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COMPARATIVE BIO-AVAILABILITY CHARACTERISTICS OF DIFFERENT BRANDS OF CHLOROQUINE AVAILABLE IN NIGERIA

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Summary

The disintegration, dissolution and bio-availability characteristics of six of the most popular brands of chloroquine used in Nigeria were determined in order to test the hypothesis that there are no significant differences among the different brands. The disintegration times of all the six brands ranged from 8·9 to 40·4 min while the dissolution times ranged from 17·5 to 60 min. All passed the U.S. Pharmacopoiea (USP) XX disintegration test. Bio-availability studies done on two brands, Avloclor (ICI) with the fastest dissolution rate and Pfizerquine (Pfizer) with the slowest dissolution rate, showed similar areas under the curve (AUC). Furthermore, their peak height concentration (C_{max}) and time of peak height concentration (T_{max}) did not show any significant differences. Consequently, statements about any of these six brands of cloroquine having a greater efficacy than the other may be pure conjecture.

Résumé

Les characteristiques de désagrégation de dissolution et de bio-validité de six des marques les plus populaires de chloroquine utilisees au Nigeria ont été déterminées afin de verifier l'hypothese selon laquelle il n'existe pas de différences signifiantes entre ces marques. Les temps de désagrégation de chacune des six marques varierent de 8·9 a 40·4 min tandis que les temps de dissolution allerent de 17·5 á 60 min. Toutes réussirent les tests normalisés de désagrégation et de dissolution du codex américain (USP). Les études de biovalidité des produits réalisées sur deux marques. Avloclor (ICI) dont le taux de dissolution est le plus élevé et Pfizerquine (Pfizer) dont le taux de dissolution est le moins eleve, montrèrent des zônes similaires sous la courbe (AUC). En plus, il n'existe pas de différences signifiantes entre leur plus haute concentration (C_{max}) et le temps d'acherer cette concentration (T_{max}). Par conséquent, les affirmations comme quoi chacune de ces six marques aurait une plus grande efficacité que les autres ne peut être uqe pure conjecture.

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Introduction

In Nigeria, official standards and enforcement agencies for the quality control of drugs manufactured or imported into the country are not very effective. Consquently, there is a proliferation of different brands of the same drug and the bio-equivalence of these products is unknown. In the last 10 years there has occurred a flooding of the Nigerian pharmaceutical market with different brands of chloroquine. At the present time, there are at least eighteen different brands of chloroquine in Nigeria (Medipharm, 1978).

It is generally known that even small differences in the manufacturing process may considerably alter the disintegration, dissolution and consquently the bio-availability of the active ingredients in a product (WHO, 1974). Consequently, such small differences can produce brands of the same generic compound which are therapeutically non-equivalent. This has, in fact, been well demonstrated for a number of drugs

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like chloramphenicol (Glazko *et al.*, 1968) and oxytetracycline (Brice & Hammer, 1969).

The view has often been expressed, usually based on clinical impressions, that some brands of chloroquine give better prophylaxis or suppression than others. It is usually assumed that this variability in response is due to the therapeutic non-equivalence of these different brands. A search through the literature has shown that there are, in fact, no studies on the comparative bio-availability of the different brands of chloroquine. Consequently, there is ample justification for undertaking such a study, especially in view of the importance of cloroquine in the treatment of malaria in Africa where the widespread eradication programmes do not appear to be succeeding.

Therefore, it was decided to examine the disintegration and dissolution characteristics of six of the most widely prescribed brands of chloroquine in Nigeria. From the results thus generated, the relative bio-availabilities of the fastest-dissolving Avloclor (ICI) and the slowest-dissolving Pfizerquine (Pfizer) as determined by their peak height concentration (C_{max}), time of peak height concentration (T_{max}), and concentration time curves were then compared.

Materials and methods

Disintegration time

Six oral brands of chloroquine (Table 1) were tested for their disintegration characteristics. All the brands except for Aralen were purchased off the counter in Ibadan. Aralen (Winthrop) was the kind donation of Winthrop Laboratories, New York, U.S.A.

The disintegration test was performed according to the procedure in the U.S. Pharmacopoiea (USP) XX test. Six tablets were tested at a time, and altogether three sets of tablets were tested for each brand of chloroquine. The disintegration medium was 1 l de-ionised distilled water in a bath maintained at 37·5(±1)°C. All brands except Aralen (Winthrop) disintegrated in water within the time limit of 30 min set for uncoated tablets (USP XX). Aralen (Winthrop) did not disintegrate in water within this time limit. Consequently, it was subjected to the disintegration procedure outlined for coated tablets (USP XX).

