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Short Communication

Measurement of quinine in filter paper-absorbed blood by high-performance liquid chromatography

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ABSTRACT

An adaptation of an existing high-performance liquid chromatographic assay is described for the measurement of quinine, within the therapeutic concentration range, in whole blood. This method, in particular the use of small blood samples which have been dried onto filter paper strips, has advantages for clinical and pharmacokinetic studies in the tropics on children with malaria.

INTRODUCTION

At standard treatment doses, the antimalarial drug quinine (QN) attains concentrations in the blood at steady state between 10 and 15 mg/l [1]. An assay for QN over a concentration range of approximately 1–20 mg/l thus has application in studies to assess the effect on drug disposition of primary infection, secondary diseases, age, race, route of administration and dose. The collection of blood onto filter paper strips has been described for the determination of chloroquine [2], has

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advantages over liquid blood in terms of safety, and ease of storage and transportation, and is a potentially useful sampling method for other drug studies. We have adapted an existing high-performance liquid chromatographic (HPLC) technique [3] to permit the measurement of QN in filter paper-absorbed samples of blood, which facilitated sample collection in a clinical study of children with severe malaria [4].

EXPERIMENTAL

Reagents

Filter paper strips (Whatman grade 17) were a gift from Dr. F. Churchill, Centers for Disease Control (Atlanta, GA, USA). QN and quinidine (QD; internal standard) were obtained from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade), orthophosphoric acid, ammonia and ethyl acetate (all AnalaR grade) were obtained from BDH (Poole, UK). Hexane (HPLC grade) was supplied by Fisons (Loughborough, UK). Ethyl acetate was re-distilled before use.

Sampling handling

Blood was drawn by venepuncture and mixed in plastic tubes containing lithium heparin. Aliquots of 100 μl were transferred in duplicate to filter paper strips using a 200-μl pipette (Gilson Pipetman). The contents of the pipette tip were expelled as a band crossing the 2 cm $\times$ 7 cm strip. The strips were air-dried and stored at room temperature, protected from direct sunlight, until assayed. Alkaloids are stable chemicals; QN and QD can be stored at room temperature for long periods with no chemical change, both in the dry state and as liquid pharmaceutical dosage forms [5].

Extraction procedure

To the filter paper-absorbed blood QD (50 ng; 10 μl of a 5 μg/ml aqueous solution) was added using a 25-μl capacity "Microlitre" glass syringe (SGE, Ringwood, Australia). The strips were then re-dried at 37°C (0.5 h). The bands of dried blood were cut into small pieces and transferred to 10-ml silanised glass culture tubes, to which ammonia (0.5 ml) was added. After vortex-mixing and incubation at room temperature (5 min), samples were extracted into hexane–ethyl acetate (1:1, v/v; 5.0 ml) by vortex-mixing (30 s). Following centrifugation (*ca.* 2000 *g*; 5 min) and separation, the organic phase was evaporated to dryness under nitrogen (37°C). Samples were reconstituted in mobile phase (120 μl) and aliquots of 50 μl were injected into the system.

Chromatography

Chromatography was performed using an Isochrom Delivery System (Model No. A0099/314, Spectra Physics, San Jose, CA, USA) fitted to a Rheodyne valve injector (equipped with a 50-μl loop) and connected to a stainless-steel column

(Ultrasphere ODS, 15 cm × 4.6 mm I.D., 5 μm particle size, Beckman, Geneva, Switzerland) preceded by a guard column (CN precolumn, Waters Assoc., Milford, MA, USA). Column effluent was monitored by a variable-wavelength UV detector (Model No. A009/307A, Spectra Physics) set at 254 nm. Mobile phase consisted of water–acetonitrile (9:1, v/v) containing triethylamine (1%) adjusted to pH 2.8 with orthophosphoric acid and flowing at 2.0 ml/min; this generated an operating pressure of *ca.* 138 bar.

