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Note

Determination of chloroquine in blood by gas chromatography with nitrogen-selective detection using an internal standard

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For determining chloroquine in biological media, ultraviolet spectrophotometry and spectrofluorimetry are the commonest techniques, but they can exhibit a lack of sensitivity or selectivity. Several workers have used gas chromatography (GC) with a flame-ionization detector (FID) [1] and possible coupling with a mass spectrometer [2, 3] or with a ^{63}Ni electron-capture detector and O-ethyl O-(*p*-nitrophenyl) phenylphosphonothioate as an internal standard [4]; a few nanograms of chloroquine can be determined in rabbit urine by the last procedure. We obtained good sensitivity using a nitrogen-selective detector (NP FID) and medazepam as an internal standard [5], but interferences sometimes occurred and the volume of the samples was often very large. We attempted to overcome these problems by using another internal standard and improving the operating conditions. Whilst we were carrying out these studies, a technique for the determination of chloroquine and its monodesethyl metabolite in plasma and urine by high-performance liquid chromatography (HPLC) has been developed, with a sensitivity of 0.5 nmol/l using fluorescence detection [6].

EXPERIMENTAL

Reagents and standards

All reagents were of analytical-reagent grade. Diethyl ether and 1,2-dichloroethane were purified by using "Solvants, Documentation, Synthèses" (Peypin, France). Standard solutions of chloroquine sulphate (10 and 1 ng/μl of chloroquine base) and of papaverine (internal standard) (20 ng/μl of papaverine base) were prepared in methanol. They remain stable for at least 1 month when stored in a refrigerator at 4 °C.

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Blood samples

Blood samples were taken with potassium oxalate as anticoagulant and analysed immediately or stored at 4°C; in the latter instance the analysis must be carried out within 1 week.

Procedure

A whole blood sample (1—2 ml) was placed in a cylindro-conical centrifuge tube and 1 ml of distilled water and 1 ml of 60% potassium hydroxide solution were added. The mixture was boiled for 3 min with shaking, in order to destroy the protein bonds of chloroquine. After cooling, 100 μl of the papaverine standard solution (2 μg of internal standard) and, after vibrating for 14 sec, 10 ml of diethyl ether were added. The mixture was shaken mechanically for 15 min and centrifuged. The ether phase was transferred into another centrifuge tube and 1.2 ml of 0.1 *N* sulphuric acid were added. After shaking for 15 min and centrifuging, the ether phase was discarded. The aqueous phase, transferred in a centrifuge micro-tube, was alkalinized with one drop of 60% potassium hydroxide solution and 50 μl of a 1,2-dichloroethane—isoamyl alcohol (9:1) mixture were added. After shaking for 15 min and centrifuging, the upper aqueous phase was discarded and 2—3 μl of the organic phase were injected into the gas chromatograph.

Gas chromatography

A Hewlett-Packard Model 5170A gas chromatograph equipped with a nitrogen-specific detector was used. The glass column (1.2 m × 4 mm I.D.) was packed with 3% of OV-1—OV-17 mixture (1:3) on Gas-Chrom Q (100—120 mesh). The column temperature was 235°C and the injection port and detector temperatures were 300°C. The gas flow-rates were carrier gas (nitrogen) 50 ml/min, hydrogen 4 ml/min and air 30—40 ml/min. Under these conditions, chloroquine and the internal standard (papaverine) had retention times of 3 min 50 sec and 13 min 10 sec, respectively.

The chloroquine concentration was calculated from a calibration graph constructed by analysing blood samples spiked with chloroquine (20—300 ng/ml) and a fixed amount (2 μg/ml) of internal standard. The chromatographic response was linear in this range. The peak-area ratio of chloroquine to internal standard was plotted against the concentration of chloroquine. It would be possible in routine operation to incorporate only two standard points in each series of determinations.

RESULTS AND DISCUSSION

Papaverine was chosen as the internal standard because this quinoline alkaloid, in addition to its structural similarity to chloroquine, also has similar extraction characteristics. Moreover, papaverine is not often associated with chloroquine in therapeutic applications.

When applied to whole blood the technique gives an average recovery (mean of six determinations) of 67 ± 3.5% for 100 and 150 ng/ml of chloroquine and 65 ± 7% for 200 and 300 ng/ml. The recoveries from plasma and urine were 68 ± 3% and 78 ± 4%, respectively, for 200 ng/ml of chloroquine.

