Journal of Chromatography, 225 (1981) 139—149
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 929

SENSITIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS FOR CHLOROQUINE IN BODY FLUIDS APPLICATION TO STUDIES OF DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM*

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(First received January 20th, 1981; revised manuscript received April 8th, 1981)

SUMMARY

A high-performance liquid chromatographic method has been developed for the sensitive determination of chloroquine in body fluids. The method has been applied to quality-control assay of World Health Organization (WHO) In-Vitro, Macro-Test Kits for the assessment of susceptibility of *Plasmodium falciparum* to chloroquine. Experiments utilizing [^{14}C]chloroquine demonstrated that water was not capable of efficiently desorbing chloroquine from the inside surfaces of kit vials. The addition of blood to the vials effectively desorbs chloroquine. Subsequent addition of the blood to aqueous base followed by hexane extraction permits quantitation by reversed-phase, ion-pair high-performance liquid chromatography utilizing ultraviolet detection at 344 nm. The method is capable of determining as little as 20 ng of chloroquine per vial. This method, utilizing the methyl ether of 9-anthracenemethanol as internal standard, can quantify chloroquine in 1 ml of blood or urine with a minimum detection limit of 20 ppb* (ng/ml). Measurement of blood levels of chloroquine in persons contracting falciparum malaria while following a prophylactic regimen complements in-vitro drug susceptibility measurements in characterizing resistant strains of the parasite.

INTRODUCTION

Resistance of certain strains of *Plasmodium falciparum* to chloroquine has been reported from a number of malarious areas in the world [1]. In order to effectively monitor the occurrence and spread of resistant parasites, the World Health Organization (WHO) has developed standardized test kits for assessment of the response of *P. falciparum* to chloroquine. The WHO In-Vitro, Macro-Test

*Throughout this article, the American billion (10^9) is meant.

0378-4347/81/0000—0000/$02.50

Kits, based on the work of Rieckmann and Lopez-Antunano [2], each contain fifteen series of vials, each series containing seven vials with 5 mg of glucose and 0.25—3.0 nmol of chloroquine. In addition there are 30 control vials and 9 vials containing 1.25 nmol for use in special investigations. In use, 1 ml of defibrinated blood from an infected patient is added to each of the vials and incubated at 37°C for 24 h, followed by assessment of the effect of increasing levels of chloroquine on the maturation of the parasites. Growth at the 1.5-nmol level of chloroquine and above indicates parasite resistance.

The use of the WHO test kits can standardize assessments of *P. falciparum* resistance to chloroquine around the world, but to do so the kits must be carefully quality-controlled. Although a number of other approaches have been used in the past to quantify chloroquine in blood, including ultraviolet (UV) spectroscopy [3], fluorescence spectroscopy [4] and gas chromatography [5], the use of high-performance liquid chromatography (HPLC) with UV detection at 344 nm appeared to promise the best combination of convenience, sensitivity and specificity for the desired assay.

Another aspect of the evaluation of chloroquine resistance in *P. falciparum* is the measurement of blood levels of chloroquine in persons contracting falciparum malaria while following a prophylactic regimen. The demonstration of normally effective blood levels of chloroquine coincident with the presence of parasitemia suggests the presence of a resistant strain of the parasite. Further, a method used for measuring blood levels should be capable of measuring as little as 10 ppb chloroquine since this level represents the lower end of the effective range [6, 7] in plasma. Corresponding whole-blood values are substantially higher [8, 9].

The present study details the development of methodology which provides accurate quality-control assay of the small quantities of chloroquine used in the test kits. This methodology can be used also to determine blood levels of chloroquine in cases of suspected chloroquine-resistant malaria, to establish whether therapeutic levels have indeed been attained. The urine levels corresponding to therapeutic blood levels are in the ppm range and thus easily measured by the method.

The potential for adsorptive losses of chloroquine during analysis has been studied, and the method includes precautions designed to make such losses negligible.

