Effects of artemether on the pharmacokinetic parameters of quinine in wistar rats.

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ABSTRACT

The effect of artemether on the pharmacokinetic parameters of Quinine(QN) was carried out in this study. The parameters were investigated using adult Wistar rats. The rats were divided into three groups; A, B and C. Group A served as the control which was administered intramuscularly (i.m) 0.9w/v normal saline while group B was administered QN and artemether (i.m) at a dose of 5.14mg/kgbody weight(b..w). and (0.5mg/kgb.w respectively while Group C was administered QN alone (i.m) at a dose of 5.14mg/kgb.w .Blood samples were collected from each group of animals at 30 min. interval for 8 hr after the injection. The investigation was based on a one-compartment model with first order absorption. Using HPLC method with a limit of detection of 0.5µg/mL, the plasma concentration of quinine was monitored and quantified. The area under the blood concentration –time curve (AUC_∞) of QN was not significantly different ($P \geq 0.05$) when compared QN alone with QN in the presence of artemether being 124 µg/mlhr and 136 µg/mlhr for groups B and C, respectively. The half life ($t_{1/2}$) and T_{max} of QN in the groups B and C were the same being 8hr and 2hr respectively for both while Cmax showed a significant difference ($P \leq 0.05$) being 54.63µg/ml and 90.55µg/ml in the presence and absence of QN, respectively. This work justifies the fact that these drugs can be concomitantly administered since pharmacokinetic parameters like Tmax and the (AUC_∞) of quinine were not significantly affected by artemether.

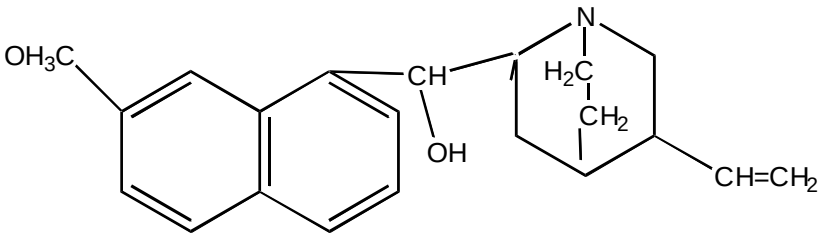
KEYWORDS: Quinine, Artemether, pharmacokinetic parameters.

INTRODUCTION

Artemether and quinine are antimalaria drugs used in the treatment of malaria infections in the tropical countries of the world. Malaria is one of the tropical diseases that is associated with both human and economic loss. Malaria is reported to cause the death of children in Africa, killing nearly one million children each year. Every day approximately 3,000 children die from the disease. According to the Centers for Disease Control and Prevention (CDCP), malaria is also known to be the 5th cause of death from infectious diseases worldwide (after respiratory infections, HIV/AIDS, diarrhea diseases, and tuberculosis). Malaria is the 2nd leading cause of death from infectious diseases in Africa, after HIV/AIDS. (Schild, 1980) Monotherapy was formerly being used for the treatment of malaria but the incidence of resistant strain of Plasmodium species has

led to the introduction of combination therapy

Quinine(QN) is an alkaloid isolated from Cinchona bark in 1820 (Ajibola, 2000). It contains a quinoline and a quinolidine group joined by a secondary alcohol group Fig.1.



Fig, 1. Quinine

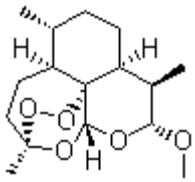


Fig 2. Artemether

QN is laevo-rotatory, but its isomer quinidine is dextro-rotatory. The laevo-rotatory isomer is more efficacious as an antimalaria agent compared to its dextro-rotatory isomer (Schild, 1980; Ajibola, 1998). Artemisinin (qinghaosu) and its derivatives are antimalaria drugs derived from a Chinese plant. Artemisinin derivatives are an important new class of anti-malaria agents. These compounds contain endoperoxide bridges, which are essential for anti-malaria activity (William, 1998 Meshnick, 2002). Artemisinin acts via a two-step mechanism. It is first activated by intraparasitic heme-iron, which catalyzes the cleavage of the endoperoxide . A resulting free radical intermediate may then kill the parasite by alkylating and poisoning one or more essential malaria protein(s) (Meshnick, 2002).

