Sensitive Radioimmunoassay and Enzyme-Linked Immunosorbent Assay for the Simultaneous Determination of Chloroquine and Its Metabolites in Biological Fluids

C. ESCANDE*[x], P. CHEVALIER[‡], F. VERDIER[§], AND R. BOURDON*

Received January 12, 1989, from the *Laboratoire de Biochimie-Toxicologie, Hôpital Fernand Widal, rue du Faubourg Saint-Denis, 75475 Paris Cedex 10, France, the ‡Rhône-Poulenc Santé, Institut de Biopharmacie, Unité d'Immunologie Quantitative, 92165 Antony Cedex, France, and the §Institut National de la Santé et de la Recherche Médicale, Unité 13, Hôpital Claude Bernard, 75944 Paris Cedex 19, France.
Accepted for publication May 12, 1989.

Abstract □ Two new methods for the simultaneous determination of chloroquine and its two main metabolites (monodesethylchloroquine and bisdesethylchloroquine) in biological samples, radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), are described. Antiserum is produced in rabbits immunized with *N*-(2-carboxyethyl)desethylchloroquine:protein conjugate. Besides chloroquine, this antiserum recognizes with good affinity the two main metabolites, monodesethylchloroquine and bisdesethylchloroquine (70 and 40% of crossreaction, respectively). Amodiaquine cross reacts by 4.5%; cross reactions with monodesethylamodiaquine, bisdesethylamodiaquine, and other antimalarial drugs are <1%. No extraction step or sample preparation is required for either system. Sensitivity limits are, respectively, 0.70 nM (3 pg of chloroquine sulfate measured in 10 μL of plasma sample) for RIA, and 10 nM (22 pg of chloroquine sulfate measured in 5 μL of plasma sample) for ELISA. The interassay coefficients of variation are, respectively, <10 and <16% for RIA and ELISA in the range 14–410 nM (6–180 ng/mL). The results of both methods are well correlated (r = 0.97) and correlate with spectrophotometry (r = 0.98) and HPLC results (r = 0.93). Because of their high sensitivity, both methods can be used in the case of chloroquine poisoning and in the control of malaria prophylaxis and treatment.

For over 30 years, chloroquine (7-chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline) has been the most commonly used antimalarial drug both as a therapeutic and a prophylactic agent.[1] It is also used in the treatment of chronic rheumatoid diseases.[2] The major problem concerning the use of chloroquine in prophylaxis and treatment of malaria now comes from the developing resistance of *Plasmodium falciparum* to this compound.[3] To confirm that a *P. falciparum* strain is chloroquine resistant and not that other factors such as insufficient blood concentrations of the drug (malabsorption, differences in drug metabolism, incorrect dosage schedules) are responsible for failure to obtain radical cure of malaria infection or adequate protection, the clinical inefficiency of the drug must be confirmed by in vitro and in vivo tests, and blood levels of the drug must be determined.[4,5]

Present analytical methods, such as the Dill-Glazko test,[6] the Haskin's test,[7] the Wilson-Edeson test,[8] the ion-pair extraction method,[9] and the spectrophotometric method,[10] are either not sensitive enough and not very selective, or they are sensitive and selective but unsuitable for carrying out measurements in the field since they require sophisticated instrumentation (HPLC).[11] All these methods require an extraction step which leads to a long, complicated, and laborious assay.

A radioimmunoassay has been described using a monoclonal antibody against the 7-chloro-4-aminoquinoline conjugated to keyhole limpet hemocyanin.[12] This antibody is not specific to chloroquine since it recognizes all compounds bearing the 4-aminoquinoline moiety. In particular, amodiaquine is recognized 10 times more often than chloroquine and its metabolites. Recently, an ELISA method has been described using sheep-produced antisera to a chloroquine derivative (2-aminoethylamino group in the 7 position instead of Cl) conjugated to keyhole limpet hemocyanin.[13] This antiserum is specific to chloroquine and does not recognize the metabolites, mono- and bisdesethylchloroquine.

We report here simple, sensitive, and specific RIA and ELISA methods to determine chloroquine and its two metabolites (monodesethyl- and bisdesethylchloroquine) in biological samples.