EXPERIMENTAL*

Standards

Standard chloroquine base, desethylchloroquine base and chloroquine diphosphate were supplied by Sterling-Winthrop Research Institute (Rensselaer, NY, U.S.A.).

The methyl ether of 9-anthracenemethanol, used as internal standard, was synthesized by methylation of the alcohol using sodium hydride and methyl iodide in dimethyl sulfoxide. A 1-g quantity of 9-anthracenemethanol was

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

dissolved in 4 ml of dimethyl sulfoxide in a screw-cap test tube, and about 50 mg of hexane-washed sodium hydride and 0.3 ml of methyl iodide were added. The mixture was shaken for 30 min. A 10-ml quantity of water was added followed by extraction using four 4-ml quantities of benzene. Evaporation of the combined benzene extracts followed by vacuum drying yielded slightly over a gram of the product. The melting point range of the crude product was 82—87°C. The structure of the product was verified using proton nuclear magnetic resonance spectroscopy and gas chromatography—mass spectrometry. HPLC analysis using the reversed-phase, ion-pair conditions of the chloroquine analysis showed the presence of a small impurity peak eluting immediately after the main peak. Recrystallization from hexane raised the melting point to a range of 86—88°C but did not completely remove the impurity. The latter has no effect on the analysis.

[^{14}C] Chloroquine used in the adsorption studies was kindly provided by Dr. Coy D. Fitch, St. Louis University Medical Center. It had been purchased from New England Nuclear (Boston, MA, U.S.A.) and had a specific activity of 1.66 mCi/mmol.

Reagents

Non-radioactive chloroquine diphosphate used in the [^{14}C] chloroquine work was from Sigma (St. Louis, MO, U.S.A.). Sodium hydride (50% dispersion in mineral oil), methyl iodide and 9-anthracenemethanol were obtained from Aldrich (Milwaukee, WI, U.S.A.). Hexane and methanol were glass-distilled solvents, available from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Water was deionized and passed through a SEP-PAK C_{18} cartridge, available from Waters Assoc. (Milford, MA, U.S.A.), prior to use. Sodium heptanesulfonate was from Eastman Organic Chemicals (Rochester, NY, U.S.A.). The blood used for the standards and test-vial incubation was freshly collected and preserved using acid citrate dextrose (ACD).

Equipment

The liquid scintillation counting was performed using Beckman Models LS-230 and LS-7500 liquid scintillation spectrometers.

Fluorescence measurements were performed using a Perkin-Elmer Model MPF-2A spectrophotofluorometer. The excitation and emission wavelengths were set at 332 nm and 385 nm, respectively, while a 10-nm slit-width was set for both entrance and exit slits.

The HPLC apparatus consisted of two Waters Model M6000A solvent delivery systems and a Waters Model 660 solvent programmer, coupled to a Waters Model U6K loop injector, a Varian Vari-chrom variable-wavelength absorbance detector and a Whatman Partisil-10 ODS-3 (25 cm × 4.6 mm) reversed-phase column.

Analysis of test-kit vials using water extraction and fluorescence quantification

Each test-kit batch contained fifteen series of seven vials per series, with vials nominally containing 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 3.00 nmol of chloroquine. Each was also to contain 5 mg of glucose. Five series were analyzed for each test-kit batch. A spectrofluorometric method from the literature [4]

was adapted for assay of chloroquine in the WHO test-kit vials. One milliliter of demineralized water was added to each vial, followed by vortexing and the subsequent addition of 1.0 ml of pH 9.5 borate buffer. Each vial was vortexed and the contents transferred to a 0.5-ml micro-cuvette for measurement of fluorescence intensity. The quantity originally present in each vial was determined by comparison with a standard curve which was established by determining the fluorescence intensity of solutions made by the dilution of 3.0-ml aliquots of each standard with 3.0 ml of buffer. Standard curves were linear over the range of interest.

