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Note

High-performance liquid chromatographic method for the determination of amopyroquine in biological fluids

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The 4-aminoquinoline amopyroquine has been available for antimalarial therapy for nearly 30 years [1] and has also been successfully employed in the treatment of discoid lupus erythematosus [2]. Interest in amopyroquine is now renewed, as its close structural analogue amodiaquine (Fig. 1) has been effective in cases of chloroquine resistant *P. falciparum* malaria [3]. However, due to a lack of suitably selective and sensitive methods of analysis, the pharmacokinetics of amopyroquine are virtually unknown.

This report describes a high-performance liquid chromatographic (HPLC) method for analysis of amopyroquine in biological fluids. This method has been applied to the determination of the half-life of amopyroquine in the rat.

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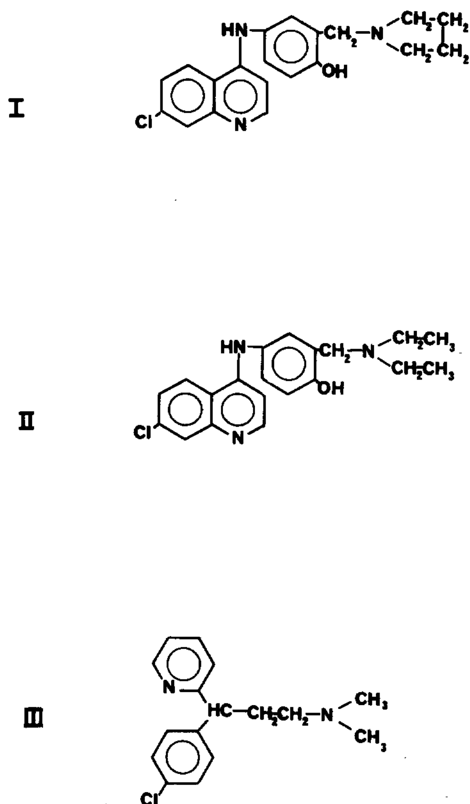

Fig. 1. Structural formulae of amopyroquine (I), amodiaquine (II) and internal standard (III).

EXPERIMENTAL

Chemicals

Amopyroquine [4-(7-chloro-4-quinolylamino)-α-1-pyrrolidyl-*o*-cresol dihydrochloride] was supplied by Warner Lambert (Ann Arbor, MI, U.S.A.). Chlorpheniramine maleate, the internal standard, was supplied by ICI Pharmaceuticals (Alderley Edge, U.K.). Ammonia solution (0.88 specific gravity) and orthophosphoric acid were obtained from British Drug Houses (Poole, U.K.). All other reagents and solvents were of HPLC grade (Fisons, Loughborough, U.K.).

Chromatography

The method was developed on a Spectra-Physics liquid chromatograph. The system consisted of an SP 8770 solvent delivery system, an SP 8750 organiser module equipped with a Rheodyne valve injection system and an SP 8300 fixed-wavelength UV absorbance detector fitted with a 254-nm mercury source. The separation was carried out on a reversed-phase plastic column obtained pre-packed, maintained at room temperature (25°C) (μBondapak Rad-Pak Phenyl; 10 μm particle size; 10 cm$\times$8 mm I.D.; Millipore/Waters, Harrow, U.K.) and housed in a radial compression module (Z module; Millipore/Waters). The mobile

phase consisted of water–acetonitrile–methanol (90:8:2, v/v/v) containing triethylamine, buffered to pH 2.55 with orthophosphoric acid, flowing at 4 ml/min.

Sample treatment procedure

To samples of plasma or urine (0.2–0.4 ml) containing an aqueous solution of the internal standard (1500 ng, 15 μl added) was added acetonitrile (1 ml). The mixture was centrifuged (1500 *g*, 5 min) and following the addition of ammonia solution (2 ml) the supernatent was decanted and extracted twice with 5 ml of hexane–ethyl acetate (9:1) by mechanical tumbling.

After separation, the combined organic phases were evaporated to dryness under nitrogen at 45°C and reconstituted in methanol (40 μl). Up to 20 μl was injected onto the column. All glassware was pretreated with dichlorodimethylsilane (5%, v/v) in order to minimise amopyroquine adsorption. To avoid photodecomposition of the drug, extract tubes were wrapped in aluminum foil during the sample treatment procedures.

