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Note

High-performance liquid chromatographic—electrochemical assay method for primaquine in plasma and urine

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Primaquine, an 8-aminoquinoline, is currently the only available antimalarial drug which is a true tissue schizontocide. In 1950 it was first reported to be the drug of choice for the radical cure of vivax malaria [1] and a true causal prophylactic agent not only against vivax malaria but also the frequently lethal falciparum malaria. More than a quarter of a century later a less toxic replacement for primaquine has not been forthcoming nor have the mechanisms of its antimalarial and hemolytic effects been elucidated [2]. Although several methods have recently been reported using gas—liquid chromatography—mass spectrometry [3], gas—liquid chromatography—electron-capture detection [4] and high-performance liquid chromatography (HPLC)—UV [5—7] techniques, they require extraction, derivatization with partial recovery or lack sensitivity for monitoring therapeutic dosage regimens. Here we would like to report a simple, sensitive assay for primaquine, and its metabolites utilizing HPLC with electrochemical detection (ED).

MATERIALS AND METHODS

Chemicals

Primaquine (PQ) was obtained from Aldrich (Milwaukee, WI, U.S.A.) as the diphosphate salt. 6-Methoxy-8-aminoquinoline was obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.). PQC, 8-(3-carboxy-1-methyl-propylamine)-6-methoxyquinoline (Fig. 1) was graciously provided by Dr. J.D. McChesney, University of Mississippi, U.S.A. The chemicals used in preparing the buffer are reagent-grade anhydrous citric acid (Sigma, St. Louis, MO, U.S.A.) and potassium hydroxide (Fisher Scientific, Fair Lawn, NJ, U.S.A.). Acetonitrile and

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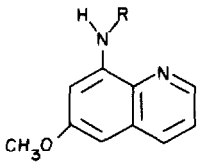

PRIMAQUINE R = $-CH(CH_3)-CH_2-CH_2-CH_2-NH_2$

8-(4-amino-1-methylbutylamino)-6-methoxy quinoline

PQC R = $-CH(CH_3)-CH_2-CH_2-COOH$

8-(3-carboxy-1-methylpropylamino)-6-methoxy quinoline

Fig. 1. Structures of primaquine (PQ) and the oxidative deamination product PQC.

methanol are HPLC grade obtained from Fisher Scientific. Amberlite XAD-2 is obtained from Bio Rad Labs. (Richmond, VA, U.S.A.). Deionized water is prepared with a Milli RO/Milli Q water purification system from Millipore (Bedford, MA, U.S.A.).

Instrumentation

The HPLC system (Fig. 2) consists of a Waters WISP (Waters Intelligent Sample Processor) (Waters Assoc., Milford, MA, U.S.A.), a Waters 6000A pump and a Waters μ Bondapak CN (250 mm × 3.9 mm I.D., particle size 10 μm) reversed-phase column. Mobile phase is acetonitrile—0.08 *M* citrate (77:23, pH 5.0). Flow-rate is 1 ml/min at ambient temperature.

Detection is by ESA Model 5100 dual-electrode electrochemical detector (ESA, Bedford, MA, U.S.A.) and with an ESA guard cell placed between the pump and injector. There are in line ESA 0.2-μm graphite filters before the guard and analytical cells (Fig. 2). The analytical cell contains two electrodes in series configuration, with the upstream electrode being designated D_1 and the downstream electrode being designated D_2. The potentials used are 0.56 V at the guard cell and 0.3 V and 0.50 V at D_1 and D_2. The gain at D_2 is 50 × 10 with a response time of 10 sec. The signal from D_2 is recorded on a Fisher Recordall Series 5000 dual-pen recorder.

Procedure

Preparation of plasma and urine samples are carried out according to a previously reported procedure [7] except that smaller amounts of standard were used in construction of the standard curve. PQ and PQC, 10 ng/ml to 1.0 μg/ml and 0.20 μg/ml to 2.0 μg/ml, respectively, were added to control plasma and urine samples.

Quantitation

The calibration curves are obtained each day by plotting detector response against the concentration for each of the standard solutions. Slope of the calibration curves and coefficients of determination are then calculated and utilized in quantitating PQ and PQC in unknown samples.

