Journal of Chromatography, 377 (1986) 307—318
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3040

ISOLATION, CHARACTERIZATION AND STANDARDIZATION OF A MAJOR METABOLITE OF AMODIAQUINE BY CHROMATOGRAPHIC AND SPECTROSCOPIC METHODS

FREDERICK C. CHURCHILL*, DWIGHT L. MOUNT and LESLIE C. PATCHEN

Division of Parasitic Diseases, Center for Infectious Diseases, Centers for Disease Control, U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA 30333 (U.S.A.)

and

ANDERS BJÖRKMAN

Parasitology Department, National Bacteriological Laboratories, S-10521 Stockholm (Sweden)

(First received September 11th, 1985; revised manuscript received November 15th, 1985)

SUMMARY

The amodiaquine metabolite 2-hydroxydesethylamodiaquine (designated metabolite II), one of the two major human metabolites of this antimalarial prodrug, is characterized by chromatographic and spectroscopic methods. This metabolite has been isolated in milligram quantities from the urine of an amodiaquine-dosed individual by extraction and preparative high-performance liquid chromatography (HPLC) and standardized using nuclear magnetic resonance spectroscopy with internal standardization. Aliquots of this standard provided accurately known amounts of the compound for spectroscopic characterization, for use as an HPLC standard and for assessment of in vitro activity against malaria parasites. Knowledge of the structure of the two major metabolites of amodiaquine (the other is desethylamodiaquine) permits speculation as to the presence of three additional human metabolites, chromatographic confirmation for one of which is demonstrated. The in vitro activity of metabolite II is shown to be 1% that of amodiaquine for two chloroquine-sensitive *Plasmodium falciparum* strains. Should this relationship hold generally, desethylamodiaquine is the only chemical species resulting from oral dosing with amodiaquine which contributes significantly to antimalarial activity in the blood.

INTRODUCTION

In *Plasmodium falciparum* malaria the incidence of resistance to chloroquine

0378-4347/86/$03.50

continues to increase in large areas of the developing world, constituting a public health problem of substantial proportions [1]. Treatment with amodiaquine (Am), a 4-aminoquinoline as is chloroquine, has been therapeutically effective for certain resistant strains [2] and has recently been tested in areas of chloroquine resistance in East Africa [3—5] and Southeast Asia [6].

Previous work in our laboratories [7] has demonstrated, by high-performance liquid chromatographic analysis (HPLC) with ultraviolet spectroscopic detection, that oral administration of Am results in appreciable blood levels of two Am metabolites, but that little or no Am is apparent in whole blood. One of the metabolites, desethylamodiaquine (DEAm), designated metabolite I [7], was shown to have an in vitro activity from one to one third that of Am against several *P. falciparum* isolates. The other metabolite seen in blood, designated metabolite II [7], was tentatively identified as 2-hydroxydesethylamodiaquine (HDEAm) based on proton nuclear magnetic resonance (NMR) spectroscopy. Quantities of HDEAm isolated during this previous investigation were not sufficient to permit in vitro comparisons of antimalarial activity with that of Am.

A study by Mihaly et al. [8] assayed plasma levels of Am in two Thai patients from whom blood samples were taken over a period of 20 h following a 10 mg/kg intravenous infusion of Am given over a 4-h period. In both cases Am levels, determined by HPLC, dropped to about 10 ng/ml within 12 h after the infusion was completed. The study of Mihaly et al. [8] provides useful information regarding the rapid clearance of Am, but did not address the question of Am metabolism.

In our study we set out to isolate and standardize a useful quantity of metabolite II, to delineate the primary metabolic pathways of Am, to characterize the metabolites in evidence and to infer the presence of metabolites not detected by the present methodology.

EXPERIMENTAL*

Standards

Amodiaquine base, desethylamodiaquine dihydrochloride and bidesethylamodiaquine (bDEAm) dihydrochloride hemihydrate were supplied by Parke-Davis Division of Warner-Lambert (Ann Arbor, MI, U.S.A.). The *p*-nitrotoluene used as an NMR internal standard was a Fisher TherMetric standard, m.p. 51.54 ± 0.05°C (Pittsburgh, PA, U.S.A.).

