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Failure to detect amodiaquine in the blood after oral administration

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1 Plasma and red blood cell concentrations of amodiaquine (AM) and its metabolite, desethylamodiaquine (DAM), were monitored at intervals up to 21 days after a single oral dose of 600 mg amodiaquine in five normal African subjects

2 Concentrations of AM and DAM were determined using a high performance liquid chromatography technique with a lower limit of sensitivity of 10 ng ml^{-1} for both compounds.

3 AM was not detectable in plasma and red blood cell samples withdrawn from any of the subjects.

4 DAM was detectable in both media. It appeared in the blood 0.5–1 h after administering AM, reached a peak in 1.5–2 h and subsequently declined slowly.

5 We conclude that AM is rapidly converted to DAM and its blood concentration, compared with that of DAM, is very low if not negligible.

Keywords amodiaquine desethylamodiaquine pharmacokinetics

Introduction

The increasing spread of chloroquine-resistant *P. falciparum* in different parts of the world has stimulated interest in alternative drugs for the treatment of acute falciparum malaria. Amodiaquine, itself a 4-aminoquinoline like chloroquine (CQ), has been found in some recent studies to be active against *P. falciparum* with R_I and R_{II} levels of resistance to chloroquine (Spencer *et al.*, 1984; Watkins *et al.*, 1984). This drug may therefore be subject to increasing use in areas of CQ-resistant malaria. Until now very little pharmacokinetic study of amodiaquine has been undertaken, the assumption being that its disposition in the body is exactly like that of chloroquine. In view of the possibility of increasing use of this drug, we thought it desirable to commence a characterization of its pharmacokinetic profile. Data so generated, we believe, should contribute substantially towards optimising the therapeutic use of the drug.

Methods

Five adult African male volunteers aged between 25 and 35 years took part in the study. They were all healthy with normal haematological tests and normal biochemical tests of kidney and liver function. None of them had had malaria or taken amodiaquine or any other antimalarial drugs in the previous 3 months. They voluntarily agreed to participate after the nature of the study and the procedure had been carefully explained to them. The study protocol was vetted and approved by the Ethics Committee of the University of Ibadan, College of Medicine

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where the study took place. The subjects were advised to refrain as much as possible from taking any drugs throughout the duration of the study and for 1 week previously, and to report to the investigators if they had to. None of the subjects had cause to take any drug throughout the study period.

On day 0 of the study, each subject received 600 mg amodiaquine (Camoquin tablets, Parke-Davis) orally at about 09.00 h after an overnight fast. The tablets were swallowed with about 200 ml of water. Subjects were allowed to take fluids subsequently as desired but food was not allowed until 4 h after the tablets. Venous blood (10 ml) was taken before giving the drug and at 0.5, 0.75, 1, 1.5, 2, 4, 8 and 24 h after. In some of the subjects blood was taken again at 3, 7, 14 and 21 days. The blood was collected into a heparinised tube and was immediately centrifuged at 1000 *g* to separate the plasma and red blood cells which were then stored frozen at −20°C until analysed. The samples were subsequently transported frozen in polyurethane insulation-boxes from Ibadan, Nigeria, to the Centres for Disease Control, Atlanta, Georgia, USA, where they were analysed for amodiaquine (AM) and its desethyl metabolite, desethyl-amodiaquine (DAM). The total storage time was less than 3 months.

Extraction of AM and DAM from plasma, red blood cells or urine

To a 2.0 ml aliquot of plasma (or red blood cells or urine) in a teflon-lined screw-cap test tube was added 1.0 ml of distilled water and 1.0 ml of 50% aqueous solution of dipotassium hydrogen phosphate (K_2HPO_4). After adding 10 ml of 1, 2-dichloroethane the mixture was shaken at 280 oscillations/min for 20 min and then centrifuged at 200 rev/min for 15 min. The aqueous layer was removed with a pasteur pipette. An 8.0 ml aliquot of the organic extract was transferred to another clean teflon-lined screw-cap test tube and extracted with 3.0 ml of 0.1 M HCl for 1 min on a vortex mixer. The organic layer was removed with a pasteur pipette and discarded. The aqueous layer was basified with 2.5 ml of 0.1 M NaOH and 1.0 ml of 10% K_2HPO_4 solution and then extracted twice with 3.0 ml portions of methyl-*t*-butylether by shaking the mixture on a vortex mixer for 1 min. The ethereal layer was removed after each extraction, using a pasteur pipette, and the combined extracts were evaporated by keeping the test tube in a water bath at 60°C and under a stream of nitrogen. The residue was dissolved in 15 μl of mobile phase and a 10 μl aliquot injected onto the chromatograph.