Experimental Section

Reagents—The parent compound chloroquine sulfate and the derivative desethylchloroquine base were supplied by Rhône-Poulenc, Saint Fons, France. Amodiaquine dihydrochloride and derivatives were gifts from Institut de Médecine et d'Epidémiologie Tropicales, Hôpital Claude Bernard, Paris, France. Mefloquine base, hydroxychloroquine sulfate, 7-chloro-4-amino-(3-aminopropyl)quinoline, 7-chloro-4-hydrazinoquinoline, and 6-8-dichloroquine were from Unité de Biologie Clinique et Expérimentale, Institut Gustave Roussy, Villejuif, France. The carrier-free Na ^{125}I (in NaOH), donkey anti-rabbit immunoglobulins horseradish peroxidase conjugated, and ABTS substrate [2,2'-azino-di-(3-ethylbenzthiazoline sulphonate)] were supplied by the Radiochemical Center, Amersham, U.K. Quinine sulfate, quinidine sulfate, quinacrine dihydrochloride, bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, poly-L-lysine (PM = 35 000) hydrochloride, tyrosine methyl ester base, dicyclohexyl carbodiimide, and *N*-hydroxysuccinimide were from Sigma. All other reagents were of the best grade available.

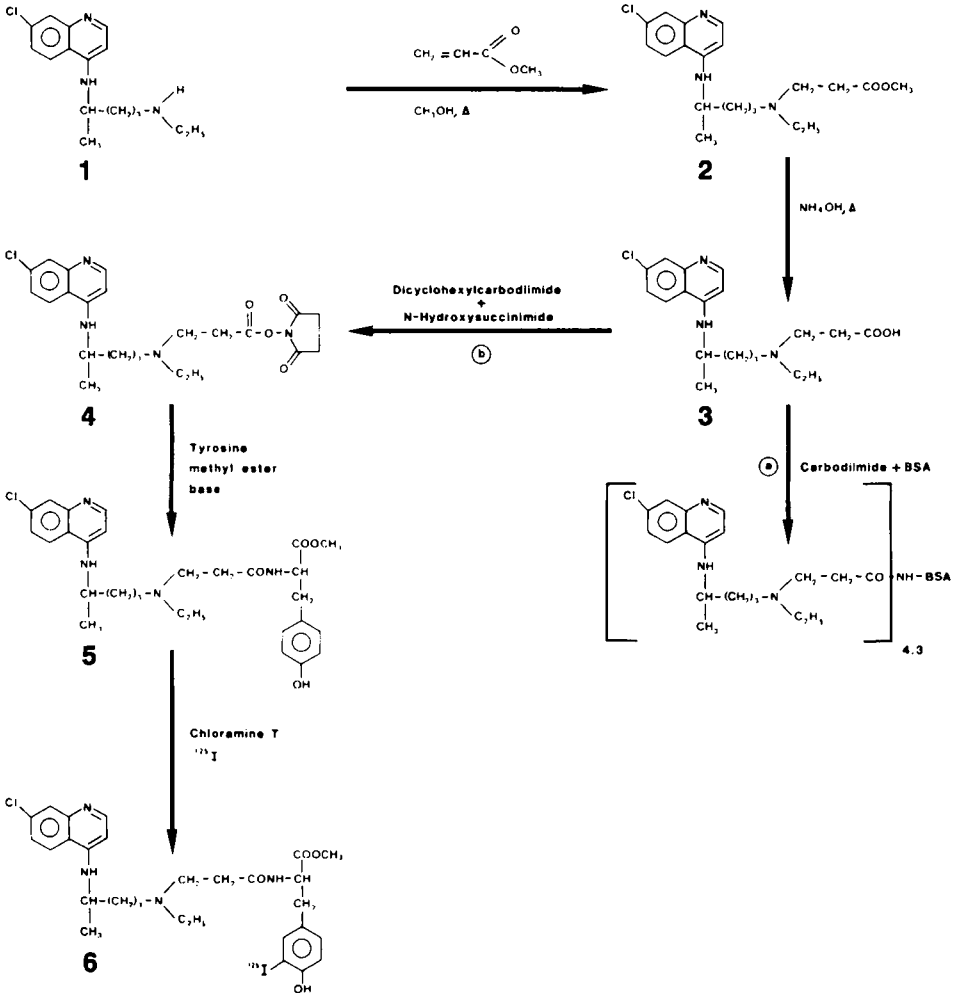
Apparatus—Radioactivity was measured by a solid scintillation counter (model gammamatic Kontron). Dynatech M 129A polystyrene 96-wells microtiter-plates were read by a Dynatech MR700 Microplates Reader. A centrifuge (model GR2000 SX Jouan), microtiter-plates shaker incubator (model AM89 Dynatech), and processor-controlled sample distributor (model Tecan 505 Berthold) were used. Biological concentrations were obtained by using internal programs of gamma counter and ELISA soft of Microplates Reader.