Reagents and solvents

Methanol and methyl *tert.*-butyl ether (MTBE) (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) were glass-distilled. d_4-Methanol (99.6 atom%) was from Merck, Sharpe and Dohme (Kirkland, Canada). [^{2}H]Chloroform was purchased from Stohler Isotope Chemicals (Rutherford, NJ, U.S.A.). 1,2-Dichloroethane was obtained from Aldrich (Milwaukee, WI, U.S.A.). All other chemicals used were of reagent grade or better.

*Use of trade names and commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Dosing of volunteer

The male volunteer weighed 90 kg and received a 600-mg dose (as base) of Camoquine (amodiaquine dihydrochloride dihydrate) on two separate occasions several weeks apart. The Camoquine was produced by Parke-Davis Division of Warner-Lambert. Three 8-h collections of urine were made within the first 24-h period after each dose.

Isolation and characterization of metabolite II

Extraction from urine. Quantities of urine equal to about 700 ml, sampled within 24 h of a 600-mg (as base) dose of Camoquine, were extracted as described previously [7] except that the concentration of dipotassium hydrogen phosphate was doubled for extractions into MTBE to increase extraction efficiency.

Analytical HPLC. HPLC with UV detection was employed to evaluate metabolite concentrations in urine extracts and to monitor the purification of metabolite II. The HPLC apparatus used for these purposes consisted of a Spectra-Physics Model SP8700 ternary-gradient solvent delivery system, a Rheodyne Model 7125 loop injector, an Altex Ultrasphere-ODS C_{18} reversed-phase column (5 μm particle diameter; 15 cm $\times$ 4.6 mm), an LDC/Milton Roy SpectroMonitor III variable-wavelength absorbance detector monitoring UV absorption at 254 nm, (detector sensitivity 0.05 a.u.f.s.) and a Hewlett-Packard Model 3390A digital recorder–integrator. The mobile phase was methanol–water–acetic acid (63:36:1) containing sodium heptanesulfonate at a concentration of 0.025 *M*. The solvent flow-rate was 1.0 ml/min, and the solvent was routinely recycled to moderate solvent consumption.

Preparative HPLC. Metabolite II was separated from Am and other metabolites by reversed-phase HPLC using a different chromatographic system than that employed for analytical HPLC. The equipment used for this purpose included an Altex Model 110A isocratic pump, a Rheodyne Model 7125 loop injector, a Varian Micropak CN-10 cyano-bonded preparative HPLC column (10 μm particle diameter; 50 cm $\times$ 8 mm), an LKB 2138 Uvicord S absorbance detector monitoring UV absorption at 254 nm (detector sensitivity 1.0 a.u.f.s.) and a Varian Model 9176 strip-chart recorder. The mobile phase was methanol–water (20:80) containing 0.025 *M* methanesulfonic acid adjusted to an apparent pH of 2.6 with ammonium hydroxide. The flow-rate was 3.0 ml/min.

Four separate urine extractions were each followed by preparative HPLC isolations of metabolite II. In each case the eluate fraction containing the metabolite was extracted from the aqueous phase which had been made basic with dipotassium hydrogen phosphate and then partitioned three times with MTBE. The MTBE layer was separated each time, and the extracts were combined. The MTBE was evaporated using a 60°C water bath and a gentle flow of dry nitrogen. Each of the four products purified in this way was dissolved in several milliliters of methanol. The four samples were combined in a 25-ml volumetric flask and diluted to volume with methanol. Four 6-ml aliquots were transferred to separate containers, two to 15-ml centrifuge tubes and two to 10-ml volumetric flasks. One of these aliquots was taken for NMR quantification and the other three set aside for use in spectroscopic characterization, HPLC standardization and evaluation of in vitro antimalarial activity.

Spectroscopic characterization of the purified sample of metabolite II

The methanol was evaporated from one of the aliquots in a 15-ml centrifuge tube. A [^{2}H]chloroform addition—evaporation step, followed by a similar [^{2}H]methanol treatment, served to remove the last of the methanol. A 100-μl quantity of 1.97 mg/ml Fisher TherMetric-grade *p*-nitrotoluene in [^{2}H]methanol was added as an internal standard compound and the sample diluted to 0.7 ml with [^{2}H]methanol. The sample was vortexed and transferred by Pasteur pipet to a 5-mm NMR sample tube. The NMR spectrum was acquired in 128 scans using a Nicolet NB-360 NMR spectrometer. One such spectrum acquisition utilized a delay between 90° pulses of 7 s, another, also with 128 scans, of 21 s. In both instances, the aromatic portion of the spectrum was integrated. The integral of the high-field *p*-nitrotoluene doublet was compared with that of the 5-position doublet in the metabolite for each spectrum. The quantity of metabolite in this one fourth aliquot was calculated by the Barcza equation [9].