Calibration, analytical precision and recovery

Standard curves in the range 0.25–10 μg/ml were prepared by adding known quantities of amopyroquine to drug-free plasma or urine containing the internal standard (750 ng/ml). Samples were analysed as described above and the peak-height ratio of compound to internal standard was plotted against the corresponding weight ratio. Peak-height ratios of experimental samples were also determined and the concentrations calculated from the standard curves.

Recovery of amopyroquine and the internal standard were estimated by comparing the peak height obtained from an extracted plasma sample with that from an aqueous solution containing the same amount of each compound. The inter- and intra-assay precision data were determined for amopyroquine by replicate assays of the same sample.

Animal studies

Two groups, A and B, of four rats (male Wistars mean weight 500 g) were provided with an Oxoid 41B diet and drinking water ad libitum. Rats in group A were anaesthetized with pentobarbitone sodium (60 mg/kg) and the trachea, jugular vein and carotid artery exposed and cannulated. Heparin was then administered [400 U/kg in saline intravenously (i.v.)]. The animals then received 120 mg/kg amopyroquine intraperitoneally (i.p.). Rats in group B were dosed with 60 mg/kg amopyroquine i.p., then housed separately in metabolism cages throughout the study period of five days to facilitate complete urine collection.

In group A blood samples (750 μl) were removed from the carotid artery predose, then at 15, 30, 60, 120, 180, 240, 300, 360, 420 and 540 min into Microcap tubes (L.I.P. Equipment and Services, Shipley, U.K.).

Total urine was collected serially for five days from the rats in group B, the volume recorded and an aliquot retained for analysis. All samples were stored at −20°C until time of analysis.

Calculations

Coefficients of variation (C.V.) for calculation of assay precision were calculated from the ratio of the standard deviation to the mean. Plasma elimination half-life was calculated by regression analysis of the post distributive log-linear portion of the plasma concentration versus time curve. Data are presented as mean ± S.D.

RESULTS AND DISCUSSION

Chromatograms of extracts of drug-free plasma, spiked plasma and plasma from a rat dosed with amopyroquine are shown in Fig. 2. The corresponding chromatograms of urine extracts are shown in Fig. 3. Amopyroquine and the internal standard chlorpheniramine were resolved with retention times of 4 and 6 min, respectively. The minimum detectable concentration (defined as a peak three times baseline noise at 0.0025 a.u.f.s. in a 1-ml plasma sample) was 5–10 ng/ml.

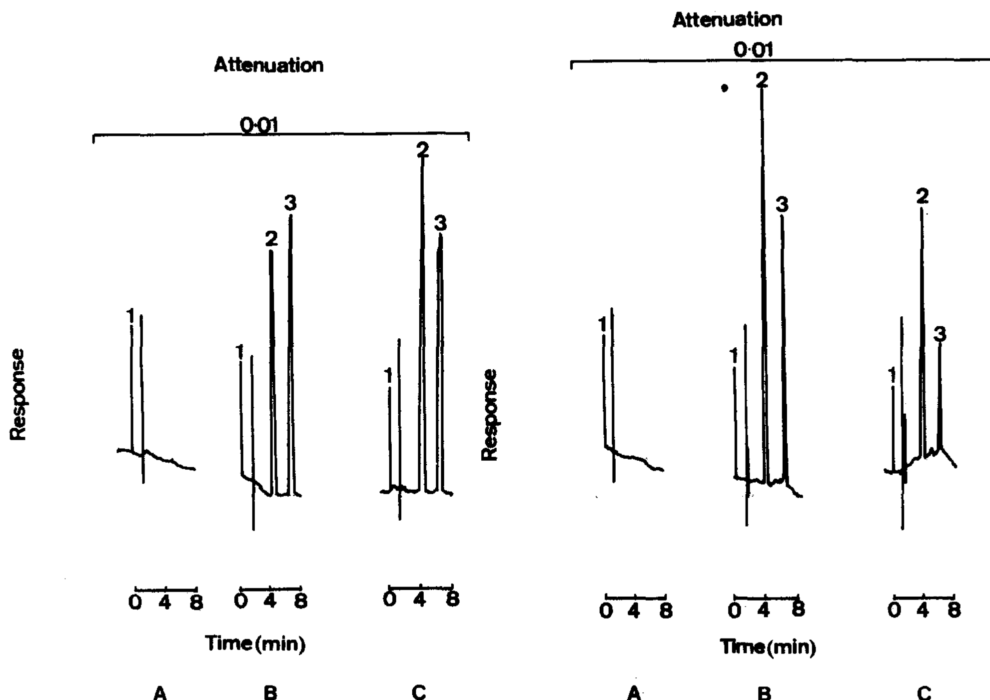

Fig. 2. HPLC profiles for (A) blank plasma extract, (B) spiked plasma extract (amopyroquine concentration, 0.78 μg/ml) and (C) extract of a plasma sample taken from a rat dosed with amopyroquine at 120 mg/kg (amopyroquine concentration, 1.4 μg/kg). Peaks; 1 = injection event; 2 = amopyroquine; 3 = internal standard.

