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RELATIVE BIOAVAILABILITY AND PHARMACOKINETIC PARAMETERS FOLLOWING ADMINSTRATION OF SINGLE ORAL DOSE (2x150 mg) OF CHLOROQUINE TABLET IN HEALTHY SUBJECTS

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ABSTRACT

The bioavailability of Chloroquine phosphate (150 mg base) film coated tablet (manufactured by Pars - Darou Co. Iran) was compared with that of the Resochin tablet (Bayer, Germany) in seven healthy male and female volunteers. Blood samples were collected up to 14 days after oral dosing of 300 mg and chloroquine serum concentrations were determined by means of High Performance Liquid Chromatography (HPLC). From the analysis of serum samples the

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following pharmacokinetic parameters were obtained: the absorption rate of test tablet and that of reference were 0.25 h^{-1} and 0.34 h^{-1} respectively; maximum serum concentration of both tablets was nearly equal, 81.6±25 and 81.4±27 ng/ml (for test and reference tablets respectively); time to reaching C_{max} (t_{max}) was also very similar, 4±1.6 for test and 4±1.0 h for reference; the area under the serum concentration-time curve [AUC(0 - ∞)] of 6976 $ng.ml^{-1}.h$ ±1967 for test and 6446 $ng.ml^{-1}.h$ ± 2460 for reference tablets were found using trapezoidal rule. By evaluating of the r=(T/R)x100 for each parameters, the test tablet was found to be bioequivalent to Resochin at p=0.05 .

INTRODUCTION

Chloroquine (CQ) is a 4-aminoquinoline which has been used for the treatment and prophylaxis of malaria for more than 40 years (1). It is the most widely prescribed antimalarial in the world (2), and is the drug of choice for malaria caused by plasmodium vivax, p.malariae, p. ovale and sensitive strains of p. falciparum (1). CQ is also used in rheumatoid arthritis and other collagen diseases (3). Despite this long experience, its pharmacokinetics have not been fully characterised. CQ is well absorbed after oral adminstration in healthy subjects and also in children with uncomplicated malaria. The time to peak concentration is variable, and the solution of CQ has shown lower bioavailability than that of tablet (4). The V_d value ranged from 116 - 1000 l/kg (4, 5, 6), confirming extensive tissue distribution. Estimated terminal half life is also reported 3 hours (7), 7-14 days (4) and 1-2 months (5), which may be due to multicompartmental system of distribution. Binding of CQ to plasma proteins is about 50-65% and there are no difference between healthy subjects, patients with rheumatoid arthritis and patients with kwashiorkor (8, 9). With this variable charachterstics, therapeutic

serum concentration in treatment of malaria is reported between 15-30 ng/ml (9).

Since South and South east of Iran is reported as an endemic area for malaria, it is important to evaluate the performance of Chloroquine tablet (which is manufactured by Pars - Darou Co. Iran). Therefore the objective of current study is to determine the relative bioavailability of Chloroquine tablet (test) with Resochin, and to evaluate the pharmacokinetic parameters following a single oral dose (2x150mg) in healthy volunteers.

MATERIALS AND METHODS

Materials

Pure Chloroquine phosphate obtained from Pars-Darou Co. and Hydroxychloroquine sulfate powder was kindly donated by Sterlig-Winthrop (Sweden), HPLC grade Acetonitril and Methanol, and analytical grade KH_2PO_4 were used throughout the analysis. To prevent CQ binding, all glasswares coming into contact with CQ solutions was treated with Aquasil silanising agent.

Methods

1. Dissolution studies

Dissolution studies were performed according to USP XXII and percent dissolved were determined by UV spectrophotometer (Shimadzo 160 A) at sampling times. The dissolution rate profiles for test and reference tablets are shown in Fig 1.

2. Human studies

2.1. Drug analysis

To 0.5 ml of serum added 50 microliter of HCQ solution (as internal standard), vortex-mixed for 20 S and allowed to stand for 30

