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Absolute bioavailability of quinine formulations in Nigeria

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Summary

This study compared the absolute bioavailability of quinine sulphate as capsule and as tablet against the intravenous (iv) infusion of the drug in twelve male volunteers. Six of the volunteers received intravenous infusion over 4 h as well as the capsule formulation of the drug in a cross-over manner, while the other six received the tablet formulation. Blood samples were taken at predetermined time intervals and plasma analysed for quinine (QN) using reversed-phase HPLC method. QN was rapidly absorbed after the two oral formulations with average t_{max} of 2.67 h for both capsule and tablet. The mean elimination half-life of QN from the iv and oral dosage forms varied between 10 and 13.5 hr and were not statistically different ($P > 0.05$). On the contrary, the maximum plasma concentration (C_{max}) and area under the curve (AUC) from capsule were comparable to those from iv ($P > 0.05$), while these values were markedly higher than values from tablet formulation ($P < 0.05$). The therapeutic QN plasma levels were not achieved with the tablet formulation. The absolute bioavailability (F) were 73 % (C.I., 53.3 – 92.4 %) and 39 % (C.I., 21.7 – 56.6 %) for the capsule and tablet respectively and the difference was significant ($P < 0.05$). The subtherapeutic levels obtained from the tablet form used in this study may cause treatment failure during malaria and caution should be taken when predictions are made from results obtained from different formulations of QN.

Keywords: *Quinine, capsules, tablets, bioavailability, malaria*

Résumé

Cette étude comprarée la disposition absolue de la quinine sulfate orale et intraveneux chez 12 males volontier. Six recevaieent l'injection intraveneux(IV) pendant 4 heures ainsi que les six autres recevaient les capsules orale. Les échantillons du sang étaient prelevés pouir determiner l'intervalle de temps de disposition et la concentration du plasma en quinine(Qn) utilisant la phase reverse de la méthode de la chromatographie liquide(HPLC). La quinine était rapidement absorbée après les 2 formalutions prise. Avec le temps moyen Tmax de 2.6 h pour les comprimes et capsule. La démi-vie d'elimination de la Qn par voie Intraveneux et voie orale variait entre 10-13.5 hr et n etaient pas statistiquement significatif (P>0.05) Au contraire, la concentration max dans le plasma(Cmax) et la surface sous la courbe (SSC) pour les capsules étaient comparable à celles par IV (P<0.05). Les taux de plasma therapeutique de la Qn n'étaient pas achevées avec les comprimés. La disposition absolu(F) etait 73% (CI:53.3-92.4%) et 39%(CI:21.7-56.6%) pour les capsules et les comprimes respectivement et la difference etait significative(P<0.05). Les taux sous-thérapeutique obtenus des comprimés pourraient causer d'échec thérapeutique et les les précautions doivent etre prise dans la prédiction des résultants dans different formulations de quinine.

Introduction

Quinine (QN) is employed in the treatment of multi-drug resistant malaria and is a drug of choice for the treatment of severe and complicated malaria [1,2]. It is administered by slow intravenous (iv) infusion in severe and/or complicated malaria and orally (as capsule, tablet, or syrup) in uncomplicated malaria as well as following iv therapy as patient conditions improve [3,4]. Earlier studies on absolute bioavailability (BA) of QN report that QN is completely absorbed (BA ranging from 64 – 90 %) after oral administration of tablets [4-8], while information on the capsule formulation is lacking.

Due to the assumption that quinine is completely absorbed after oral intake, similar doses of quinine are employed in malaria treatment via both iv and oral routes and pharmacokinetic parameters such as total clearance and volume of distribution have been computed assuming complete absorption [1,9].

QN has a narrow therapeutic index and exhibits considerable intra- and inter- individual variations in its kinetics [9-12], therefore BA studies of the formulations is necessary as required by WHO for such drugs [13]. The increasing use of QN, the increasing availability of many generic forms as well as the "fake drug syndrome" in Africa, where malaria is an important disease deem it necessary to evaluate the bioavailability of QN formulations circulating in any country.

In this present study, the absolute BA of two types of oral formulations of QN (capsule and uncoated tablet) were evaluated against iv administration of the drug in healthy subjects.