The time taken for all six tablets to disintegrate completely such that no solid particle was left within the wire mesh used for suspending the tablets was taken as the disintegration time. Three disintegration tests were done for each brand, the mean of the three readings being taken as the disintegration time.

Dissolution test

Dissolution test was performed using the rotating basket apparatus described in the USP XX test. The dissolution time was defined as the time taken for 50% of the drug to go into solution, there is no USP specification for dissolution of chloroquine.

The dissolution medium employed was 0·1 N HCl placed in a 1-l bath maintained at 37·5(±1)°C. The rate of revolution of the wire basket was maintained at 50 rev/min. Sampling was done for a total of 2 h. Samples were obtained from the same depth of the water bath every 2½ min for 10 min, every 5 min for 20 min, every 10 min for 30 min and every 30 min for 1 h. Five-millilitre samples were taken out and immediately filtered to remove insoluble excipients, the clear filtrate being collected in a glass test tube. Following the removal of each 5-ml sample, 5 ml dissolution medium (0·1 N HCl) at a temperature of 37·5° was returned to the bath so as to maintain the volume of the bath constant at 1 l. The

TABLE 1. The six brands of chloroquine tested

Formulation brand	Salt	Lot No.	Base (mg chloroquine)	Tablet coating
Aralen (Winthrop)	Phosphate	AW 122D	300	Coated
Avloclor (ICI)	Phosphate	DA 186	150	Uncoated
Malarex (Dumex)	Phosphate	54860-3	150	Coated
Nivaquine (M&B)	Sulphate	IL 203	150	Uncoated
Pfizerquine (Pfizer)	Phosphate	92420104	150	Coated
Resochin (Bayer)	Phosphate	3181D	150	Uncoated

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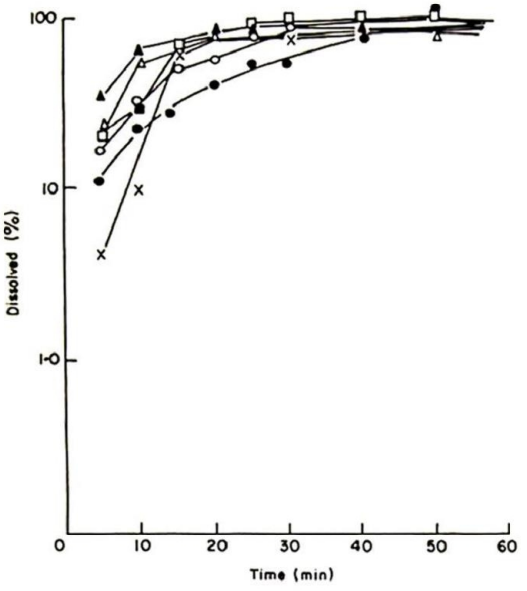

FIG. 1. The dissolution rate of six brands of chloroquine. The ordinate (% dissolved) is in a log scale while the abscissa (time) is in an arithmetic scale. ●, Pfizerquine; ×, Aralen; ○, Malarex; □, Resochin; ▲, Avloclor; △, Nivaquine.

samples were assayed for chloroquine immediately afterwards and the chloroquine concentration estimated from standard curves. Under these conditions, over 50% of each brand went into solution within 60 min (Fig. 1).

Chloroquine assay

Chloroquine was assayed by a method originally described by McChesney (1962) and later modified by Vogel and Konigk (1975) but using ether in place of heptane (Adelusi & Salako, 1980). Basically, two techniques were used (1) the macrotechnique for analysing chloroquine concentrations above 1 μg/ml, and (2) the microtechnique for analysing chloroquine concentrations below 1 μg/ml.

(1) Macrotechnique. One millilitre of biological sample was added to an equal volume of 1 N NaOH in 10% NaCl and the mixture was shaken briefly in a Vortex mixer. Thirty millilitres of ether was added in a 40-ml centrifuge tube. The mixture was shaken for 30 min in an Eberbach shaker. This was then followed by centrifugation at 1500 *g* for 10 min in a Sorvall RC — 3B refrigerated centrifuge at 4°C. Twenty millilitres of the ether layer was removed into a 45-ml centrifuge tube containing 3 ml 0·1 N HCl. The mixture was again shaken for 5 min to back-extract the chloroquine into 3 ml HCl. The ether layer was aspirated off through a water pump. Two millilitres of the acid phase was then aspirated into a fluorimeter tube containing 0·5 ml 0·3 N NaOH and 0·5 ml 0·3 M borate buffer (pH 9·5). The pH of the resultant solution was 9·8-10·0. The fluorecence was measured at 25°C (wave length of excitation 310 nm, wave length of emission 405 nm) using the Aminco-Bowman Fluorescence Spectro-fluorometer at exit slit size 2 mm.