Chromatographic system

A modular h.p.l.c. system was used consisting of a Spectra-Physics SP8700 Extended Range Pump, A Rheodyne 7125 injector fitted with a 10 μl loop, an LDC/Milton Roy Spectromonitor III variable wavelength detector set at 251 nm, a Hewlett Packard 3390A Integrator and a stainless steel column, 15 cm × 4.6 mm i.d. containing 5 μ ultrasphere-ODS (Altex).

The mobile phase was a mixture of solvents A and B, the composition of which were as follows:

Solvent A: methanol: acetic acid (99 : 1, v/v) containing heptane sulphonic acid sodium salt (0.005 M).

Solvent B: water: acetic acid (99 : 1, v/v) containing heptane sulphonic acid sodium salt (0.005 M).

The two solvents were placed in separate reservoirs and the pump was programmed to mix the solvents in a ratio of 75 : 25 (A : B). This corresponds to an isocratic mobile phase composition of methanol: water: acetic acid (75 : 24 : 1, v/v/v) containing 0.005 M heptane sulphonic acid sodium salt.

Quantification of AM and DAM was by comparison of peak areas or peak weights from unknown samples with peak areas or peak weights obtained when standard solutions of AM and DAM (238 ng, 476 ng and 952 ng 2 ml^{-1}) in drug free plasma (or urine) were processes as described above. Standard solutions in whole blood were employed for the determination of red blood cell samples of unknown concentrations.

Results

AM was not detectable in any of the plasma or red blood cell samples. On the other hand DAM was detectable in all samples from 0.75 h onwards. The possibility that AM might have disappeared from the samples as a result of storage was investigated by administering 600 mg amodiaquine to two other volunteers (ORI and FCC). Blood was collected at 0, 1 and 4 h and analysed immediately (without prior storage) for AM and DAM content in whole blood, plasma and red blood cells. The results (Table 1) show that only DAM, and no AM, was detectable in the various samples. It is therefore concluded that absence of AM from the study samples was not due to loss through storage. Urine samples were also collected from one of the latter two volunteers (ORI) and analysed for AM and DAM. As with blood samples only DAM was detectable in the urine samples.

Table 1 Concentration of desethylamodiaquine (DAM) in unstored blood (two subjects) and urine (one subject) following a single oral dose of 600 mg amodiaquine (AM). Amodiaquine assayed for at the same time was negative in all samples.

		Blood		
		Concentration of DAM (ng ml^{-1})		
	Time (h) after dosing	*Red-blood cells*	*Whole blood plasma*	
Subject 1	1	41.9	233.2	179.1
	4	147.7	194.1	372.5
Subject 4	1	150.9	168.5	138.5
	4	90.1	49.6	92.1

Urine (Subject 1 only)		
	Desethylamodiaquine excreted	
Duration of sampling (h)	*Total (μg)*	*% of administered AM*
8	6589.7	1.1
16	14588.9	2.4
24	16881.9	2.8

Figures 1 and 2 show the log concentration-time plots for DAM in plasma and red blood cells respectively for the individual subjects and Figure 3 shows the mean plasma and red blood cell concentration-time plots for all the five subjects. DAM was detectable in plasma and red blood cells from 0.75 h. Its concentration reached a peak at 1.5–2 h and subsequently declined slowly such that at 24 h the concentration was still about 30% of the peak concentration. In all subjects for whom samples were available beyond 24 h, levels higher than 10 ng ml^{-1} were detectable. No definite relationship between plasma and red blood cell DAM concentrations was found.