The within-run reproducibility (coefficient of variation 2%) was established using blood samples that contained 200 ng/ml of chloroquine. The determinations were repeated seven times. The between-run reproducibility (coefficient of variation 6.3%) was tested on plasma samples that contained 200 ng/ml of chloroquine. The samples were frozen for 4 weeks. The determinations were carried out each week.

The sensitivity limit for a quantitative determination was ca. 20 ng/ml in whole blood.

The specificity was tested with respect to possible interferences from other antimalarial drugs and from chloroquine metabolites. The peaks of amodiaquine (Flavoquine), pyrimethamine and quinine did not interfere in the determination of chloroquine. Sulfadoxine, associated with pyrimethamine in the commercial drug Fansidar, was not extracted. Chloroquine metabolites were extracted from the urine of a patient under chloroquine therapy, after enzymatic hydrolysis, separated by thin-layer chromatography and eluted from the spots with methanol. One of the metabolites isolated had a similar retention time to chloroquine, but on lowering the column temperature to ca. 220°C two separated peaks were obtained. The other urinary metabolites did not

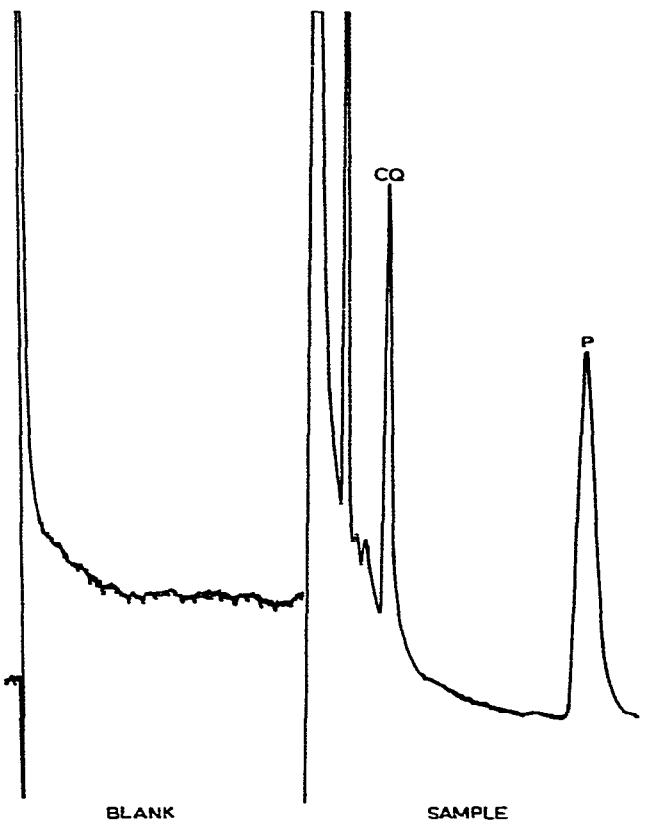

Fig. 1. Chromatogram obtained from the whole blood extract of a patient who had received 600 mg of chloroquine in tablets 24 h previously (attenuation = 16; recorder speed = 0.5 cm/min). CQ = chloroquine; P = papaverine (internal standard).

interfere. However, on analysing blood from patients on chloroquine treatment, no interfering substances (natural or metabolites of chloroquine) were found in the determination of the unchanged drug (Fig. 1).

The procedure is more sensitive and selective than spectrophotometry and spectrofluorimetry. It is less sensitive than the HPLC method described by Bergqvist and Frisk-Holmberg for human plasma and urine [6]. Its sensitivity is similar to that of the GC technique developed by Murayama and Nakajima for rabbit urine [4]. It gives a result within 1.5 h and seems to be more rapid than the other GC or HPLC methods.

The method is particularly suitable for the routine determination of chloroquine in whole blood from patients under treatment. In blood samples from two patients who had ingested 24 h earlier 600 mg of the drug in a single dose, we found 0.5 and 0.6 μg/ml of chloroquine. It can also be used for pharmacokinetic studies and therapeutic control. The method can be applied to the determination of chloroquine in plasma and urine; hydrolysis with potassium hydroxide solution is not necessary for urine samples, but the column temperature must be lowered to 220°C in order to prevent interference from one of the chloroquine metabolites. Its extension to the analysis of tissues should be possible. Finally, it is suitable for use in toxicology, for diagnostic or prognostic purposes in possible cases of poisoning.

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