(2) Microtechnique. While the macrotechnique was sensitive enough to estimate chloroquine concentrations about 500 ng/ml very accurately with a coefficient of variation of 5%, it was not accurate enough to reproducibly measure chloroquine concentrations below 500 ng/ml. In fact, it could not differentiate between concentrations of 50 ng/ml and blanks. Consequently, the microtechnique was used for concentrations of 500 ng/ml and less. This was based on the same procedure but with the ether extract of chloroquine being back-extracted into only 300 μl HCl so as to concentrate the chloroquine better for reading on the fluorimeter. After aspiration of the organic phase, 200 μl acid phase was combined with 50 μl 0·3 N NaOH and 50 μl borate buffer and transferred to a 3 × 2·4 mm quartz cuvette (American Instrument Co., U.S.A.). This method detected up to 5 ng/ml with a coefficient of variation of 10% at 10 ng/ml, and less at higher concentrations.

Bio-availability studies

Two sets of beagle dogs were used in the bio-availability experiments. Each set consisted of four dogs. Each dog was approximately 6 months old and 10 kg in weight. All eight dogs had an initial dose of intravenous chloroquine, approximately 2 mg/kg as part of another experiment, followed 28 days later by an oral dose of chloroquine. Dogs 1 to 4 were each dosed with one tablet of Avloclor (150 mg base) while Dogs 5–8 were each dosed with one tablet of Pfizerquine (150 mg base).

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Analysis of results

The area under the curve (AUC) was obtained using the linear trapezoidal rule up to the last data point. The remaining AUC beyond the last data point was estimated using the equation.

$$\int_{t_{last}}^{\infty} C_{dt} = \frac{C_{last}}{\lambda_2}$$

where t_{last} = time of last sampling.
C_{last} = concentration of last sample.
λ_2 = terminal rate constant.

Lambda$(\lambda)_2$ was determined using a non-linear least squares regression programme.

Values are given in the text and tables as means ± s.e.mean. Difference between means are evaluated using Student's *t*-test and *P* values less than 0·05 are taken as significant.

Results

The results of the disintegration and dissolution studies were as shown in Table 2. Avloclor had the fastest disintegration and dissolution times of 8·9 and 17·5 min respectively. Resochin, Malarex and Nivaquine had an intermediate position. Their disintegration times were 11·4, 12·7 and 14·0 min respectively and dissolution times 27·5, 27·5 and 25 min respectively.

Malarex, in spite of being a coated tablet, fell into the intermediate group, while Pfizerquine and Aralen, the two other coated tablets, had the slowest disintegration and dissolution. Their disintegration times were 26·2 and 40·4 min respectively while their dissolution times were 60 and 50 min respectively. All of the six tablets passed the USP specification test for disintegration.

The peak height concentrations (C_{max}) and the time of peak height concentration (T_{max}) were shown in Table 3. Mean C_{max} for Avloclor was 581·1 (±48·1) ng/ml while that for Pfizerquine was 461·6 (±46·9) ng/ml. The mean T_{max} for Avloclor was 2·76 (±0·01) h, while that for Pfizerquine was 2·66 (±0·03) h. There were no significant differences between these two parameters. The concentration time curves for the two formulations are virtually superimposable as shown in Fig. 2.

The AUCs obtained in Dogs 1–4 dosed with Avloclor were as shown in Table 3. The mean

TABLE 2. Disintegration and Dissolution Times

Brand name (maker)	Disintegration time (min)	Dissolution time (min)
Avloclor (ICI)	8·9	17·5
Resochin (Bayer)	11·4	27·5
Malarex (Dumex)	12·7	27·5
Nivaquine (M&B)	14·0	25·0
Pfizerquine (Pfizer)	26·2	60·0
Aralen (Winthrop)	40·4	50·0

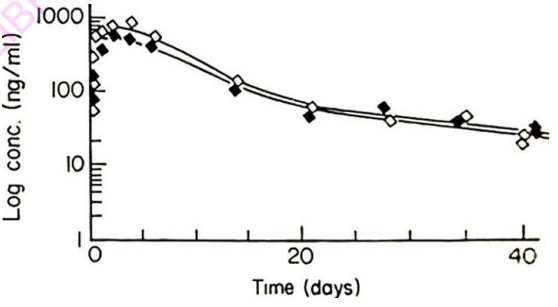

FIG. 2. Computer-generated log concentration time curves for Avoclor (◇) and Pfizerquine (◆). The curves represent data for the first of each group of dogs.

