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Full Length Research Paper

Disposition of quinine and its major metabolite, 3-hydroxyquinine in patients with liver diseases

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Quinine, extensively metabolized by CYP 3A4, has 3-hydroxyquinine as the major metabolite which also contributes to its antimalarial activity. This study assessed the impact of various liver diseases on the disposition of quinine and 3-hydroxyquinine. Ten adult patients with liver diseases ranging from cirrhosis, primary liver carcinoma, hepatitis, ascites, and amoebic liver disease as well as six healthy subjects received single oral dose of 600 mg quinine sulphate tablets. Venous blood and urine were collected over 48 h. Quinine and 3-hydroxyquinine were determined from the matrices by a validated high performance liquid chromatography (HPLC) method. Wide inter-individual variations were observed in the subjects especially those with liver diseases. Cmax (4.47 vs 2.41mcg/ml) and AUC (73 vs 51mcg.h/ml) values were significantly increased in liver disease patients. Clearance was reduced by 30% from 3.27 to 2.31 (p = 0.009). Metabolic ratio of quinine/3-hydroxyquinine in plasma decreased from 5.5 to 1.42 over 4 to 48 h. Cumulative amount of quinine and 3-hydroxyquinine produced in urine were 38 mg (8%) and 32 mg (7%) respectively. Compromised metabolism of quinine in liver disease patients suggests the necessity of reviewing the dosage of quinine in liver disease patients who come up with malaria, a situation that further reduces liver function.

Key words: Quinine, 3-hydroxyquinine, liver diseases, CYP3A4.

INTRODUCTION

The cinchona alkaloids has been an important antimalarial drugs for more than 350 years and its principal constituent, quinine (QN), still remains effective against chloroquine-resistant falciparum malaria. It is still widely used for the treatment of cerebral and complicated malaria as well as leg cramps (Roy et al., 2002). Development of quinine resistance in *Plasmodium falciparum* has been relatively slow and incomplete by comparison with those of the other notable antimalarial drugs such as chloroquine, mefloquine, and sulphadoxine-pyrimethamine (Pukrittayakamee et al., 2003). In areas with multidrug-resistant strains, 7 day regimens of quinine and tetracycline still provide cure rates well over 90% in patients with uncomplicated falciparum malaria (Looareesuwan et al., 1992). Reports show that the pharmacokinetic properties of and therapeutic responses to quinine vary with age, pregnancy, immunity, and disease severity (Pukrittayakamee et al., 1997; White, 1997). Approximately 80% of QN is systemically cleared by hepatic biotransformation and the major metabolite 3-hydroxyquinine (3-OHQN), which contributes 5 to 12% of antimalarial activity is formed by cytochrome P450 3A4 (Muralidharan et al., 1991; Zhang, 1997; Wilairatana, 1994).

The clearance of QN is reported to be significantly reduced during malaria, liver and renal diseases (Purkrittayakamee et al., 1997; Auprayoon, 1995; Newton, 1999). Its reduction in liver disease condition has been shown to be predominantly as a result of disease-induced dysfunction in hepatic mixed-function oxidase activity (majorly CYP3A) which impairs the conversion of quinine to its major metabolite, 3-OHQN, but the effect on 3-OHQN disposition is unknown.

QN is a low clearance drug with a narrow therapeutic index therefore changes in disposition due to liver lesions

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Table 1. Pharmacokinetics of quinine in patients with liver disease and healthy subjects.

Parameter	Liver disease (n=10)	Healthy (n=6)	P value	Significance
C_{max} (mcg/ml)	4.47 ± 2.81 (2.22 -11.06)	2.41 ± 0.76 (1.45 – 3.16)	p<0.05	Significant
t_{max} (h)	3.60 ± 0.84 (2.0 – 4.0)	2.67 ± 0.51 (2.0 – 3.0)	p<0.05	Significant
AUC (mcg.h/ml)	73.39 ± 35.98 (35.63 – 39.70)	50.82 ± 24.27 (29.37 – 93.64)	p<0.05	Significant
$t_{1/2}$ (h)	15.00 ± 6.03 (8.77 – 28.06)	13.64 ± 4.01 (7.00 – 17.32)	p>0.05	Not significant
Cl/F (ml/min/kg)	2.31 ± 0.92 (1.02 – 3.84)	3.27 ± 1.46 (1.31 – 4.72)	p<0.05	Significant
V_d/F (l/kg)	3.01 ± 1.43 (0.89 – 5.00)	3.55 ± 1.63 (1.96 – 6.30)	p>0.05	Not significant