Chemical modifications of Artemisinin (reduction plus etherification) have enabled more potent and more soluble derivatives to be developed. Among these different products is Artemether (Paluther®) (fig. 2), the methyl ether form of dihydroartemisinin ((Luo *et al.,* 1984).

QN is the mainstay for the treatment of multi-drug resistant malaria and in severe malaria in many countries. It is still effective despite reports of reduced sensitivity (Pukrittayakamee *et al.,*1994). The World Health Organization (WHO) has recognized the use of Artemisinin Combination Therapies in the treatment of malaria, as a long-term measure to control spread of the disease under its Roll Back Malaria program (WHO, 2003).

The incidence of resistance to single dosage regimen in malaria treatment has led to combination therapy which has drastically reduced the incidence of resistance. The combinational therapy has led to synergistic

actions of some drugs or in some other cases has led to exacerbations of effects of drugs or reduction in activity. The systemic bioavailability of drugs is an essential process that precedes any pharmacological activity (Rang *et al*, 2003). The objective data of the use of drugs i.e. the relationship between plasma concentration and the intensity of therapeutic/ toxic action , plasma half lives , relative efficacy of different medications etc. are being obtained with the aim of optimizing therapy (Tripathi , 2001). The purpose of this study was to ascertain whether artemether has an effect on pharmacokinetic parameters of quinine in Wistar rats .

MATERIAL AND METHODS

Fifteen adult Wistar rats, weighing between 200-270g were obtained from the animal house of the Department of Veterinary Physiology , University of Ibadan. The rats were kept in standard cages and acclimatized for a period of seven days in the animal house of the Faculty of Pharmacy, Olabisi Onabanjo University , Ogun State , Nigeria . Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were duly followed throughout the experimental procedure. The experimental guideline was in conformity with the Faculty of Pharmacy, Olabisi Onabanjo University rules guiding the use of rats for scientific studies. The rats were kept five per cage and fed daily with standard rat diet produced by Bendel's Feed Limited, purchased from Ijokun, Sagamu, Ogun State, Nigeria. All rats were allowed water *ad libitum*. The drugs Artemether (80mg/ml) i.e. 80 mg Artemether preserved in 1ml of 125 x 4mm) column as stationary phase and 0.05% v/v triethylamine in acetonitrile Arachis oil (vehicle) (May and Baker Ltd, Nigeria), QN (600mg/2ml) (Medreich) and normal saline (0.9% w/v) (Unique Ltd, Nigeria) were purchased from Adun-Ade Pharmacy Limited, Sagamu, Ogun State, Nigeria. A 70kg human takes a maximum dose of 160mg Artemether i daily for three days in the treatment regimen for m,alaria while maximum dose of 1800mg per day of QN is given .. Therefore, extrapolating from human doses each adult wistar rat received corresponding doses of 0.5mg/kg/b.w. of Artemether and 5.14mg/kg/b.w. of QN administered intramuscularly. Rats in control Group A received 5.14ml/kg/bodyweight of normal saline. Group B was administered 0.5mg/kg/b.w. of Artemether and 5.14mg/kg/b.w. of QN while Group C was administered 5.14mg/kg/b.w. of QN alone. The tail of each rat was cut at the tip with a sterile scissors and blood was collected at 0 and at 30mins interval for 8 hours into heparinised tubes. The blood samples were then centrifuged within 24 hr at 3000g for 10 mins to obtain plasma

.The plasma was analysed for QN by adapting the method of quinine extraction from biological fluids described by (Babalola, *et al*., 2004). QN was extracted from plasma (1 ml) by addition of 200 µl of perchloric acid to precipitate plasma proteins, followed by addition of 1 ml of 5 M NaOH and 4 ml of diethyl ether for solvent extraction. After mixing using a vortex mixer, the organic layer was aspirated and back extracted into 0.05 M H_2SO_4 . The extracted drug was then chromatographed by HPLC (Buchi Lab. Switzerland) utilizing hypersil BDS C18 (5µm, buffered with Potassium hydrogen phosphate (KH_2PO_4) as the mobile phase.

