In vitro bioequivalence study of nine brands of artesunate tablets marketed in Nigeria

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Abstract

Background & objectives: The availability of numerous brands of artesunate in our drug market today places clinicians and pharmacists in a difficult situation of choice of a suitable brand or the possibility of alternative use. The aim of the present study was to predict the bioequivalence of nine brands of artesunate tablets marketed in Nigeria using *in vitro* tests.

Methods: The *in vitro* dissolution study was carried out on the nine brands of artesunate tablets using the basket method according to US Pharmacopoeia (USP) guidelines. Other general quality assessment tests like hardness and disintegration time were also determined.

Results: All the brands tested passed the British Pharmacopoeia (BP) standard for disintegration time. Only AT2, AT4, AT6 and AT9 passed the standard for hardness. There were significant differences in the dissolution profiles of the nine brands. All the brands except AT1, however, released >70% of artesunate within 30 min. Four of the brands AT5, AT6, AT7 and AT8 exhibited >90% dissolution in <10 min. The other brands AT1, AT2, AT3, AT4 and AT9 (innovator brand) have calculated similarity factors of 23.8, 59.8, 50, 54.8 and 100.

Interpretation & conclusion: Based on the *in vitro* tests, AT5, AT6, AT7 and AT8 are considered bioequivalent and interchangeable, while AT2, AT3 and AT4 are considered bioequivalent and interchangeable with the innovator brand (AT9). AT1 has very low dissolution rate, which will likely result in poor bioavailability. The results show the need for constant monitoring of new brands of artesunate introduced into the drug market to ascertain bioequivalence and conformity with pharmacopoeia standards.

Key words Artesunate tablets – bioequivalence – disintegration time – hardness – *in vitro* dissolution

Introduction

Widespread resistance of *Plasmodium falciparum* to quinoline-based drugs has made the disease situation difficult to manage in malaria endemic areas[1]. Artemisinin and its derivatives are a major advance in antimalaria treatment[2]. These drugs are increasingly used for the treatment of multi-drug resistant *P. falciparum.* With the adoption of artemisinin combination therapy (ACT) by WHO as the first line drugs, the average consumption of these artemisinin-based drugs has increased resulting in the influx of numerous brands into the global drug market. Artesunate is the most widely available and used drug. Oral artesunate can be used alone or in combination, usually with mefloquine or amodiaquine[3].

The availability of numerous brands of artesunate in

our drug market today places clinicians and pharmacists in a difficult situation of choice of a suitable brand or the possibility of alternative use. Besides, there are growing concerns that various artesunate formulations may have different bioavailability and that development of resistance will accelerate if suboptimal doses are used[4,5]. Despite the considerable use in Nigeria, there are no reports on the bioavailability and bioequivalence of the various brands of artesunate tablets marketed in Nigeria. Hence the present investigation has been carried out.

Oral artesunate is hydrolysed rapidly back to the metabolite dihydroartemisinin (DHA), which is intrinsically more active as antimalaria agent. Oral artesunate may, therefore, be considered as mainly a prodrug for DHA, as this metabolite is the main contributor to the overall antimalaria activity[6-8]. The bioavalability of artesunates from different formulations of the drug is thus an important parameter to assess when comparing the clinical performance of various brands. Prediction of *in vivo* bioavailability in most oral drugs has been shown to depend on the *in vitro* dissolution studies[9-11].

In the present study, we set out to assess the *in vitro* dissolution of nine brands of artesunate tablet marketed in Nigeria. The results of the study will provide a rationale for the interchangeability or otherwise of the selected brands with the innovator brand. Other general quality assessments of the tablets are also determined.

Material & Methods

A total of nine brands of artesunate designated as AT1, AT2, AT3, AT4, AT5, AT6, AT7, and AT8 were compared with the reference drug, AT9. Pure sample of artesunate was kindly donated by Kunimed Pharmaceutical Co., Lagos, Nigeria. UV-Visible PC Spectrophotometer (Model Unico 2102, U.S.A.), Mosanto tablet hardness tester (Mosanto, U.K.), Erweka disintegrating chamber, Erweka DT-D dissolution test (Erweka, U.K.) were used for analysis.

