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Research Article

Comparative bioavailability study of a new quinine suppository and oral quinine in healthy volunteers

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Abstract

Purpose: *There is the need for alternative and more convenient route of quinine (QN) administration in complicated and severe malaria. The purpose of this study is to compare the bioavailability (BA) of a new quinine suppository made from theobroma oil to that of an existing tablet formulation in healthy volunteers.*

Methods: *Six healthy volunteers were administered with 300 mg of QN sulphate as suppository and tablet in a crossover manner. QN concentrations in both plasma and urine at predetermined time points were determined spectrofluorimetrically.*

Results: *Absorption was slower, more variable and lower with the suppository than with the tablet. The time of maximum concentration (T_{max}), maximum concentration (C_{max}), area under the curve (AUC) and cumulative urinary excretion (Du^{∞}) for the two formulations were also significantly different, with no changes in elimination half-life ($t_{1/2}$). The respective C_{max} and AUC values were 4 to 5 times higher with the tablet (2.32 $\pm$ 0.22 μg/ml, 36.31 $\pm$ 10.06 μg.h/ml) than with the suppository (0.52 $\pm$ 0.37 μg/ml, 7.69 $\pm$ 5.79 μg.h/ml). The Du^{∞} were 9.17 $\pm$ 1.11 mg and 2.56 $\pm$ 0.55 mg for the tablet and suppository respectively. The relative BA of the suppository was 21.24 $\pm$ 16.00 % (95 % C. I., 8.44 – 34.04%) from plasma levels and 26.14 $\pm$ 7.80 % (95 C.I., 19.90 – 32.38 %) from urine excretion.*

Conclusion*: Absorption of this new QN suppository is poor; therefore it may not be therapeutically expedient to substitute it for the tablet form at the same dose. Improving the suppository formulation or increasing the dose in order to increase its BA may be necessary.*

Key words: *Quinine, suppository, bioavailability.*

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Introduction

Quinine (QN) is one of the least expensive and most effective and available drug for the treatment of severe and multi-drug resistant malaria. It is still effective against *Plasmodium falciparum* strains in Africa[1-3]. Recent reports reveal that QN is still as effective as artemisinin and derivatives in treating cerebral malaria in children[3, 4].

In severe and complicated malaria, intravenous (i.v.) injections of QN are usually recommended until the patient is able to take oral formulations[5, 6]. However, this route of administration is not often applicable in rural areas due to lack of trained health personnel as well as inaccessibility to health facilities[7]. Another route of QN administration, which is intramuscular (i.m.), is a common source of complication in children leading to pain, local inflammation, abscess, tetanus and lower extremity disability[8, 9]. The oral route is effective but is unsuitable for nauseous and comatose patients. There is therefore the need for alternative and more convenient route of administration of QN.

The rectal route is commonly used in paediatric practice and is widely assessed as an alternative to parenteral administration[10]. At present, artemisinin and its derivatives are available as suppositories and have been found to be as effective as i.v. and i. m. formulations in treating severe malaria[4, 11, 12]. Barenness and other workers have discovered that intrarectal administration of a QN cream, Quinimax®, and injectable soluble QN salts are effective in treatment of severe and complicated malaria in children in some French-speaking parts of Africa[7, 8, 13-16]. These workers observed that intrarectal QN (IRQ) is well tolerated and safe. They also observed that their efficiency was comparable to i.m. and i.v. treatments despite their very poor and erratic bioavailability [7, 13-16]. However, some of the rectally injected QN exhibited such side effects as early rejection, intestinal transit problems, watery stool, and insufficient product retention requiring re-administration [8, 15]. The IRQ requires adequate dilution to reduce acidity and so requires trained personnel to do so[8]. The only quinine rectal formulation so far tested, which is the rectal cream [13], also requires trained personnel for its handling and administration. This practice can defeat the benefit of rectal administration of the drug in rural areas and can affect self-administration and compliance by the patients themselves or their caregivers. It is therefore necessary to produce a formulation specifically adapted to the rectal route and optimise dosing hence the need to formulate a proper QN suppository that is simple to use and requires no trained personnel or manipulation such as is the case with artemisinin suppositories. Presently no QN suppository is available for use.

In this study, we formulated QN suppository and compared its bioavailability with the tablet formulation using healthy adult volunteers.