Adsorption studies using [^{14}C] chloroquine

Standard dilutions, prepared in water, contained ^{14}C-labeled chloroquine and non-radioactive chloroquine (NRC) in appropriate concentrations for vial fortification. Control vials from a WHO test-kit batch, containing glucose only, were charged with 0.25—3.0 nmol of [^{14}C] chloroquine, using 50 μl of the appropriate standard solution per vial in each case. Counting of 50-μl aliquots of each standard was performed using a xylene—dioxane—2-ethoxyethanol (1:3:3) counting solution [10] to verify the activity of the standards.

In analysis of the fortified vials, a gentle flow of dry nitrogen was used to remove water from each vial. A 1.0-ml quantity of either defibrinated blood or demineralized water was added to vials at each level, followed by 37°C incubation for 2 h.

Silanized vials were fortified and incubated similarly to the control vials. These vials had been acid-washed, water-rinsed, acetone-rinsed and dried, followed by silanization using 5% dimethyldichlorosilane in toluene. Rinsing with methanol and subsequent drying completed the treatment. For these tests 50 μl of 100 mg/ml glucose was added prior to fortification with the 50 μl of [^{14}C] chloroquine solution.

Analysis for [^{14}C] chloroquine in the vials was essentially by the method of Fitch [11]. For each incubated vial the blood or water was quantitatively transferred to a screw-cap test-tube containing 0.5 ml of 5 *N* sodium hydroxide and 0.1 ml of 4.09 mg/ml aqueous NRC. A 1.0-ml quantity of water was used as a rinse. The pipet was pre-rinsed using about 0.5 ml of 0.32 mg/ml NRC. A 5-ml quantity of 1.5% isoamyl alcohol in heptane was added, followed by capping (PTFE insert) the test tube, 20 min reciprocal shaking and centrifugation for 5 min at 270 *g*. A 4.0-ml aliquot of the heptane layer from each was then transferred to a corresponding scintillation vial, 15 ml of toluene-based cocktail added and the samples counted.

To each of the original vials was added 0.10 ml of 4.09 mg/ml NRC and 1.0 ml of water. Each solution was then quantitatively transferred to a corresponding test tube containing 0.5 ml of 5 *N* sodium hydroxide and 0.1 ml of 0.32 mg/ml NRC and the sample extracted and counted as outlined above.

Analysis of test-kit vials and clinical samples using blood incubation followed by HPLC—UV quantification

In the analysis of vials, standards were prepared by fortifying clean vials with known quantities of chloroquine diphosphate and 5 mg glucose each and carrying these through the procedure to yield a standard curve. A 1.0-ml quantity of

whole blood with ACD as anticoagulant/preservative was added to each vial, standard and sample alike, and the vials incubated for 20 min at 37°C. Each sample was then quantitatively transferred by silanized Pasteur pipet to a silanized screw-cap test tube containing 0.5 ml of 5 *N* sodium hydroxide. A 2.5-ml quantity of demineralized water was used as a rinse in the transfer. A 3.0-ml quantity of hexane was added to each sample tube. Each tube was capped and shaken for 30 min using a reciprocal shaker. A 50-μl quantity of octanol was added, followed by centrifugation, to aid in the separation of layers. Internal standard solution was then added (200 μl of a 5.6 μg/ml solution of the methyl ether of 9-anthracenemethanol in ethyl acetate) to a silanized 3-ml Reacti-vial (Pierce, Rockford, IL, U.S.A.) after which a 2.0-ml aliquot of the hexane layer was also placed in the Reacti-vial. The sample in each vial was evaporated using a heating block held at 45°C to leave only residual octanol. The hexane refluxed during evaporation, continually washing the sides of the Reacti-vial. A 20-μl quantity of mobile phase concentrate [methanol—water—acetic acid (72.5:25.0:2.5) with 0.0125 *M* sodium heptanesulfonate] was added to the cooled vial. Injections of 15-μl quantities of standards and samples on the chromatograph using UV detection permitted quantitation.