Synthesis of Immunogen—The hapten *N*-(2-carboxyethyl)desethylchloroquine (3) was prepared by alkylation of monodesethylchloroquine free base (1) with methyl acrylate followed by alkaline hydrolysis of the resultant ester, *N*-(2-methoxycarbonylethyl)desethylchloroquine (2). The hapten was then coupled to BSA using the water-soluble carbodiimide method[14] (Scheme I).

N-(2-Methoxycarbonylethyl)desethylchloroquine (2)—Methyl acrylate (300 μL, 3.3 mmol) was added at once to a solution of monodesethylchloroquine free base 1; 0.6 g, 2 mmol) in 10 mL of methanol. The mixture was maintained at a gentle reflux for 2 h at 60 °C in a silicone oil bath. Reaction advancement was monitored by thin-layer chromatography (TLC) on silica gel 60 $CHCl_3$:CH_3OH:NH_4OH (90:10:1; R_f = 0.95). The solvent was then evaporated under reduced pressure at room temperature.

N-(2-Carboxyethyl)desethylchloroquine (3)—Distilled water (10 mL), 3.9 mL of 1 M HCl to obtain pH 3–4, and 3 mL of 3 M ammonia

0022-3549/90/0100-0023$01.00/0



Scheme I—(a) Synthesis of immunogen. (b) Synthesis of ^{125}I labeled radioligand: (**1**) monodesethylchloroquine free base; (**2**) *N*-(2-methoxycarbonylethyl)desethylchloroquine; (**3**) *N*-(2-carboxyethyl)desethylchloroquine; (**4**) *N*-hydroxysuccinimide activated carboxyl ester; (**5**) Methyltyrosinate-*N*-(2-carboxyethyl)desethylchloroquine derivative; (**6**) ^{125}I-labeled radioligand.

were successively added to the previously dried residue. The mixture was stirred and maintained at a gentle reflux for 2 h in a silicone oil bath at 110 °C. Reaction advancement was monitored by TLC with the same conditions as described above ($R_f = 0$); IR(KBr): 1730 cm^{-1} (C = 0); ^1H NMR (DMSO-d_6): 1.1 (t, CH_2-CH_3), 1.3 (d, CH_3-C), 1.75 (m, -CH_2-CH_2), 2.6 (t, CH_2-CH_2-COOH), 3.1 (m, CH_2-CH_2-N), 3.1 (m, N-CH_2-CH_3), 3.3 (t, N-CH_2-CH_2-COOH), 4 (m, N-CH), 6.8 (d, H_2), 7.55 (dd, H_6), 7.8 (d, H_8), 8.3 (d, H_4), and 8.5 (d, H_5) ppm, MS: *m/z* 363 (M^+).

Conjugation of Hapten (3) to Bovine Serum Albumin—The carboxylic acid derivative (**3**) was then coupled to BSA as described below. The following were successively added to a flask: 2 mL of aqueous solution of **3** (0.223 g, 6.1×10^{-4} mol), 7 mL of BSA (0.035 g, 5.2×10^{-7} mol) in 0.05 M morpholino ethane sulfonate buffer (pH = 5.5), 2 mL of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.1 g, 5.2×10^{-4} mol) in distilled water. The solution was mixed by swirling for 2 h at room temperature. It was then dialyzed exhaustively against distilled water and stored frozen at −20 °C in 1 mL aliquots. The number of hapten residues coupled per mole of BSA was calculated spectrophotometrically to be 4.3.

Conjugation of Hapten (3) to Poly-L-lysin for ELISA Procedure—The quantitative ELISA method was based on competition between solid phase-bound antigen and free antigen. Bound antibodies were detected by a peroxidase-labeled second antibody. Chloroquine does not show significant binding to polystyrene even at high concentration. However, polystyrene can be coated with hapten that has been covalently coupled to poly-L-lysine using carbodiimide.[15]

The ratio of poly-L-lysine to hapten in the conjugate can greatly affect the sensitivity of the immunoassay. It is therefore important to determine the optimal ratio for each hapten to give the best combination of assay sensitivity and antibody binding. The conjugation procedure is described below. Briefly, 2.5 mL of an aqueous solution of **3** (20.5 mg), 1 mL of poly-L-lysine (10 mg) in 0.01 M morpholino ethane sulfonate buffer (pH = 6), and 0.4 mL of an aqueous solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (7.5 mg) were successively added in a glass vial. The mixture was allowed to rock for 2 h at room temperature. The resulting conjugate did not need to be purified and could be stored for up to 6 months at 4 °C without loss of antibody binding activity. One preparation is usually sufficient to coat several thousand wells.