Materials and Methods

Subjects

Nine healthy adult male Nigerian subjects were recruited into the study but six subjects complied fully with the protocol. The six volunteers were aged 21 – 27 years (24 ± 2.68 years, mean ± SD) and weighed 55 – 69 kg (59 ± 5.20 kg, mean ± SD). All the volunteers were non-smokers and none was receiving any other drugs at least two weeks before commencement of the study and no other drugs or alcohol or caffeine was permitted throughout the duration of the study.

Preparation of the Quinine Suppository

The new QN suppository was prepared in the Drug Research and Production Unit (DRPU) of Obafemi Awolowo University Ile-Ife, Nigeria by fusion method using a blend of theobroma oil and beeswax as the suppository base. The suppository (1 g)

contained 300 mg QN sulphate (248.6 mg QN base). Uncoated QN sulphate tablets - (Generic, Poole, UK, Lot # PL 4569/0089) were obtained from a local pharmacy at Ibadan. The QN sulphate suppository and tablets were analysed by non-aqueous titration as described in BP 1998[17]. The chemical contents of the suppository and the tablets were 96.60% w/w and 98.28% w/w, respectively. These values are within the official specifications[17].

Drug administration and sample collection

All the subjects observed an overnight fast prior to drug administration and remained without food until 4 h after drug intake and thereafter meals were taken. Water was allowed to be taken freely during the study.

The design of drug administration was a simple crossover. On the day of study each subject received 300 mg QN sulphate in the form of one tablet with a glass of water. After a one-month washout period, the subjects received one suppository of QN sulphate (300 mg) through the rectum.

Venous blood samples (5 ml) were collected by venipuncture from the forearm just before and at 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24 and 48 h following administration of rectal and oral doses of the drug. The blood samples were placed in heparinised tubes, centrifuged immediately at 3,000 *g* for 10 min to obtain the plasma. Total urine voided was collected just before and at intervals of 0-4, 4-8, 8-12, 12-24 and 24-48 h after drug administration. The volume was measured and aliquot of 10 ml stored. All plasma and urine samples were stored at –20 ^{o}C until analysed.

Sample analysis

The plasma and urine samples were analysed for QN spectrofluorimetrically by adapting the method of quinine extraction from biological fluids described previously[18]. 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 analysed by a fluorimeter (Perkin Elmer Fluorescence Spectrometer Model 204 Uberligen, Germany). The wavelengths of detection were 355 nm for excitation, and 450 nm for emission. Analysis of the drug from urine samples was as described for plasma except that 0.2 ml of urine was diluted to 1 ml with water before extraction process and analysis.

The intra-day and inter-day precision of the method ranged from 1 to 4.7 % (CV%) in plasma and 3 to 9 % in urine. Percent recovery ranged from 96.5 to 98 % in plasma and 95 to 100 % in urine. The limit of detection was 20 ng/ml. The accuracy of the method assessed by the deviation of determined concentrations from the actual concentration was less than 8 % at various concentrations tested for both fluids.

Pharmacokinetic analysis

Peak plasma concentration (C_{max}) and time to reach peak concentration (T_{max}) were obtained from the plot of plasma concentration versus time profile. The area under the plasma concentration time curve (AUC) was calculated by linear trapezoidal method with extrapolation to infinity using Ct/β where Ct is the last determined concentration and β is elimination rate constant calculated from the slope of the terminal phase of plasma concentration-time curve.

From urine levels, pharmacokinetic parameters such as total amount excreted unchanged (Du^{∞}), maximum peak of excretion $[(dDu/dt)]_{max}$, time of maximum peak excretion (t_{max}) and elimination half-life ($t_{1/2}$) were evaluated from excretion rate plots.

Bioavailability (F) of the suppository with respect to the tablet was estimated as AUC_{rectal}/AUC_{oral} x 100 % and from urine as $Du^{\infty}_{rectal}/Du^{\infty}_{oral}$ x 100 %.

Data are presented as mean ± SD and compared using Student's t-test for paired observation; p value < 0.05 was considered significant at 95% confidence interval.

Ethical Issues

The Joint University of Ibadan and College of Medicine Ethics Committee approved the study protocol. Written informed consent was obtained from all the subjects.

Results

The test medications (QN tablets and QN suppositories) were well tolerated by all subjects. No adverse effects were observed in any of the volunteers. The suppositories were never expelled and no rectal irritation or diarrhoea was reported.

