prevent the recrudescent infection.

Conclusion: The overall pharmacokinetic profile of lumefantrine in Pakistani healthy volunteers appears to be comparable to other ethnic groups reported from various countries and the dose regimen used is adequate for the treatment and prevention of recrudescence.

Keywords: lumefantrine, pharmacokinetics, artemether-lumefantrine.

INTRODUCTION

Pharmacokinetic profile of a drug provides an understanding of absorption, distribution, metabolism excretion as well as possible interactions with other drugs. The knowledge about the pharmacokinetic behaviour of a drug is required for its safe and effective use and for determining the relationship between size and frequency of dose administration. Environmental factors like climate, smoking, alcohol consumption and diet may have profound effect on drug metabolism and disposition. Differences in diet may considerably alter the metabolism rate or drug blood levels among different ethnic populations[1].

Although the pharmacokinetics of artemether-lumefantrine has been studied in Thais, Malaysians, Chinese and Africans but to date there is no study conducted on Pakistanis. The studies on different groups report inter subject variability in pharmacokinetic parameters. The half life of lumefantrine was estimated at 87 hours in two Chinese trials, 74 and 107 hours in two Thai trials and only 30 hours in a European trial[2]. This is explained on the basis of difference in food intake and the metabolism of the drug by cytochrome 3A4 (CYP3A4). The wide variation in total CYP3A4 content seen among individuals has been attributed to both environmental and genetic factors which mean drugs cleared by this isoform are expected to have highly variable pharmacokinetics[3]. Apart from half life there is variation in other pharmacokinetic parameters like volume of distribution (Vd), it is 217 litres in a Thai study[4] and 159.2 litres in Malaysian volunteers[5]. A wide variation of plasma

Correspondence: Dr Nusrat Jafery, Pharmacology Department, Margala Institute of Health Sciences, Quaid e Azam avenue Gulrez III, Rawalpindi

Received: 11 Nov 2010; Accepted: 11 Apr 2011

clearance is apparent from studies published by various researchers. In Chinese study[6] plasma clearance was 6.6 litres / hour whereas it was 7.2 litres /hour in a Thai study[7].

This study was designed with a view to assess the pharmacokinetics of lumefantrine in Pakistani healthy subjects and to compare it with the documented pharmacokinetics of lumefantrine. Variations in the pharmacokinetics of drugs are the effect of differences in the rate of drug absorption, distribution, metabolism and elimination. These factors appear to be determined mainly by genetic predisposition and also probably by environmental factors such as diet and exposure to chemical agents[8]. It therefore is all the more important to conduct pharmacokinetic studies on local population.

MATERIALS AND METHODS

This quasi experimental study was conducted in the department of Pharmacology and therapeutics, Army medical College, Rawalpindi from Dec 2006 to Dec 2007. Twelve healthy Pakistani non-smokers adult male volunteers, were incidentally from different regions of the country to include Punjab, Khyber Pakhtoonkhwa, Northern Areas and Azad Kashmir, with age ranging from 20 to 36 (Mean 26.8 + 5.63) years and weight from 56 to 73 (Mean 64.4 + 5.58) Kilograms participated in the trial. The study excluded subjects with a history of drug sensitivity or allergy, liver disease, heart disease or significant electrocardiograph abnormalities, psychiatric disturbances and subjects who required regular medication. Subjects were excluded if they had taken any prescription medication within 4 weeks, or over-the-counter medications within two weeks of the start of the study. The nature and the rationale of the study were explained to the volunteers and those who consented with their own freewill to participate were inducted in the study. Ethics Committee approval of the protocol, consent form, and volunteer information document was obtained from the National University of Sciences and Technology's Review Board.

The chemicals and the solvents used were of analytical and HPLC grade from Merck, Dermastadt, Germany. Halofantrine used as internal standard was gifted by Glaxosmithkline Karachi, Pakistan and the external standard lumefantrine was obtained from the Hilton Pharma, Pakistan. Four tablets of artemether-lumefantrine (Exafal, Novartis Pakistan), each tablet containing 20mg of artemether and 120mg of lumefantrine equivalent to 80mg of artemether and 480mg of lumefantrine as a single dose were administered to each of the volunteers with a glass of water after an overnight fast. A normal breakfast was allowed after about an hour. Blood samples, for lumefantrine estimation in plasma, were taken by performing venipuncture and putting indwelling canula, for the first 12 hours and by direct venipuncture subsequently. Blood samples were taken at predose (0 hr) and at 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120, 168, 264 and 336 hours after administration of the drug. The blood sample was centrifuged without delay at 4°C at 2200 g for 10 minutes. The separated plasma was then transferred into screw-capped plastic cryovials and stored at -80°C to prevent breakdown of lumefantrine, until analysed[9]. The analysis was completed within 3 months after sampling.

