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Single Dose Bioequivalence Study of Two Brands of Chloroquine Phosphate Tablets Using Salivary Kinetics

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The present study was undertaken to evaluate the feasibility of salivary kinetics of chloroquine as an approach to biopharmaceutical and pharmacokinetic assessment and to test the bioequivalency of two marketed brands of chloroquine phosphate tablets using noninvasive saliva sampling technique. Pharmacokinetic parameters were computed following oral administration of a single dose of 600 mg of chloroquine base (250 mgx4 chloroquine phosphate tablets) to ten human healthy volunteers. All volunteers received medication in a two period, two way cross-over design. The difference between the formulations were statistically insignificant. In all the cases the relative bioavailability of the test formulation, B was found to be in the range of 84-115%. No adverse reactions were observed during the entire study except for mild dizziness in 70% of the volunteers.

Malaria is by far the world's most important tropical parasitic disease and kills more people than any other communicable disease except tuberculosis, claiming between 1.5 to 2.7 million every year[1,2]. It is most serious in economically backward countries. The advantage of chloroquine, which comprises of its relatively low cost compared with the new antimalarial drugs, ready availability and relatively few and milder side effects, emphasize need for its retention as a mainstay in the control of malaria. Chloroquine is a highly effective blood schizonticide and is the 4-amino quinoline most widely used to prevent or terminate attacks of vivax, ovale, and sensitive strains of falciparum malaria[3]. The drug policy of the national malaria eradication program envisage a 600 mg chloroquine adult dose as the Indian strain of *plasmodium vivax* has been highly sensitive to chloroquine[4].

Many factors related to the formulation or manufacture or compression may be the important ones in drug bioavailability from compressed tablets[5]. Biopharmaceutical and physicochemical factors leading to inadequate drug delivery to the systemic circulation often may result in reduction of the blood levels of the active free drug below the effective concentration and since the therapeutic blood level for sensitive *P. falciparum* is about 0.03 mcg/ml, any brand of chloroquine should provide adequate drug levels for the treatment of sensitive strains of *P. falciparum*[6]. Commendable clinical value of chloroquine phosphate in India, availability of several brands and a necessity of noninvasive body fluid sampling technique for therapeutic drug monitoring and comparative evaluation of biological performance prompted us to take up the present study.

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MATERIALS AND METHODS

Chloroquine phosphate was procured from Sarpin Pharmaceuticals, Nadiad, Gujarat, India. Two commercial chloroquine phosphate brands were procured from the local market. All other chemicals and reagents were of analytical grade.

Dissolution study:

Dissolution test was performed using the USP basket method (Electrolab-TDT-06 P, India). The medium employed for the dissolution was 900 ml of 0.1 N hydrochloric acid, pH 1.2 stirred at 75 rpm[7]. Samples were periodically withdrawn, replacing with the fresh medium.

TABLE 1 - SALIVA KINETICS FOLLOWING ORAL ADMINISTRATION OF SINGLE DOSE OF CHLOROQUINE PHOSPHATE EQUIVALENT TO 600 MG BASE

	Time (h)Saliva concentration in ng/ml													
Volunteers	0	1	2	3	4	5	6	8	10	12	24	AUC (µg/mlxh)	C_{max} (ng/ml)	T_{max} (h)
I	00	180	188	205	185	163	149	124	108	96	83	2.7	205	3
	00	196	260	213	190	182	176	161	141	104	98	3.1, 87	260	2
II	00	199	221	203	188	172	155	139	126	102	89	2.9	221	2
	00	172	244	207	194	168	157	143	129	114	94	3.1, 94	244	2
III	00	198	211	186	170	157	141	122	110	98	81	2.7	211	2
	00	211	279	207	184	159	145	116	102	90	85	2.73, 99	279	2
IV	00	170	192	201	196	176	168	145	133	116	102	3.0	201	3
	00	203	273	229	201	180	163	139	124	110	92	3.07, 98	273	2
V	00	174	188	211	180	164	147	133	122	112	94	2.7	211	3
	00	178	192	215	198	180	166	145	131	118	106	3.2, 84	215	3
VI	00	188	217	207	186	170	155	139	124	108	90	2.9	217	2
	00	198	252	211	196	174	153	120	98	81	73	2.6, 112	252	2
VII	00	182	199	209	198	172	157	149	133	120	102	3.1	209	3
	00	178	184	213	180	157	145	124	112	100	87	2.7, 115	213	3
VIII	00	161	205	178	166	153	139	124	104	92	73	2.5	205	2
	00	168	188	201	192	166	153	114	102	89	79	2.58, 97	201	3
IX	00	180	213	201	184	166	149	126	114	100	89	2.99	213	2
	00	198	209	201	180	166	149	118	102	89	75	2.59, 115	209	2
X	00	168	182	215	199	184	170	157	145	133	116	3.4	215	3
	00	188	201	233	219	207	184	166	141	118	101	3.28, 104	233	3
Average	00	180	202	201	185	168	153	136	122	108	92	2.9	211	2.5
	00	189	228	213	193	174	159	135	118	101	89	2.89, 100	238	2.4