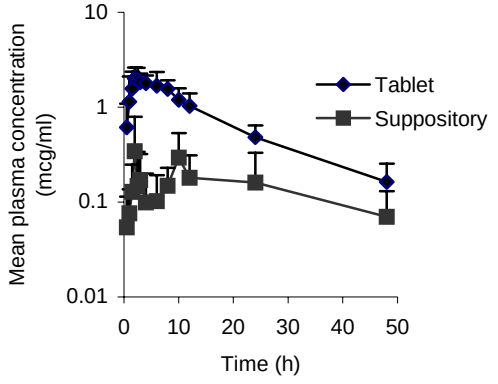

Figure 1: Mean plasma concentration versus time profiles of quinine (QN) following single oral and rectal administration of 300 mg of QN sulphate as tablet and suppository to healthy volunteers

The mean plasma concentration versus time profiles of QN in the volunteers, after single oral and rectal doses of QN sulphate are shown in Figure 1. Comparative pharmacokinetic parameters derived from plasma and urine are shown in Tables 1 and 2, respectively. The plasma profiles after rectal route was biphasic in almost all the subjects producing two peaks around 2 and 10 h (Fig. 1). The T_{max} of absorption in plasma after suppository intake (7.25 ± 4.50 h) was significantly longer (p = 0.0336) than the T_{max} after tablet intake (2.67 ± 1.67 h). However, the t_{max} of urinary excretion were similar for both formulations (p = 0.667) (Table 2). The elimination half-life ($t_{1/2}$) following rectal administration was longer and more variable than after oral administration but the difference was not significant in both plasma and urine (p > 0.1).

There were wide inter-individual variations in drug levels in both plasma and urine samples. QN levels as assessed by C_{max}, AUC and Du^{∞} in plasma and urine, after oral administration were approximately 4 to 5 times higher (p = 0.0039) than QN levels obtained after administration of the suppository. The bioavailability of the suppository relative to the tablet was calculated as 21.24 ± 16.00 % (95 % C. I., 8.4 to 34.04 %) in plasma. The total amount excreted in urine (Du^{∞}) were 9.17 ± 1.11 mg for the tablet and 2.56 ± 0.55 mg for the suppository, giving a relative BA of 26.1 ± 7.8 % (95 % C.I. 19.9 to 32.4%). There was no significant difference between these relative BA values. .

Discussion

Rectal QN administered as injectable solution or cream has proven to be effective for the treatment of both uncomplicated and complicated malaria despite their poor bioavailability and the side effect of early rejection [8, 15]. The need to improve the performance of this alternative route and improve its administration and compliance has necessitated the formulation of QN suppositories, which are more adaptable to the rectal route. Comparing the bioavailability of the formulation with other formulations will provide a guide for those who may want to make use of the

Table 1: Pharmacokinetic parameters obtained from plasma after administration of single dose of 300 mg Quinine sulphate as tablet and suppository to healthy volunteers

Volunteers	Age (yr)	Weight (kg)	C_{max} (μg/ml)		T_{max} (h)		$T_{1/2}$ (h)		AUC (μg.h/ml)		F (%)[a]
			Oral	Rectal	Oral	Rectal	Oral	Rectal	Oral	Rectal	
BC	27	60	1.68	1.19	2.0	2.0	7.29	8.79	24.70	2.43	9.84
BD	27	69	1.96	0.66	2.5	10.0	14.20	14.07	35.76	18.28	51.12
BF	22	58	2.34	0.47	1.5	10.0	10.25	14.34	3463	9.63	27.81
BG	22	55	2.74	0.31	6.0	12.0	13.27	7.59	51.44	7.26	14.11
BH	21	61	3.17	0.16	2.0	1.5	9.33	26.01	43.98	4.88	11.10
BI	25	55	2.05	0.31	2.0	8.0	10.32	21.16	27.33	3.68	13.47
Mean	24.0	59.7	2.32	0.52	2.67	7.25	10.78	15.33	36.31	7.69	21.24[b]
SD	2.68	5.20	0.55	0.37	1.67	4.45	2.56	7.11	10.06	5.79	16.00
			p = 0.004		p = 0.034		p = 0.224		p = 0.001		

[a] F is bioavailabilty = AUC_{rectal}/AUC_{oral} x 100 %; [b]95 % confidence limit is 8.44 –34.04 %

Table 2: Pharmacokinetic parameters obtained from urine after administration of single dose of 300 mg Quinine sulphate as tablet and suppository to healthy volunteers.