Different brands of artesunate studied were selected based on frequency of prescription, use and availability in hospital and community pharmacy shelves. Drugs were obtained from pharmacies located in four different major towns in Nigeria. The towns were selected to ensure adequate geographical spread. All the brands used were registered by the National Agency for Food Drug Administration and Control (NAFDAC) and were manufactured within six months of the study. A simple analytical procedure based on UV spectrophotometry was developed and adopted for quantitation of the drug in solution. Bioequivalence was assessed based on comparison of parameters like T_{50} (50% dissolution time) and T_{90} (90% dissolution time) and by calculating similarity factor, f_2[12].

Serial diluted solutions of 10, 20, 30, 40, 50, 60 mg% of artesunate were prepared from a stock solution of 100 mg% in sodium hydroxide and monobasic potassium phosphate solution (SIF). Absorbance readings were taken at 287 nm using spectro-photometer. A plot of absorbance *vs* concentration of artesunate was made from which the regression equation was calculated.

The dissolution tests were carried out using the basket method according to US Pharmacopoeia (USP) guidelines[13], operated at 100 rpm in a dissolution bath containing SIF, with sink condition maintained at a temperature of 37 ± 0.5°C. One tablet chosen randomly from each of the tablets was put into the basket suspended in the dissolution medium. Samples (2 ml) were withdrawn at intervals for a total of 120 min. At each withdrawal 2 ml of fresh dissolution medium was used to replace the withdrawn sample. Each sample was filtered, diluted and the absorbance reading determined at 287 nm using UV spectrophotometer against the blank, SIF. The concentration was thereafter determined from the calibration curve of pure artesunate.

In vitro bioequivalence was demonstrated by comparison of the dissolution profile after fitting into the mathematical model, similarity factor, f_2. The similarity factor, f_2 used as the mathematical model for comparing the bioequivalence of the nine brands was calculated using the following formula.

$$f_2 = 50 \bullet \log \left[\frac{100}{\sqrt{1 + \frac{\sum\limits_{t=1}^{t=n} \left[\overline{R}(t) - \overline{T}(t) \right]^2}{n}}} \right]$$

Where, f_2 = Similarity factor; n = Number of time points; R *(t)* = Mean percent drug dissolved e.g. a reference product; T *(t)* = Mean percent drug dissolved of e.g. a test product. Not more than one mean value of >85% dissolved for each formulation. An f_2 value between 50 and 100 suggests that the two dissolution profiles are similar[12].

The results of crushing strength and disintegration time tests were analyzed using Student's *t*- test (SPSS 11) and expressed as mean ± SEM. Differences between the means of the brands and that of the innovator drug, AT9 were considered statistically significant at p <0.05.

Results

The nine brands of artesunate tablet showed significant variation in crushing strength and disintegration time (Table 1). Five brands, AT1, AT3, AT5, AT7 and AT8 have crushing strength values <5 kgf and are considered suboptimal, while the other brands, AT 2, AT4, AT6 and AT9 with values >5 kgf are considered optimal[14]. All the brands tested disintegrated in <16 min (Table 1). The calibration curve as shown in Fig. 1 has good correlation (R^2 = 0.9891).

The dissolution profiles for brands AT1, AT2, AT3, AT4 and AT9 indicate that all the brands except AT1 released >70% of the active ingredient within 30 min (Fig. 2a). Similarly, the dissolution profiles of AT5, AT6, AT7 and AT8 as shown in Fig. 2b indicate that

Table 1. Results of hardness and disintegration time tests

Formulation	Hardness (kgf)	Disintegration time (min)
AT1	3.0 ± 0.29*	8.0 ± 0.57*
AT2	5.0 ± 0.58*	5.9 ± 0.03#
AT3	4.5 ± 0.29	0.58 ± 0.006#
AT4	8.5 ± 0.29	7.0 ± 0.28#
AT5	1.65 ± 0.057#	1.15 ± 0.029#
AT6	11.5 ± 0.30	1.0 ± 0.14$
AT7	2.5 ± 0.28*	0.3 ± 0.03#
AT8	3.0 ± 0.57*	4.0 ± 0.14#
AT9	7.0 ± 0.28	15 ± 0.35

*p <0.05; #p <0.01; $p <0.001 all compared with the innovator brand, AT9; Values are given as mean ± SEM; n = 5.

all the brands released >90% of the active ingredient within 10 min.