The ultraviolet spectra of metabolites I and II were run on a Beckman Model DU-7 UV—visible spectrophotometer. The samples were run in 0.001 *M* hydrochloric acid at a concentration of 4.16 mg/ml for metabolite I and 3.56 mg/ml for metabolite II.

Fast atom bombardment mass spectrometry (FAB-MS) was performed in the positive-ion mode on a 70-μg sample of metabolite II. The spectrum was run at the Midwest Center for Mass Spectrometry, Department of Chemistry, University of Nebraska-Lincoln (Lincoln, NE, U.S.A.), a National Science Foundation Regional Instrumentation Facility (Grant No. CHE 8211164). Measurements were made on the MS-50 system equipped with a DS-55 data system. The sample was dissolved in a mixture of dithiothreitol and dithioerythritol. Ionization was by FAB using 8—10 kV argon atoms.

Electrochemical properties of Am and its major metabolites

An HPLC system with electrochemical detection was set up to evaluate the sensitivity of Am and its metabolites to electrochemical detection. This system consisted of a Spectra-Physics Model SP8700 ternary-gradient solvent delivery system, a Rheodyne Model 7125 loop injector, an Alltech Absorbosphere C_{18} reversed-phase column (5 μm particle diameter; 15 cm × 2.1 mm), a Bioanalytical Systems Model LC-4B amperometric detector with a glassy carbon electrode set at various potentials within the oxidative range and a Spectra-Physics Model SP4270 digital recorder—integrator. The mobile phase was prepared by mixing 32 parts methanol with 68 parts of an aqueous solution which was 0.025 *M* in ethanesulfonic acid, 0.01 *M* in diethylamine and 0.0002 *M* in disodium ethylenediaminetetraacetic acid (EDTA). The flow-rate was 0.4 ml/min. We varied the potential applied to the glassy carbon electrode in the oxidative range to determine the most selective setting commensurate with sensitive detection of the analytes. The sensitivities of Am, DEAm, and HDEAm to electrochemical detection were determined.

In vitro tests of the activity of Am and metabolite II against P. falciparum

We conducted in vitro tests to measure the response of isolates of the malaria parasite *P. falciparum* essentially as described previously [7]. Quantities of

parasites from one strain of *P. falciparum* from Tanzania (F32) and one clone from Honduras (HB3), both sensitive to chloroquine, were obtained from continuous cultures maintained in the laboratory. The parasites were incubated in either normal culture medium (RPMI 1640 supplemented with 30 m*M* Hepes buffer and 10% human serum) or in culture medium containing various concentrations of Am or metabolite II. For comparative experiments on each of the two isolates, parasite suspensions at 2% hematocrit were made with a starting parasitemia of 0.16 and 0.30% infected erythrocytes. Aliquots of 200 μl were distributed into the wells of microtiter plates which had been predosed with amodiaquine hydrochloride or metabolite II hydrochloride in dilute hydrochloric acid to give ten different concentrations by serial dilution from 3.6 mg/l to $1.2 \cdot 10^{-4}$ mg/l Am or metabolite II in buffered medium. Additional wells had been predosed with dilute hydrochloric acid alone followed by serial dilution in buffered medium. The plates were incubated for 48 h at 37°C, the cultures were harvested, Giemsa-stained thin smears were made and the number of parasites per 10^4 erythrocytes were counted. The end point for inhibition was the concentration of the compound that prevented any increase in parasite count during the incubation period.

