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Note

High-performance liquid chromatographic analysis of quinine and its diastereoisomer quinidine

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There has been widespread development of resistance by the malaria parasite to synthetic antimalarial drugs such as chloroquine, particularly in South East Asia. This has prompted a reappraisal of the cinchona alkaloids, used either singly or in combination, for the treatment of malaria [1]. In Thailand, for example, severe infection in obstetric patients is now treated with quinine [2], despite there being little known of the extent of its placental transfer [2]. Quiniacene has traditionally been the preferred antimalarial in the family of cinchona alkaloids. However, as quinidine is at least as potent, and possibly more potent than quinine as an antimalarial [3], this isomer is now gaining wider use [1].

Quinine and quinidine have usually been measured in biological fluids by extraction fluorescence methods that lack selectivity and sensitivity [4, 5]. These limitations have been overcome by high-performance liquid chromatographic (HPLC) methods which enable one or other of these isomers to be measured separately [6–9]. Only one method has been described, utilizing combined gas chromatography–mass spectrometry (GC–MS), which allows both quinine and quinidine to be analysed concurrently, and this method is complex [10].

The present report describes a rapid, sensitive and selective method for the determination of quinine and/or quinidine, either separately or simultaneously. The assay method has been applied to the measurement of maternal and foetal samples obtained from a pilot study in a chronically cannulated pregnant sheep.

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EXPERIMENTAL

Reagents

Quinine hydrochloride and quinidine hydrochloride monohydrate were obtained from Sigma (St. Louis, MO, U.S.A.) and monoethylglycinexylidide (MEGX) was supplied by Astra Pharmaceuticals (North Ryde, Australia). Ammonia solution (specific gravity 0.88), triethylamine and orthophosphoric acid were obtained from BDH Chemicals (Poole, U.K.) whereas hexane, ethyl acetate and HPLC-grade acetonitrile were supplied by Waters Assoc. (Sydney, Australia).

Instrumentation

The method was developed on a constant-flow high-performance liquid chromatograph (Waters Assoc.) which consisted of a solvent delivery system (Model 6000A), an injector (Model U6K) and a variable-wavelength ultraviolet absorbance detector operating at 254 nm (Model 481-Lambdamax). The reversed-phase plastic column was obtained prepacked (Rad-Pak μBondapak C_{18}, 10-μm particles, 100 mm$\times$8 mm I.D., Waters Assoc.) and was housed in a Z module (Waters Assoc.). An in-line precolumn (packed with μBondapak C_{18}, 10-μm particles, Waters Assoc.) was used to protect the analytical column.

Chromatography

The mobile phase consisted of water–acetonitrile (91:9, v/v) containing triethylamine (1%) adjusted to pH 2.5 with orthophosphoric acid. Chromatography was carried out at a flow-rate of 3.5 ml/min which gave a back-pressure of 190 kPa.

Plasma treatment procedure

To samples of plasma (1 ml), containing MEGX as internal standard (15 μg; 150 μl aqueous solution), was added ammonia solution (1 ml). This mixture was extracted with 10 ml hexane–ethyl acetate (9:1) by vortex mixing (60 s). After centrifugation (1000 *g* for 10 min) the separated organic phase was evaporated under a gentle stream of nitrogen at 35°C. The dry residue was reconstituted in the mobile phase (100 μl) and 40 μl of this were injected on the column.

Analytical recovery and assay precision

The analytical recoveries of quinine, quinidine and MEGX were estimated by comparing the peak heights obtained from an extracted plasma sample containing known amounts of the substances, with the peak heights obtained from an aqueous solution containing the same amount of each compound. The intra- and inter-assay precision were determined at two concentrations by replicate assays of samples from pools of plasma spiked to 10 and 1000 ng/ml.

Chronically cannulated pregnant sheep study

The assay was applied to a pilot study in which the disposition of quinine and quinidine was examined in a pregnant sheep (weight, 54 kg) during the last two