Discussion

Until recently there has been little interest in studying the pharmacokinetics of AM because of the limited used of the drug and because of the presumption that it probably behaves exactly like CQ. In addition, there was no sensitive or specific method for the assay of the drug in biological samples, the material assayed by the earlier method described by Trenholm *et al.* (1974) being 7-chloro-4-aminoquinoline which would be yielded by any 4-aminoquinoline differing from chloroquine only in the side chain. However, with the expected increasing use of AM, the need to study its pharmacokinetics has

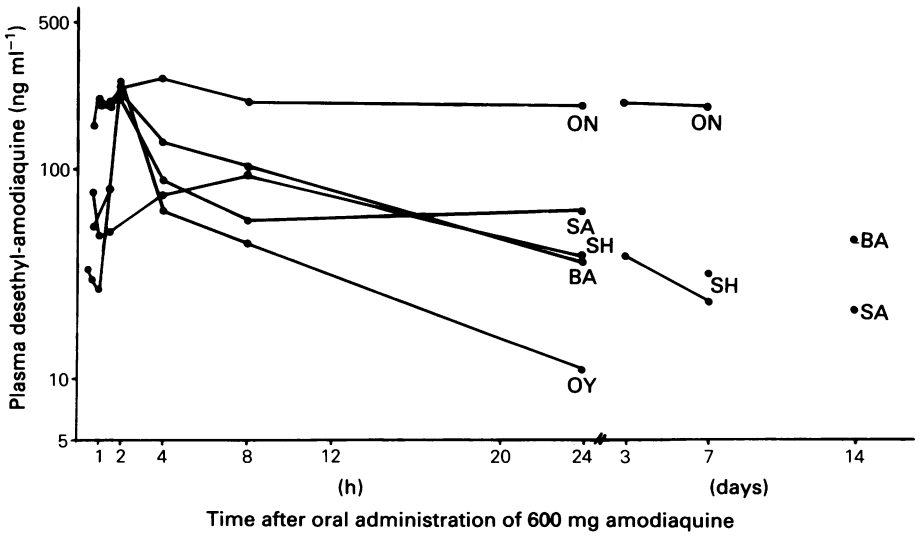

Figure 1 Plasma desethylamodiaquine concentration-time curve for five individual subjects after a single oral dose of 600 mg amodiaquine.

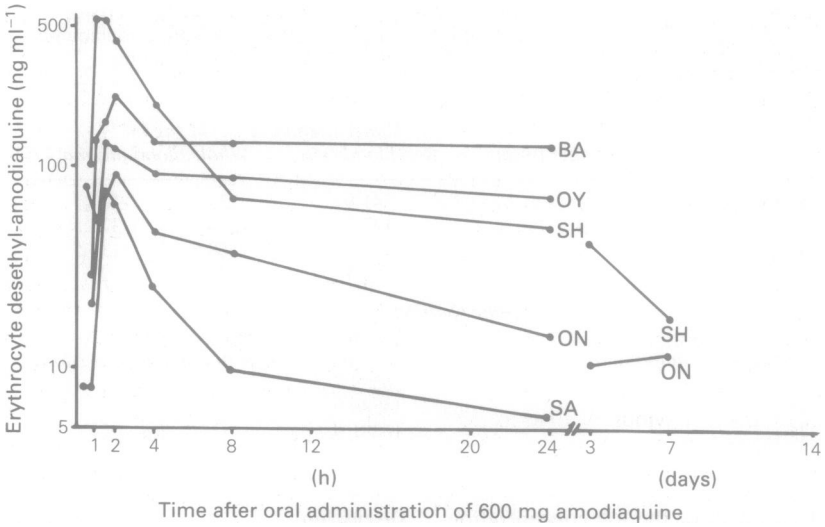

Figure 2 Red blood cell desethylamodiaquine concentration-time curves for five individual subjects after a single oral dose of 600 mg amodiaquine.

