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Note

Quantification of quinine in human serum by high-performance liquid chromatography

MICHAEL EDSTEIN*

Army Malaria Research Unit, Milpo, Ingleburn, Sydney, NSW 2174 (Australia)

JOHN STACE

Princess Margaret Hospital for Children, Perth (Australia)

and

FRANK SHANN

Royal Children's Hospital, Melbourne (Australia)

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The emergence of strains of *Plasmodium falciparum* resistant to chloroquine and other antimalarial drugs has resulted in a renewal of interest in the use of quinine to treat severe cases of falciparum malaria. Because of the side effects of quinine physicians should consider serum quinine concentrations when using this drug. Therefore, a rapid and accurate method for assaying quinine in biological fluids is desirable.

The most common methods for the determination of quinine levels in biological fluids are based on fluorescence procedures. Brodie and Udenfriend [1] described a protein precipitation method using metaphosphoric acid followed by testing of the supernatant with fluorescence detection. Cramer and Isaksson [2] reported a benzene extraction procedure of alkalinized plasma with fluorescent measurement of a sulphuric acid layer. Recently, gas chromatographic (GC) methods have been developed for the quantification of quinine. Bonini et al. [3] reported a sensitive and selective GC procedure for the determination of quinine added to drug-free urine and blood. Furner et al. [4] described a gas chromatographic—mass spectrometric (GC—MS) method for the quantification of quinine in human urine with a detection limit of 5 ng of quinine.

The aim of the present work was to develop a rapid, sensitive and selec-

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tive method for the determination of quinine in human serum using high-performance liquid chromatography (HPLC). The method was subsequently used to quantify quinine in serum collected from children. Reversed-phase ion-pair chromatography was used with pentanesulphonic acid as the counter-ion. A comparison was made between the HPLC method and the widely used fluorescence procedure of Cramer and Isaksson [2]. A comparison of capillary and venous blood levels of quinine in these children was also made.

MATERIALS AND METHODS

Chemicals and standards

HPLC grade acetonitrile, methanol and 1-pentanesulphonic acid were obtained from Waters Assoc. (Sydney, Australia). All aqueous solutions were prepared using deionized glass-distilled water. A stock solution of quinine dihydrochloride was prepared containing 100 μg of quinine base per ml of water and stored at 4°C in an amber glass bottle. Working solutions were prepared by appropriate dilution of the stock solution with water or drug-free serum.

Instrumentation and chromatographic conditions

The liquid chromatograph consisted of a Pye Unicam LC-XPD pump, a WISP Model 710B autosampler (Waters Assoc.), and a Whatman Partisil 10 ODS 2, particle size 10 μm (250 × 4.6 mm I.D.) reversed-phase column, protected by a LiChrosorb RP-18 guard column, particle size 10 μm (30 × 4.6 mm I.D.) (Brownlee Labs., Santa Clara, CA, U.S.A.). The detector was a Schoeffel Model FS 970 fluorometer operated at an excitation wavelength of 350 nm with a 418-nm emission cut-off filter. The fluorometer sensitivity was set at 5.6, the range at 0.2 or 0.5 μA full scale with a time constant of 6.0 sec. Peak areas were measured using a Pye Unicam DP 88 integrator. The mobile phase consisted of methanol—acetonitrile—water (30:30:40, v/v) with 0.005 *M* 1-pentanesulphonic acid (pH 3.75) pumped at a flow-rate of 1.5 ml/min (back pressure approximately 140 bar) at ambient temperature.

A Farrand Model 801 spectrofluorometer equipped with xenon lamp and 10-nm slit widths was used for the method of Cramer and Isaksson [2]. The fluorescence intensity was monitored at the excitation wavelength of 350 nm with emission wavelength set at 450 nm.

Analytical procedure for HPLC

To precipitate serum proteins a 100-μl sample (serum standards, biological sample) was mixed with 100 μl of acetonitrile in a 1.5-ml polypropylene centrifuge tube. After mixing for 15 sec, the proteins were separated by centrifugation at 12,000 *g* for 5 min. An aliquot (10 μl) of this supernatant was injected. Serum samples were tested in duplicate. Standard curves for quinine concentrations expressed as the free base were determined using peak areas.

Recovery and reproducibility

Quinine recovery was determined at concentrations of 1.25, 2.5, 5.0, 7.5

and 10 μg quinine per ml of serum by comparing peak areas with areas obtained by direct injection of pure standards. Within-day reproducibility was evaluated by analysis of serum standards (n = 5) containing 5.0 and 10.0 μg quinine per ml. Day-to-day reproducibility was determined by assaying serum standards containing 1.25, 2.5, 5.0, 7.5 and 10 μg quinine per ml on seven occasions.