The reversed-phase column was operated in the ion-pair mode using a mobile phase of methanol—water—acetic acid (80:19:1) with 0.005 *M* sodium heptanesulfonate. The flow-rate was 1.0 ml/min (55 bar). UV absorbance of eluting compounds was measured at 344 nm with a detector sensitivity of 0.05 a.u.f.s. Peak heights from the corresponding recorder trace were used for quantitation. The ratio of the chloroquine peak height to the internal standard peak height was plotted against quantity of chloroquine for each standard to establish a curve. Comparison of that calculated ratio for each sample to the standard curve was then made.

WHO In-Vitro, Macro-Test Kits were assayed for chloroquine upon receipt from the facility in which they were manufactured in Manila, The Philippines.

Clinical samples containing chloroquine in whole blood, plasma and urine were analyzed as above with the sample added to the 5 *N* sodium hydroxide in a screw-cap test tube and then carried through the remainder of the procedure. A 2.5-ml quantity of demineralized water was added to give the desired concentration of sodium hydroxide before extraction. For urine samples, final dilution was to 1.0 ml, and quantitation was by external standard comparison with fortified urine standards similarly treated.

RESULTS AND DISCUSSION

Desorption of chloroquine from glass by water and by blood

Earlier studies have demonstrated the adsorption of organic bases on untreated glass [12—15], even in dilute aqueous acid [14]. The indications of chloroquine adsorption on glass in the present study, seen in the fluorescence assay of test-kit vials, were confirmed by the experiments utilizing ^{14}C-labeled chloroquine. Blood is clearly much better able to desorb chloroquine from untreated vials than is water (Table I). These results suggest the necessity of adding blood to test-kit vials prior to analysis for chloroquine.

Silanized vials also have an appreciable tendency to adsorb chloroquine in

TABLE I

BLOOD/VIAL AND WATER/VIAL DISTRIBUTION OF CHLOROQUINE FOUND USING [^{14}C]CHLOROQUINE FORTIFIED (A) WHO IN-VITRO MACRO-TEST KIT VIALS (BATCH 10) AND (B) PRETREATED (SILANIZED) VIALS

Quantity of chloroquine (nmol)	(A) Macro-test kit vials				(B) Silanized vials			
	Percent of added counts found	Distribution of found counts (%)			Percent of added counts found	Distribution of found counts (%)		
		Blood	Water	Vial		Blood	Water	Vial
0.25	96.4	98.2		1.8	98.1	98.9		1.1
0.25	95.6	97.2		2.8	97.9	98.8		1.2
1.25	91.1	97.4		2.6	95.1	99.4		0.6
1.25	90.7	97.6		2.4	95.2	99.7		0.3
3.00	90.5	97.1		2.9	95.1	99.4		0.6
3.00	88.9	97.4		2.6	94.7	99.3		0.7
0.25	89.6		12.4	87.6	91.9		91.7	8.3
1.25	86.4		33.2	66.8	100.0		98.3	1.7
3.00	88.5		52.0	48.0	101.0		99.1	0.9

competition with water (Table I). The approximately 0.02 nmol adsorbed as indicated by the data in Table I represents 6.5 ng of chloroquine. This demonstrates the care necessary to avoid or compensate for losses of chloroquine when analyzing at the ppb (ng/ml) level.

Characterization of the HPLC method for chloroquine in blood

Standard curve data for the method are summarized in Table II. Fig. 1 shows representative HPLC—UV traces. The data demonstrate that the linearity of the system is quite good as is the precision at the 0.82 nmol per vial (263 ppb in blood) level. The range represented encompasses that corresponding to the test-kit vials and to concentrations normally found in the whole blood and plasma of treated individuals. Taking a 3.0-ml sample and a larger fraction of the extract for injection permits accurate detection of as little as 5 ppb (ng/ml) chloroquine.