may have implications in treatment outcome. Liver diseases or lesions are varied and tend to contribute differently to the disposition of drugs. Previous animal studies show that liver lesions affect clearance of QN and its metabolite differently and it is been reported that *in vivo* function of CYP3A is relatively disease sensitive in human malaria (Purkrittayakamee et al., 1997). Previous studies on QN disposition in liver disease did not monitor the parent active metabolites therefore in this study we have carried out disposition of QN and its major metabolite, 3-OHQN in patients with various kinds of liver diseases.

Objectives

(i) To determine the pharmacokinetics (PK) of QN in patients with various liver diseases.
(ii) To compare the PK of QN in liver disease patients with data from healthy subjects.
(iii) To determine the disposition of the major and active metabolite of quinine, 3-OHQN in patients with liver diseases.

METHODS

Subjects

Ten adult patients with different liver diseases aged between 31 and 65 years and weighing 43 to 89 kg plus six healthy adult subjects aged 20 to 30 years and weighing 53 to 68 kg were recruited into the study. Liver diseases were diagnosed by clinical and pathological findings. Diseases were classified as liver cirrhosis, primary liver carcinoma, hepatitis, ascites and amoebic liver disease. The study was approved by Ethics Committees of Obafemi Awolowo University Teaching Hospital, Ile-Ife and UI/UCH, and informed consent was obtained from each participant.

Treatment and sample collection

Following an overnight fast, subjects were given a single dose of 600 mg QN sulphate (equivalent to 500 mg QN base) tablet (acf Chemiafarma, Maarsen, Holland) with a glass of water. Venous blood (5 ml) was collected from the forearm vein at predetermined times: 0, 1, 2, 3, 4, 6, 12, 24, and 48 h. Total urine voided were collected at time intervals from 0 to 24 h in patients with liver diseases.

Drug analysis

QN and 3-OHQN concentrations in plasma and urine were analysed by a validated high performance liquid chromatography (HPLC) method developed in our laboratory with retention times of 7.8 and 3.4 min respectively (Babalola et al., 1993). A reversed-phase C18 column was used with an UV detector at a wavelenght of 254 nm. Primaquine was employed as internal standard. The mobile phase was a mixture of 0.02 M potassium dihydrogenphosphate, methanol and acetonitrile (75:15:10 v/v/v) containing 74 mM perchloric acid as the counter ion. Blank plasma collected prior to QN administration showed no endogenous sources of interference with the assay. 3-OHQN was not assessed in healthy subjects.

Pharmacokinetic analysis

Pharmacokinetic parameters of QN (such as tmax, Cmax, $t_{1/2}$, AUC, CL/F and Vd) in both groups were analyzed by model-independent method (Gibaldi, 1991). Cmax and tmax were noted directly from concentration-time data. $t_{1/2}$ was calculated from terminal plasma drug concentrations. [AUC] $0\rightarrow\infty$ was calculated from linear trapezoidal method. Apparent oral clearance (Cl/F) was estimated from dose/AUC while apparent Vd/F was calculated from clearance ($Cl \times t_{1/2}/0.693$). Comparison of pharmacokinetic parameters obtained from patients with liver disease and healthy subjects was made by using student t-test and F-test and a $p<0.05$ was regarded as significant.

RESULTS

Quinine sulphate at a single oral dose was well tolerated by both patients and healthy subjects. Plots of mean plasma concentration calculated in microgram per milliliter (mcg/ml) vs time (h) for QN in both groups are shown in Figure 1, indicating higher levels of QN in patients than volunteers. The plasma profiles of QN and 3-OHQN are shown in Figure 2. Pharmacokinetic parameters are summarized in Table 1.

