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Note

High-performance liquid chromatographic analysis of amodiaquine in human plasma

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The substituted 4-aminoquinoline, amodiaquine, has been available for antimalarial therapy for 40 years [1]. Although more potent both in vitro and in vivo than chloroquine [2], amodiaquine has only recently assumed significance

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as an antimalarial agent consequent to its successful treatment of chloroquine-resistant *Plasmodium falciparum* [3].

In spite of the long standing availability of this compound, there is a dearth of information concerning its pharmacokinetics in man. This is in part due to the lack of suitably selective and sensitive methods of analysis [4, 5] and also until very recently to the limited clinical interest in amodiaquine [3].

This report describes the high-performance liquid chromatographic (HPLC) analysis of amodiaquine in human plasma. In addition. we have applied the method to the measurement of amodiaquine in samples derived from two patients in Thailand undergoing antimalarial therapy with this drug.

EXPERIMENTAL

Reagents

Amodiaquine hydrochloride was supplied by Parke Davis & Co. (Pontypool U.K.) and the internal standard, 6-methoxy-8-aminoquinoline by Aldrich (Gillingham, U.K.). Ammonia solution (specific gravity 0.88), triethylamine and orthophosphoric acid were obtained from BDH (Poole, U.K.). Pronalys AR grade diethyl ether and HPLC-grade methanol were supplied by Fisons (Loughborough, U.K.).

Instrumentation

The method was developed on a constant-flow high-performance liquid chromatograph (Spectra Physics, St. Albans, U.K.). This system consisted of a solvent delivery system (Model SP 8700) with an organiser module (Model SP 8750) equipped with a Rheodyne valve injector and was coupled to a fixed-wavelength ultraviolet absorbance detector operating at 340 nm (Waters Assoc., U.K.; Model 441). The reversed-phase plastic column was obtained prepacked (μBondapak Rad-Pak Phenyl, 10-μm particles; 10 cm × 8 mm I.D.; Waters Assoc.) and was housed in a radial compression module (Z module; Waters Assoc.).

Chromatography

The mobile phase consisted of water—methanol (73:27, v/v) containing triethylamine (1%), adjusted to pH 2.8 with orthophosphoric acid. Chromatography was carried out at a flow-rate of 3 ml/min which was associated with a back-pressure of 190 kPa.

Plasma treatment procedure

To samples of plasma (1.0 ml) containing internal standard (200 ng) was added ammonia solution (2 ml). This mixture was extracted twice by mechanical tumbling for 15 min with diethyl ether (5 ml). After centrifugation (1000 *g* for 10 min) and separation, the organic phases were combined and evaporated to dryness under a steady stream of nitrogen at 25°C. The residue was reconstituted in the mobile phase (55 μl) and 50 μl of this were injected on the column.

All glassware was pretreated with dichlorodimethylsilane in toluene (5%, v/v) in order to minimise amodiaquine adsorption. To avoid photodecomposition

of the drug, extraction tubes were wrapped in aluminium foil throughout the sample treatment procedures.

Analytical recovery and assay precision

The analytical recovery of amodiaquine and the internal standard was estimated by comparing the peak height of an extracted plasma sample containing a known amount of the substance with the peak height of an aqueous solution containing the same amount of each compound. The intra- and inter-assay precision were determined by replicate assays of samples from a pool of spiked plasma.

Clinical study

Two Thai patients (24 and 25 years, weighing 47 and 75 kg, respectively) with proven infection with uncomplicated *Plasmodium falciparum* malaria, were admitted to the intensive care ward of the Pra Pikklao Hospital (Chantaburi, Thailand). Each subject received an initial dose of 10 mg/kg amodiaquine followed by 5 mg/kg daily for three days. All doses were given as 4-h intravenous infusions. Venous blood samples (10 ml) were withdrawn prior to the first infusion, and then after this infusion at 0, 10, 30 min and at 2, 6, 12 and 20 h. Blood was collected into plastic, heparinized tubes, centrifuged and the plasma transferred to plain plastic vials and stored at −20° C until assayed.

Calculations

Coefficients of variation were calculated from the ratio of the standard deviation to the mean. The pharmacokinetic parameters, elimination half-life, systemic plasma clearance and volume of distribution were calculated by standard model independent pharmacokinetic formulae [6].

RESULTS AND DISCUSSION

Previous analytical methods for estimation of amodiaquine have been limited to spectrophotometric and fluorometric procedures which lacked both sensitivity and selectivity for this compound [4, 5]. In addition, these methods involved cumbersome sample preparation and non-selective chemical treatment procedures aimed at enhancing either the absorbance or fluorescence of the parent drug. The proposed assay method utilizes selective and sensitive HPLC analysis of plasma extracts obtained by a rapid and simple sample preparation.