Originally, 1.5% isoamyl alcohol in heptane was used as the extracting solvent for the HPLC method. The use of hexane, followed by the addition of 50 μl of octanol prior to centrifugation, was substituted for several reasons. Hexane is more volatile and thus more easily evaporated from the sample than heptane. Octanol serves as well as isoamyl alcohol to break emulsions during extraction and to minimize adsorption of chloroquine on glass surfaces. Further, octanol serves as a "keeper" for the internal standard during evaporation. With isoamyl alcohol the last of the alcohol must be removed to avoid an interference in the chromatogram, although heating of the sample appreciably beyond dryness causes sublimation of the internal standard and correspondingly high results for the method. The presence of the residual octanol yields a negative dip in the chromatogram, but at a point (retention time = 4.6 min)

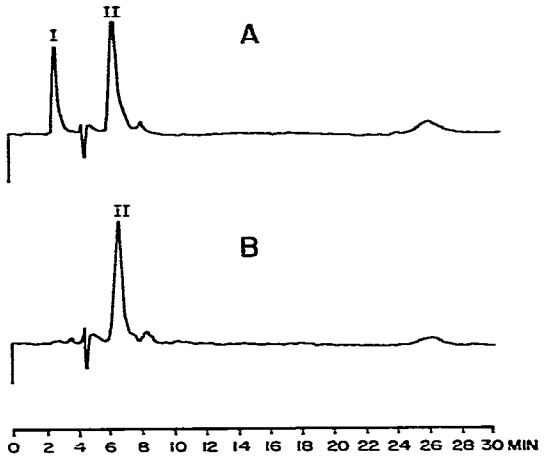

Fig. 1. Representative traces for the analysis of chloroquine by reversed-phase, ion-pair HPLC using the methyl ether of anthracenemethanol as internal standard. (A) Standard containing 262.9 ng/ml chloroquine base in blood; (B) blood blank. Peaks: I, chloroquine; II, internal standard.

which does not interfere with the analysis. Also, the HPLC peaks are sharper than is the case when isoamyl alcohol is used.

The solvent used to reconstitute the samples prior to injection is designed to provide adequate solubility for both chloroquine and the internal standard along with mobile phase compatibility, to ensure good peak shapes.

It was demonstrated that, after reconstitution, the samples could be stored for no longer than one day prior to injection to avoid some loss of precision. The samples were injected on the same day that they were reconstituted; in this way 14—16 samples can be processed at a time.

Cleaning and silanizing the glassware between each glassware use was found to be important. Inadequately deactivated surfaces adsorb appreciable amounts of chloroquine, and a beading test using water showed that silanized surfaces lost much of their hydrophobic character after one use. Further, rinsing of glassware with demineralized water and acetone sometimes failed to remove all of the chloroquine from the previous analysis. Treating the glassware between each use guarantees removal of the last traces of chloroquine by the 6 *N* hydrochloric acid together with the formation of a fresh, inert, hydrophobic surface by silanization for the subsequent analysis.

A typical chromatogram, illustrated in Fig. 1A, exhibits several interesting features. The chromatogram is free from interfering peaks even though the amount of extract injected represents the quantity of chloroquine in 0.20 ml of blood. Indeed, the hexane extraction provides sufficiently specific extraction that a 3.0-ml blood sample may be taken and an injection of extract equivalent to 1.2 ml of blood made when the concentration in the sample requires and quantity of sample permits. This reduces the minimum detection limit for the method to 5 ng/ml. The retention times of chloroquine and internal standard are 2.6 and 6.4 min, respectively. Other characteristics of the chromatogram include the negative dip due to octanol at 4.6 min, the small quantity of impurity in the internal standard appearing at 8.2 min and the broad peak at 26.0