Wide inter-individual and inter-disease variations were observed especially in liver disease patients. In comparison with healthy subjects, there were four significant changes in pharmacokinetic parameters of QN in patients with liver disease. Cmax and AUC were significantly larger: 4.47 ± 2.81 vs 2.41 ± 0.76 mcg/ml and73.4 ± 36.0 vs 50.82 ± 24.27 mg.h/ml respectively ($p<0.05$). tmax was prolonged: 3.6 ± 0.84 vs 2.67 ± 0.51 h, while

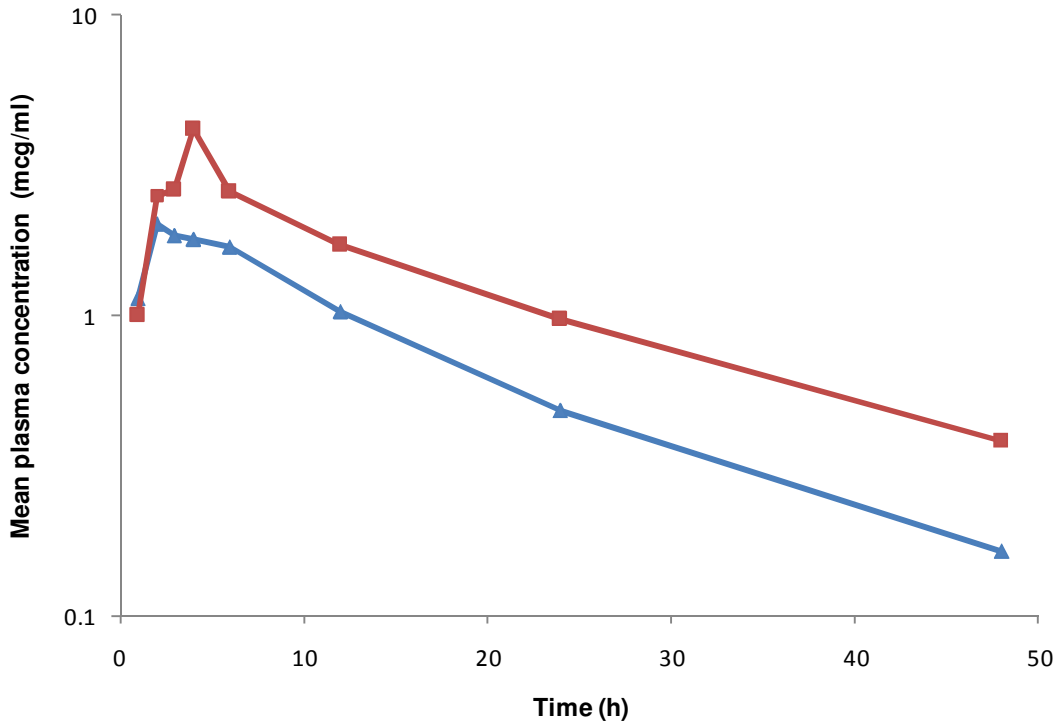

Figure 1. Plot of mean plasma concentrations of quinine after a single oral dose of 600 mg in patients with liver disease (■) and healthy subjects (▲).