TABLE 3. Oral bio-availability

	Dog No.	Wt (kg)	Dose (mg/kg)	AUC(0–∞) (ng/ml. day)	C_{max} (ng/ml)	T_{max} (h)
Avloclor	1	10·90	13·76	7526·3	555·3	3·0
	2	13·20	11·36	5063·2	501·8	3·0
	3	12·00	12·50	7804·0	546·2	3·0
	4	11·70	12·82	7778·1	721·2	2·0
Mean ± s.e.mean		11·95(±0·24)	12·61(±0·25)	7042·9(±331·5)	581·1(±48·14)	2·8(±0·01)
Pfizerquine	5	11·2	13·39	8101·7	410·6	2·0
	7	10·4	14·42	6661·5	418·9	2·0
	8	9·5	15·79	9053·8	555·3	4·0
Mean ± s.e.mean		10·37(±0·28)	14·53(±0·40)	7939·0(±401·4)	461·6(±46·9)	2·7(±0·028)

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AUC for Avloclor was 7042·9 (±331·5) ng/ml. day.

The results of only three dogs (5, 7, and 8) were analysed for Pfizerquine, Dog 6 was eliminated because it was not possible to accurately estimate the terminal elimination phase because some drug levels near the sensitivity of the assay appeared to be underestimated. Hence, the AUC calculations for Dog 6 were deemed unreliable. The mean AUC for Pfizerquine was 7939·0 (±401·4) ng/ml. day. There was no significant difference between the two AUCs.

Discussion

The disintegration times for these six brands of chloroquine fell into three main categories — the fast category comprising Avloclor, the intermediate category comprising Resochin, Malarex and Nivaquine, and the slow category comprising Pfizerquine and Aralen. Malarex, Pfizerquine and Aralen are coated tablets. This would explain the slow disintegration of Pfizerquine and Aralen, but Malarex, despite its coating, had a fairly fast disintegration time (12·7 min). Malarex, though a coated tablet, had a shorter disintegration time than Nivaquine, an uncoated tablet.

The dissolution times paralleled the disintegration times being shortest for Avloclor, intermediate for Resochin, Malarex and Nivaquine and longest for Pfizerquine and Aralen. These differences among the six brands do not appear significant since all six passed the USP standard specification tests for disintegration, and their dissolution characteristics were reasonably similar. Furthermore bio-equivalency (as shown by superimposable concentration time curves and comparable C_{max} and T_{max}) has been demonstrated between the two formulations with the fastest and slowest disintegration and dissolution characteristics. It is well known that poor bio-availability may be associated with poor disintegration/dissolution characteristics (WHO, 1974). Since minor differences in the fastest and slowest disintegrating and dissolving preparations did not appear to influence the bio-availability of chloroquine it seems reasonable to expect no significant difference in the bio-availability of the other four brands of chloroquine. Any differences observed in the therapeutic responses to the six brands studied is unlikely to be due to bio-availability differences.

With the recent emergence of chloroquine resistance of *P. falciparum* in Africa (Campbell *et al.*, 1979; Fogh, Jepsen & Effersøe, 1979; Kean *et al.*, 1979) and the suspicion of chloroquine resistance in Nigeria (Olatunde, 1977; Eke, 1979) it is imperative that the issue of therapeutic equivalence or non-equivalence of these brands of chloroquine be resolved. Otherwise there would always be the uncertainty as to whether or not inadequate treatment with consequent recrudescence was being interpreted as chloroquine resistance.

Absence of any demonstrable differences in the relative bio-availabilities of the two chosen extremes of these six brands of chloroquine contradicts the widely held belief that non-equivalence exists among these brands. Such belief based purely on uncontrolled observations or so-called clinical impressions can be very misleading for obvious reasons. Any number of other reasons apart from unequal bio-availability could be responsible for variable responses to treatment. The bitter taste of the drug could lead to poor compliance. Vomiting, a common clinical feature of acute malaria, could bedevil absorption. Furthermore, ingestion of inadequate doses of the drug due to self-prescribing is extremely common. Finally, the possibility of chloroquine resistance in Nigeria which has been suspected (Olatunde, 1977; Eke, 1979) should always be borne in mind, especially as *in vitro* resistance has been induced by continuous propagation of a West African strain in the absence of chloroquine (Jensen *et al.*, 1981). Consequently, there is the need for continued vigilance so as not to miss cases of true chloroquine resistance in this community.

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