Stock solutions of lumefantrine with strength of 1.0-20 ng /µl and the internal standard halofantrine (100ng/µl) were prepared by dissolving each substance in dimethylsulphoxide and stored at -20˚C. Calibration curve was prepared by spiking drug free plasma samples with standard solutions of lumefantrine and the internal standard halofantrine. The samples were then taken through the extraction procedure and the peak height ratio/area of the drug was plotted against the corresponding concentration of the drug. The method described[5] was used for the determination of concentration of lumefantrine in plasma. The extraction of lumefantrine was carried out in 15 ml glass test tubes. The test tubes were treated with dichlorodimethyl silane in toluene (5%, v/v). This was done to minimize the drug adsorption. A sample of 1 ml of plasma was taken and 50 µl halofantrine (5000 ng/ ml) and 1 ml of 0.1 M phthalate buffer (pH 2.0) was added and the mixture was

vortex mixed for 30 seconds. The resultant mixture was then extracted with 7 ml of hexane diethyl ether (70:30, v/v) by mechanical tumbling at the speed of 10 revolutions per minute for 30 minutes. This was then centrifuged at 1440 g, for 15 min. The aqueous phase was then frozen and the organic phase was separated. After separation the organic phase was evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was reconstituted in 50 µl of methanol- water-glacial acetic acid-diethyl amine (93:6:1:0.03) 20 µl of this was injected on to the column for analysis.

High Performance Liquid Chromatography (HPLC) Model LC-10AD (Shimadzu Corporation, Japan) with ultraviolet detector (SPD-.10A VP wave length range 190-370nm, Shimadzu Corporation, Japan) operated at 335 nm was used to measure the plasma lumefantrine concentration. Chromatographic separations were achieved on a 150 mm x 4.6 mm I.D., 10µm particle size C18 reverse phase stainless steel column (Shim pack, VP ODS, Shimadzu). The mobile phase was acetonitrile - 0.1 M ammonium acetate (HPLC grade, Merck). The pH of the mobile phase was adjusted to 4.9 with galacial acetic acid. The pumping ratio of 90:10 acetonitrile to 0.1M-ammonium acetate was maintained with a flow rate of 1.5 ml per minute. The oven temperature was maintained at 25˚C. The chromatograms were recorded on the connected computer (Pentium 4). Twenty micro litre of the extract of the sample was introduced into the column through the injector. All the samples were analysed in duplicate and the results have been expressed as means. The limit of quantification was 5ng/ml and the within day and day to day coefficients of variations were below 5%. Peak heights / areas were measured and concentration of lumefantrine was computed with the help of computer software APO, MW Pharm version 3.60 Mediware Holland. A two-compartment open model was assumed to derive the pharmacokinetic parameters. The assumption is based on a central compartment of small apparent volume and a peripheral compartment of larger apparent volume.

RESULTS

A total of 216 plasma samples including the predose (0hr) sample were drawn from the volunteers for the pharmacokinetic analysis. The plasma concentration-time curves of lumefantrine on linear (figure 1) and log scale were used to calculate the variety of pharmacokinetic parameters (Table I) of the drug. Lumefantrine was absorbed from the gastrointestinal tract with a mean lag time of 1.01+ 0.24 hours. The absorption rate constant of lumefantrine ranged from 0.10 to 0.31(mean 0.17 + 0.064) litres / hour. The absorption half-life was mean 4.5 + 1.4 hours. The results show that the plasma lumefantrine concentration increased with time and the mean peak concentration (Cmax) was 4.600 + 2.280 mg/l. The time (Tmax) at which peak concentration (Cmax) was achieved ranged from 4.8 to 11.7 (mean 8.5 + 2.15) hours. The apparent volume of distribution (Vd) that was estimated on the basis of a two-compartment model was 132.8 to 532 (mean 276.5 + 124.1) litres. Phase 1 (during first 24 hours) half-life of lumefantrine ranged from 2.23 to 6.95 (mean 4.5 + 1.4) hours. Half-life phase 2, which denotes the elimination half-life of lumefantrine, was calculated on the basis of fourteen days sampling. It ranged from 61.84 to 130.2 (mean 89.45+21.24) hours. Clearance of lumefantrine in the volunteers ranged from 0.84 to 5.23 litres / hour with a mean value of 2.44 + 1.34 litres /hour. In our study all the subjects except subject number two, had a plasma lumefantrine concentration well above the required level of 0.28 mg/l on day seven to prevent recrudescence. The mean concentration on day seven was 0.53 mg/l.