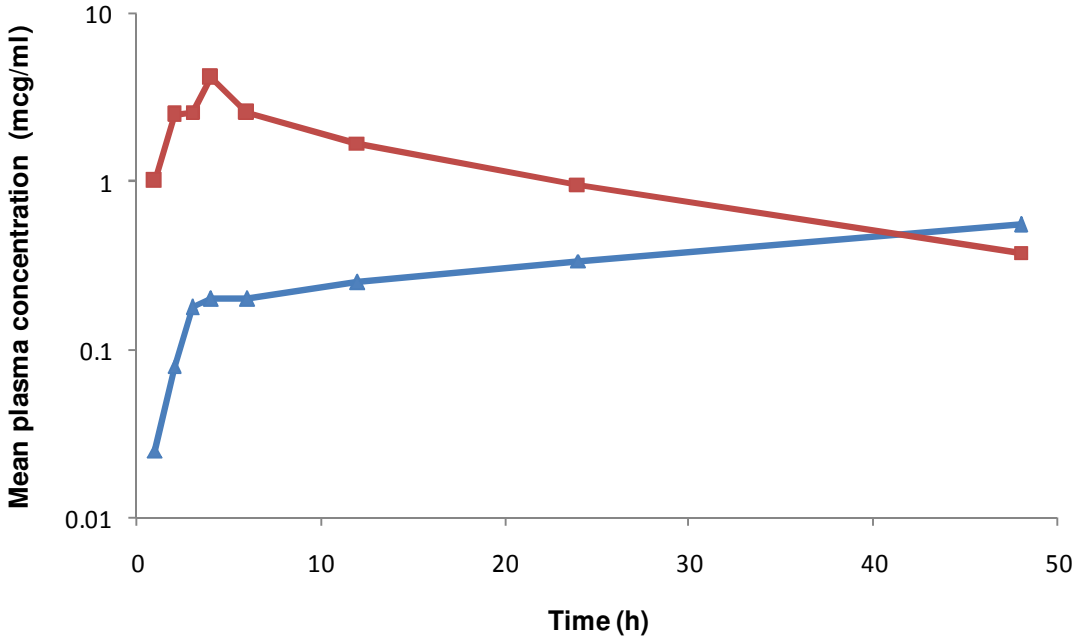

Figure 2. Plot of mean plasma concentrations of quinine (■) and 3-hydroxy quinine (▲) after a single oral dose of 600mg in patients with liver disease.

clearance was reduced (2.31 ± 0.92 vs 3.27 ± 1.46 ml/min/kg ($p<0.05$). Metabolite ratio (QN/3-OHQN) in plasma decreased in patients from 4 to 48 h ranging from 5.5 to 1.42. Cumulative QN and 3-OHQN excreted in urine in the patients were 37.69 ± 27.05 mg (7.5%) and 32.90 ± 20.96 mg (6.6%) respectively (Table 2). The limit

Table 2. Quinine/3-hydroxyquinine plasma and urine profile in varied liver disease conditions.

Disease	Subject	QN/3-OHQN plasma ratio		Urine analysis			
		4 h	48 h	Cumulative amount of QN (mg)	%	Cumulative amount of metabolite (mg)	%
Liver cirrhosis	1	0	2.13	60.45	12.09	65.89	13.18
	2	6.25	0	6.09	1.22	6.51	1.30
Primary cell carcinoma	3	6.67	1.27	49.44	9.89	31.7	6.34
	4	2.63	0	19.4	3.88	29.8	5.96
Hepatitis	5	7.14	1.82	42.75	8.55	12.92	2.58
	6	6.67	0	20.8	4.16	31.6	6.32
Ascites	7	3.23	1.52	98.46	19.69	66.78	13.36
	8	12.5	2.63	34.72	6.94	17.22	3.44
	9	6.25	0	14.76	2.95	19.8	3.96
Amoebic liver disease	10	3.57	1.96	30.03	6.01	46.8	9.36
Mean		5.49	1.42	37.69	7.5	32.9	6.6
SD		3.38	0.96	27.05	5.41	20.96	4.19
Data range		(2.63-12.5)	(0-2.63)	(6.09-98.46)		(6.51-66.78)	

Mean quinine/3-hydroxyquine ratio from 4hr to 48 h = 5.49 ± 3.38 to 1.42 ± 0.96, Mean cumulative quinine excreted = 37.69 ± 27.05 (~ 8%), Mean cumulative 3-hydroxyquinine excreted = 32.9 ± 20.96 (~ 7%).

of detection was 10 ng/ml. The between-day precision for this method averaged between 1.4 and 6% over the concentration range of 0.4 to 10 µg/ml while the recovery ranged between 91 and 98%.

DISCUSSION

The results obtained exhibited wide intra and inter individual variations especially in liver disease patients. Out of the 10 patients with liver diseases, 2 had liver cirrhosis, primary liver cell carcinoma (2), hepatitis (2), ascites (3) and 1 with amoebic liver disease. There was no correlation between the type of liver disease and the pharmacokinetic parameters obtained, rather there was wide intra and inter disease variation. Liver function and mixed function oxidase activities including the activity of CYP3A4 and liver blood flow are all compromised during hepatitis and malaria, (Wilairatana et al., 1994) and therefore can alter the disposition of drugs such as QN, which are majorly cleared by the liver. In the presence of malaria, patients with liver disease may experience more reduction in QN clearance and metabolism of its major metabolite, 3-OHQN, although this can be counteracted in the presence of higher levels of QN and longer half-life. As a result of impaired hepatic biotransformation, plasma levels of QN were higher than levels observed in healthy subjects – with AUC and Cmax of patients being 44 and 86% higher respectively than healthy subjects.