Preparation of the ^{125}I Label (6)—*Synthesis of Chloroquine-Tyrosine Methyl Ester Derivative (5; Scheme I)*—The carboxylic acid derivative (**3**) was reacted with *N*-hydroxysuccinimide (NHS) via dicyclohexylcarbodiimide (DCC) in the solvent dimethylformamide (DMF) to give an acylamino ester of NHS (**4**) which could then react with the primary amine group of tyrosine methyl ester (**5**).[16] Briefly, a mixture of 23 mg of **3**, 9 mg of NHS, and 16 mg of DCC in 1.2 mL of DMF was stirred at room temperature for 1 h and then allowed to stand at 4 °C overnight. The reaction product was obtained after filtering off the dicyclohexyl urea. Reaction advancement was monitored by silica gel chromatography using $CHCL_3$:CH_3OH [75:25, v/v; **3** $R_f = 0.1$, NHS derivative (**4**) $R_f = 0.5$]. Yield was ~50%. Then, 10 mg of tyrosine methyl ester base in 350 μL of DMF was added to the previous solution. After mixing, the solution was allowed to stand at room temperature for 15 min. Purification was then carried out by preparative silica gel chromatography under the previously described conditions (tyrosine methyl ester $R_f = 0.8$, NHS derivative $R_f = 0.5$, conjugation product $R_f = 0.25$).

Radioiodination of 5—The conjugate **5** was easily labeled with ^{125}I at the ortho position of the aromatic nucleus:[17] 20 μL of 0.1 M phosphate buffer (pH = 7.4), 5 μL of carrier-free ^{125}I corresponding to

0.5 mCi, and 10 μL of a freshly prepared aqueous solution of chloramine T (1 mg/mL) were rapidly added to 10 μL of an ethanol solution of 5 (1 mg/mL). After a reaction time of 2 min, 20 μL of a freshly prepared aqueous solution of sodium metabisulfite (6 mg/10 mL) was added. The tracer 6 was purified immediately on gel filtration on a 1 × 60-cm column of Sephadex G25 Fine (Pharmacia, Sweden) equilibrated with phosphate buffer (0.05 M, pH = 6.6; 0.15 M NaCl, 1 g/L of NaN_3, 2 g/L of gelatine).

Immunization Procedure—The hapten:protein conjugate (1 mg/rabbit) was emulsified in complete Freund's adjuvant and injected intradermally at several sites on the backs of three New Zealand white rabbits. Conjugate in incomplete Freund's adjuvant was administered as a booster immunization at approximately one-month intervals for three months. Serum was obtained from blood collected from rabbit marginal ear veins and stored frozen at −20 °C or lyophilized.

Radioimmunoassay Procedure—Phosphate buffer (0.05 M, pH = 6.6) containing 0.1% sodium azide, 0.9% sodium chloride, and 2 g/L of gelatine (PGB) was used as the diluent throughout. Seven standard solutions from 0.075 to 5 ng/mL of chloroquine sulfate were prepared in PGB for the establishment of a standard curve (Figure 1). Standards (100 μL) or plasma samples (10 μL completed with PGB or 100 μL of appropriate dilution) were added in duplicate to 200 μL of PGB in disposable polystyrene tubes and combined with antiserum (100 μL at the working dilution 1:40 000) and ^{125}I tracer 100 μL (60 000 cpm/100 μL). Each tube was vortexed briefly and incubated at 37 °C for 1 h. Thereafter, 0.5 mL of ice cold dextran-coated charcoal suspension (35 mg of dextran T70 and 350 mg of activated charcoal in 100 mL of assay buffer) was added to each tube. After incubation for 15 min at 4 °C and centrifugation, the supernatants were eliminated and the radioactivity of the free fraction was counted in a gamma counter. Concentrations of analyte in unknown samples were determined by computer interpolation from a logit/log plot of the standard data.

ELISA Procedure—Prior to the assay, the Dynatech plate (96 wells Immulon M129 A) was coated with 0.4 μg of the hapten poly-L-lysine conjugate per well in 0.1 mL of tris(hydroxymethyl)aminomethane buffer (0.01 M, pH = 7.4; TB). The plate was covered with sealing tape, and incubated for 2 h at 37 °C and then overnight at 4 °C before use. After emptying the wells by aspiration, the plate was blocked against nonspecific binding by incubation at 37 °C for 30 min with 200 μL per well of PGB (described above). After the washing step with TBS:TW (TB containing 0.15 M NaCl and 0.1% Tween 20), the coated plates, ready for use, were stored at 4 °C for at least four to six months. For the assay, six standard solutions from 0.015 to 0.5 ng/50 μL of chloroquine sulfate were prepared in TBS:TW:BSA (TBS:TW containing 1% bovine serum albumin) in order to establish the standard curve (Figure 1). Standards (50 μL) or plasma samples (5 μL completed with TBS:TW:BSA or 50 μL of appropriate dilution) were assayed in triplicate. This was immediately followed by the addition of the antichloroquine antiserum (100 μL at the working dilution 1:40 000). Plates were shaken at 37 °C for 1 h, then washed and aspirated four times with TBS:TW. Thereafter, 200 μL of a 1:4000 dilution (in TBS:TW:BSA) of a donkey antirabbit immunoglobulin peroxidase conjugate was added to every well of the plate. The plate was incubated and washed as before. Then 100 μL of substrate solution ABTS and 100 μL of TBS:TW were added to each well. After a 30-min incubation in the dark at room temperature, the absorbance was read at 405 nm.