The calculated similarity factors, f_2, for AT1, AT2, AT3, and AT4 are shown in Table 2. Apart from AT1, all the other brands fall within the acceptable range of 50–100.
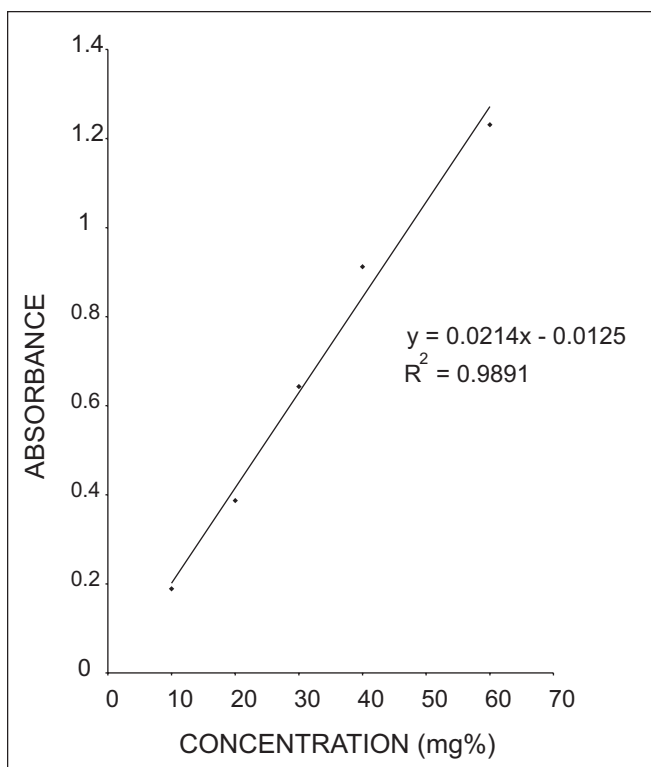

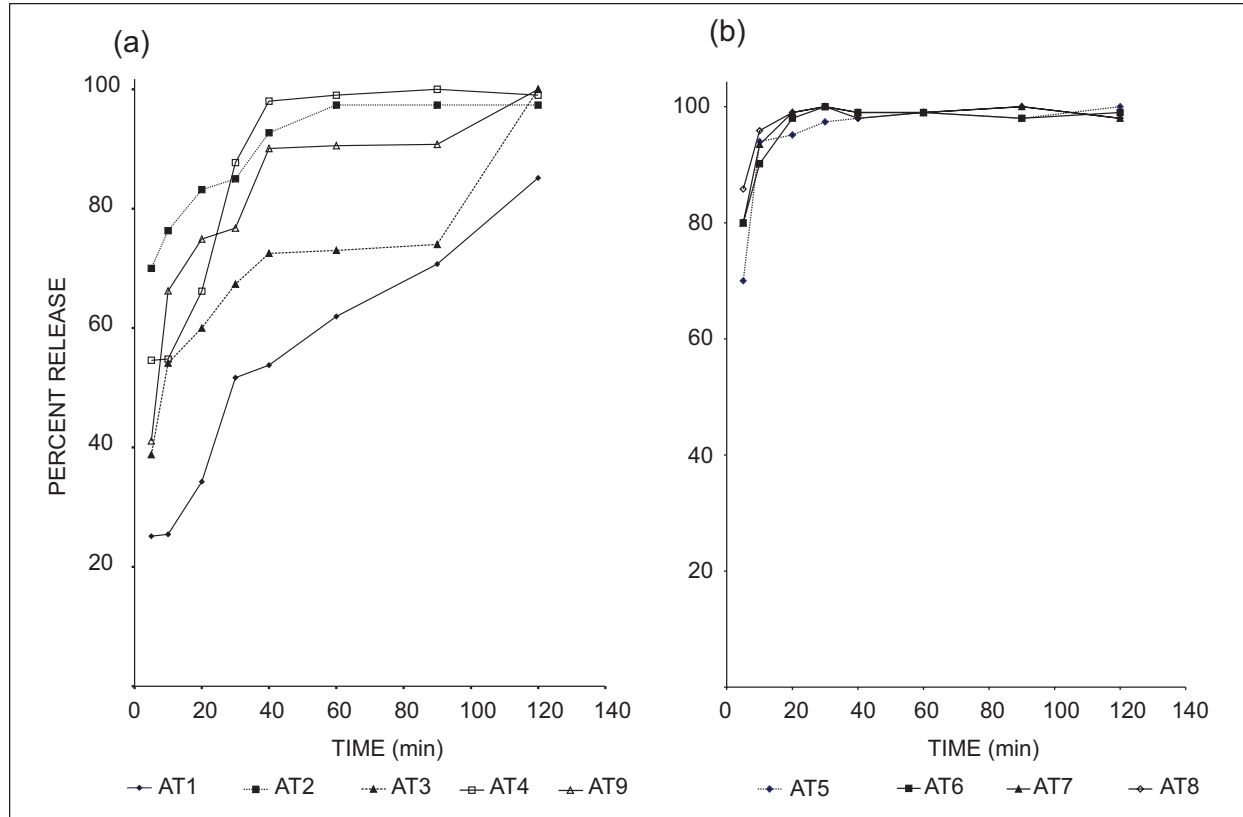*Fig. 1:* Calibration curve of artesunate in SIF