Profile of 3-OHQN in plasma shows a steady increase with time compared to the decline of the parent drug QN (Figure 2). Considering the varied hepatic dysfunction, it will be expected that the profile of the metabolite be at most similar in pattern to the parent compound. Reason for this deviation may likely be the high protein binding property of QN (to α–acid glycoprotein AAG) as compared to its major metabolite, 3-OHQN, which is ~ 50% bound to plasma proteins thereby leading to reduced amount of free QN. Plasma protein binding is established as an important determinant of QN clearance (Orlando, 2009). In all of the patients there was a continuous increase in the metabolite level with time with a value as high as 62% of that of the parent compound (median 30%, range 22 to 62%) at 24 h post dose. A similar result was observed by Newton et al. (1999) in a study of 3-OHQN in patients having severe malaria coupled with acute renal failure. This incremental observation of 3-OHQN can be advantageous during malaria treatment with the metabolite adding to the antimalarial activity of the parent drug and this contribution could be greater in conditions of renal failure as it is seen in severe malaria (Purkrittayakamee et al., 1997; Newton et al., 1999). On the other hand it can also be contributory to adverse effects that occur with QN

intake if it contributes to parent drug activity like 3-hydroxyquinidine (the diastereomer) does to quinindine and as noted by Newton et al. (1999) 3-OHQN should be monitored routinely when QN is administered for malaria treatment in liver compromised patients (Newton et al., 1999).

From its disposition profile (Figure 2) we assume that the elimination pattern of 3-OHQN is completely different from that of QN and therefore follows another pathway but the results obtained by Orlando et al. (2009) differs with a formation rate-elimination that virtually mimics that of the parent compound both in the presence as well as absence of a CYP3A4 inhibitor – erythromycin. The diversity of liver disease conditions could account for this difference as there was wide intra and inter individual variations in this study and the results reflected are a mean of these variations.

Mean percentage of QN excreted unchanged in patients' urine (8%) observed in this study (Table 2), is higher than earlier reports of 5% obtained for healthy subjects (Babalola et al., 1998) and further confirms compromised clearance**.** Although QN is majorly cleared by the liver, the percentage contribution of 3-OHQN to this amount is unknown; approximately 7% was reported in this study in patients with liver disease (Table 2). The clearance of QN is intrinsically low and appears sensitive to disease. These data are consistent with earlier observations suggesting impaired metabolic clearance of QN in patients with liver diseases and malaria (Auprayoon et al., 1995; Karbwang et al., 1993); the difference being that in the present study, the major and active metabolite, 3-OHQN was monitored. Although other hydroxylated metabolites (e.g, 2-OHQN) are also eliminated but 3-OHQN has approximately one tenth (1/10) antimalarial activity of the parent compound and is also indicative of the activity of the hepatic metabolizing enzyme CYP3A4 (Mirghani et al., 2003).

Conclusion

The apparent reduction in QN clearance and its major metabolite in liver disease patients is from disease-induced dysfunction in hepatic mixed function oxidase activity especially CYP3A. Such conditions will impair the biotransformation of QN to its major and active metabolite 3-OHQN and at the same time will have benefits in the combined antimalarial activity of both parent drug and metabolite. There will therefore be the need for routine monitoring of both quinine and 3-hydroxyquinine to ensure that the administered dose of quinine is such that will not lead to pronounced adverse effects should it be assumed that 3-hydroxyquinine exhibits similar adverse effects as quinine just as its diastereomer, 3-hydroxyquinidine does to its own parent drug, quinindine.

This data suggests the necessity to review current dosage regimen of QN when used in treatment of falciparum malaria in patients with most forms of liver diseases especially since the activity of the liver and kidney are reported to be compromised during falciparum malaria.

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