Data Analysis—The ELISA absorbance measurements for each plate were collected and stored on a floppy disk using an IBM PC interfaced directly with the microplate reader. Concentrations of analyte in unknown samples were determined by computer interpolation from a logit/log plot of the standard data using an interactive computer program (EIA soft from Dynatech).

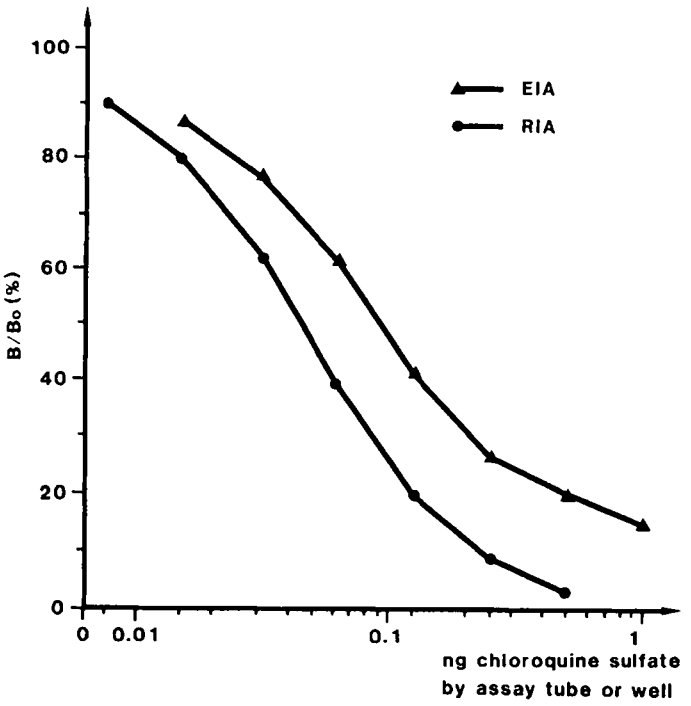


Figure 1—Standard curves.

Results

Characterization of Antisera—Antisera were titrated by dilution in the assay buffer and incubation of 0.1 mL of each dilution with either ~30 000 cpm of [^{125}I]chloroquine:tyrosine methyl ester or 0.4 μg of chloroquine:lysine conjugate coated in wells. The best working titer (rabbit 170) obtained was 1:40 000 (1:100 000 in the assay tube or in the well) for RIA or ELISA.

Properties of the Methods—*Limit of Quantitation*—The sensitivity of the two immunoassays for chloroquine in terms of the quantity of drug contained either in the assay tube or in the well is shown in Figure 1. The ELISA system was somewhat less sensitive. The possible interference due to plasma matrix difference was also examined. In the RIA procedure, endogenous materials did not interfere with the assay in human plasma or whole blood until 20 μL of volume. In the ELISA procedure, plasma interfered starting at a volume of 5 μL. The detection limit was 8 pg of chloroquine sulfate per assay tube for RIA, corresponding to ~0.70 nM of plasma for 10 μL of sample. For ELISA, the detection was 22 pg of chloroquine sulfate per well, corresponding to ~10 nM of plasma for 5 μL of sample. The range of the standard curve (2 to 1145 nM of sample) was adequate for the determination of plasma chloroquine concentrations for prophylactic schedule. One microliter of plasma is sufficient for therapeutic chloroquine concentrations.

Specificity—The specificity of the antisera (rabbit 170) was assessed according to Abraham's criteria.[18] With the exceptions of monodesethylchloroquine and bisdesethylchloroquine [hydroxychloroquine and 7-chloro-4-amino-(3-aminopropyl)quinoline are not found as metabolites], the cross reactivity of the antisera with quinoline and other antimalarial drugs with the quinoline ring, such as amodiaquine, mefloquine, quinine, and quinacrine, was minimal (Table I).