Recovery, standard curves and reproducibility

Recovery of QN and QD (internal standard) was assessed by adding 1 μg of QN and 50 ng of QD to each of five 100-μl aliquots of drug-free blood, which were dried onto filter paper and extracted as above. Recovery was defined as the ratio of the peak heights for QN and QD from the extract to those produced by direct injection of 1 μg of QN and 50 ng of QD. Standard curves were prepared by the addition of QN (2.5–20 μg/ml) to drug-free blood, which was transferred onto filter paper strips and extracted with the unknowns in each run. Intra-assay reproducibility was assessed by the addition of QN to drug-free blood at the concentrations 1.0, 1.5, 2.0, 2.5 and 20 μg/ml (n = 6 in all cases). Inter-assay reproducibility was assessed weekly over six weeks using 2.5 and 20 μg/ml QN.

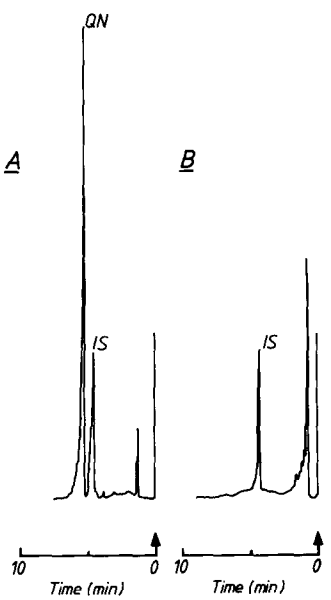

Fig. 1. Chromatograms from (A) 100 μl filter paper-absorbed blood containing 20.0 μg/ml quinine and (B) a pre-dose blood sample from the same patient. Peaks: QN = quinine; IS = internal standard (quinidine, 50 ng).

RESULTS

Chromatography

QN and QD were resolved to baseline, at QN concentrations up to 20 μg/ml, with retention times of 5.5 and 4.5 min, respectively (Fig. 1). There was no interference from chloroquine (retention time 3.5 min) or from the antimalarials sulfadoxine, pyrimethamine, proguanil, cycloguanil, chlorproguanil or chlorcycloguanil, none of which were detected on chromatography. The lowest detectable concentration of QN from a 100-μl sample was 0.1 μg/ml, which at 0.01 a.u.f.s. consistently produced a peak $> 4 \times$ background noise. However, coefficients of variation (C.V.) rose above 10% at concentrations < 1.0 μg/ml, which was therefore taken as the limit of determination.

Recovery, standard curves and reproducibility

The recovery of QN was 46 ± 4% (mean ± S.D.) and that of QD was 42 ± 17% ($n = 5$). Standard curves were linear ($r^2 \geq 0.9925$). Intra-assay C.V. were 7.0, 5.0, 5.0, 7.2 and 3.5%, respectively. Inter-assay C.V. were 12.5 and 9.2%, respectively.

DISCUSSION

Our modification to the method of Edstein *et al.* [3] permits the estimation of QN from filter paper-absorbed blood with high specificity. With a limit of determination of 1 μg/ml, our method is less sensitive than that of Edstein *et al.* (100 nmol/l), although quite adequate for the estimation of concentrations within the therapeutic concentration range, and has been used to measure QN in venous blood after parenteral administration to children with severe malaria [4]. Most investigations of QN disposition conducted in Africa are hampered by difficulties of venous access, although capillary blood is easily obtained and appears to have the same QN concentration as venous blood [6]. Venous blood was used during the development of this assay (indwelling cannulae were needed in the severely ill children from whom blood was drawn), although capillary blood could equally well have been analysed. Furthermore, limited freezer space, difficulties in transportation and scarcity of dry ice frequently complicate the storage of liquid samples in the tropics. The collection of blood samples dried onto filter paper strips eliminates these problems. An additional benefit of the storage of dry samples is the inactivation of human immunodeficiency virus [7], which may reduce the risk of infection during sample handling.

The modifications described here, which facilitate the collection, storage and transport of samples for the estimation of QN, should be applicable to other drugs of interest in the tropics. The feasibility of such modifications to assay methods should be explored before clinical work starts.

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