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DETERMINATION OF QUINACRINE (MEPACRINE) IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORIMETRIC DETECTION

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SUMMARY

Quinacrine (mepacrine, Atebrine) is an antiparasitic acridine derivative that is currently used as an intrapleural sclerosing agent. It was determined in plasma by reversed-phase high-performance liquid chromatography of a dichloroethane extract. The mobile phase was methanol–phosphate buffer (65:35) with 0.1 m*M* decylammonium hydrochloride and 8 m*M* ethanolammonium hydrochloride. Strong base was added post-column, and the detection of quinacrine was by fluorescence at 270/495 nm. Use of ethacridine lactate as internal standard was feasible but without significant advantage. The coefficient of variation for eight determinations of quinacrine was 4% at 3.4 ng/ml and 5% at 17 ng/ml. The stability of quinacrine in blood, plasma, water and dichloroethane solution was satisfactory for practical work. The usefulness of the analytical procedure for pharmacokinetic studies could be demonstrated.

INTRODUCTION

Quinacrine (mepacrine, Atebrine) is an acridine derivative synthesized as a substitute for quinine in the treatment of malaria [1,2]. It was extensively used as an antiparasitic agent in the decades around World War II. More recently, quinacrine has come into fairly widespread use as a sclerosing agent for intrapleural administration, in patients who suffer from accumulation of pleural exudate [3]. Probably by eliciting a local inflammatory reaction, the drug causes the lung to adhere to the chest wall, with obliteration of the pleural cavity.

Blood, plasma and urine concentrations of quinacrine on oral, intramuscular and intravenous administration have been extensively investigated by direct fluorimetry [4–8]. The therapeutic plasma concentration in the treatment of malaria is 25–100 ng/ml [2,8]. The pharmacokinetics of the drug on intrapleural instillation has not been described, and no chromatographic method seems to exist for the determination of quinacrine in body fluids. For the purpose of phar-

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macokinetic work, we decided to develop a high-performance liquid chromatographic (HPLC) assay for quinacrine in blood plasma.

The pK_a values of quinacrine dihydrochloride are 7.7 and 9.5 [9], and its distribution coefficient in octanol–pH 7.4 buffer is $10^{1.9}$ [10]. Acridine derivatives are strongly fluorescent, and the excitation and emission maxima of quinacrine are 285 (or 420) nm and 500 nm in water at pH 11 [7] and 285 nm and 500 nm in 85% lactic acid solution [11]. In aqueous solutions, a pH-dependent fluorescence yield, with a maximum at pH 12.5, has been reported [4]. Quinacrine is unstable in alkaline [9] as well as in strongly acidic aqueous solutions [12]. Moreover, quinacrine binds very strongly to cells and cell constituents [8,13], and to glass surfaces [6]. These findings had to be considered in the design of the HPLC method.

EXPERIMENTAL

Reagents and chemicals

Quinacrine dihydrochloride was purchased from Boots (Nottingham, U.K.) and ethacridine lactate from Takeda (Osaka, Japan). The compounds were dissolved in a 0.1 m*M* aqueous solution of decylamine hydrochloride adjusted to pH 3. All stock solutions were prepared by dilution with the decylamine hydrochloride solution and kept in the refrigerator. Their quality was checked by direct injection into the chromatograph. Freshly made solutions were compared with old ones. Decylamine was purchased from Fluka (Buchs, Switzerland) and ethanolamine from BDH (Poole, U.K.). Methanol (E. Merck, Darmstadt, F.R.G.), dichloromethane (Merck), diethyl ether (BDH), propanol (Nordic Pharmacopoea) and dichloroethane (Fisher, NJ, U.S.A.) were used without further purification. The water was freshly distilled and collected in a stainless-steel vessel.

Instrumentation and chromatographic conditions

The liquid chromatography system consisted of an LDC/Milton Roy (Riviera Beach, FL, U.S.A.) Constametric III pump, a Rheodyne 7125 loop injector with a 20- or 100-μl loop, a Minipuls 2 (Gilson, Villiers Le Bel, France) peristaltic pump for post-column reagent addition and a Shimadzu (Kyoto, Japan) RF-530 variable-wavelength fluorescence monitor. A LiChroCart (Merck) RP-18 7-μm column (250 × 4 mm I.D.) was used, and the mobile phase was a mixture of methanol–10 m*M* phosphate buffer (65:35) with 0.1 m*M* decylamine and 8 m*M* ethanolamine, adjusted with hydrochloric acid to an apparent pH of 3.0. The flow-rate was 1.0 ml/min. Post-column, methanol–0.5 *M* sodium hydroxide solution (65:35) was added through a low-dead-volume tee connection at a flow-rate of 0.3 ml/min. The apparent pH of the eluate was thus increased to 12.2. The excitation wavelength was 270 nm and the emission wavelength was 495 nm.