RESULTS AND DISCUSSION

Isolation, standardization and characterization of metabolite II

The extraction of urine was carried out similarly to that in our earlier study [7], except for employment of a higher concentration of dipotassium hydrogen phosphate in the aqueous phase for the MTBE extraction step, which was found to improve extraction efficiency. Later work (unpublished

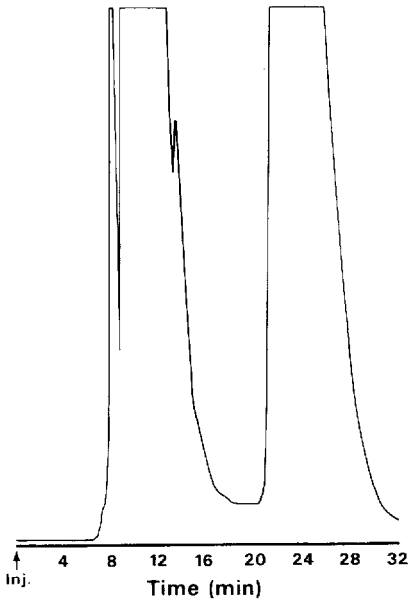

Fig. 1. Chromatogram resulting from preparative HPLC on an extract of an 8- to 16-h urine collection from the volunteer after dosing with Camoquine (600 mg as base). Collection of metabolite II was begun at 21 min and terminated at 28 min.

results) identified ethyl acetate as a more effective solvent than 1,2-dichloroethane for extraction of metabolite II in the initial extraction from blood or urine.

The preparative HPLC procedure proved a substantial improvement over the preparative thin-layer chromatography (TLC) used in the previous work [7]. Separation efficiency at preparatively useful sample loadings permitted efficient separation of HDEAm from DEAm. The use of methanesulfonate for ion-pairing resulted in an inversion in elution order relative to that found using the earlier published HPLC conditions [7]. The elution order was also unexpected based on polarities of the two metabolites themselves without consideration of ion-pair interactions. The elution order was the same, with methane sulfonate as an ion-pairing agent, whether we used a C_{18}-bonded or cyano-bonded column. Fig. 1 shows a chromatographic trace corresponding to the isolation of the HDEAm in the extract of a 700-ml quantity of urine collected between 8 and 16 h after a volunteer ingested an amount of Camoquine equivalent to 600 mg of amodiaquine.

Analytical HPLC, using heptanesulfonate anion for ion-pairing, resulted in elution of HDEAm before DEAm. Therefore choice of hydrocarbon chain length for the alkylsulfonate mobile phase modifier offers the opportunity to select elution order and degree of separation over a range of solvent strengths, a useful approach in tailoring analytical methodology to optimize the detection of either of the two analytes for a specific set of samples. Fig. 2 includes an analytical HPLC—UV trace of a urine extract from a volunteer who had received the drug. Identity of the numbered peaks was inferred by comparison

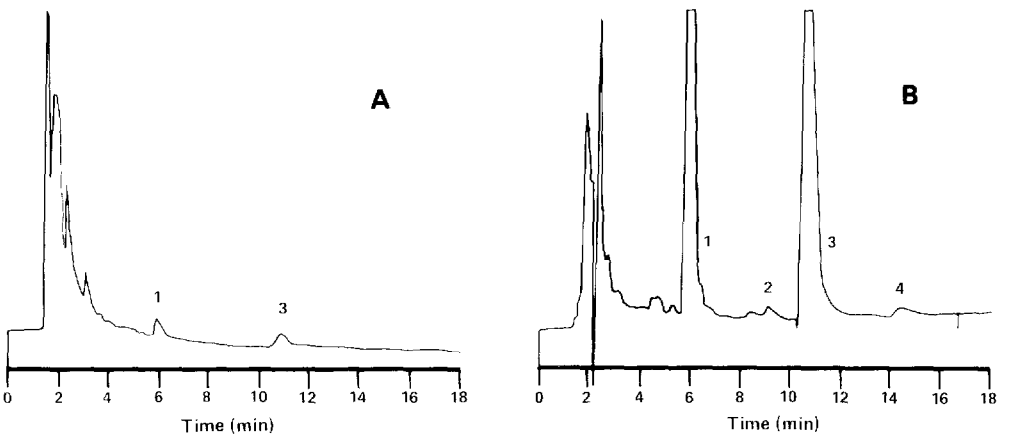

Fig. 2. Chromatograms resulting from the application of analytical HPLC to (A) a 500-μl quantity of a predose urine in the volunteer (the last previous dose had been four months earlier) and (B) a 500-μl quantity of urine from the volunteer collected between 16 and 24 h after a dose of Camoquine (600 mg as base). Each chromatogram represents the quantity of material extracted from 25 μl of urine. Peaks 1—4 were identified based on comparison with standards as detailed in the text. Peak 1 is due to metabolite II (HDEAm), peak 2 to metabolite III (bDEAm), peak 3 to metabolite I (DEAm) and peak 4 to amodiaquine (Am). Note the measurable amounts of metabolites I and II remaining in urine samples four months after the most recent dose of Am. A chromatogram resulting from analysis of a 500-μl quantity of urine from a person who had never taken Am showed no peaks beyond a retention time of 4 min.