Accuracy and Precision: Intra- and Interassay Variations—Both intra- and interassay variations were estimated by using plasma standards spiked only with chloroquine sulfate. The intra-assay variance was calculated from assay volumes obtained from a single day, whereas the interassay variance was assessed on the basis of assay values from 10 consecutive days. The results are summarized in Table II. Note that both intra- and interassay variances were <10% for RIA and <16% for ELISA at all the chloroquine concentrations studied. Nearly 100% of chloroquine was recovered.

Comparison of the RIA and ELISA Procedures—The values obtained with the RIA procedure were compared with those determined for the same plasma samples (n = 48) using the ELISA method. There was good correlation (r = 0.97;

Table I—Cross Reactivity of the Antiserum for Chloroquine

Compound	Cross Reactivity Relative to Chloroquine, %
Chloroquine	100
Monodesethylchloroquine	70
Bisdesethylchloroquine	40
Hydroxychloroquine	230
Amodiaquine	4.5
Desethylamodiaquine	<0.1
Bisdesethylamodiaquine	<0.1
7-Hydroxydesethylamodiaquine	<0.1
Mefloquine	<0.1
Quinine	<0.1
Quinidine	<0.1
Quinacrine	0.13
7-Chloro-4-amino(3-aminopropyl)quinoline	180
7-Chloro-4-hydrazinoquinoline	0.4
6-8-dichloroquine	0.12

Table II—Intra- and Interassay Accuracy and Precision[a]

Assay	Added Concentrations of Chloroquine Sulfate		Plasma Sample Volume, μL	Accuracy, %	CV, %
	ng/mL	nM			
RIA					
Intra-assay	6	13.7	10	106	5.4
	18	41.3	10	99	6.4
	60	137.5	1	101	6.5
	180	413	1	106	4.6
Interassay	6	13.7	10	96	7.7
	18	41.3	10	101	9.9
	60	137.5	1	101	8.0
	180	413	1	99	8.0
ELISA					
Intra-assay	6	13.7	5	112	15.0
	18	41.3	5	105	10.0
	60	137.5	1	106	11.0
	180	413	1	113	12.6
Interassay	6	13.7	5	108	16.0
	18	41.3	5	106	13.0
	60	137.5	1	99	15.7
	180	413	1	101	13.0

[a] n = 10.

Figure 2) between the assay values obtained by these two methods.

Comparison of ELISA with the High-Performance Liquid Chromatographic Method[5]—The new RIA or ELISA procedures have adequate sensitivity to measure chloroquine in plasma samples from all dosage schedules (until 0.70 nM with 10 μL of plasma), whereas for the HPLC method, only concentrations >10 nM could be estimated (UV detection). The plasma concentrations of chloroquine and monodesethylchloroquine (70% of cross reactivity) determined by the new ELISA procedure were plotted against those (sum of chloroquine and monodesethylchloroquine) determined by the HPLC method for the same plasma samples (n = 19). Under these conditions there was a good correlation (r = 0.93; Figure 3). When the values obtained by the new ELISA procedure were compared with those determined by the HPLC method, the slope of the least square line was 1.04. There were not enough biological samples to compare the RIA with the HPLC method.

Comparison of the RIA with the Spectrophotometric Method[10]—The plasma concentrations of chloroquine from

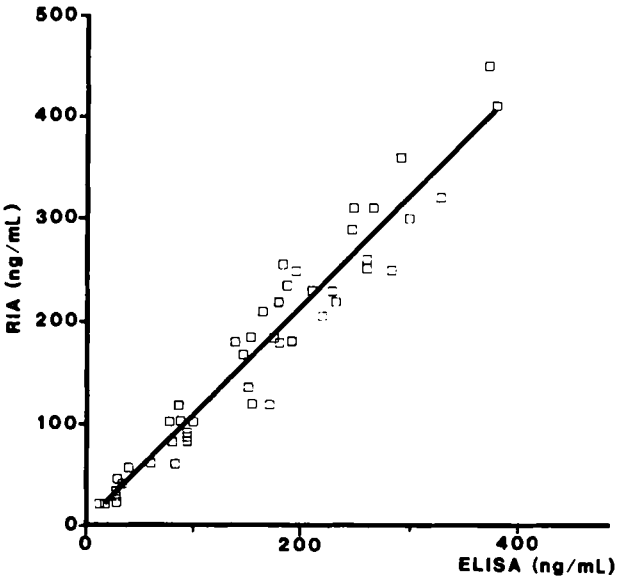


Figure 2—Comparison of the plasma chloroquine sulfate concentrations as determined by new RIA and ELISA methods (n = 48; r = 0.97; slope = 1.06; intercept = 2.07).