Second value in each cell corresponds to Brand A

Third value in AYC column is relative bioavailability of Brand B to A

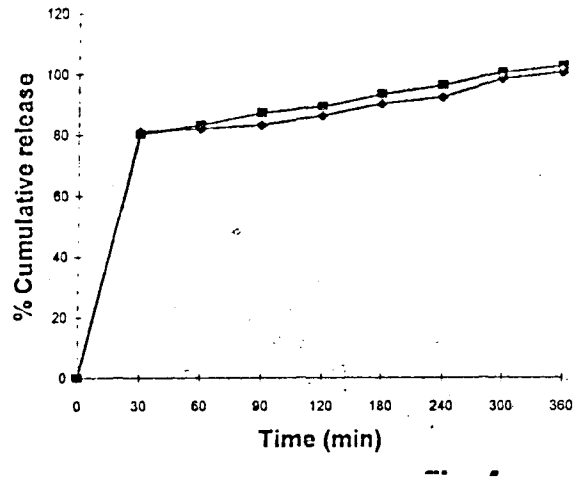

Fig. 1 : Dissolution profile of Chloroquine phosphate tablets

—●— represents brand A and —▫— represents brand B

Samples were filtered, suitably diluted and analyzed at 344 nm using a UV-visible spectrophotometer-160A (Shimadzu, Japan).

Analysis of chloroquine phosphate in saliva :

Blank or spiked or volunteer's saliva samples were made alkaline with 0.1 ml of 5 N sodium hydroxide, kept in contact with 20 ml dichloroethane with frequent gentle mixing for a period of 1 h. Clear organic layer was separated, dried on a water bath, residue reconstituted in 0.1 N hydrochloric acid and absorbance measured against blank at 344 nm.

Pharmacokinetic and bioavailability study :

Ten healthy male volunteers 22-25 y old, participated in this study. Chloroquine phosphate was administered (250 mgx4 tablets) orally as a single dose equivalent to 600 mg base along with 200 ml of water on an empty stomach preceded by an overnight fast. Volunteers had neither taken chloroquine chronically for the past four weeks and were instructed not to take any drug before or during the study. Quality, quantity and time of intake of fluids and solid food were kept uniform as much as possible amongst the subjects. Two commercial brands were used. All volunteers received a single oral dose of the medication in a two period, two way crossover design. Volunteers were advised to clean the buccal cavity thoroughly prior to saliva sampling. Saliva samples of 10 ml were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h postdosing. Wash out period of three weeks were employed between the study. The area under the concentration-time curve (AUC_{0-24}) was calculated by trapezoidal method. The maximum saliva concentration was directly obtained from concentration-time curve. Paired t-test was utilized to test the statistical significance of bioequivalency results.