Fig. 2 a & b: Percent release of artesunate with time in SIF

Discussion

Oral artesunate is widely used, very well tolerated and highly effective antimalaria drug[8]. Consequently, several brands have been introduced into the Nigerian

Table 2. Results of some parameters from dissolution profiles of the nine brands

Formulation	T_{50} (min)	T_{90} (min)	Similarity factor, f_2
AT1	30*	>120#	23.8
AT2	<10	<40	59.8
AT3	<5	<40	50.0
AT4	<5	<40	54.8
AT5	<5	<10	ND
AT6	<5	<10	ND
AT7	<5	<10	ND
AT8	<5	<10	ND
AT9	<10	<40	100

*p <0.05; #p <0.01, significantly higher when compared with the innovator drug (AT9); n = 5; ND = Not determined.

drug market in recent times. This multiplicity of brands often put clinicians and pharmacists into a difficult situation of choice, and the possibility of interchangeability among brands. To prove two or more drugs (same active ingredient) bioequivalent, a similarity in the rate and extent to which the drug in the dosage form becomes available for absorption need be demonstrated[15]. Drugs from oral dosage forms only become available for absorption following the process of disintegration and dissolution. In this study, parameters like T_{50}, T_{90} and f_2[12] derived from the dissolution profiles of the nine brands of artesunate were used as estimators for the bioavailability of artesunate, hence their bioequivalence.

Our results based on the *in vitro* dissolution show that significant variation exists in the bioavailability of artesunate from the nine brands of artesunate tablets. However, all the brands except AT1 released >70% artesunate within 30 min and as such passed the

British Pharmacopoeia[16] standard for dissolution test of uncoated tablets. Four brands, AT5, AT6, AT7 and AT8 exhibited >90% dissolution in <10 min (Table 2 and Fig. 2b). This high rate of dissolution precludes any possibility of bioavailability problem resulting from drug dissolution and hence justifies interchangeability among the four brands. In cases where >85% of the drug is dissolved within 15 min, dissolution profiles are usually accepted as similar without further mathematical evaluation[12]. The other five brands AT1, AT2, AT3, AT4 and the innovator drug, AT9, did not meet the criterion of 85% dissolution and as such were subjected to further mathematical evaluation to demonstrate bioequivalence. The T_{50} and T_{90} values of AT3 and AT4 are similar, while that of AT2 and the innovator drug, AT9 are similar (Table 2). AT1 has a T_{50} and T_{90} values of 30 and >120 min respectively indicating poor dissolution, hence poor bioavailability. The result of statistical comparison of the five brands using similarity factor as estimator, showed that AT2, AT3 and AT4 are bioequivalent with the innovator drug, AT9.

An oral dosage form is normally composed of a drug substance and excipients and the proportion between them, the type of excipients and the manufacturing method of the final product are chosen based on the content, the physicochemical and the bulk properties of the drug and its absorption characteristics. Taken as a whole, this gives each product certain dissolution characteristics, which varies from one brand to the other. It is not surprising, therefore, the observed variation in the *in vitro* dissolution of the nine brands of artesunate included in this study. Oral artesunate may be considered mainly a prodrug for dihydroartemisinin (DHA), as the metabolite is the main contribution to the overall antimalarial activity[6,7]. The rate and extent of dissolution in the gut, and hence the absorption is a very critical step in demonstrating the bioequivalence, since the absorbed drug molecule readily converts to DHA.

Comparison of the therapeutic performance of two or more medicinal products containing the same active substance is a critical means of assessing the possibility of alternative use between the innovator and any essentially similar medicinal product[12]. Our results so far show that the four brands of artesunate, AT5, AT6, AT7 and AT8 can be interchanged in clinical settings. More so, AT2, AT3, and AT4 are interchangeable with the innovator brand, AT9 based on the calculated similarity factor.