DISCUSSION

The present project was planned to study pharmacokinetics of lumefantrine in healthy volunteers after oral administration of a single dose of artemether–lumefantrine combination (Exafal tablets, Novartis) equivalent to 480 mg of lumefantrine and 80 mg of artemether under local environmental conditions. Healthy volunteers were selected because of the convenience of studying the single dose pharmacokinetics; moreover the single dose produces the therapeutic plasma levels, which

are maintained by the subsequent doses. The sampling was carried out up to the fourteenth day (336hrs) after drug administration to establish the elimination half-life of lumefantrine. In our study, lumefantrine was slowly absorbed from the gastrointestinal tract (GIT) with a mean lag time of 1.01 hrs. The absorption of lumefantrine from the gut was slow the peak plasma concentration was achieved slowly and resulted in a maximum concentration (Cmax) of 4.6mg/l. The mean time to reach the peak (Tmax) was 8.5 hrs. In eight out of twelve volunteers the peak was observed in the plasma sample taken after six hours of drug administration. In a study of healthy Chinese volunteers[10] the mean Tmax was 6.1 hrs and Cmax was 2.034 mg/l, which is lower as compared to our study. Since the peak plasma levels in a single dose drug study are largely determined by the rate of drug absorption from the gastrointestinal tract, the difference in time of peak plasma concentration in our study may be explained on the basis of intestinal motility and pH level in the GIT which may in turn be related to chronic effects of different dietary habits. Another source of considerable inter-individual variability is the activity of the enzyme CYP3A4 which causes metabolism of lumefantrine. Since it is also expressed in the human small intestine, it contributes to the first pass effect (11). The wide variation in the total CYP3A4 content seen among individuals, which has been attributed to both environmental and genetic factors, means that the drugs cleared by this isoform are expected to have highly variable pharmacokinetics[3].

The lumefantrine concentration and area under curve (AUC) values measured in Pakistani volunteers by us were unexpectedly high compared to a study in healthy Caucasian volunteers[12]. Absorption half-life of lumefantrine was estimated to be 4.5 hours with absorption rate constant of 0.043 hours in Pakistani volunteers. Absorption half-life reported in a Thai trial[4] was 5.3 hours while another study[2] reports 3.2 hrs of absorption half-life. The mean half-life phase-1 of lumefantrine in Pakistani volunteers was 4.5 hours. The mean elimination half-life in our study was 89.45 hours as compared to 30 hours in a European trial[12], 87 hours in Chinese trial[5], 74 and 107 hours in two Thai trials[4,6]. An exceptionally large variation in this regard is apparent from the review of the available pharmacokinetic data of the drug. Lefvere and Thomsen (1999) calculated the terminal half-life 30.9 hours after sampling for 168 hours while it was 69.5 hours in a Chinese study[10] after sampling for 336 hours. The terminal half-life and duration of sampling in our study is close to the Chinese trial. The terminal half-life determined in our study is within the range described in the literature, which is 3-4.5 days[13]. Since there is an extensive variation in terminal half-life in different ethnic groups it may be obligatory to carry out the sampling for longer period to ascertain a realistic value of the parameter.

Lumefantrine has a large volume of distribution (Vd). In our volunteers the mean Vd was 276.5 litres as compared to 159.2 litres in Malaysian volunteers[5]. The Volume of distribution in our study is comparable to the reported value of 217 litres in a Thai study[4]. Acute malaria does alter the apparent Vd of some antimalarial drugs e.g. quinine[14]; although the very large changes in AUC from dose to dose imply that changes in oral bioavailability rather than the Vd are the main contributor to inconsistency in plasma concentration profiles. In our subjects, total plasma clearance of lumefantrine was 2.44 litres /hour. Lumefantrine has an apparent low plasma clearance[2]. A wide variation of this parameter is apparent from studies published by various researchers. In Chinese study[6] plasma clearance was 6.6 litres / hour whereas it was 7.2 litres /hour in a Thai study[7].