Fig. 3. HPLC profiles for (A) blank urine extract, (B) spiked urine extract (amopyroquine concentration, 1.4 μg/ml) and (C) extract of a urine sample from a rat which received amopyroquine at 60 mg/kg (amopyroquine concentration, 2.2 μg/ml). Peaks; 1 = injection event; 2 = amopyroquine; 3 = internal standard.

Analytical recoveries for amopyroquine and the internal standard were 88.0 ± 9.3 and 97.8 ± 3.9%, respectively. All calibration curves showed linearity ($r=0.99$) for amopyroquine in both plasma and urine. The intra- and inter-assay precision data for amopyroquine are summarized in Table I. The application of the method to plasma spiked at 50 and 250 ng/ml revealed the respective assayed values to be 46.8 ± 4.1 and 233.2 ± 20.5 ng/ml ($n=5$ per concentration), i.e., within approximately 7% of the spiked concentration.

TABLE I

INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION FOR AMOPYROQUINE

Sample	Concentration (ng/ml)	Coefficient of variation (%)	
		Intra-assay	Inter-assay
Plasma	50	6.8	8.9
	250	9.3	6.2
Urine	200	7.7	8.5
	1000	4.3	9.1

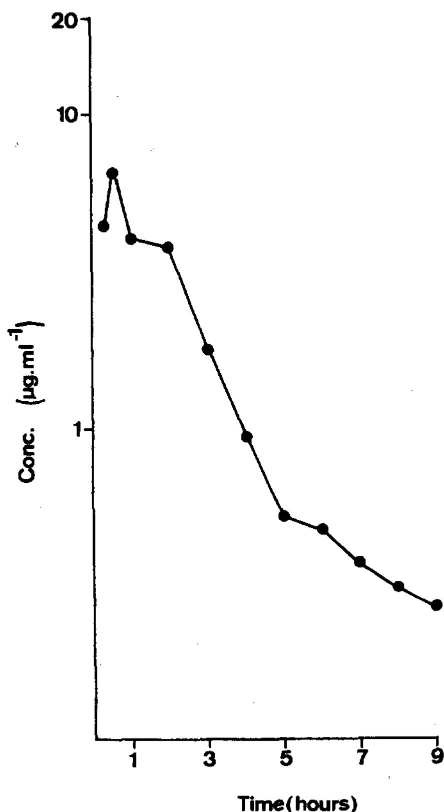

Fig. 4. Representative semilogarithmic plot of plasma levels of amopyroquine against time from a single rat following 120 mg/kg amopyroquine.

The assay was shown to be selective as it was free from chromatographic interference from endogenous material and the following antimalarials: primaquine, chloroquine, proguanil, cycloguanil, chlorcycloguanil, chlorguanil, amodiaquine, pyrimethamine, sulphadiazine, sulphadoxine and mefloquine. In addition neither desethylchloroquine nor desethylamodiaquine interfered with the assay. Hence the proposed method would be ideally suited to amopyroquine determination where these other compounds were being co-administered or had formed part of a previous treatment.

Sample preparation is significantly more rapid than in a previous assay for amodiaquine [4], where a heat-labile internal standard greatly prolonged the evaporation under nitrogen of the combined organic phases. This is not the case in the present study.

The assay method was employed to examine the pharmacokinetics of amopyroquine in the rat, a useful animal model for the study of malaria. A typical log plasma concentration versus time curve is shown on Fig. 4. Mean plasma levels of amopyroquine were observed to reach a maximum of 7.8 μg/ml at 30 min after dosage, after which they decayed with a terminal phase half-life of 2.0 ± 0.5 h. The only previous report in rats could not detect the drug in plasma [5]. Urinary excretion of amopyroquine over 120 h was $6.8 \pm 6.4\%$ of the dose.

In summary, the proposed assay has been shown to be selective, sensitive and suitable for the determination of amopyroquine in biological fluids.

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