Samples

Blood samples were drawn from patients who received intrapleural quinacrine dihydrochloride. A test dose of 100 mg in 20 ml of physiological saline was given, followed by the therapeutic dose of 500 mg in 100 ml of saline. Blood samples

were drawn from a peripheral vein into heparinized Venoject® blood-collecting tubes. The plasma was collected on centrifugation of the samples for 15–20 min at 1200 *g* and stored at −20 °C until analysis.

Blank, leucocyte-free CPD (citrate, phosphate, dextrose) plasma was a gift from the Blood Transfusion Center of Malmö General Hospital.

Sample work-up, without internal standard

To 1.00-ml samples (or less) of plasma were added 0.30 ml of 0.2 m*M* decylamine in 0.2 *M* disodium hydrogenphosphate solution (pH 8). The samples were extracted with 4.00 ml of dichloroethane on a Hook and Tucker rotamixer, and the solvent layers were separated by centrifugation at 1200 *g*. Of the organic layer, 3.00 ml was transferred to another tube, and the solvent was evaporated at room temperature under a stream of dry air. The residue was taken up in 200 μl of mobile phase, and 40 μl of this solution were injected into the chromatograph. During the procedure, the samples were protected from strong light.

Standard curves and precision, without internal standard

Standard curves were drawn, using absolute peak heights, from the chromatograms of plasma samples spiked with 2.5–1000 ng/ml of quinacrine dihydrochloride (5.3–2114 n*M*). The quinacrine concentration of eight samples spiked with 4.0 ng/ml of quinacrine dihydrochloride (8.5 n*M*), and of another eight spiked with 20 ng/ml (42.3 n*M*), were quantitated against them.

Sample work-up, with internal standard

To 1.00-ml samples of plasma were added 0.50 ml of internal standard solution (ethacridine lactate 100 ng/ml). To each sample was added 1.0 ml of 0.05 *M* sodium hydroxide solution, giving a pH of 11, whereupon it was immediately extracted with 4.0 ml of 1-propanol–dichloromethane (1:9). The work-up then proceeded as described above.

Standard curves and precision, with internal standard

Standard curves were drawn in the range 12.5–100 ng/ml of quinacrine dihydrochloride, and also in the range 125–1000 ng/ml, with a ten-fold higher amount of internal standard. The precision was checked as described above, at 20 and 200 ng/ml.

Extraction yields

The extraction yields of quinacrine or ethacridine from plasma were investigated at pH 8 and 11, using duplicate samples, each spiked with quinacrine dihydrochloride or ethacridine lactate, 20 or 500 ng/ml. The solvents tried were dichloroethane, mixtures of 1-propanol with dichloromethane or dichloroethane, or diethyl ether. Also, the extraction yield of quinacrine from freshly drawn blood was determined, with haemolysis by freezing and thawing before pH-adjustment and extraction.

Stability of quinacrine and ethacridine

Effect of pH. Quinacrine dihydrochloride solution (70 µl, 100 µg/ml) was added to 7.0 ml of 0.01 *M* sodium hydroxide solution, or 7.0 ml of 0.05 *M* citrate buffer (pH 7), or 7.0 ml of 0.05 *M* citrate buffer (pH 3). The screw-cap tubes used had been washed with ethanol, and the buffer solutions were passed through 0.22-µm Millipore® filters. The solutions were kept in an incubator at 40 °C, and aliquots were withdrawn at appropriate times. The pH of these aliquots was adjusted to 3.5, and they were then frozen at −20 °C until analysis by direct injection.

The stability of ethacridine was checked analogously, but only over a period of 24 h.

Influence of light. Six samples of quinacrine base, 80 ng/4 ml, in dichloroethane were prepared by extraction from an aqueous solution. Six ethacridine base samples were prepared in the same way. Three samples of each were kept 40 cm under a 15-W fluorescent lamp continuously on for 4 days. The other samples were kept in the dark, at room temperature. On evaporation of the solvents, the samples were analysed without internal standard.

Stability of quinacrine in plasma and blood

Quinacrine dihydrochloride solution (70 µl, 10 µg/ml), was added to 7.0 ml of plasma from freshly drawn, heparinized blood. Six aliquots of 1.00 ml each were kept for 4 days before analysis. Three of them were kept frozen at −20 °C, while the other three were kept at room temperature in the dark.