Since lumefantrine is slowly and erratically absorbed followed by slow elimination it persists for much longer time than artemether in the blood after oral administration of the combined preparation (Exafal). The mean residence time (MRT) of lumefantrine, calculated from plasma concentration-time data of our study, was 98.17

hours. This indicates the average time of stay of the drug introduced into the body. This parameter has not been reported in other studies. The fixed drug combination ensures that no parasites are exposed to artemether alone and therefore there is no individual selective pressure to the emergence of artemisinin resistance. As few parasites are exposed to lumefantrine alone and that too to a relatively high concentration of the drug, the chances that a lumefantrine resistant parasite would be present and survive is reduced by approximately 10-fold as compared to that if lumefantrine were used alone to treat the disease. This shows that the lumefantrine AUC is associated with cure and when co administered with artemether to prevent recrudescence[2].

Plasma lumefantrine concentration of 0.28 mg/l (280µg/l) on day seven was found to be an alternative but useful discriminating cut-off to determine the likelihood of subsequent recrudescence[4]. A study[2] shows that if plasma lumefantrine concentrations on day seven were over 0.5 mg/l then 94 percent of the patients were cured, whereas if the concentrations were less than 0.28 mg/l then 49 percent had recrudescent infection. In a Thai study[7] 75 percent of the patients with plasma lumefantrine concentration above 0.28 mg/l on day seven were cured. In our study all the subjects had a plasma concentration on day seven well above the cut off value of 0.28 mg/l except subject number two who had a plasma concentration of 0.20mg/l. The mean residence time of lumefantrine and the half life phase-1 was reported first time in our study.

CONCLUSION

The overall pharmacokinetic profile of lumefantrine in Pakistani healthy volunteers, with some variations like Cmax and Tmax evaluated in our study are different from other studies, appears to be comparable to other ethnic groups reported from various countries. This shows that the dose advised to the patients of uncomplicated malaria in the beginning of the therapy produces adequate levels of the drug in plasma to exert antiplasmodial action, which may be maintained by administration of the subsequent doses. The single dose of artemether-lumefantrine combination (Exafal tablets, Novartis) equivalent to 480 mg of lumefantrine and 80 mg of artemether administered to our subjects produces plasma concentration which is sustained well above the median cut off value of 0.28 mg/l required to prevent the recrudescent infection.

ACKNOWLEDGMENTS

We are grateful to the staff of Centre for Research in Experimental and Applied Medicine for extending laboratory facilities, in particular, Mr. Tatheer. We are obliged to National University of Sciences and Technology Rawalpindi, Pakistan for the financial assistance.

REFERENCES

1. Burrougs VJ, Maxey RW and Levy RA. Racial and ethnic differences in response to medicines: Towards individualized pharmaceutical treatment. J Natl Med Assoc 2002; 94 (10):1-26

2. White NJ, Vugt MV and Ezzet F. Clinical pharmacokinetics and pharmacodynamics of artemether-lumefantrine. Clin Pharmacokinet Aug: 1999; 37(2): 105-125.

3. Wilkinson GR. Cytochrome P4503A (CYP3A) metabolism: Prediction of in vivo activity in humans. J Pharmacokinet Biopharm: 1996; 24(5):475-90.

4. Ezzet F, Mull R and Karbwang J. Population pharmacokinetics and therapeutic response of CGP56697 (artemether-benflumetol) in malaria patients. Br.J. Clin Pharmacol:1998; 46:553-561

5. Mansor SM, Navratnam V, Yahaya, N, Nair NK, Wernsdorfer WH. and Degen PH. Determination of a new antimalarial drug benflumetol, in blood plasma by reverse phase high performance liquid chromatography with ultraviolet detection. J Chromatogr: 1996; 682:321-325.

6. Jiao X, Liu GY, Shan CO, Zhao X, Li XW, Gathmann I and Royce C. Phase II trial in China of a new, rapidly-acting and effective oral antimalarial, CGP 56697, for the treatment of Plasmodium falciparum malaria. Southeast Asian J Trop Med Publ Hlth: 1997; 28: 476-481.

7. Vugt MV, Brockman A, Gemperli B, Luxemburger C, Gathmann I, Royce C. Slight T, Looareesuwan S, White NJ and Nosten F. Randomized comparison of artemether-benflumetol and artesunate-mefloquine in treatment of multidrug-resistant falciparum malaria. Antimicrob Agents Chemother: 1998; 42: 135-139.