RESULTS

Pharmacokinetic Analysis.

The mean plasma concentration versus time profiles of QN in the various blood samples after the HPLC analysis are as shown in the graph (fig.3). Each point showing the mean of the plasma concentrations and standard deviation (SD) in Wistar rats at each collection time for each group.

These pharmacokinetic parameters like peak plasma concentration (C_{max}), the time of peak plasma concentration (T_{max}) and the area under the blood concentration time curve (AUC) were calculated from the individual plasma concentrations time curve of the two groups. Half life ($T_{1/2}$) was calculated by using the elimination rate constant (k_e) ($T_{1/2} = 0.693/k_e$) and k_e was determined from the slope of the terminal portion of the log concentration time curve . The area under the curve up to last data point (AUC_t) was calculated by trapezoidal rule method and the area from last data point up to infinity(AUC_∞) was calculated by dividing terminal time drug concentration by k_e. .(Nicholas, 1993) as shown in table 1. The other parameters were calculated using appropriate pharmacokinetic equations. (Ajibola, 2000).

Pharmacokinetic Parameters

Data Analysis: Data are expressed as Mean ± SD. Student's't' test with 95% confidence level was used for statistical analysis of the results.

DISCUSSION

This work seeks to determine the effect of artemether on the pharmacokinetic parameter of QN. The investigation was based on a one-compartment model with first order of absorption (Na-Bangchan, *et al*, 2000). Following i.m administration of artemether, the concentration of QN in the plasma after HPLC analysis differed from those administered with QN alone after 1hr, 1hr 30mins, 2hrs and 8 hrs intervals which were 58.53, 85.34, 90.55, 9.84ng/ml ± SD. respectively. The group A animals were administered 0.9w/v of concentration 90.55ng/ml ± 7 for QN in the absence of artemether in comparison with 54.63ng/ml ± 4 of QN in the presence of artemether. The area under the curve (AUC) depicts the bioavailability of the drug in the systemic circulation and as obtained from concentration versus profile fig. 3, AUC of the two groups vary slightly. The AUC of QN was 136.3 ng/mlhr ± 6 while that of QN in the presence of artemether was 124.8 ng/mlhr ± 11 . The AUC of QN was reduced by 8.4%. in the presence of artemether and this is in agreement with the work of Oliver Burk *et al,* (2005). Artemether has a comparatively high recrudescence rate which has been attributed to the auto induction of CYP2B6- mediated metabolism (Oliver Burk *et al,* 2005). Artemether induces cytochrome P-450 and MDRI expression by activation of Xenosensors Pregnane X receptor and constitive Androstane Receptor which mediate induction of drug metabolizing enzymes and drug transporters (Bertam, 2001, Martin *et al* 2005).