Stability and storage of quinine

To assess the stability of frozen samples, 0.5-ml aliquots of serum standards were stored at −15°C in polypropylene centrifuge tubes for seven months and subsequently assayed by the HPLC method.

Patients

The subjects in this study consisted of 29 hospitalized children (15 males and 14 females) with an age range of one month to ten years. Blood was collected by venipuncture, centrifuged and the serum was stored at −15°C in polypropylene or glass tubes until analysed. Finger-prick capillary blood was collected in microtainers (Becton and Dickinson) with gentle squeezing of the finger to maintain blood flow.

Comparison of HPLC and fluorescence methods

The HPLC method developed in this study and the method of Cramer and Isaksson [2] were compared by quantifying the quinine in sera from seventeen children after administration of 10 mg/kg quinine intramuscularly. The HPLC method was also applied for comparison of venous and capillary serum quinine levels from twelve children after administration of 3 mg/kg intramuscular quinine.

RESULTS AND DISCUSSION

HPLC analysis of quinine

The minimum volume of serum required for the HPLC method was 10 μl. The speed of the method was such that three standards and an unknown sample could be assayed within 30 min. The limit of detection was 0.3 μg quinine per ml of serum (3 ng per 10 μl injected). This sensitivity should be able to be increased by injecting larger sample volumes.

Endogenous substances in serum were found not to interfere with the assay. A typical chromatogram of a patient's serum sample is shown in Fig. 1a. Chromatograms of the patient's drug-free serum and spiked serum containing 5.0 μg quinine per ml are shown in Fig. 1b and Fig. 1c, respectively. The retention time for quinine was 4.2 min with an unidentified compound appearing at 2.8 min. Pentane sulphonic acid was an essential component of the mobile phase for the separation and elution of quinine from the column.

Calibration curves (range: 1.25—10.0 μg quinine per ml of serum) prepared on seven different days were linear with correlation coefficients of not less than 0.996. The range studied encompassed the therapeutic range of quinine of 3.0—7.0 μg/ml [5]. Analysis of quinine standards in serum and water yielded identical standard curves. The recovery of quinine was excellent with

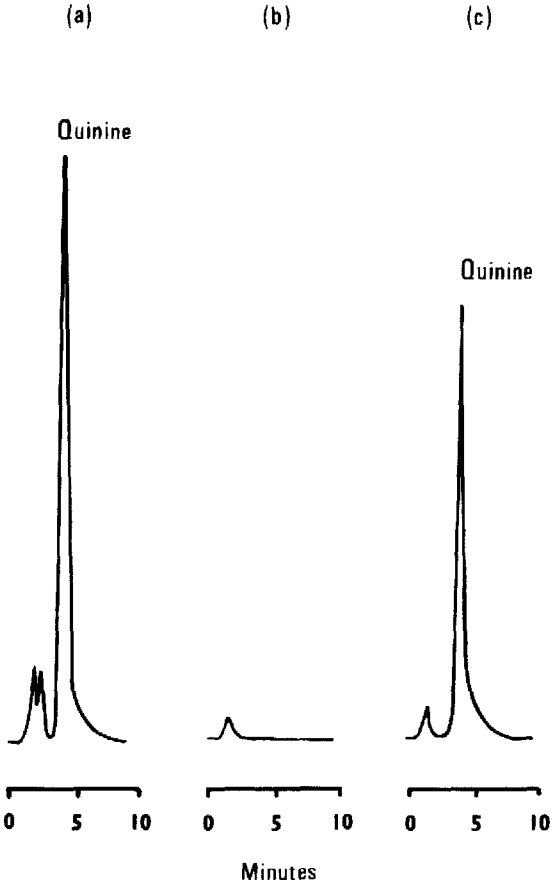

Fig. 1. Chromatograms of a patient's serum sample 6 h after receiving quinine (a), a serum sample from the same patient before receiving quinine (b), and human drug-free serum spiked with 5 μg/ml quinine (c).

TABLE I

REPRODUCIBILITY OF QUININE ESTIMATION IN SERUM

	Concentration (μg/ml)	No. of samples	Coefficient of variation (%)
Within-day	5.0	5	5.4
	10.0	5	3.0
Day-to-day	1.25	7	9.4
	2.5	7	7.7
	5.0	7	7.1
	7.5	7	7.0
	10.0	7	7.1

a mean recovery of spiked serum of 99± 2.5% over the range studied. Within-day and day-to-day coefficients of variation averaged 4.2% and 7.7%, respectively (Table I).

The principal advantages of our HPLC method over GC procedures [3, 4] are ease of sample preparation, avoidance of solvent extraction and speed of analysis. Furthermore, the GC—MS equipment used by Furner et al. [4] is expensive to buy and maintain, and requires a highly skilled operator.