8. Pi EH, Simpson GH and Cooper TB. Pharmacokinetics of desipramine in caucasian and Asian volunteers. Am J Psychiatry:1986; 143:1174-1176

9. Lefevre G, Polly C, Heinz S, Souppart C, McLean M, and Stypinski D. Pharmacokinetics and electrocardiographic pharmacodynamics of artemether-lumefantrine with concomitant administration of ketoconazole in healthy subjects. Br.J. Clin Pharmacol: 2002; 54:485-492

10. Zeng MY, Lu ZL, Yang SC, Zhang M, Liao J, Liu SL and Teng XH. Determination of benflumetol in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Appl: 1996; 681: 299-306

11. Fitzsimmons ME and Collins JM. Selective biotransformation of the human immunodeficiency virus protease inhibitor saquinavir by human small intestine cytochrome P4503A4; potential contribution to

high first pass metabolism. Drug metab dispos: 1997; Feb 25(2):256-266

12. Lefevre G. and Thomsen M.S. Clinical pharmacokinetics of artemether and lumefantrine (Riamet). Clin Drug Invest : 1999: 18:467-480

13. Annex 3, Pharmacology of antimalarials drugs. In:Guidelines for the treatment of malaria. World Health Organisation. 2006.

14. White N.J. Antimalarial pharmacokinetics. Br J Clin Pharmacol: 1992; 34: 1-10.

.....

Table: Pharmacokinetic parameters of lumefantrine in healthy subjects after oral administration of 480 mg of the drug

Pharmacokinetic parameters	Mean	SD	Min	Max
Area Under the Curve (AUC)[h.mg/l]	262.5	129.6	91.65	567.8
Clearance (CL) [l/h]	2.44	1.34	0.84	5.23
Volume of distribution Vd [l]	276.5	124.1	132.8	532.0
Half-life phase 1 [h]	4.50	1.40	2.23	6.95
Half-life phase 2 [h]	89.45	21.24	61.84	130.2
Absorption rate constant (ka) [1/h]	0.17	0.064	0.10	0.31
Absorption half-life [h]	4.50	1.40	2.24	6.96
Lag-time [h]	1.01	0.24	0.91	1.8
Time to peak Tmax [h]	8.50	2.15	4.84	11.70
Peak concentration Cmax [mg / l]	4.60	2.28	1.80	9.86
Mean residence time MRT[h]	98.17	17.18	62.5	125.6

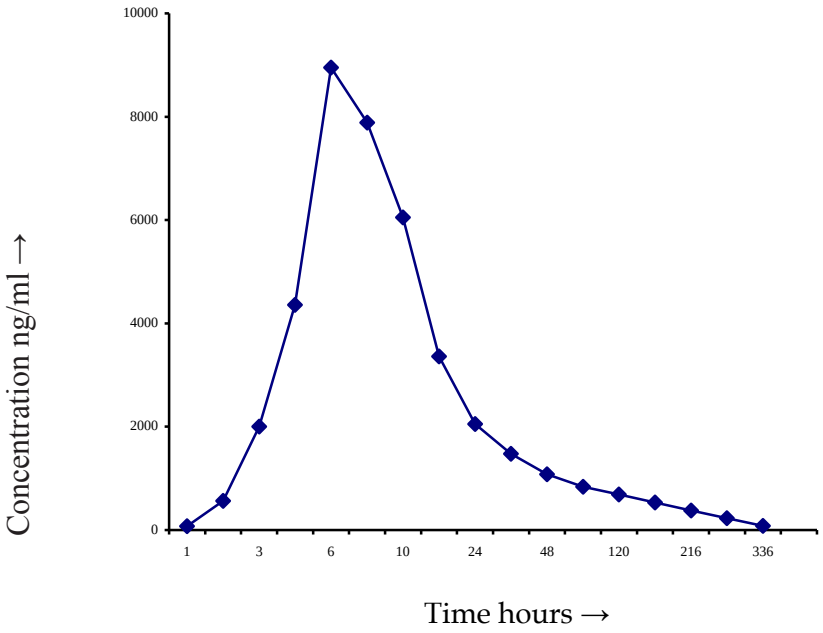

Figure: Mean plasma concentration-time curve of lumefantrine (Linear scale)