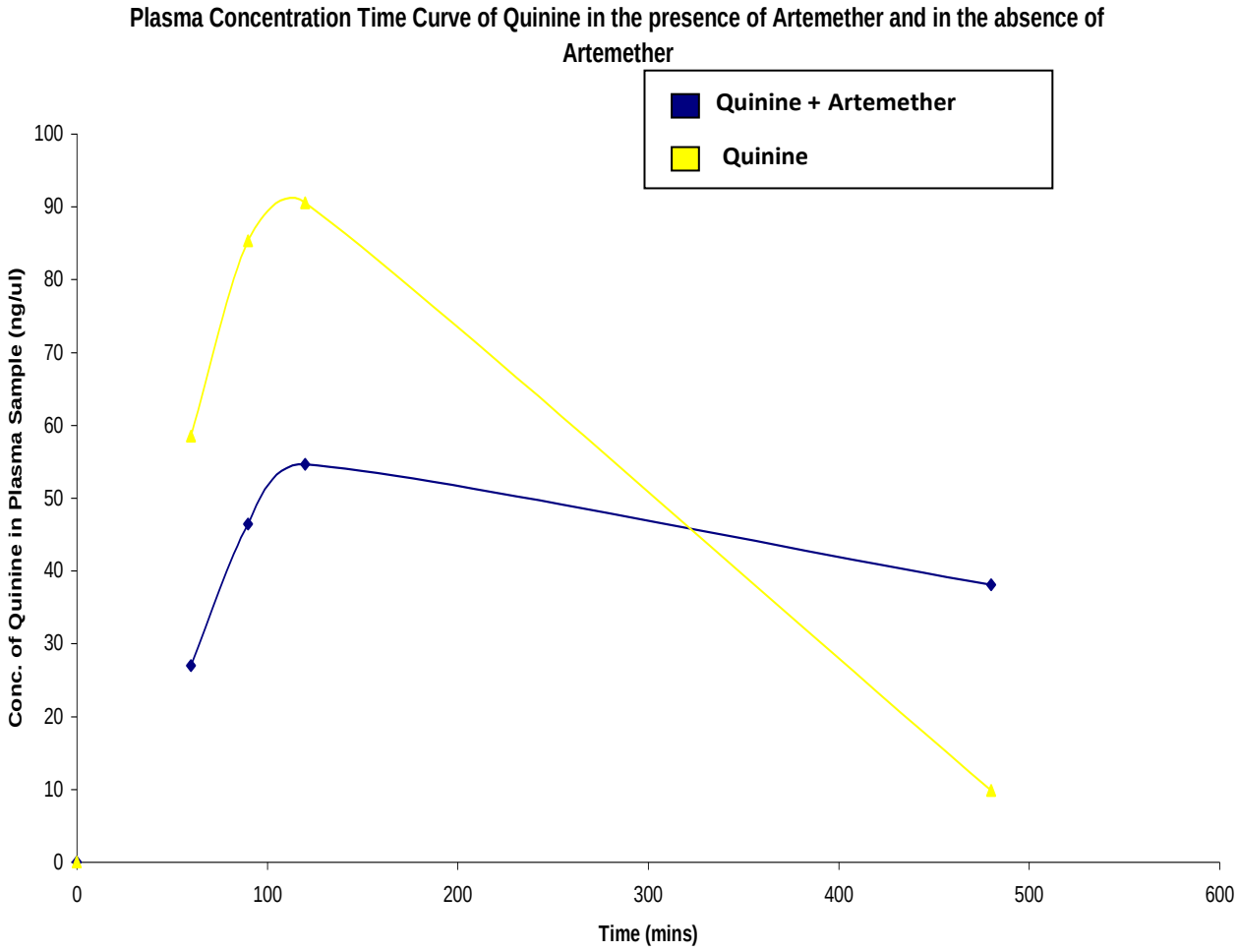


Fig. 3. The mean plasma concentration versus time profiles of QN in the various blood samples of the presence and absence of Artemether. Each point showing the mean of the plasma concentrations and standard deviation (SD)

TABLE 1. Pharmacokinetic parameters obtained from plasma concentration time curve of Quinine plus Artemether and Quinine alone.

Parameters	Quinine plus Artemether	Quinine alone
C_{max} (ng/ml)	54.63µg/ml ± 4.0	90.55µg/ml ±7.0
T_{max} (minutes)	120mins ± 8.0	120mins ± 8.0
$T_{1/2}$ (minutes)	8hrs ± 0.5	8hrs ± 0.5
$AUC_{0-\infty}$ (ng/ml/hr)	124.8 µg/mlhr ± 11.0	136.3 µg/mlhr ± 6.0
K_a	0.3465 hr^{-1}	0.3465 hr^{-1}
K_e	0.085662hr^{-1}	0.085662hr^{-1}
V_d	0.0902L	0.00014L
C_l	4.0x10^2ml/hr	3.7 x10^2ml/hr

The induction of these enzymes increases metabolism of quinine which leads to reduction in the bioavailability of QN in the blood. (Oliver Burk *et al,* 2005). However from the calculations as obtained in the graph fig.3, the peak plasma concentrations (Cmax) were significantly different (P≥0.05) but the lower plasma levels obtained from the two groups are not lower than the therapeutic window for QN, which assures therapeutic efficacy *(Babalola et al, 2004) The* plasma peak concentrations after administration of QN alone and with artemether were 54.63ng/ml and 90.55ng/ml respectively and this is in conformity with the work of Na-Bangchan, *et al*,(2000) and Babalola *et al,*(2004).