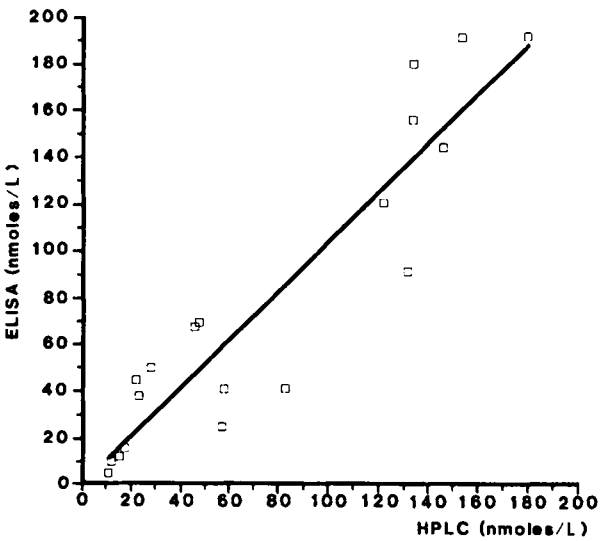


Figure 3—Comparison of the plasma chloroquine sulfate concentrations as determined by high-performance liquid chromatography (HPLC) and new ELISA methods (n = 19; r = 0.93; slope = 1.04; intercept = 0.75).

toxicological study in pigs,[19] determined by a spectrophotometric method, were plotted against those determined by the RIA method for the same plasma samples (n = 25). There was good correlation (r = 0.98; Figure 4). The same result was obtained from the comparison between these two methods on tissues concentrations of chloroquine obtained from the same toxicological study in pigs (Table III). A 100-mg sample of tissue was minced with scissors and then added to 10 mL of cold PGB and crushed in a Potter's crusher. The homogenates were centrifuged and chloroquine was determined by RIA in supernatant diluted 1:100 or 1:1000. Comparison of the ELISA procedure with the spectrophotometric method could not be carried out because the ELISA method was not yet available.

Discussion

The development of the present immunoassay procedures was based on a successful approach taken to develop RIA

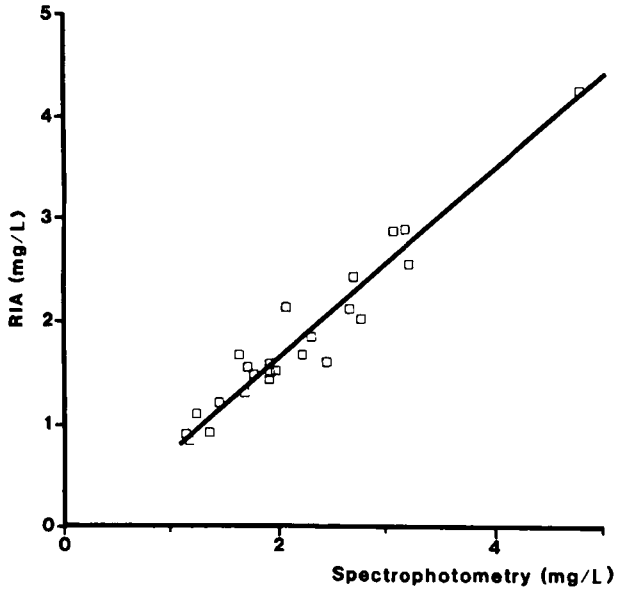


Figure 4—Comparison of the plasma chloroquine sulfate concentrations as determined by spectrophotometric and new RIA methods (n = 25; r = 0.98; slope = 0.91; intercept = −0.16).

Table III—Comparison of the Tissue Chloroquine Sulfate Concentrations as Determined by Spectrophotometry and New Radioimmunoassay[a]

Tissue	Number	RIA, μg/g	Spectrophotometry, μg/g
Heart	1	139	111
	2	132	124
	3	130	150
Liver	1	228	285
	2	349	262
	3	379	333
Muscle	1	28	30
	2	23	24
	3	19	24

[a] Samples from overdosed pigs.

procedures for chlorpromazine and its metabolites[14] in which the drug molecule was linked to the carrier protein through a two-carbon bridge bound to the N-10 side chain of the drug. All the immunized animals responded well and large amounts of antiserum, possessing high titers, were obtained. Thus, the antiserum produced by such an immunogen is specific both to the quinoline ring portion of the drug molecule and the side aliphatic chain. The antiserum did not cross react markedly with any of the other antimalarial agents or with either of the different substituents on the quinoline ring or the different side chain, such as amodiaquine. In fact, the only compounds tested that showed high cross reactivities, according to Abraham's criteria, with the antiserum developed here were the two metabolites monodesethylchloroquine and bisdesethylchloroquine. This lack of specificity towards these two metabolites is due to the coupling position of the hapten to the carrier protein. The ELISA procedure was compared with the HPLC method by determining plasma concentrations of chloroquine and monodesethylchloroquine. There was good correlation between the assay values (n = 19) determined by the ELISA and HPLC (chloroquine and monodesethylchloroquine; r = 0.93), and the slope of the regression line was not significantly different from 1 (1.04). The two immunoassay methods also showed a good correlation with each other.