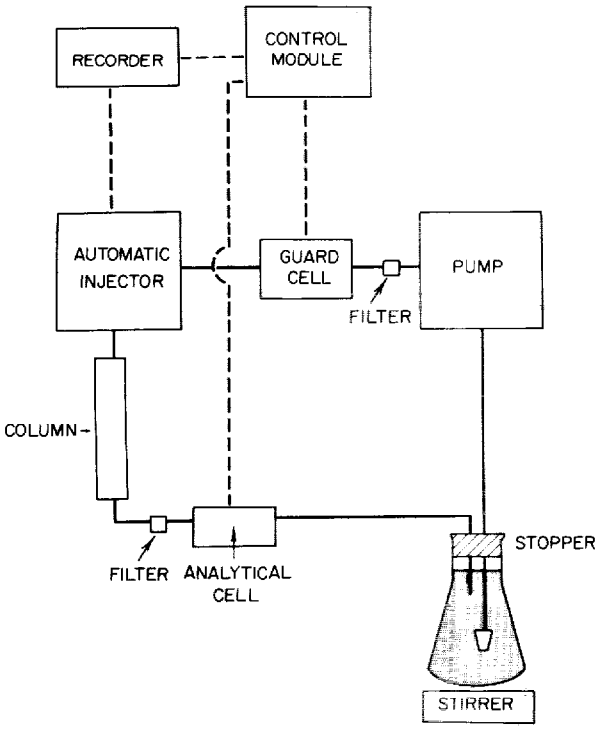

Fig. 2. Schematic presentation of the HPLC—ED system used. The fluid path is shown by the solid line and the electrical connections are shown by the hatched line.

Recovery

Recoveries of PQ and PQC at different concentrations are determined by comparison of spiked plasma R_1 to direct injection of PQ and PQC in mobile phase solvent R_2. Recovery (%) = $R_1/R_2 \times 100$.

Recovery of spiked urine primaquine samples are determined by passing known solutions of primaquine through the Amberlite XAD column and analyzing the methanol eluent for primaquine content. The detector responses are then compared using the above equation.

Dosing and specimen collection

After Institutional Review Board approval, single doses of primaquine diphosphate were administered with four ounces of water to normal healthy male volunteers. Doses given ranged from 30 to 180 mg calculated as the base. At 3 h after the dose the volunteers were allowed to eat and drink ad libitum.

Venous blood was collected at 0, 0.5, 0.75, 1, 2, 2.5, 3, 4, 6, 8, 10, 12, 15, 18 and 24 h. Urine is collected at time zero and at each void for up to 48 h.

RESULTS

Standard curves are linear over a concentration range of 10 to 1000 ng/ml and 0.2 to 2.0 μg/ml for PQ and PQC, respectively. The coefficients of determination (r^2) of the calibration graphs are 0.995 ± 0.004 (n=10). The lower

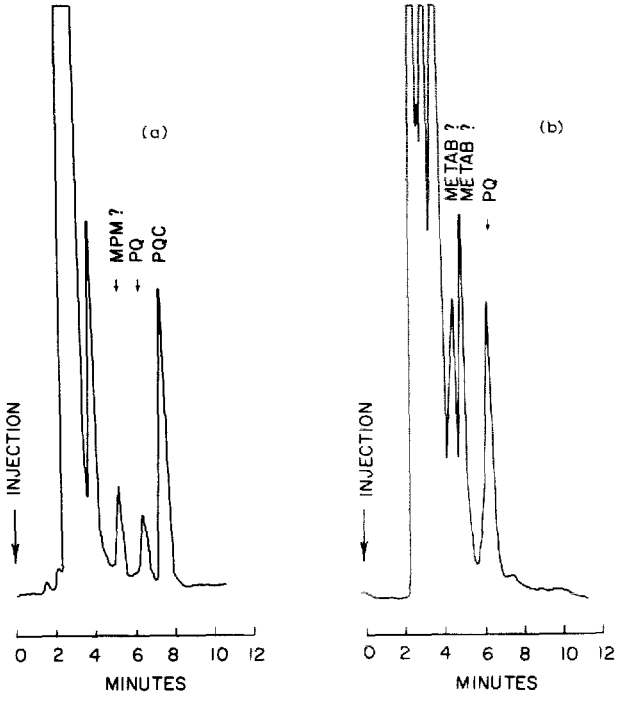

Fig. 3. Chromatograms of (a) plasma sample; and (b) urine extract done on a 0—24 h urine pool. Peaks: MPM = more polar metabolite.