TABLE II

STANDARD-CURVE DATA FOR CHLOROQUINE IN WHOLE BLOOD BY HPLC—UV

Relative standard deviation (n = 4) at 0.822-nmol level = 2.32%.

x = Chloroquine added		y = Chloroquine/internal standard peak height ratio	x' = Chloroquine calculated* (ng)
nmol	ng		
0.00	0.00	0.00	0.72
0.205	65.73	0.206, 0.231	62.2
0.822	262.9	0.931, 0.955, 0.938, 0.903	262.7
1.541	493.0	1.794, 1.753	499.4
3.082	986.0	3.487, 3.500	983.1

*Calculated from the least squares straight line $y = mx + b$ for which m = 0.00356, b = −0.00255; r^2 = 0.9997.

min, due apparently to blood lipids. In practice an injection is made and then a second sample introduced after 10 min. No further injection is made until the lipid peak from the second injection has eluted, requiring a total of 40 min from the time of the first injection. Another peak due to a blood component appears in some samples and is seen at a retention time of 5.0 min in Fig. 1.

The determination of chloroquine in WHO test-kit vials

In comparison of analyses of vials from a WHO test-kit batch by the water extraction—fluorescence and blood incubation—HPLC methods, results by the former method are quite low for the lower nominal value vials where the effect of chloroquine adsorption is amplified (Table III). HPLC quantification of blood-incubated, test-kit vials was chosen over fluorescence because in our hands the former provides better precision and greater selectivity and is proce-

TABLE III

COMPARISON OF RESULTS OF ANALYSES OF CHLOROQUINE TEST-KIT VIALS BETWEEN METHOD A USING WATER EXTRACTION WITH FLUORESCENCE QUANTIFICATION AND METHOD B USING BLOOD INCUBATION WITH HPLC—UV QUANTIFICATION

Average of 5 values except where noted.

Batch 3, nominal (nmol)	Quantity of chloroquine (nmol)	
	Method A	Method B
0.25	0.056 ± 0.009	0.234 ± 0.093
0.50	0.098 ± 0.120	0.457 ± 0.102
0.75	0.49 ± 0.09	0.735 ± 0.289
1.00	0.66 ± 0.08	1.02* (1.09, 0.94)
1.50	1.20 ± 0.13	1.41**
2.00	1.83 ± 0.26	1.84* (1.65, 2.03)
3.00	2.81 ± 0.41	2.85* (2.96, 2.73)

*Two values.

**Single value.

durally simpler than a fluorescence method designed to provide comparable sensitivity.

HPLC analysis of chloroquine in clinical samples

The method used in clinical analysis is the same as that for the kits except for the elimination of blood incubation and subsequent transfer to the extraction test tube. The application of the method to clinical samples, however, requires some conjecture as to the interpretation of the analytical results. McChesney et al. [16] state that, of identifiable chloroquine-related species in the urine of treated human subjects, an average of 70% was unchanged drug and 23% was desethylchloroquine. An independently developed HPLC method [17], published subsequent to the completion of the present study, includes plasma analysis data for a patient in which the chloroquine concentration was found to be approximately ten times that of desethylchloroquine.

In experiments employing the present method, authentic desethylchloroquine proved to have a retention time and sensitivity comparable to that for chloroquine. Efficiency of extraction is 70% for the metabolite compared to about 85% for chloroquine. Since the compensating standards contain chloroquine alone, the value determined by the method will represent slightly less than the sum of the two compounds, expressed as chloroquine, if desethylchloroquine is present.