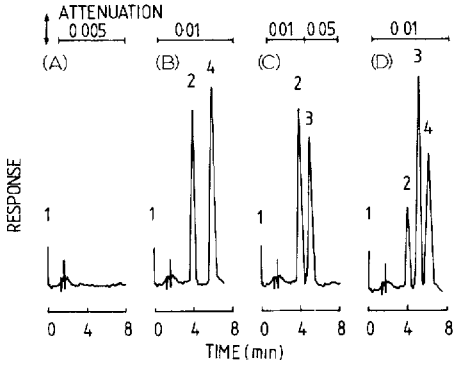

Fig. 1. HPLC profiles of sheep plasma obtained predose, i.e. blank plasma (A), sheep plasma 4 h post-quinine (concentration = 576 ng/ml) (B), sheep plasma 4 h post-quinidine (concentration = 1300 ng/ml) (C) and aqueous stock solution of quinidine (100 ng), quinine (100 ng) and MEGX (1000 ng) (D). Peaks: 1 = the injection event; 2 = MEGX; 3 = quinidine; 4 = quinine.

weeks of gestation (full term = 147 days). Under general anaesthesia, cannulae were inserted into carotid and jugular vessels of mother and foetus as previously described [11], and the animal then allowed to recover for three days prior to experimentation. This experimental model allows for drug administration (via jugular vein) and also permits chronic blood sampling (via carotid artery) from both mother and foetus in a conscious, non-anaesthetised preparation. In this pilot study, quinine was infused (10 mg/kg) into the maternal jugular vein over 5 min. Blood samples (6 ml) were withdrawn from the maternal carotid artery predose and again at 4, 6, 8, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 420 min after the end of drug infusion, whereas foetal arterial samples (4 ml) were collected predose and again at 4, 10, 30, 60, 90, 120, 180, 240, 300, 360 and 420 min. Three days later, the study was repeated following administration of quinidine (10 mg/kg) and blood was sampled as above.

After separation of blood cells, plasma samples were stored at $-20\,^{\circ}C$ until assayed for quinine or quinidine.

Calculations

Coefficients of variation were calculated from the ratio of the standard deviation to the mean. Pharmacokinetic parameters were calculated by standard model-independent pharmacokinetic formulae [12].

RESULTS AND DISCUSSION

Chromatograms of maternal sheep plasma obtained predose, i.e. blank plasma (A), and again 4 h after quinine dosage (B) or quinidine dosage (C) are shown in Fig. 1 along with a chromatogram of an aqueous stock solution of MEGX (retention time, $t_R = 4$ min), quinidine ($t_R = 5$ min) and quinine ($t_R = 6$ min) (Fig. 1D). The chromatogram of blank plasma was free from all chromatographic interference, all three compounds were completely resolved to baseline, and samples could be injected at 7-min intervals.

Calibration curves were linear ($r > 0.99$) in the range 0–20 000 ng/ml for both quinine and quinidine and the analytical recovery from plasma was 63, 65 and 35% for quinine, quinidine and MEGX, respectively. The minimum detectable level (defined as a peak four times that of baseline noise) was 10 ng/ml for both quinine and quinidine on the highest detector sensitivity used (0.005 a.u.f.s.).

The within-day and day-to-day coefficients of variation for both drugs were less than 5% at 1000 ng/ml and within-day variation was less than 10% at 10 ng/ml (corresponding to the lower limit of assay sensitivity). Extracted plasma samples could be stored if needed, as dry residues at $-20°C$ until reconstitution with chromatography mobile phase prior to analysis. The assay method was shown to be free of chromatographic interference from the 3-hydroxy metabolite of quinidine ($t_R = 2.4$ min) and from the other antimalarial drugs, chloroquine, mefloquine, halofantrine, primaquine, pyrimethamine and proguanil. A number of polar metabolites of quinine and quinidine have been identified and chromatographed on reversed-phase HPLC [8]. Under the conditions used in the present assay, these metabolites would be expected to elute with or near the void volume [8].

The use of MEGX as internal standard allowed the simultaneous determination of both quinine and quinidine. In the event that quinine was to be measured alone, quinidine could be used as internal standard (and vice versa). Under such circumstances, assay specifications for linearity, variance and sensitivity were comparable to those obtained when MEGX was used as internal standard. Compared to other published liquid chromatographic assays, the present method has the advantages of rapid simultaneous analysis, using a straightforward sample treatment procedure, the use of an internal standard and lower limits of assay sensitivity.