Materials and methods

Subjects

Twelve male healthy volunteers divided into two groups of six participated in the study after giving their informed consent. Group A subjects (capsule group) were aged between 18 and 30 years and weighed between 47 and 66 kg, while group B (tablet group) were aged between 20 and 25 years and weighed between 53 and 68 kg. They were all

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adjudged healthy on the basis of history, clinical examination, biochemical and electrocardiographic screening prior to entry to the study. All the volunteers were non-smokers, none had taken quinine or any other medication at least one week before the study. They all abstained from other drugs, coffee, alcohol and beverages such as soft or carbonated drinks throughout the duration of the study. The study protocol received approval from the joint University College Hospital and University of Ibadan (UCH/UI) Ethics Committee..

Drug administration and sample collection

The twelve subjects were randomly allocated to two groups of six (A & B). On the first day of study after observing an overnight fast, subjects in group A received oral dose of 600 mg quinine sulphate capsules (Eli – Lily and Co., Indianapolis, USA), while subjects in group B received oral dose of 600 mg quinine sulphate plain (uncoated) tablets (acf Chemiafarma, Maarsen, Holland) with 200 ml of water. The subjects remained fasted until 4 h post dose after which food and water were allowed freely. Venous blood (5 ml) was collected from the forearm vein before and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 24, 36 and 48 h after drug intake.

Two weeks after, intravenous infusion was given to subjects in group A after an overnight fast. 600 mg quinine dihydrochloride injection (Avion Pharma, Hamburg, Germany) was added to 0.9 % of normal saline and infused over 4 h. Venous blood (3 ml) was collected by indwelling catheter before and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 4 h during infusion and at 0.5, 1, 2, 3, 4, 5, 6, 8, 20, 32 and 48 h after the infusion. All blood samples were placed in heparinised tubes and centrifuged immediately for 10 min at 1000 rpm to separate plasma, which was stored at –20°C until analysis.

Both the 600 mg quinine sulphate and the dihydrochloride used in the study were all equivalent to 500 mg base of QN. All the three formulations (injection, capsule and tablets) were subjected to some pharmaceutical assessment such as weight uniformity and chemical assay following British Pharmacopoeial (B.P.) standards [14]. They all gave active content of between 97.3 and 100 % w/w, which fell within the limits of 95 to 105 % specified in B.P.

Sample and data analysis

The plasma samples were analysed for QN by ion pair reversed-phase HPLC method developed in our laboratory by Babalola et al, 1993 [15] after validation. A C-18 column was used with fixed UV detector at 254 nm. Mobile phase was a mixture of 0.02M potassium dihydrogen phosphate, methanol and acetonotrile (75:15:10 %v/v) containing 74 mM perchloric acid. The limit of detection of this assay method was 10 $ngml^{-1}$ using 1 ml plasma. Interassay coefficient of variation (CV %) was < 6 % for both within run and in-between run. Recovery was more than 90 % over the concentration range analysed. Plasma collected prior to QN administration (blank) showed no endogenous source of interference with the assay.

Pharmacokinetic parameters were evaluated from plasma QN concentration versus time data by non-compartmental methods [16]. The peak plasma concentration (C_{max}) and time of peak plasma concentration (t_{max}) were observed directly from plasma concentration time data of each subject. The area under the curve (AUC) was calculated by trapezoidal method. The extrapolated AUC was estimated by using Ct/β, where β is the elimination rate constant. β was determined by linear regression of the post-distribution phase of the concentration – time profile. Absolute BA (F) was determined as AUC_{iv}/AUC_{oral}. The clearance (total clearance, CL_T for iv infusion and oral clearance, CL/F for capsule and tablet) were determined as Dose/AUC; while volume of distribution (V_d for iv and V_d/F for oral) were calculated as Clearance/β.

Results were recorded as mean ± S.D. Analysis of variance (ANOVA) was used to perform statistical evaluations amongst the three formulations and this was further subjected to the Student Newman – Keuls (SNK) procedure for comparison of differences between individual means and pair groups. In all, a value of $p < 0.05$ was considered statistically significant. The absolute bioavailability parameter (F) was further analysed using 90 % confidence interval (C.I.) approach of Westlake [17].

Results

A few of the subjects experienced some form of side effects from mild to moderate, all corresponding to times of high plasma concentrations. One subject complained of headache of 30 minutes duration at the third hour of iv infusion, while another complained of dizziness and abdominal pain 4 h after oral intake of the capsule. No complaints were received from the subjects after tablet intake. However, all symptoms experienced were reversible and none was serious enough to stop any participant from completing the study.