become more urgent and the recent description of a sensitive and specific h.p.l.c. method for the analysis of AM and DAM in biological samples by Churchill *et al.* (1985) has substantially eased the analytical problem. Using this new h.p.l.c. method with a lower limit of sensitivity of 10 ng ml^{-1} we were unable to detect AM in the plasma or red blood cells of subjects given 600 mg AM orally at any time from 30 min onwards. The fact that DAM was detectable in all subjects from 45 min onwards suggests that AM is rapidly absorbed from the intestinal lumen but is immediately converted to DAM and possibly other metabolites (Churchill *et al.*, 1985) in the intestinal wall and liver. The peak plasma concentration of DAM observed in this study (approximately 240 ng ml^{-1}, mean) is about 10 times the peak concentration of the main chloroquine metabolite, desethylchloroquine, after a similar dose of chloroquine (Gustafsson *et al.*,

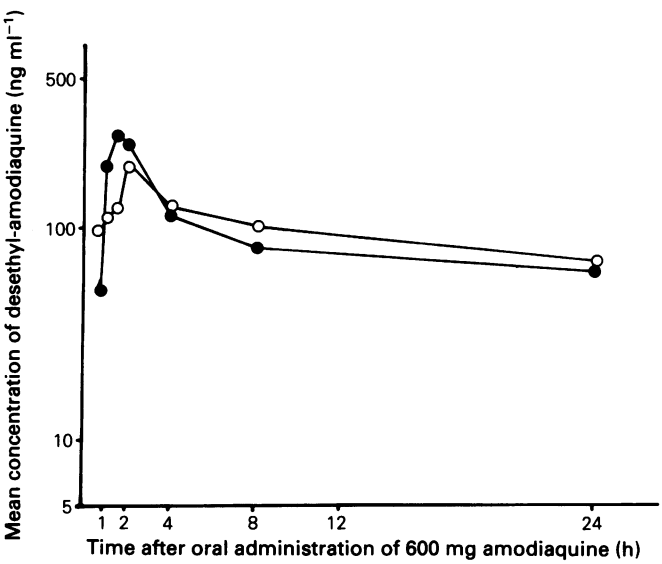

Figure 3 Mean plasma (○) and red blood cell (●) desethylamodiaquine concentration-time curves for the five subjects after a single oral dose of 600 mg amodiaquine.

1983) suggesting a more quantitative metabolism of amodiaquine than chloroquine to their respective desethyl metabolites.

Our study has also shown that unlike in the case of chloroquine and desethylchloroquine, DAM is not preferentially concentrated in the red blood cells. The blood schizontocidal activity of chloroquine (CQ) has been adduced, in part, to the preferential concentration of the drug in red blood cells. The finding with DAM thus raises the possibility that preferential concentration in red blood cells is not, after all, necessary for the blood schizontocidal activity of 4-aminoquinolines.

The slow decline of DAM concentration is reminiscent of the behaviour of certain other antimalarials including the 4-aminoquinoline chloroquine and its metabolite desethylchloroquine, the quinolinemethanol, mefloquine, and the carboxylic acid derivative of primaquine, an 8-aminoquinoline. Chloroquine is extensively bound to tissues (Adelusi & Salako, 1982) and the gradual release from these binding sites might be responsible for the slow decline of the plasma concentration-time curve. The disposition of DAM in the body is not known but could well be similar to that of chloroquine.

On the basis of our results, two important points can be made. The first is that the two main 4-aminoquinoline antimalarial drugs (CQ and AM) differ substantially in their pharmacokinetics after a single oral dose and data obtained for one cannot be extrapolated to the other. It would therefore be necessary to study the pharmacokinetics of AM after single and multiple doses by different routes of administration to the same kind of depth that CQ has been studied. The second point relates to the observation that the concentration of AM in blood after a standard oral antimalarial dose is so low as to be practically negligible when compared with that of DAM. This implies (i) that AM behaves as a pro-drug and probably takes no part in the *in vivo* antimalarial effect after a single oral dose of 600 mg in a semi-immune adult and (ii) that levels of DAM and not those of AM reflect the antimalarial activity when AM is administered for the treatment or prophylaxis of malaria. Studies by Churchill *et al.* (1985) have shown both AM and DAM to be active *in vitro* against all isolates of *P. falciparum* tested, with DAM having a range of activity between 1 and 0.33 that of AM. The use of AM in *in vitro* assay kits for measuring the susceptibility of malaria parasites to AM regimens may therefore need to be reappraised since DAM might be the more logical compound to use for that purpose.

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