None of the following drugs interfered with the determination of quinine in serum: amodiaquine, chloroquine, chloramphenicol, Fansidar®, Maloprim® and mefloquine. However, quinidine, the diastereoisomer of quinine, has a retention time similar to that of quinine and interfered with quinine assays in the present study.

Stability of serum quinine samples

There was no significant difference in quinine concentration of spiked serum samples tested before and after storage at −15°C for seven months.

TABLE II

COMPARISON OF SERUM QUININE LEVELS MEASURED BY HPLC AND FLUORESCENCE METHODS

Quinine levels are given in μg/ml.

Patient No.	Quinine at 2 h*		Quinine at 4 h		Quinine at 2 h	Quinine at 4 h
	Fluor.**	HPLC	Fluor.	HPLC	Fluor./HPLC	Fluor./HPLC
1	9.6	7.6	7.4	6.4	1.26	1.16
2	6.6	6.3	5.2	4.2	1.05	1.24
3	7.2	5.1	5.3	3.4	1.41	1.56
4	7.7	5.2	5.6	3.9	1.48	1.44
5	13.5	7.3	8.8	6.4	1.85	1.38
6	4.6	4.0	3.7	2.6	1.15	1.42
7	10.1	7.1	7.4	5.6	1.42	1.32
8	6.3	4.6	6.5	3.4	1.37	1.91
9	9.3	6.5	10.2	5.9	1.43	1.73
10	9.5	5.9	8.5	5.1	1.61	1.67
11	10.9	8.3	9.8	8.8	1.31	1.11
12	11.2	11.1	10.4	10.5	1.01	0.99
13	14.1	13.4	13.0	11.9	1.05	1.09
14	7.9	7.7	8.9	6.1	1.03	1.46
15	12.5	6.2	10.4	4.8	2.02	2.17
16	6.6	5.6	4.3	4.0	1.18	1.08
17	8.1	8.5	7.7	7.6	0.95	1.10
Mean ± S.D.	9.2± 2.7	7.1± 2.4	7.8± 2.5	5.9± 2.6	1.33± 0.30	1.40± 0.33
Paired *t*-test					$t = 4.38$	$t = 5.13$

*h represents hours after receiving quinine.
**Fluor. = fluorescence method of Cramer and Isaksson [2].

Comparison of quinine concentration measured by HPLC and fluorescence method

The fluorescence method yielded on average 37% higher quinine values than those obtained by HPLC (Table II). The serum levels of quinine measured by the two methods were statistically different ($P < 0.001$). This difference may be due to interference by metabolites of quinine in serum in the fluorescence method. In previous analyses of quinidine [6—10] determined by fluorescence methods spurious estimates of quinidine were obtained and were attributed to interference from metabolites and other components of plasma.

The difference between HPLC and the fluorescence method for the measurement of quinine suggests that HPLC is more selective. Consequently, the HPLC method would be a more appropriate method for monitoring quinine levels in man, particularly as quinine has a narrow therapeutic index.

Capillary and venous serum quinine levels

Because of the capability of the HPLC method to analyse small serum volumes, we investigated the collection of capillary serum for monitoring quinine levels. Capillary and venous serum quinine concentrations for twelve patients are presented in Table III. Capillary serum quinine concentrations average 63% of venous serum quinine concentrations. The lowered levels of serum quinine measured in capillary blood compared with that measured in venous blood may be attributed to dilution of blood with tissue fluids. Also, squeezing the finger may have caused a reduction in plasma protein in capillary blood compared to the plasma protein level in venous blood.

TABLE III

COMPARISON OF CAPILLARY AND VENOUS SERUM QUININE LEVELS

Patient No.	Serum quinine concentration (μg/ml)		Capillary/venous
	Capillary (2 h*)	Venous (2 h)	
1	1.3	2.0	0.65
2	0.8	0.8	1.00
3	1.2	1.7	0.71
4	1.3	3.6	0.36
5	1.0	3.0	0.33
6	2.6	4.0	0.65
7	0.9	2.7	0.33
8	1.2	1.7	0.71
9	2.4	4.1	0.59
10	0.8	1.0	0.80
11	0.7	0.8	0.88
12	2.2	3.7	0.59
Mean ± S.D.	1.4±0.7	2.4±1.3	0.63±0.21
Paired *t*-test			$t = 4.52$

*h = hours after receiving quinine.

As approximately 70% of total quinine is bound to plasma protein [11], any reduction of plasma protein concentration in blood could result in lower quinine levels. The use of capillary blood obtained by gently squeezing the finger, therefore, was not suitable for measuring quinine concentrations for clinical assessment.

CONCLUSION

In summary, a simple, rapid and accurate HPLC procedure for the estimation of quinine in serum has been developed, suitable for the routine monitoring of quinine levels in patients. A commonly used fluorescence method gave significantly higher concentrations of quinine than the HPLC procedure. This difference may be related to greater selectivity of the HPLC.

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