To 10 ml of freshly drawn, heparinized blood was added 350 µl of quinacrine dihydrochloride solution, 4.0 µg/ml. Three samples of 1.0 ml were immediately frozen, another three were kept for 2 days at room temperature in the dark, and three more were kept for 4 days in the same way before freezing. All samples were then thawed and analysed by the ordinary procedure, without internal standard.

RESULTS

Representative chromatograms are given in Fig. 1. The capacity factors (k') of quinacrine and ethacridine were 0.93 and 2.2. The limit of detection for quinacrine (peak-to-noise ratio 3) was ca. 0.2 ng/ml (injected amount 80 pg). When no base was added post-column, the sensitivity was ca. three-fold lower. In this latter measurement, the excitation and emission wavelengths were 285 and 500 nm, respectively [11].

Standard curves and precision, without internal standard

The standard curves were linear (generally with $r = 0.999$) up to quinacrine dihydrochloride concentrations of 500 ng/ml. The first eight samples showed a mean quinacrine base concentration of 3.17 ± 0.13 (S.D.) ng/ml (theoretically 3.38 ng/ml), giving a coefficient of variation (C.V.) of 4.0%. The other eight plasma samples showed a mean concentration of 16.2 ± 0.87 ng/ml (theoretically 16.9 ng/ml), giving a C.V. of 5.3%.

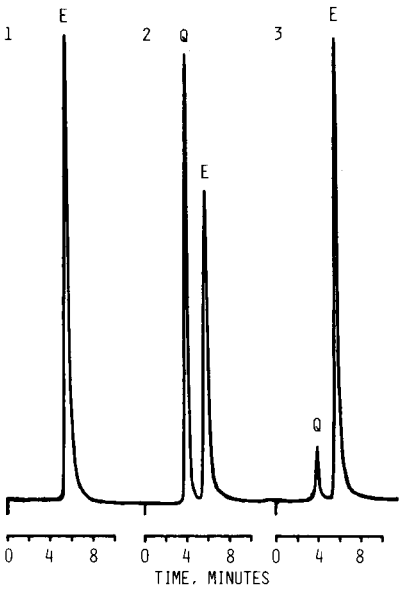

Fig. 1. Chromatograms of plasma samples from a patient (same as in Fig. 2), with ethacridine added as internal standard: (1) a sample taken before administration of quinacrine; (2) and (3) samples taken 2 and 24 h after the administration. The quinacrine base concentrations are 261 and 20 ng/ml, respectively. The injected sample volumes were 40, 30 and 40 μl. Fluorescence monitor: attenuation H32. Recorder: 1 mV, paper speed 0.25 cm/min. Peaks: Q = quinacrine; E = ethacridine.

Standard curves and precision, with internal standard

The standard curves were reproducibly linear up to a quinacrine dihydrochloride concentration of 500 ng/ml. At higher concentrations, the slope of the curves increased. When the two series of spiked samples were analysed, the found quinacrine base concentrations were 95 ± 4% and 96 ± 5% of the expected ones.

Extraction yields

Quinacrine was extracted satisfactorily with dichloroethane at pH 8, the yields being 98% at 20 ng/ml and 93% at 500 ng/ml. On the other hand, the extraction of ethacridine was very poor under these conditions (2–5%). The extraction yield of quinacrine tended to decrease at a higher pH, whereas that of ethacridine increased markedly. Owing to the rather different characteristics of quinacrine and ethacridine, no solvent or solvent mixture could be found that extracted both quantitatively. With propanol–dichloromethane (1:9) at pH 11, the extraction yields of quinacrine were 67% at 20 ng/ml and 69% at 500 ng/ml, and those of ethacridine were 91% and 81%, respectively.

Since CPD plasma was used in the standard curves whereas actual blood samples were drawn in heparinized tubes, the possible influence of heparin (500 or 2000 I.U./sample) on the extraction yield of quinacrine was investigated and found to be negligible.

The extraction yields of quinacrine from haemolysed blood, with dichloro-

ethane at pH 8, were 88, 93 and 89%, from samples spiked with 20, 100 or 400 ng/ml of quinacrine dihydrochloride, respectively.

Stability of quinacrine and ethacridine, effect of pH

At 40 °C and in the dark, quinacrine was degraded with a pseudo-first-order half-life of 112 h in 0.01 *M* sodium hydroxide solution, 27 days in 0.05 *M* citrate buffer of pH 7, and ca. 250 days in citrate buffer of pH 3. There was, within the limits of error of the method, no appreciable degradation of ethacridine over 24 h in the dark at 40 °C and pH 12, 7 or 3.