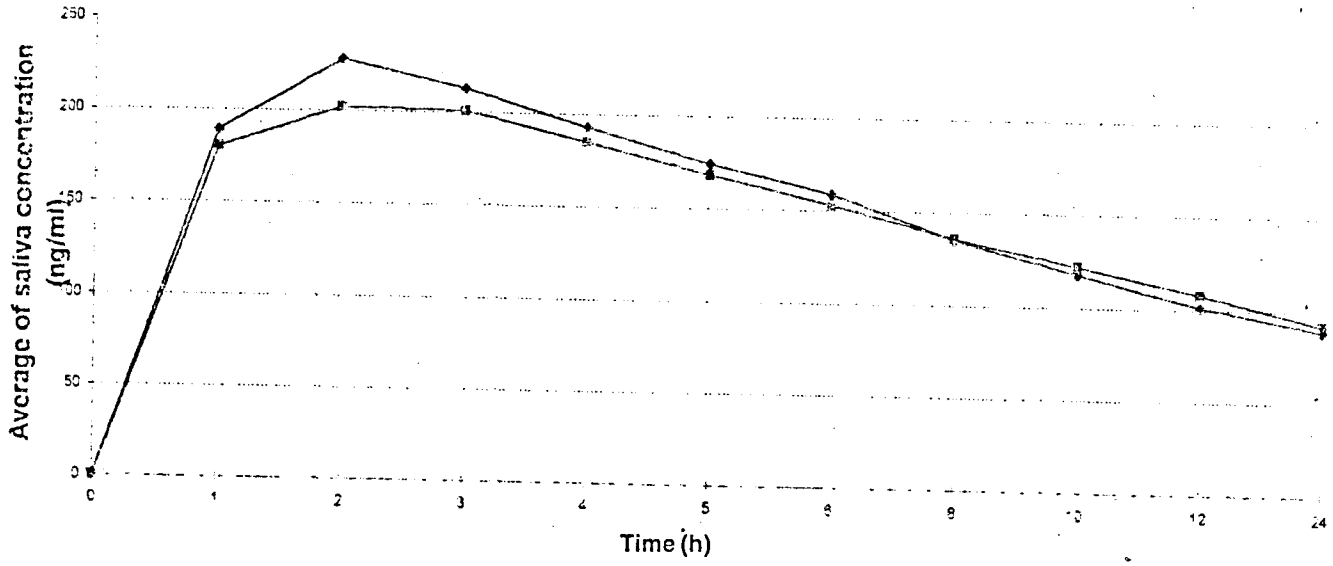

Fig. 2 : Saliva kinetics of chloroquine phosphate tablets

Chloroquine Phosphate levels were determined spectrometrically at different time intervals from volunteers administered a single dose of 600 mg of chloroquin tablets of either brand A (●—●) or brand B (▫—▫)

RESULTS AND DISCUSSION

The two commercial chloroquine phosphate brands have exhibited equivalent dissolution profiles (Fig. 1) and comply with the official requirements (70% in 45 min). Dissolution studies are the *in vitro* models routinely employed to compare and evaluate the biological performance of formulations and also act as an important quality control/assurance tool.

Bioequivalence testing of brands marketed in India against the innovators brand or reference is an absolutely essential *in vivo* demonstration to assure the therapeutic performance of pharmaceutical products. Following the establishment of *in vitro* and *in vivo* correlation using blood profiles as a standard practice, we advocate saliva sampling as the noninvasive body fluid sampling procedure to monitor and compare pharmacokinetic and bio-pharmaceutical characteristics of the products. The relationship between plasma and salivary concentrations of chloroquine phosphate following oral administration was established and chloroquine concentrations in saliva was found to be half of that of plasma[8]. A summary of pharmacokinetic parameters such as AUC, C_{max}, T_{max}, plasma concentrations and their mean values are given in Table 1. Relative bioavailability values of B formulations have been found to be in the range of (84-115%) displayed in Table 1. Fig. 1 depicts the dissolution profiles and Fig. 2 shows the mean saliva profiles of two brands of chloroquine phosphate. The results of our study indicate that the two brands are bioequivalent ($p<0.001$). C_{max} and T_{max} of the formulations are also similar to that reported in the literature[8]. Saliva sampling techniques are increasingly being used in biopharmaceutics, forensic medicine and other diagnostic sciences. Chloroquine phosphate was well tolerated by the volunteers with no adverse effects throughout the study except for mild dizziness in the first four hours in 70% of the volunteers[9].

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