Mean plasma QN concentrations after administration of the three formulations (iv infusion, capsule and tablet) are illustrated in Fig. 1, while derived pharmacokinetic parameters are summarised in Table 1. The declining phases of the plasma concentration versus time curves were approximately parallel to one another. T_{max} varied between 2 and 4 h (mean, 2.7 h) after oral administartion of both capsule and tablet and there was no significant difference between them ($P > 0.05$). The elimination half – lives ($t_{1/2}$) obtained after the three formulations (mean range, 10.2 - 13.5 h) were comparable ($P > 0.05$). On the contrary, the AUC and C_{max} obtained after tablet intake were significantly lower ($P < 0.05$) than those obtained after iv infusion and capsule intake. The absolute BA, F from the capsule was 73 % and from the tablet, 39 % ($P < 0.01$). Analysis of the data using Westlake

Table 1: Pharmacokinetic parameters of quinine derived after intravenous infusion and oral administration of single dose of 500 mg of the drug to volunteers.

Parameters	IV infusion	Capsule	Tablet
t_{max} (h)		2.67± 0.76	2.67 ± 0.51
		(2.0-4.0)	(2.0-3.0)
C_{max} (mgl^{-1})	9.43 ± 3.26	6.68 ± 3.00	2.41 ± 0.76[c]
	(8.27-15.26)	(4.52 12.70)	(1.45-3.16)
AUC ($mg.hml^{-1}$)	129.85 ± 32.20	94.58 ± 31.96	50.82 ± 24.27[c]
	(81.20-181.60)	(65.30-148.40)	(29.37-93.64)
Bioavailability, F (%)		73	39
		(C.I., 53.3-92.4)	(C.I., 21.7-56.6)
$t_{1/2}$ (h)	12.18 ± 4.33	10.20 ± 2.87	13.64 ± 4.01
	(7.00-19.97)	(7.70-15.64)	(7.00-17.32)
CL ($mlmin^{-1}kg^{-1}$)[a]	1.19 ± 0.29	1.71 ± 0.62[c]	3.27 ± 1.46[c,e]
	(0.78-1.60)	(0.98-2.64)	(1.31-4.72)
V_d (lkg^{-1})[b]	1.31 ± 0.77	1.44 ± 0.40[d]	3.55 ± 1.63[d,e]
	(0.73-1.77)	(0.79-2.00)	(1.96-6.30)

Values are expressed as mean ± S.D. and (range)
[a] Total clearance (CL_T) for iv; oral clearance (CL/F) for capsule and tablet
[b] V_d for iv; Vd/F for capsule and tablet
[c] CL corrected for F = 1.25 ± 0.45 (0.71-1.93) for capsule; 1.27 ± 0.58 (0.51-1.84) for tablet
[d] V_d corrected for F = 1.07 ± 0.34 (0.57-1.61) for capsule; 1.38 ± 0.65 (0.77-2.44) for tablet
[e] significantly different from iv and capsule ($p < 0.05$)

90 % C.I. approach showed F range of 53.3 – 92.4 % for the capsule and 21.7 – 56.6 % for the tablet.

Oral clearance (CL/*F*) and volume of distribution (V_d/F) obtained after capsule intake were not significantly different ($P > 0.05$) from total clearance (CL_T) and V_d after iv infusion, while those obtained after tablet intake were significantly greater ($P < 0.05$). However, when correction was made for F, the CL and V_d estimates were similar for the three preparations ($P > 0.05$).

Discussion

The rapid absorption of QN from both capsule and tablet formulations (t_{max} of 2 – 4 h) is in agreement with findings of earlier studies by Salako *et al*, 1992 [5]; Paintaud *et al*, 1993 [6]; Jamaludin *et al*, 1988 [11] and Babalola *et al*, 1997 [12]; who have all reported t_{max} of between 1 and 4 h. The rapid absorption of QN is expedient in therapy when therapeutic levels are desired within a very short time interval. QN was also rapidly eliminated from the body and by 48 h after drug administration most of the drug has been eliminated. The fact that the plasma level curves after iv and oral doses were essentially parallel (Fig. 1); the t_{max} from oral doses were similar (Table 1) and the $t_{1/2}$ did not vary significantly confirm that the route of administration of QN does not affect its pharmacokinetics as reported by earlier workers [3, 5, 9-12].