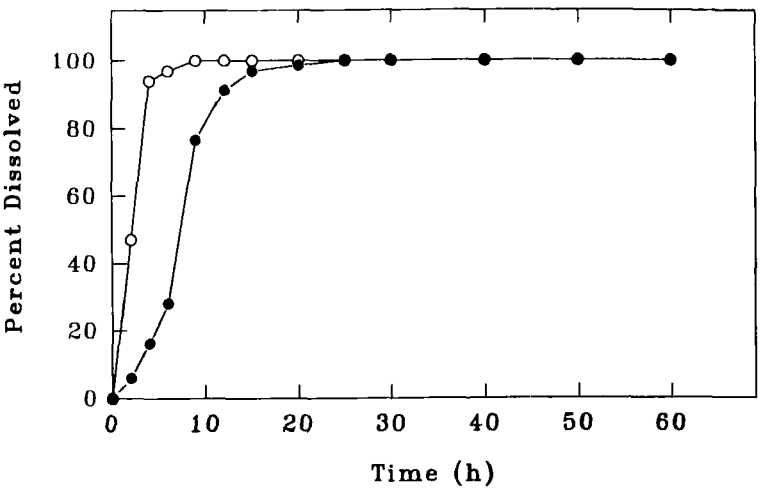

Fig 1 : Percentage of Dissolved CQ vs Time Profiles for Chloroquine Test (●) and Reference (O) Tablets.

minutes, then centrifuged at 8000 rpm for 10 minutes. The clear supernatant was separated and filtered with 0.45 μm filter (Milex HV, Millipore). then 100 microliter of filtrate was injected into HPLC.

The HPLC system was consisted of μBonda-pack C_{18} column (30 cm, 10 μm), mobile phase of Acetonitril-Phosphate buffer (20 :100) pH=3 with 0.9 ml/min flow rate and the UV detection of 343 nm. In this system the retention times of HCQ and CQ were 5.5 and 7.0 minutes respectively. The limit of detection for CQ was 4.0 ± 0.2 ng/ml.

2.2. Subjects

Five female and two male volunteers with mean age of 27 ± 2 years and mean weight of 67 ± 10 kg participated in this study. Non of them had never taken Chloroquine, and the subjects who had received medication recently were excluded. all subjects were selected after successful completion of physical examination and tests consisting of

blood chemistry examination, complete blood count and urinalysis. All volunteers gaved their written informed consent after given full details of the research to participate in the study.

2.3. Study design and sampling

The volunteers were given two doses of Chloroquine or Resochin tablet after a standard breakfast in a double-blind two way cross-over design. Venous blood samples (5ml) were collected before and at 0.5, 1, 2, 3, 4, 5, 7, 9, 24 and 48 hours, also 1 and 2 weeks after dosing. Blood samples were taken into SST venoject tubes (Becton Dickinson, U.K.), and centrifuged at 4000 rpm for 15 min (Heraeus), the serum was transfered to plastic tubes and all samples were stored at -20°C until the time of analysis.

3. Calculation and statistical analysis

The log CQ concentrations of test and reference tablets after adminstration of two doses (300mg base) were ploted against time. The maximum serum concentration (C_{max}) and the time to reach C_{max} (t_{max}) were obtained from the concentration-time profiles, the linear slope (β) was calculated from the linear part of profiles using ordinary Least Square method. The half life ($t_{1/2}$) was found from the quotiont $0.693/\beta$. The total area under the curve (AUC) was estimated using trapesoidal rule and the oral clearance (CL/F) was also obtained from equation CL/F=D/AUC. The relative bioavailability F was calculated from the ratio of AUCs obtained following adminstration of test tablet to that of the reference tablet.

Means and standard deviations for all the parameters, expressed as mean±SD. Statistical difference in estimated pharmacokinetic parameters were tested using paired-t test at P=0.05, and the

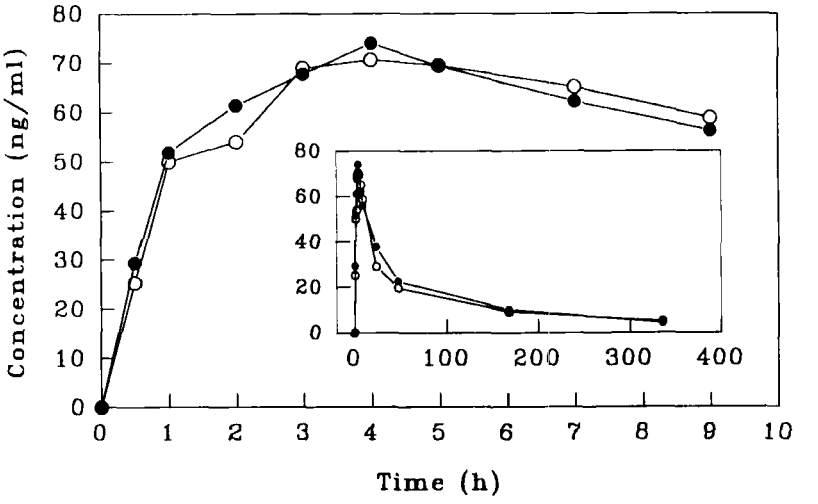

Fig 2 : Mean Serum Concentrations vs Time Profile, following Oral Adminstration of (2x150 mg) Chloroquine Test (●) and Reference (O) Tablets to Healthy Volunteers.

acceptibility of test product was also studied using confidence interval method.