The HPLC chromatograms of a stock solution of amodiaquine and internal standard (6-methoxy-8-aminoquinoline) as well as chromatograms of extracts of blank plasma, spiked plasma standard and patient's plasma, are shown in Fig. 1. Amodiaquine (t_R = 3.2 min) and the internal standard (t_R = 4.4 min) were detected as distinct peaks which were chromatographically resolved to baseline. The chromatogram of blank plasma was free of any interfering peaks. An endogenous component eluted with a retention time of 5.5 min, but it was baseline-separated from the internal standard and samples could be injected at 6-min intervals. It was necessary to adjust the mobile phase pH to precisely 2.8 in order to achieve the chromatographic separation between amodiaquine,

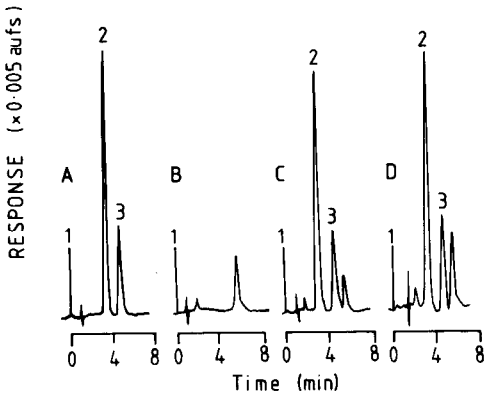

Fig. 1. HPLC chromatograms of (A) stock solution corresponding to 100 ng each of amodiaquine and internal standard; (B) blank plasma extract; (C) standard plasma extract corresponding to 150 ng/ml amodiaquine; and (D) patient's plasma extract corresponding to 142 ng/ml amodiaquine. Peaks: 1 = injection event; 2 = amodiaquine; 3 = internal standard, 6-methoxy-8-aminoquinoline.

internal standard and the endogenous component, as shown in Fig. 1. The chromatography of amodiaquine and the endogenous component was not influenced by varying the pH between 2.5 and 4.0; however, the retention time of the internal standard was very sensitive to changes in pH.

The assay was free of chromatographic interference from the commonly used antimalarial drugs, i.e. chloroquine, primaquine, pyrimethamine, proguanil and cycloguanil.

In the intitial assay development, column effluent was monitored at 229 nm corresponding to the λ_{max} for amodiaquine. This proved to be satisfactory for the analysis of samples derived from volunteer subjects. However, the concurrent administration of antipyretic, antiemetic and sedative agents to malaria patients resulted in a myriad of co-eluted peaks in the chromatogram, which precluded amodiaquine quantitation at this detection wavelength. Therefore an alternative λ_{max} for amodiaquine at 340 nm was preferred. Even though the molar extinction coefficient for this drug was slightly less at 340 nm than at 229 nm, peak detection for amodiaquine was more selective and therefore there was no appreciable loss in analytical sensitivity. At this higher wavelength the minimum detectable concentration of amodiaquine (as the hydrochloride salt), using 1 ml of plasma, was 5 ng/ml, and produced a peak three times the baseline noise on the highest detector sensitivity used (× 0.005 a.u.f.s.).

Calibration curves were linear ($r \geqslant 0.99$) in the range 0—1000 ng/ml and the analytical recoveries from plasma were 60% for amodiaquine and 50% for the internal standard. The coefficient of variation for within-day assays was 5% (100 ng/ml; $n = 6$) and for day-to-day assays over a period of eight weeks 12% (100 ng/ml, $n = 7$).

Amodiaquine was found to be very unstable to light and to adsorb readily to glassware, whereas the internal standard proved to be thermo-labile. Consequently to achieve the analytical accuracy and precision we have reported, it was necessary to protect samples from light, use silylated glassware and avoid temperatures above 25°C during sample preparation.

The assay method was applied to the analysis of samples obtained from two Thai patients undergoing antimalarial therapy with parenteral amodiaquine. There was considerable inter-subject variability in the plasma level—time profiles (Fig. 2) and at the conclusion of the 4-h infusion, maximum plasma concentrations of 1020 and 106 ng/ml, respectively, were achieved. In the two patients, after an initial rapid fall, the plasma levels declined with elimination half-life values of 4.3 and 9.7 h. The systemic plasma clearance of amodiaquine can be estimated in these subjects to be 208 and 674 l/h, and the volume of distribution to be 1291 and 9434 l, respectively. This indicates that amodiaquine, as with the other commonly used 4-aminoquinoline compound chloroquine, undergoes very extensive tissue distribution [7], and also shows that the disposition of amodiaquine appears to differ appreciably between patients.

In summary, this method, which is both sensitive and selective, overcomes the limitations imposed by previous procedures and is applicable to the analysis of plasma samples derived from field studies of the clinical pharmacology of amodiaquine.

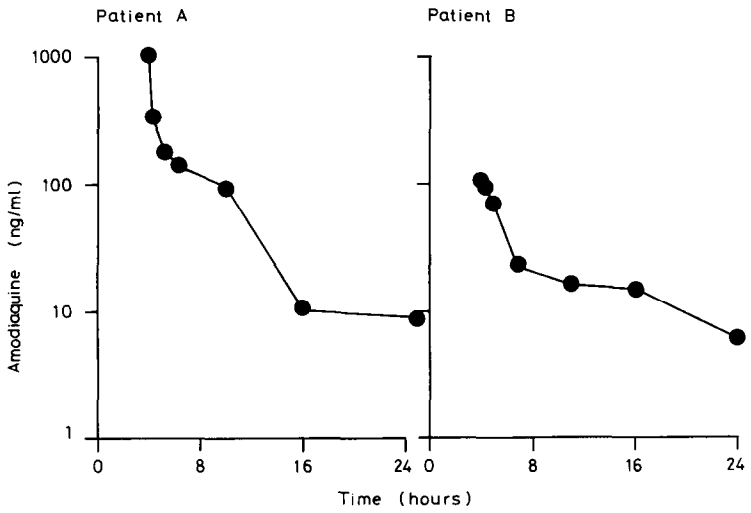

Fig. 2. Semilogarithmic plot of plasma amodiaquine concentrations (expressed as the hydrochloride salt) against time in two malaria-infected patients receiving parenteral amodiaquine, 10 mg/kg, as a 4-h infusion from time 0 to 4 h.

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