Volunteers	Age (yr)	Weight (kg)	dDu/dt_{max}		T_{max} (h)		$T_{1/2}$ (h)		Du^{∞} mg		F (%)[a]
			Oral	Rectal	Oral	Rectal	Oral	Rectal	Oral	Rectal	
BC	27	60	0.40	0.05	2	6	9.38	10.95	5.09	0.71	13.95
BD	27	69	1.09	0.31	6	6	7.57	14.03	12.43	4.37	35.16
BF	22	58	0.73	0.07	6	2	10.22	8.40	9.04	2.46	27.21
BG	22	55	0.73	0.19	6	2	11.06	12.12	12.00	3.77	31.42
BH	21	61	1.12	0.07	6	6	11.67	15.37	8.17	1.64	20.07
BI	25	55	0.76	0.31	2	2	7.14	9.71	8.31	2.41	29.00
Mean	24.0	59.7	0.81	0.17	4.67	4.00	9.51	11.76	9.17	2.56	26.14
SD	2.68	5.20	0.27	0.12	2.07	2.19	0.75	1.07	1.11	0.55	7.80
			P = 0.002		P = 0.611		P = 0.103		P = 0.00009		

[a] F is bioavailabilty = $DU^{\infty}_{rectal}/DU^{\infty}_{oral}$ x 100 %; [b]95 % Confidence limit is 19.90 – 32.38 %

suppository for treatment of malaria infections.

The results of this study demonstrate a marked difference in the extent of absorption of QN from the suppository when compared with the tablet formulations. Following administration of the suppository, the very low and variable QN concentrations observed in both plasma and urine are indicative of poor and erratic absorption. Variability was more pronounced in plasma than in urine (Tables 1 and 2) probably due to lower drug concentrations obtainable in plasma than in urine.

The marked difference in the extent of absorption of QN from suppository and tablet shows that the two dosage forms are bioinequivalent since the FDA rule in relation to confidence interval (C. I.) of 20 % was not achieved. Also the lower plasma levels obtained with the suppository, which are much lower than the therapeutic window for QN[19], may lead to therapeutic failure. Therefore higher rectal doses relative to oral may be required to achieve comparable therapeutic QN plasma levels as has been practiced by previous authors[13-15].

Several factors, including the nature of the drug substance, nature of the suppository base and the rectal environment, can influence the rate and extent of drug absorption into the body when a drug is administered as a suppository namely; the nature of the drug substance, nature of the suppository base and the rectal environment [20]. The similarity in the relative BA and elimination half-lives of the suppository and tablet formulations for plasma and urine suggests that urine may be substituted for

plasma as a non-invasive method for BA determination of QN in human. However, the poor BA of the suppository may be as a result of low colonic surface area, small rectal aqueous volume and low water solubility of QN sulphate, which can result in poor dissolution of the drug in the rectum[20]. QN sulphate was the least absorbed when compared with the dihydrochloride and bisulphate salts in humans[21]. The QN gluconate salt used by Barenness *et al*.[13] has a better water solubility hence the slightly higher BA (36 %) than obtained in the present study. Preliminary studies in our laboratory showed that the more water soluble salts produced suppositories with poor consistency even though drug release was high; however, further studies are still ongoing. Partitioning between suppository base and rectal fluid is also affected by the variable fluid volume in the rectum and QN sulphate being poorly water soluble may be retained more in the fatty cocoa butter base rather than the rectal fluid[20]. The incorporation of absorption enhancers into the suppository formulation could also improve its bioavailability.

The T_{max} and $t_{1/2}$ obtained from plasma profile after oral intake agreed with literature values [22, 23]. The longer T_{max} obtained after rectal dosing and the double peaks around 2 h and 10 h in plasma (biphasic profile) may be attributable to the erratic absorption of suppositories generally. This type of profile may be beneficial during malaria treatment because it can provide a more sustained plasma drug concentration leading to prolonged effect of the drug. QN is known to exhibit wide inter- and intra- individual variations *in vivo* [23]. Most rectally administered formulations have also been shown to exhibit poor and considerable variability in drug absorption [10] including chloroquine [24], artemisinin and derivatives [11, 12, 25] and also quinine [13, 14]. In most of the reports on rectal artemisinin and QN, despite their poor and variable BA (30-40 %), these drugs cleared malaria parasites just as their oral and parenteral counterparts although higher doses (1 to 3 times) of the rectal forms relative to the other routes were used[13-15].

Conclusion

This study indicates that the bioavailability of this newly formulated quinine sulphate suppository made with cocoa butter base is too poor compared to the existing tablet formulation. Improving the suppository formulation may be necessary and further studies are underway with the aim of developing a QN suppository that will yield optimal rectal absorption.

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