Table IV includes the results of the analyses for chloroquine in the body fluids of two patients who developed falciparum malaria after returning to the United States from separate travels in East Africa. Both had utilized a chloroquine diphosphate prophylactic regimen (300 mg as base per week) before, during and after their respective trips. A limited sample of blood from Patient 1 was available and some of this was used for in-vitro susceptibility testing of the

TABLE IV

ANALYSIS FOR CHLOROQUINE IN BODY FLUIDS OF PATIENTS DEVELOPING PLASMODIUM FALCIPARUM PARASITEMIA WHILE UNDERGOING CHEMOPROPHYLAXIS

Sample matrix	Concentration of chloroquine base, ppb (ng/ml)			
	Patient 1		Patient 2	
	Individual results	Mean	Individual results	Mean
Whole blood	108	—	198.2 205.4	201.8
Plasma	40.0	—	39.8	—
Serum	—	—	29.0 33.6	31.3
Urine	19,580 19,870 19,920 20,170	19,885 ± 242	17,510 17,360 17,600 17,360	17,460 ± 11

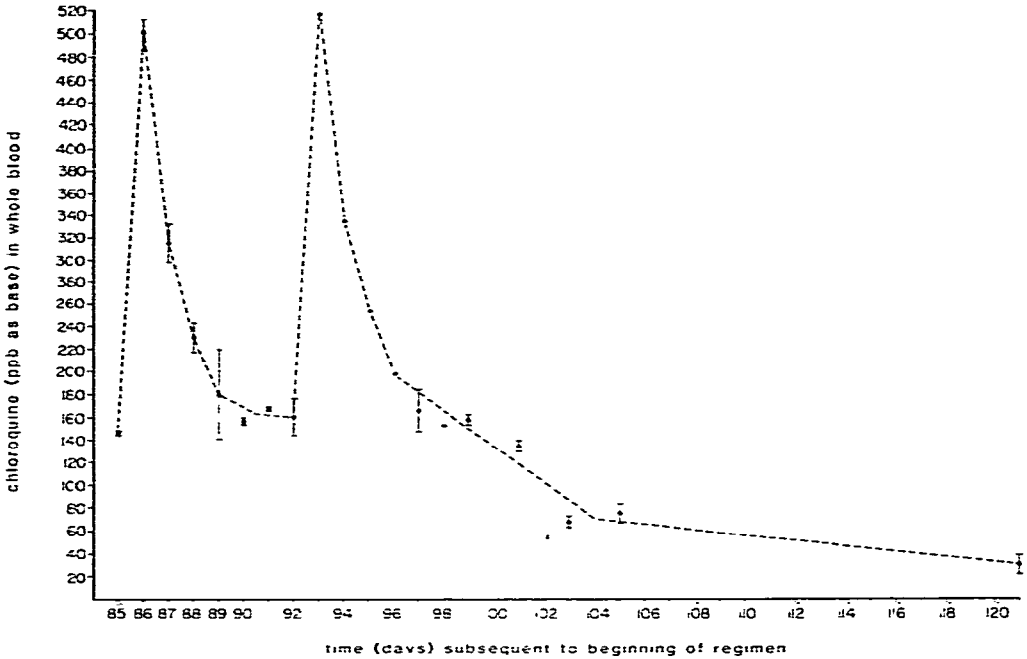

Fig. 2. Whole-blood concentrations of chloroquine in a volunteer during and subsequent to the thirteenth week of a prophylactic regimen utilizing chloroquine diphosphate (300 mg as base). Chloroquine was ingested immediately subsequent to sampling on days 85 and 92. No chloroquine was ingested subsequent to day 92. The ends of the bars extending above and below a data point represent the individual results of duplicate determinations.

parasites. The values in Table IV for Patient 1 were determined from 0.7 ml of whole blood, 1.0 ml of plasma and 1.0-ml aliquots from a urine sample.

The whole-blood drug levels in the two patients may be compared with those found (Fig. 2) in a volunteer whose blood was analyzed during and subsequent to the thirteenth week of a regimen of 300 mg as the base per week. The whole-blood chloroquine concentrations found in the patients were less than those found in the volunteer on the corresponding day subsequent to tablet ingestion, but the levels present are within a normally effective range [6] and should effectively prevent the development of chloroquine-susceptible *P. falciparum*. Detailed case studies including parasitological investigations are to be published elsewhere [18].