RESULT AND DISCUSSION

Dissolution profiles of the chloroquine phosphate test and reference tablets are presented in Fig(1), the result was within the range of USP XXII dissolution specification (Tolerance: not less than 75% should be dissolved at 45 minutes).

The means of the serum concentrations following adminstration of (2x150 mg) of test and reference tablets to healthy volunteers at each sampling time are presented in table (1). Applying paired-t test based on 95% confidence level showed that difference between concentration of test and reference tablet are not significant.

TABLE 1

Paired-t Test of Serum Concentrations following Adminstration of (2x150 mg) of Chloroquine Test & Reference Tablets

Time (h)	Serum Concentration (ng/ml) Reference Mean	SD	Test Mean	SD	Difference at 95% level
0.5	25.3	15.7	29.5	17.7	NS
1	46.9	28.8	51.9	34.9	NS
2	54.2	27.9	61.4	34.0	NS
3	69.0	32.4	67.6	26.6	NS
4	70.6	25.6	74.1	24.3	NS
5	69.5	20.0	69.4	22.1	NS
7	62.2	13.3	62.2	15.4	NS
9	58.8	17.1	56.3	18.8	NS
24	29.2	8.7	38.0	16.8	NS
48	19.6	3.9	22.6	10	NS
168	9.1	3.8	16.3	9.7	NS
336	5.3	2.5	4.7	1.3	NS

Table (2) shows the main pharmacokinetic parameters; C_{max} (test ranged from 53.3 to 124 ng/ml, reference 53.5 to 124.2 ng/ml); t_{max} (test for test ranged from 2 to 7 h and reference from 3 to 5 h); AUC(0-∞) of and 3362 to 9652 ng.h/ml and 3160 to 10055 ng.h/ml reference respectively. The oral clerance was 9.3-17.1 ml/min/kg for test and 9.6-19.7 ml/min/kg for reference product.

CQ is readily absorbed from the gut and is concentrated several folds in various tissues, from where gradually redistributed until equilibrium reaches, the consequence will be the multicompartmental behavior of the drug. In the present study although the difference in the serum concentration of the individuals based on paired-t test were not significant (table 1), but rather high standard deviation at early sampling time (up to C_{max}) and wide range of t_{max} (2-7 h) shows that

TABLE 2

Pharmacokinetic Parameters of Chloroquine following Adminstration of (2x150 mg) Test & Reference Tablets.

Subject	Cmax (ng/ml)		Tmax (h)		T1/2 (h)		CL/F (ml/min/kg)		AUC(0- ∞) (ng.h/ml)	
	Ref.	Test	Ref.	Test	Ref.	Test	Ref.	Test	Ref.	Test
1	90.9	70.9	5	7	141.0	187.3	11.1	9.3	5516	7668
2	62.4	100.0	5	3	135.9	144.4	13.9	11.1	4778	5979
3	124.2	124.0	3	2	266.5	117.5	11.5	11.3	8659	9652
4	79.4	71.5	5	4	330.0	169.0	7.6	9.3	10055	8246
5	104.9	95.2	3	5	119.5	128.3	15.8	13.8	5073	5826
6	53.5	56.4	4	4	125.0	119.5	9.6	9.7	7881	7802
7	54.0	53.3	3	3	126.0	138.6	19.7	17.1	3160	3662
Mean	81.8	81.6	4	4	177.7	143.5	12.7	11.7	6446	6978
SD	27.0	25.0	1	1	84.7	26.1	4.1	2.9	2460	1968
CV%	33.0	31.0	25	40	48.0	18.0	32.3	24.8	38.2	28.2

TABLE 3

Confidence Interval Test of Pharmacokinetic Parameters following Adminstration of Single Oral Dose.

Parameter	Ratio(T/R)	95%C.I.	90% C.I.
AUC(0-9)(ng.h/ml)	104%	86-126%	90-122%
AUC(0- ∞)(ng.h/ml)	108%	92-123%	97-128%
Cmax (ng/ml)	103%	78-128%	83-123%
Tmax (h)	103%	71-135%	78-128%

the intersubject variation exist in absorption (pre-equilibrium phase). Since the difference in initial absorption and distributi not apear to be significant (table 1) and according to pharmacokinetic parameters (ratios presented in table 3), based on 95% interval the test product lies confidence requirement well within FDA range of bioequivalence respect to AUC$(0-\infty)$ and C_{max}, while on 90% confidence interval the test product is bioequivalence respect to AUC$(0-\infty)$,C_{max} and t_{max}.

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