Variations were also observed in the result of disintegration time and non-official hardness tests. The disintegration time of all the nine brands, however, fall within British Pharmacopoeia[16] specification of disintegration time of ≤15 min. Only AT2, AT4, AT6 and AT9, which have crushing strength of ≥5 kgf are considered optimal[14]. Our result seem to support a strong correlation between disintegration time and the rate of dissolution as earlier pointed out by other workers[17], except in one case, AT3, where the drug showed low disintegration time but not commensurate high dissolution rate.

In conclusion, our results indicate that all the brands of artesunate tablet included in this study apart from AT1 seem to have high dissolution rate and hence very good bioavailability. AT5, AT6, AT7 and AT8 can be considered bioequivalent and interchangeable. More so AT2, AT3 and AT4 can be considered bioequivalent and interchangeable with the innovator brand, AT9. All the brands apart from AT1 could be substituted for one another in the therapy of malaria parasite. This study highlights among other things the need for constant monitoring of the new products introduced into our drug market with the view to ascertain bioequivalence and conformity with pharmacopoeia standards. There is need, however, to carry out *in vivo* studies to further substantiate the *in vitro* predictions.

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References

1. Facer CA**,** Tanner M. Clinical trials of malaria vaccines: progress and prospects. *Adv Parasitol* 1997; *39:* 1–68.

2. Hien TT, White NJ. Qinghaosu. *Lancet* 1993; *341:* 603–8.

3. Nosten F, Luxemburger C, ter Kuile FO, Woodrow C, Pa Eh J, Chongsuphajaisiddhi T, *et al.* Treatment of multidrug resistant falciparum malaria with a 3-day artesunate-mefloquine combination. *J Infect Dis* 1994; *170:* 971–7.

4. Na-Bangchang K, Karbwang J, Congpoung K, Thanavibul A, Ubalee R. Pharmacokinetic and bioequivalence evaluation of two generic formulations of oral artesunate. *Eur J Clin Pharmacol* 1998; *53:* 375–6.

5. White, NJ. Assessment of the pharmacodynamic properties of antimalarial drugs *in vivo*. *Antimicrob Agents Chemother* 1997; *41:* 1413–22.

6. Barradell LB, Fitton A**.** Artesunate: a review of its pharmacology and therapeutic efficacy in the treatment of malaria. *Drugs* 1995; *50:* 714–41.

7. Bethell DB, Teja-Isavadharm P, Phuong CX, Thuy PT, Mai TT, Thuy TT, *et al.* Pharmacokinetics of oral artesunate in children with moderately severe *Plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg* 1997; *91:* 195–8.

8. Newton P, Suputtamongkol Y, Teja-isavadharm P, Pukrittayakamee S, Navaratnam V Bates I, *et al*. Antimalarial bioavailability and disposition of artesunate in acute falciparum malaria. *Antimicrob Agents Chemother* 2000; *44*(4)*:* 972–7.

9. Osadebe PO, Akabogu IC. Assessment of quality control parameters and interchangeability of multisourced metformin HCl tablets marketed in Nigeria. *Boll Chim Farm* 2004; *143*(4): 170–3.

10. Osadebe PO, Esimone CO, Akabogu IC. An empirical assessment of the possibility of interchangeability between multisource ciprofloxacin hydrochloride tablets marketed in Nigeria. *Boll Chim Farm* 2003; *142*(8): 352–6.

11. Osadebe PO, Esimone CO. Qualitative and quantitative evaluation of multisource piroxicam capsules available in Nigeria. *Bio Res* 2003; *1*(2): 101–10.

12. Note for guidance on the investigation of bioavailability and bioequivalence. The European agency for the evaluation of medicinal products (*Evaluation of Medicines for Human Use*). London: Committee for Proprietary Medicinal Products 2001.

13. *United States Pharmacopoeia* XX. Easton: Mack Publishing Co. 1980; p. 958–9.

14. Ofoefule SI, Orisakwe OE, Ibezim EC, Esimone CO. Mechanisms of nifedipine release from sustained release tablets formulated with some polymeric materials. *Boll Chim Farm* 1998; *137:* 223–7.

15. Chow CS, Liu JP. Design and analysis of bioavailability and bioequivalence studies. New York: Marcel Dekker 1992; p. 1–2.

16. *British Pharmacopoeia*. London: The Stationery Office, 2001; p. 1183.

17. Proudfoot SG. Factors Influencing bioavailability and drug absorption from the gastrointestinal tract. In Aulton Mr., editor. *Pharmacokinetics: The Science of dosage forms design*, I edn. London: Churchill Linguistic 1988; p. 135.

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