There are at least two advantages in the use of whole blood, rather than plasma, for chloroquine determination in clinical studies involving chloroquine resistance. Subjects treated with chloroquine exhibit higher whole-blood than plasma levels, so that determination of the former yields more precise and accurate values at low blood concentrations. Analysis of whole blood yields a fundamental value which may be confidently compared from sample to sample, while analysis of a blood fraction, e.g. plasma, allows the potential for inaccurate results due to in-vitro drug redistribution by hemolysis or other processes [19, 20]. A leukocyte count is normally available for clinical whole-blood samples, so that this factor may be taken into account [7]. The use of the present method permits the gathering of extensive whole-blood chloro-

quine baseline data which will be useful in the characterization of future falciparum malaria cases in which resistance of the parasite to chloroquine is suspected.

ACKNOWLEDGEMENTS

We are indebted to Dr. Coy D. Fitch, Director, Division of Endocrinology, St. Louis University School of Medicine, for the [^{14}C] chloroquine, the use of his laboratory facilities and for his valuable counsel in the radiolabeled experiments. We would also like to thank Mr. Hugh Flax for careful technical assistance.

This work was supported in part by the World Health Organization (WHO) through a WHO Contract Technical Agreement and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

REFERENCES

1 W.H. Wernsdorfer and R.L. Kouznetsov, Bull. W.H.O., 58 (1980) 341.
2 K.H. Rieckmann and F.J. Lopez-Antunano, Bull. W.H.O., 45 (1971) 157.
3 L. Kazyak, in I. Sunshine (Editor), Manual of Analytical Toxicology, CRC Press, Cleveland, OH, 1971, pp. 86—88.
4 C.W. Vogel and E. Königk, Tropenmed. Parasit., 26 (1975) 278.
5 A. Viala, J.P. Cano and A. Durand, J. Chromatogr., 111 (1975) 299.
6 E.W. McChesney, W.F. Banks, Jr. and J.P. McAuliff, Antibiot. Chemother., 12 (1962) 583.
7 R.W. Berliner, D.P. Earle, Jr., J.V. Taggart, C.G. Zubrod, W.J. Welch, N.J. Conan, E. Bauman, S.T. Scudder and J.A. Shannon, J. Clin. Invest., 27 (1948) 98.
8 E.W. McChesney, W.D. Conway, W.F. Banks, Jr., J.E. Rogers and J.M. Shekosky, J. Pharmacol. Exp. Ther., 151 (1966) 482.
9 A. Durand, Thèse Pharmacie (État), Marseille, 1974.
10 G.A. Bruno and J.E. Christian, Anal. Chem., 33 (1961) 1216.
11 C.D. Fitch, Proc. Nat. Acad. Sci. U.S., 64 (1969) 1181.
12 S. Udenfriend, Fluorescence Assay in Biology and Medicine, Academic Press, New York, 1962, pp. 101—103.
13 B.B. Brodie, S. Udenfriend and J.E. Baer, J. Biol. Chem., 168 (1947) 299.
14 L.H. Bird, J. Sci. Technol., 303 (1949) 334.
15 K. Högberg, J.-E. Lindgren, T. Högberg and B. Ulff, Acta Pharm. Suecica, 16 (1979) 299.
16 E.W. McChesney, M.J. Fasco and W.F. Banks, Jr., J. Pharmacol. Exp. Ther., 158 (1967) 323.
17 Y. Bergqvist and M. Frisk-Holmberg, J. Chromatogr., 221 (1980) 119.
18 A. Gardner, R.A. Weinstein and L. Lincoln, J. Amer. Med. Assoc., (1981) in press.
19 E. Antal, S. Mercik and P.A. Kramer, J. Chromatogr., 183 (1980) 149.
20 O. Borga, K.M. Piafsky and O.G. Nilse, Clin. Pharmacol. Ther., 22 (1977) 539.