of retention times with authentic standards. Peaks 1, 2, 3 and 4 in Fig. 2 are assigned to HDEAm, bDEAm, DEAm and Am, respectively. The application of this HPLC—UV method for quantifying urine extracts has been extended to analysis of blood extracts. This methodology has been applied to monitoring of metabolite blood levels in persons under a regimen of amodiaquine chemoprophylaxis (unpublished data).

Metabolite II (HDEAm) in a one fourth aliquot of purified material was quantified using proton Fourier transform nuclear magnetic resonance (FT-NMR) spectroscopy. When a sufficiently long interval for nuclear relaxation is provided in the pulsing sequence for proton FT-NMR spectroscopy, the contribution of each proton to NMR signal intensity at its characteristic chemical shift is the same as for each other proton at its respective chemical shift. Under this circumstance a weighed quantity of an internal standard compound of known structure may be employed to quantify a sample of known structure, the result being calculated from the Barcza equation [9]:

$$W_x = \frac{M_x}{N_x} \cdot \frac{W_s N_s}{M_s}$$

where W_s = weight of standard, M_s = molecular weight of standard, N_s = number of protons per molecule under the standard peak and $W_x M_x$ and N_x are corresponding values for metabolite II. The one fourth aliquot of the isolated metabolite II was found to contain 622 μg so that the total amount isolated was 2.49 mg. The data showed that the NMR experiment with the 7-s delay between pulses discriminated by 14% against the integration intensity of the internal standard resonance chosen compared with the experiment using the very conservative 21-s relaxation delay. Integration of the spectrum using the 21-s delay was used to calculate the quantity of metabolite II.

In this way quantities of standardized metabolite are available as an HPLC standard and for accurate in vitro activity comparisons even though no synthesized primary standard is available, and the quantity of isolated material is too small to weigh accurately and/or of unknown purity. Fig. 3 shows the integration from which the quantity of metabolite II was calculated. The internal standard was TherMetric-grade *p*-nitrotoluene, which exhibits a doublet with resonances at δ 8.11 and δ 8.13 and another doublet with resonances at δ 7.40 and δ 7.42. The methyl singlet is seen at δ 2.46 ppm.

The proton NMR spectrum of metabolite II itself was acquired before the addition of the *p*-nitrotoluene standard. The following are the resonances and their assignments for this compound: NMR (d_4-methanol) δ 8.03 (d, 1, J = 8.8 Hz, position 5), δ 7.36 (d, 1, J = 2.1 Hz, position 8), δ 7.24 [d, 1, J = 8.8 Hz (J = 2.1 Hz, meta splitting) position 6], δ 7.06 [*m*, 2, positions 2′ and 6′ (unresolved)], δ 6.81 (d, 1, J = 8.5 Hz, position 5′), δ 5.60 (s, 1, position 3), δ 3.92 (s, 2, aryl—C$\underline{H}_2$—N), δ 2.73 (q, 2, J = 7.2 Hz, N—C$\underline{H}_2$—CH$_3$), δ 1.17 (t, 3, J = 7.2 Hz, N—CH$_2$—C$\underline{H}_3$). The chemical shifts of protons in the substituted benzene ring occur upfield relative to the corresponding shifts found in our earlier work [7]. In the earlier work the final step in isolation of the metabolite was elution from the TLC plate using methanol. In the present study the final step was an extraction into MTBE from aqueous base. Metabolite II isolated in the present study appears to be in a form in which the 4′-substituent