This study demonstrates a marked difference in the extent of absorption of QN from the iv, capsule and tablet formulations. The two oral dosage forms of capsule and tablet are therefore bio-inequivalent although the kinetic

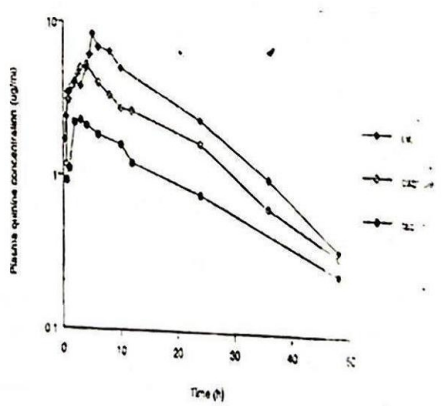

Fig. 1: *Mean plasma quinine-concentration time profiles in healthy subjects after 500 mg of the drug by intravenous infusion and oral administration of capsule and tablet formulation*

parameters from iv and the capsule formulations are statistically equivalent ($P > 0.05$). The 90 % C.I. of ± 20 % [13] approved by FDA was not achieved after both capsule and tablet intake, an indication of wide range in the bioavailability of QN from both formulations. The lower and upper limits for the F value of the tablet (21.7 - 56.6 %) were so low compared to those of the capsule (53.3 – 92.4 %). Confidence interval (C.I.) is normally used to determine if there are large differences between mean parameters. It is a function of sample size and study variability including inter- and intra- subject variability [18]. The wide margin obtained is an indication of wide inter-individual variations

of quinine already confirmed by previous workers. Although the subjects who took the tablet formulation were different from those that took iv and capsule formulations (parallel design), they were from the same locality, the same sex and did not differ significantly in age or weight. They were all subjected to the same treatment procedure and none of the entire twelve volunteers had taken QN before.

The effective therapeutic QN plasma level required for malaria chemotherapy is 4.5 – 10 mgl^{-1} also levels at which some side effects are observed [19, 20]. None of the subjects who took the tablet achieved a C_{max} up to the lower limit of the therapeutic level, while the C_{max} obtained from iv and capsule all fell within the therapeutic level (Table 1). C_{max} from tablet ranged from 1.45 – 3.16 mgl^{-1} with a mean of 2.41 mgl^{-1}. This suggests that this tablet formulation used in this study if given in the same dose as iv or capsule, may produce subtherapeutic plasma concentrations for antimalarial purpose thus allowing parasite survival and facilitating development of drug resistance. This could have serious implications for treating a life-threatening disease susch as malaria. Switchability should be practised after proper bioavailability/ bioequivalence (BA/BE) are established.

Garnham *et al*, 1976 [21] been reported that sugar coated QN tablets gave delayed and incomplete absorption, but the tablets used in the present study were plain and absorption was rapid as shown by the short t_{max} (mean < 3 h). The type of salt used (QN sulphate) could not have also contributed to poor absorption of QN from the tablet since QN sulphate capsule, which was more bioavailable were also used and QN sulphate tablets have been reported to give bioavailability of up to 90 % [4, 6, 8]. From the chemical assay, the tablet contained 97.3% of QN sulphate, which met the official specification of 95 – 105 % [14]. The compressed tablet, though complex, is one of the most widely used dosage form and many problems are encountered due to variations in manufacturing process unlike the capsule preparations [18].

QN absorption is normally assumed to be complete after oral dosage and pharmacokinetic parameters such as clearance (CL) and volume of distribution (V_d) are estimated assuming F value of 100 % [6,22]. Such an estimation should be done with caution as this may not be the case with all oral formulations of QN. The capsule form can be said to be completetly bioavailable since oral clearance (CL/F) and V_d/F estimates were comparable with total clearance (CL_T) and V_d after iv infusion. The relatively higher estimates of oral clearance and V_d after tablet intake when compared with iv and capsule, (Table 1) suggest incomplete BA of the tablet. However, when the values are corrected for F, the estimates compared favourably with those from iv and capsule formulations. This is noteworthy especially since malaria significantly alters the disposition of QN including CL and V_d [1,23]. It is advisable to take F into consideration when such parameters are being determined from oral formulations.

In summary, this study has demonstrated that QN disposition is comparable after iv and oral administration. QN is more extensively absorbed from the capsule form and very poorly absorbed from the tablet form used in the present study thus confirming the need for adequate bioavailability and bioequivalence studies for QN preparations. The clinical implication may be that of treatment failure during malaria if such tablet forms are used. This study emphasizes caution in switching tablet for iv or capsule formulations in the same doses.

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