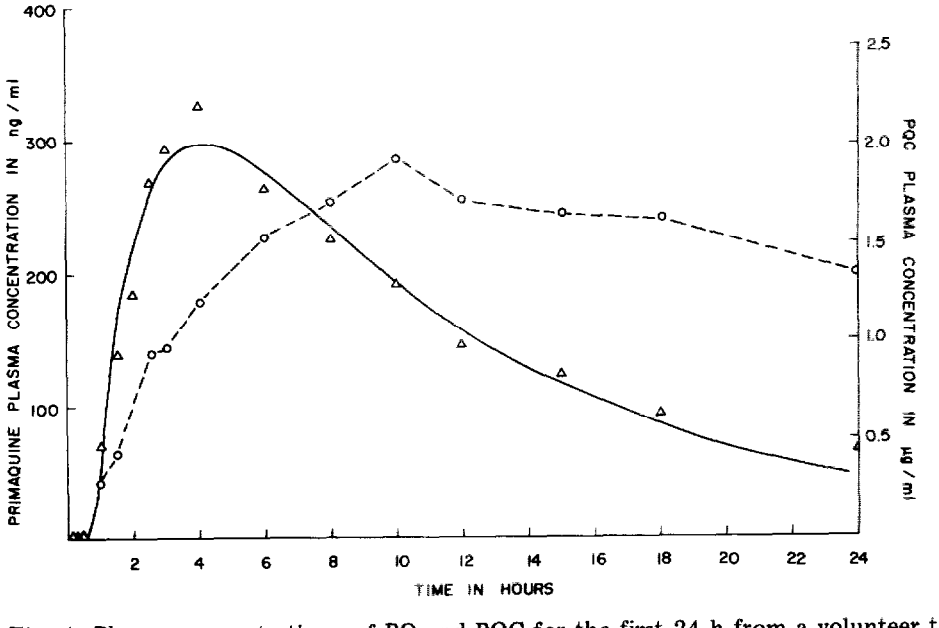

Fig. 4. Plasma concentrations of PQ and PQC for the first 24 h from a volunteer taking a single dose of 120 mg. PQ (△—△) measured concentrations with curve fitted using NONLIN; PQC (o- -o) measured concentrations with interpolated curve.

limit in routine primaquine quantitation is 0.2 ng on the column using a signal-to-noise ratio greater than 3.

Chromatograms resulting from the analysis of plasma and urine from a volunteer after ingestion of a single dose of primaquine may be seen in Fig. 3. The plasma chromatogram shows the PQ and PQC peaks as well as a more polar peak (MPM), which is ascribed to an as yet unknown metabolite. The urinary chromatogram shows the presence of PQ and two peaks which are thought to be metabolites. Using our HPLC—ED conditions PQ has a k' of 2.0 and PQC has a k' of 2.5. The known metabolite (PQC) and presumed metabolites are not present in control samples.

Fig. 4 shows typical plasma levels for PQ and PQC after a 120-mg oral primaquine dose. Data were fitted to a one-compartment open model using a non-linear least-squares regression computer program (NONLIN) [8], which estimated the following pharmacokinetic parameters: lag time, 0.78 h; absorption half-life, 1.08 h; elimination half-life, 6.72 h. These parameters were used to generate the fitted curve for PQ. Superimposed above the PQ concentration curve is the interpolated curve for the metabolite PQC.

DISCUSSION

The use of the citrate mobile phase buffer at pH 5 effects a good separation among residual plasma components, PQ, PQC and other presumed metabolites. However, it does adversely affect column life and will result in chromatograms with different retention times and even peak reversal with other manufacturers columns. Reduction of the incubation precipitation period to less than 30 min yields samples with more plasma contaminates which result in a larger signal-to-noise ratio. Effects of other substances may be minimized by setting D_1 on the electrochemical detector at 0.3 V. Thus more easily oxidizable components are oxidized before reaching the electrode at D_2.

Primaquine appears in plasma shortly after ingestion with duration of detection being dose-dependent. This period is usually less than 24 h. Visual inspection of the plasma chromatograms indicates the presence of two primaquine metabolites. One is an oxidative deamination product identified as 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline (PQC) in rats [5] and now shown by us to be a human plasma metabolite. Subsequently this was confirmed by mass spectrometry [9]. The other plasma metabolite has not been identified.

Small amounts of primaquine are found in urine (2—5% of the dose) and urinary chromatograms have two peaks in addition to primaquine. These peaks appear after oral ingestion of primaquine and are presently thought to be metabolites. PQC does not appear to be present in urine samples and attempts to find conjugation products of PQC in urine samples have been unsuccessful. Other primaquine metabolites have been reported by Strother et al. [10]. As yet we do not know if any of these correspond to our unidentified metabolites.

In contrast to our HPLC—UV method [7] the HPLC—ED technique has seven-fold increased sensitivity sufficient for measuring primaquine after oral ingestion of therapeutic as well as experimental doses. It will permit the elucidation of primaquine pharmacokinetics and allow us to follow its metabolism more closely. It is now possible to determine if there are drug—drug inter-

actions between primaquine and other antimalarials and may lead to a better understanding of primaquine antimalarial and hemolytic effects.

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