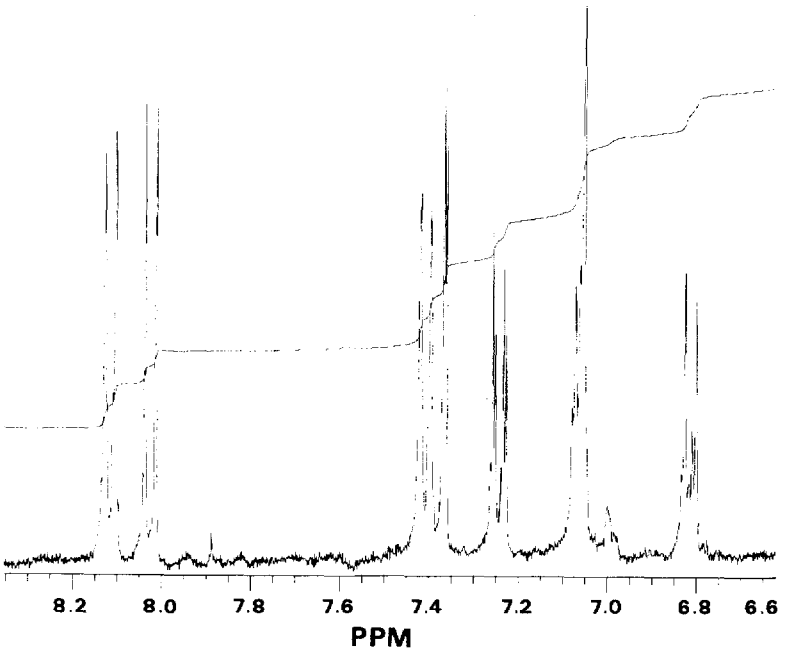

Fig. 3. Proton FT-NMR trace showing the integration of signals in the aromatic region of a mixture of an aliquot of purified metabolite II and a weighed quantity of *p*-nitrotoluene, internal standard. The doublet with resonances at δ 8.11 and δ 8.13 and that with resonances at δ 7.40 and δ 7.42 are due to the internal standard.

is the phenolate structure, shifting the 2′, 5′ and 6′ proton resonances upfield. Metabolite II was undoubtedly in its protonated form in the sample used for NMR in the earlier study [7]. The proximity of the acidic hydroxyl and the basic amine, the possibility of ring formation between the two, and the competition between intramolecular and intermolecular hydrogen bonding in metabolite II makes the acidity or alkalinity of the methanolic solution critical in determining the tautomeric form predominating in the phenolic moiety. The proton NMR spectrum for metabolite II run in this present study is directly comparable to the spectrum of DEAm run in methanol previously [7], because, presumably, in both cases the compounds were in the phenolate form. The only appreciable differences in the spectra of DEAm and metabolite II are seen in comparing quinoline ring resonances, where the effect of the presence of the hydroxyl in the quinoline ring is apparent for metabolite II.

An ultraviolet spectrum was run on metabolite II in methanol and a corresponding one of metabolite I (DEAm) overlayed for comparison (Fig. 4). It is seen that there is a hypsochromic (blue) shift of the highest wavelength λ_{max} for metabolite II compared with that in Am and DEAm. This is a reasonable expectation for the proposed structure.

The positive-ion FAB-MS results were suggestive of but not conclusive for the structure assigned to metabolite II. An *m/z* 344,346 cluster of peaks is seen which appears to correspond to the expected pseudomolecular ion containing one chlorine, but the cluster is of low intensity. Other peaks appear

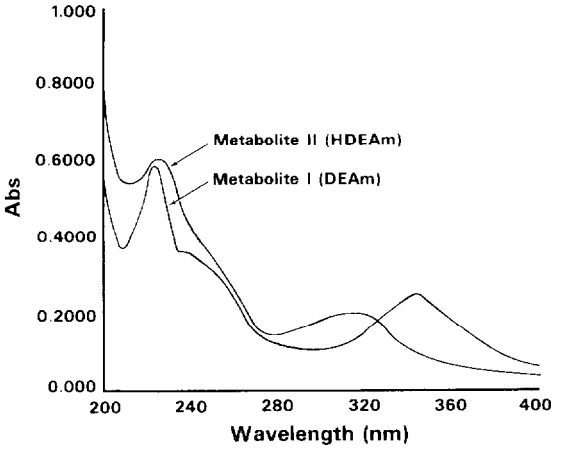

Fig. 4. Ultraviolet absorption spectra of metabolite I (4.16 μg/ml) and metabolite II (3.56 μg/ml) in aqueous 0.001 *M* hydrochloric acid.

in the spectrum which are due to impurities such as phthalates and compounds containing long-chain alkyl groups.