The assay method was applied to the measurement of quinine and quinidine concentrations in samples of maternal and foetal sheep plasma following drug administration to the mother (Fig. 2A and B). The dose level used (10 mg/kg) and the range of plasma levels seen in the adult sheep are comparable to those seen during the clinical use of these drugs [1, 2]. Plasma levels declined biexponentially in both mother and foetus for both drugs with elimination half-life values between 1.1 and 1.3 h (Table I). Drug passage across the placenta was rapid with maximal foetal levels having been achieved at the time of the first foetal sample (4 min). The elimination profile of these drugs in foetal plasma paralleled that seen in the maternal circulation (Fig. 2). Drug concentrations in the foetal circulation, however, were over an order of magnitude lower than those seen in the mother as reflected in the smaller values for foetal area under the curve ($AUC_{o \to \infty}$) (Table I).

In this pilot study, quinine appeared to have a more rapid clearance and greater volume of distribution than quinidine in maternal sheep. As a consequence, the high plasma levels of quinidine in the maternal circulation were associated with proportionally larger foetal quinidine levels than those seen for the quinine isomer. This implies that maternally administered quinidine might lead to greater foetal exposure [13] than would its diastereoisomer, quinine.

In summary, the proposed assay has been shown to be sufficiently sensitive

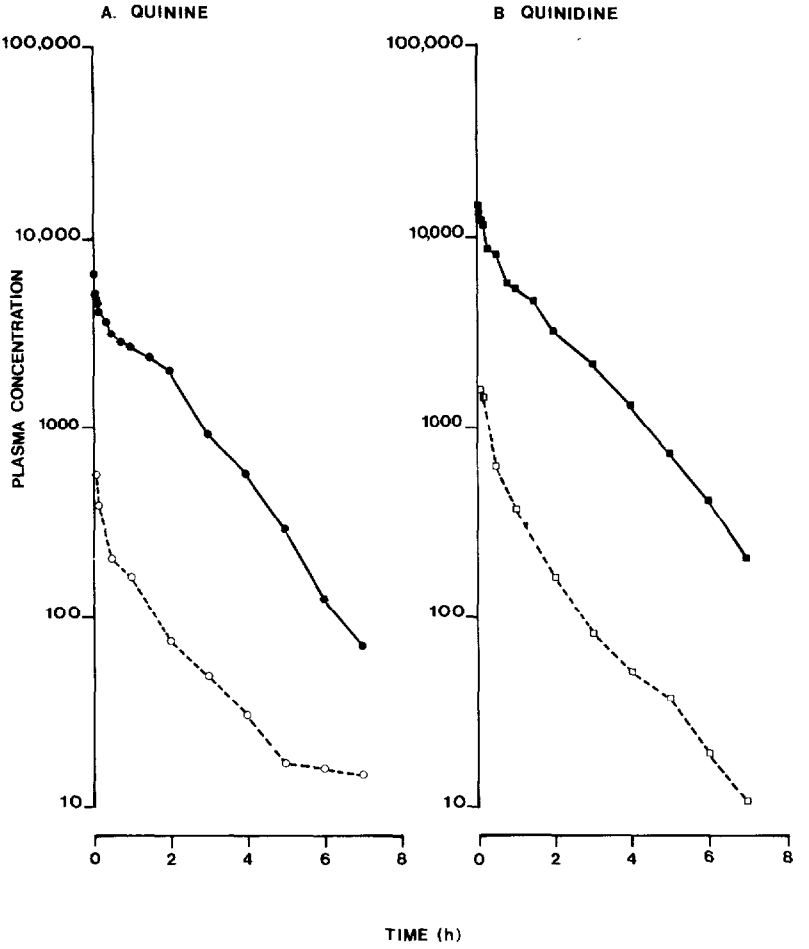

Fig. 2. Plasma disappearance profiles for quinine and quinidine from maternal and foetal plasma after intravenous dosage to the mother of either drug: (●) maternal quinine; (○) foetal quinine; (■) maternal quinidine; (□) foetal quinidine.

TABLE I

ESTIMATES OF PHARMACOKINETIC PARAMETERS FOR QUININE AND QUINIDINE IN MATERNAL AND FOETAL SHEEP

Pharmacokinetic parameter	Quinine		Quinidine	
	Maternal	Foetal	Maternal	Foetal
Half-life (h)	1.1	1.3	1.3	1.2
$AUC_{0\rightarrow\infty}$ (μg h ml^{-1})	9091	587	19 958	1411
Clearance (ml/min)	915	–	467	–
Volume of distribution (l)	86.8	–	52.9	–

and selective for measurement of quinine and quinidine in plasma samples from both maternal and foetal sheep. Preliminary results imply that stereoselective differences may exist in the disposition of these antimalarial isomers and in the nature and extent of their foetal exposure.

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