Stability of quinacrine and ethacridine, influence of light

The mean quinacrine concentration of the samples that had been kept for 4 days in normal laboratory light was 91% (range 82–96%) of the mean concentration in the samples that had been kept in the dark. In the chromatograms of the former samples, there was a small extra peak at $k' = 2.5$, that would represent a degradation product.

The mean ethacridine concentration of the samples that had been kept in the light was 91% (range 65–123%) of the mean concentration of those that had been kept in the dark. There was evidence of degradation in all samples, irrespective of storage conditions, with a high extra peak at $k' = 6.5$.

Stability of quinacrine in plasma and blood

The mean concentration of quinacrine in the plasma samples decreased by 7% in 4 days at room temperature.

The apparent concentration of quinacrine in the blood samples had decreased by 23% in 2 days at room temperature and by 31% in 4 days. There were no extra peaks in the chromatograms, so it is not clear whether the decrease in apparent concentration was due to metabolism or to progressive irreversible binding of quinacrine to blood cell constituents.

Plasma concentration curve of quinacrine in a patient

A representative plasma concentration curve is given in Fig. 2.

DISCUSSION

The main problem in the determination of the acridine derivatives was their strong adsorption to glass and other surfaces. Injections of pure water solutions of quinacrine or ethacridine into the chromatograph gave peaks of irreproducible heights, and later injections of pure water, methanol or mobile phase gave rise to strong ghost peaks. The solution to this problem was to let a competing hydrophobic amine, decylamine, be present in all solutions of quinacrine and ethacridine, including the mobile phase. Even the syringe was rinsed with mobile phase. The presence of the hydrophilic ethanolammonium ion in the mobile phase reduced peak tailing. Also, the k' values, especially that of ethacridine, could be regulated by change of the ethanolammonium concentration.

The determination of quinacrine without the use of an internal standard proved quite satisfactory. The previously reported [6] high extraction yields from plasma

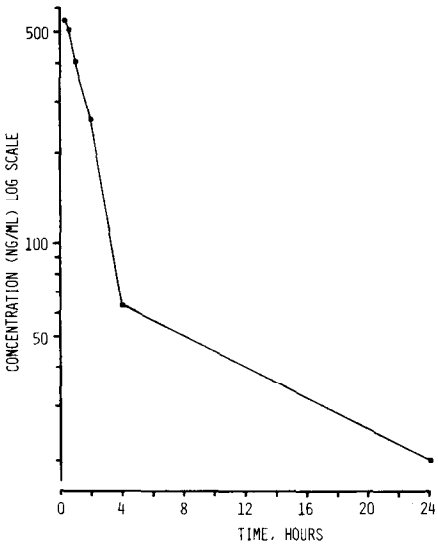

Fig. 2. Plasma concentration curve of quinacrine base in a patient (male, 96 kg) who received intrapleural quinacrine dihydrochloride as described in the text.

and blood with dichloroethane at pH 8 were confirmed. Ethacridine lactate is used as a topical disinfectant and is therefore readily available. It was tried out as an internal standard, but it showed several disadvantages. First, it proved very difficult to extract from plasma, with quite different characteristics from quinacrine, and the extraction conditions had to be a compromise with less than quantitative extraction of both compounds. Secondly, ethacridine base was only moderately stable in organic solvents.

Deviations from linearity in the standard curves were apparent for samples spiked with more than 500 ng/ml of quinacrine dihydrochloride. The deviation was strongly positive in the curves where internal standard was used and slightly positive in the ones without internal standard. At very high concentrations, the percent extraction yield of quinacrine apparently increases, markedly from the ca. 70% obtained with propanol–dichloromethane at pH 11 and slightly from the nearly 100% obtained with dichloroethane at pH 8. The peak concentrations of quinacrine after intrapleural administration went into regions where the standard curves were no longer linear. Samples taken within the first hour after the administration were therefore diluted 1:1 with blank plasma from the same patient.

Judging from the results of the stability studies, no chemical degradation of quinacrine can be expected to occur during storage or work-up. These results also confirm the findings [4,6] that aqueous solutions of quinacrine dihydrochloride can be stored for months in the refrigerator. The rapid deterioration observed [4] in very dilute solutions in pure water was most probably due to adsorption.

Quinacrine is very strongly bound to leucocytes, where it reaches concentrations more than 200 times that in plasma, whereas the concentration in erythrocytes is approximately twice the plasma concentration [6,8]. The implications of this for the sample handling and the interpretation of blood and plasma con-

centration data are discussed in detail in these references. We consequently took care to use leucocyte-free CPD plasma for all experiments and to exclude the buffy coats in the collection of plasma from blood samples. If necessary, the plasma samples were re-centrifuged before freezing.

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