The new RIA and ELISA methods have good precision in that both intra- and interassay determination showed a low coefficient of variation in the range 10–410 nM. They avoid any major sample preparation, such as extraction, and could be suitable for routine monitoring of chloroquine biological levels. A large number of assay samples of small volume can be treated rapidly; this offers an obvious advantage, especially in experimental or epidemiological studies. The present assays determine both chloroquine and its active metabolites and better correlate with the clinical toxicity than the assay of unchanged compound.

Compared with the HPLC method, the ELISA method is more sensitive and more adapted for field assays, but is less specific. Compared with colorimetric tests, the ELISA method is more sensitive and can discriminate chloroquine from the other antimalarial drugs. This represents a significant advantage for field use because, in some African communities, drugs are sometimes used indiscriminately.

References and Notes

1. Rollo, I. M. In *The Pharmacological Basis of Therapeutics*, 6th ed. Goodman, L. S.; Gilman, A. G., Eds.; Macmillan: New York, 1980; pp 1042–1045.
2. Stillman, J. S. In *Textbook of Rheumatology*; Kelley, W. N.; Harris, E. D.; Ruddy, S.; Sledge, C. B., Eds.; W. B. Saunders: Philadelphia, 1981; pp 785–795.
3. Payne, D. *Parasitol. Today* **1987**, *3*, 241–246.
4. Frisk-Holmberg, M.; Bergqvist, Y.; Termond, E.; Domeij-Nyberg, B. *Eur. J. Clin. Pharmacol.* **1984**, *26*, 521–530.
5. Verdier, F.; Clavier, F.; Deloron, P.; Blayo, M. C. *Pathol. Biol.* **1984**, *32*, 359–361.
6. Lelijveld, J.; Kortmann, H. *Bull. W.H.O.* **1970**, *42*, 477–479.
7. Haskins, W. T.; *Am. J. Trop. Med. Hyg.* **1958**, *7*, 199–200.
8. Wilson, T.; Edeson, J. F. B. *Med. J. Malaya* **1954**, *9*, 115–131.
9. Bergqvist, Y.; Hed, C.; Funding, L.; Suther, A. *Bull. W.H.O.* **1985**, *63*, 893–898.
10. Viala, A.; Durand, A.; Cano, J. P.; Jouglard, J. *J. Eur. Toxicol.* **1972**, *5*, 189–202.
11. Bergqvist, Y.; Frisk-Holmberg, M. *J. Chromatogr.* **1980**, *221*, 119–127.
12. Freier, C.; Alberici, G.; Turk, P.; Baud, F.; Bohuon, C. *Clin. Chem.* **1986**, *32*, 1742–1745.
13. Rowell, V.; Rowell, F. J.; Baker, A.; Laurie, D.; Sidki, A. M. *Bull. W.H.O.* **1988**, *66*, 211–217.
14. Hubbard, J. W.; Midha, K. K.; McGilveray, I. J.; Cooper, J. K. *J. Pharm. Sci.* **1978**, *67*, 1563–1571.
15. Gee, A. P.; Langone, J. J. In *Methods in Enzymology*; Langone, J. J.; Van Vunakis, H., Eds.; Academic: New York, 1983; pp 92, 403–413.
16. Pohlit, H. M.; Haas, W.; Von Boehmer, H. In *Immunological Methods*; Lefkovits, I.; Pernis, B., Eds.; Academic: New York, 1979; pp 181–188.
17. Massaglia, A.; Barbieri, U.; Siri-Upathum, C. *Int. J. Appl. Radiat. Isot.* **1973**, *24*, 455–462.
18. Abraham, G. E. *J. Clin. Endocrinol. Metab.* **1969**, *29*, 866–870.
19. Riou, B.; Rimailho, A.; Galliot. M.; Bourdon, R.; Huet, J. *Intens. Care Med.* **1986**, *12*, 175.

Acknowledgments

We are grateful to M. Galliot for the gifts of pig plasma and tissues assayed by the spectrophotometric method in a toxicological study. We also thank C. Freier and J. Le Bras for the gifts of compounds used in the cross reactions study, and C. Rouillard for the expert technical assistance.