The absence of QN in the sample collected at 30minutes interval might be due to minute quantity of QN in the blood sample collected from the animals which was practicably difficult to analyze after centrifugation by HPLC.

CONCLUSION

This work justifies the fact that these drugs can be concomitantly administered since pharmacokinetic parameters like T_{max} and the AUC of quinine were not significantly affected by artemether. Artemether reduces the concentrations of quinine in the blood but this invariably helps in reducing the side effects of quinine significantly. The use of combinations involving artemether and quinine analogues or derivatives is well appreciated and of significance benefit if the therapeutic effects is enhanced or remain the same.

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REFERENCES

Ajibola A .Olaniyi (1998), *Essentials of medicinal chemistry*; Antimalarias. Published by Shaneson C.I Ltd. Ibadan ,pp. 145.

Ajibola A .Olaniyi(2000), *Principles of drug quality assurance and pharmaceutical analysis*. Published by Mosuro Ltd. Ibadan . pp. 150-317

Bertam G. Katzung, (2001). *Basic and Clinical Pharmacology: pharmacokinetics and pharmacodynamics*, 8th edition. Lange Medical/ Mc Graw Hill. London , pp.35-44

Babalola C.P , Adebayo A.S , Omosoto A., Oyeyinka A.(2004). Tropical Journal of pharmaceutical research; comparative bioavailability study of a new quinine suppository and oral quinine in healthy volunteers. 3 (1) 291-297.

Luo XD, Yeh HJC, Brossi A, Flippen-Anderson JL, Gillardi R (1984). The chemistry of drugs part IV. Configurations of antimalarials derived from qinghaosu: 16 Dihydroqingyhaosu, artemether, and artesunic acid. Helv Chim Acta; 67:1515-22.

Meshnick SR (2002). Artemisinin: mechanisms of action, resistance and toxicity. Int J Parasitol ; 32:1655-60

Martin LJ, Taniguchi H, Robert NM, Simard J, Tremblay JJ, Viger RS.(2005) GATA factors and the nuclearreceptors, steroidogenic factor 1/liver receptor homolog 1, are key mutual partners in the regulation of the human 3beta-hydroxysteroid dehydrogenase type 2 promoter. Mol Endocrinol;19:2358- 2370.

Nicholas H.G, Holford M.B,(1993) Rational dosing and the time course of drug action. Clinical Pharmacokinetics. 25;495.

Na-Bangchang K, Karbwang J, Ubalee R, Thanavibul A, Saenglertsilapachai S (2000).. Absence of significant pharmacokinetic and pharmacodynamic interactions between artemether and quinoline antimalarials. Eur. J.Drug Metab. Pharmacokinetic. 25(3-4),171-3

Oliver Burk, Katja A. Arnold (2005). *Molecular Pharmacology*; antimalarial artemisinnin drugs. Erlangen , Germany, pp. 116—117

Pukrittayakamee S, Supanaranond W, Looareesuwan S, Vanijanonta, White N. (1994). Quinine in severe falciparum malaria: evidence of declining efficacy in Thailand . Trans R Soc Med Hyg; 88:324-7.

Rang. I. H.P, Dale, M.M, Ritter J.M, Moore P.K,(2003). *Pharmacology*; how drugs act, drug elimination and pharmacokinetics. 5th edition. Published by Livingstone Ltd. England, pp. 43-47,115-116.

Schild,(1980), Applied Pharmacology, twelth edition, Longman Group Limited, Singapore . pp. 442

Tripathi K.D,(2001). *Essentials of medical pharmacology.*. Fifth edition. Jaypee brothers Ltd, New Dehli. pp 12-32

World Health Organization(2003). Assessment of the safety of artemisinin compounds in Pregnancy.WHO/RBM/TDR/Artemisinin/03.1. WHO/CDS/MAL.1094

William Charles Evans(1998); Trease and Evans pharmacognosy. *Quinine and its alkaloids* Saunders Company Ltd. U.K. pp 399