Sensitivity of Am and its metabolites to electrochemical detection

Examination of the structures of Am and its metabolites suggests that these compounds are amenable to electrochemical detection in the oxidative mode. The para-relationship of the 4-amino group and the 4′-hydroxy group suggests the likelihood that a two-electron oxidation would produce a *p*-quinonimine and that such a reaction would be more facile under neutral to basic conditions than under acidic conditions. Further, it seems possible that the 2-hydroxy-4-aminoquinoline moiety of metabolite II could well oxidize under similar conditions. Preliminary experiments have shown that at a potential of +0.7 V (versus the Ag/AgCl electrode, 3.0 *M* in sodium chloride) using the mobile phase listed under Experimental with an analytical cyano-bonded or C_{18}-bonded column, DEAm can be detected at a level of 0.1 ng per injection, when eluted at a retention time (t_R) of about 4 min. Am elutes at about 6 min and has a sensitivity to electrochemical detection comparable to DEAm. The electrochemical response of metabolite II (area under the peak) is approximately three times that of DEAm in this system and at this potential. Since metabolite II elutes after DEAm in this system (t_R = 18 min), however, and its extraction efficiency is lower, its limit of detection in blood extracts is higher than for DEAm. This line of enquiry has been pursued and a report on the application of HPLC using electrochemical detection to sensitive quantitation of amodiaquine and its metabolites in blood extracts is in preparation.

In vitro activity

In vitro activity comparisons of Am and metabolite II were made using two chloroquine-sensitive strains of *P. falciparum*, the Tanzania (F32) strain and the Honduras (HB3) clone. Satisfactory growth of the parasites was obtained in the control wells (four- to five-fold). The growth in the wells which had been predosed with the highest concentration of hydrochloric acid ($1.5 \cdot 10^{-4}$

M) was not affected compared with control cultures without hydrochloric acid. For both strains the minimal inhibitory concentrations for Am and metabolite II were 0.012 and 1.2 mg/l, respectively. Thus, for these strains metabolite II has roughly 1/100 the activity of the parent drug. The results of tests against additional strains, both chloroquine-sensitive and -resistant, will be of interest, but the above preliminary results show that for the two isolates tested the contribution of metabolite II to antimalarial activity may be considered to be negligible.

Overview of primary (non-synthetic) Am metabolism

Fig. 5. summarizes the metabolic pathways and products which may be inferred from our studies. Amodiaquine may be de-ethylated or hydroxylated by oxidative enzymes with de-ethylation being the more rapid of the two processes. Metabolites I and II were identified earlier [7], and metabolite II was further characterized in the present work. Identification of metabolite III (peak 3, t_R = 9.1 min in the analytical HPLC system, Fig. 2B) as bDEAm is suggested by chromatographic comparison with standard bDEAm. Metabolite IV, the hydroxylated, bide-ethylated compound, would be expected to elute before metabolite II. Several small peaks are seen in this region of the chro-

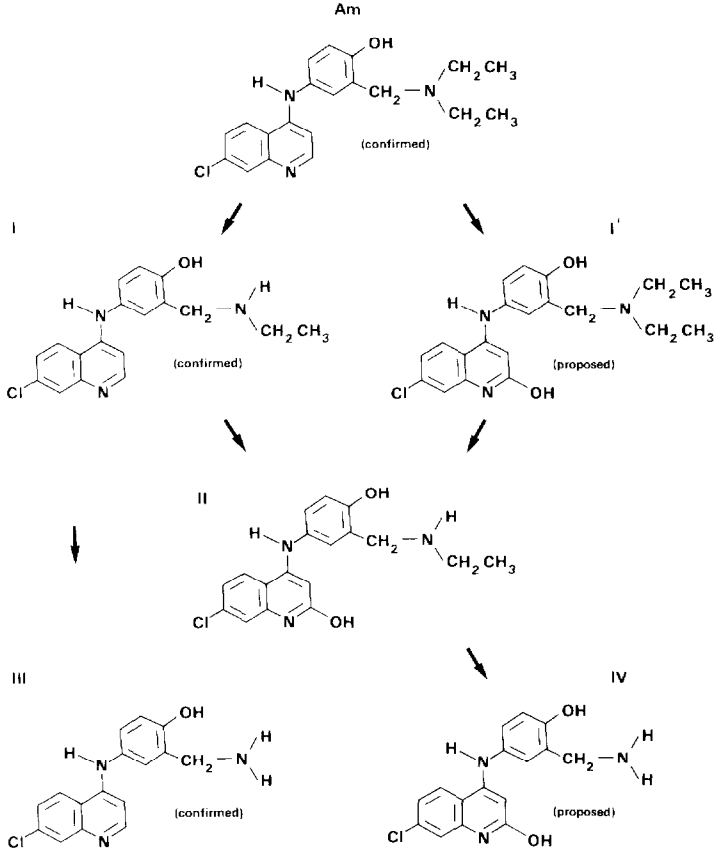

Fig. 5. Proposed primary metabolic pathways for Am in humans.

matogram (Fig. 2B). By reasoning based on comparison of the retention times of Am, DEAm and bDEAm, metabolite I′ would be expected to elute after metabolite II. The small peak at t_R = 8.4 min in Fig. 2B could be due to this compound. Alternatively, the quantity of this material may be below current detection limits and/or this compound may not be separated sufficiently from one of the major metabolites under the chromatographic conditions used. It is virtually certain that an appreciable percentage of metabolite II molecules was formed from Am through this intermediate. Only metabolites I and II appear to occur in the blood in sufficient concentrations to have any potential for impact on antimalarial activity. It is reasonable to suspect that synthetic metabolic reactions occur to produce conjugates of the various metabolites but unlikely that the concentration-activity product of these in blood would be large enough to impact overall antimalarial activity.

DISCUSSION

The isolation, purification, characterization and quantification of metabolite II of Am has provided standard material for chromatographic assay and in vitro testing against strains of *P. falciparum*. An HPLC assay with UV detection has been outlined which effectively separates Am and its known metabolites in blood and urine. An HPLC method with electrochemical detection in the oxidative mode has been shown to be sufficiently sensitive to suggest the possibility of DEAm and HDEAm assay in 100-μl finger-stick blood spots on filter paper at therapeutic levels (details of the chromatographic methods will be published separately). In vitro comparison of HDEAm and Am activity against two *P. falciparum* isolates suggests that the former (metabolite II) contributes little to the in vivo antimalarial activity found in individuals who have received Am. Further in vitro studies will test the generality of this finding. The available evidence suggests that DEAm contributes the preponderance of antimalarial activity and is the most important entity for quantification in individuals who have received Am. Moreover, DEAm is the appropriate compound for use in standardizing in vitro assays to reflect the sensitivity of malaria parasites to Am regimens.

ACKNOWLEDGEMENT

We thank Dr. Phuc Nguyen-Dinh for his interest, for facilitating the cooperative work between our laboratories and for valuable discussions regarding the work.

REFERENCES

1 W.H. Wernsdorfer, W.H.O. Chronicle, 37 (1983) 11—13.
2 K. Rieckmann, J. Am. Med. Assoc., 217 (1971) 573—578.
3 C.C. Campbell, D. Payne, I.K. Schwartz and O. Khatib, Am. J. Trop. Med. Hyg., 32 (1983) 1216—1220.
4 H.C. Spencer, T. Kipingor, R. Agure, D.K. Koech and J.D. Chulay, J. Infect. Dis., 148 (1983) 732—736.

5 H.C. Spencer, A.J. Oloo, W.W. Watkins, D.J. Sixsmith, F.C. Churchill and D.K. Koech, Lancet, ii (1984) 956—957.
6 S. Noeypatimand, S. Malikul, W. Benjapong, D. Duriyananda and M. Ungkasrithongkal, Trans. R. Soc. Trop. Med. Hyg., 77 (1983) 338—340.
7 F.C. Churchill, L.C. Patchen, C.C. Campbell, I.K. Schwartz, P. Nguyen-Dinh and C.M. Dickinson, Life Sci., 36 (1985) 53—62.
8 G.W. Mihaly, D.D. Nicholl, G. Edwards, S.A. Ward, M.L'E. Orme, D.A. Warrell and A.M. Breckenridge, J. Chromatogr., 337 (1985) 166—171.
9 S. Barcza, J. Org. Chem., 28 (1963) 1914—1915.