Journal of Chromatography, 421 (1987) 192–197
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3794

Note

Liquid chromatographic determination of amopyroquine in rabbit plasma and red blood cells

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(First received March 31st, 1987; revised manuscript received May 22nd, 1987)

Amopyroquine (APQ), 4-(7-chloro-4-quinolylamino)-α-1-pyrrolidyl-*o*-cresol, is a chemical analogue of the 4-aminoquinoline antimalarial, amodiaquine (Fig. 1).

APQ has been found to be effective against infections due to *Plasmodium falciparum* and *P. vivax* with a single intramuscular injection [1–3]. Furthermore, APQ can cure monkeys infected with chloroquine-resistant strains of *P. falciparum* [4], and it shows a good in vitro activity against chloroquine-sensitive and -resistant *P. falciparum* strains [5].

The spectrophotometric assay described by Thompson et al. [6] was not specific enough to distinguish between APQ and its possible metabolites. Very recently, Coleman et al. [7] described the plasma kinetics of APQ in the rat after a single intraperitoneal dose but did not detect any metabolites.

The purpose of the present study was to develop a rapid selective and sensitive high-performance liquid chromatographic (HPLC) method for detecting metabolites and assaying APQ in plasma and red blood cells during pharmacokinetic studies.

EXPERIMENTAL

Chemicals

Amopyroquine dihydrochloride and bisdesethylamodiaquine dihydrochloride were generous gifts from Substantia-Parke-Davis (Courbevoie, France). Stock solutions containing 10 μmol/ml APQ were prepared in distilled water. Working standards were prepared from the stock solutions. Acetonitrile and 1,2-dichlo-

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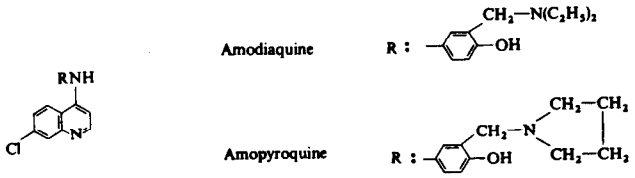

Fig. 1. Chemical structures of amopyroquine and amodiaquine.

roethane (chromatographic purity; Merck, Darmstadt, F.R.G.) were used. All other chemicals were analytical grade.

Chromatography

The high-performance liquid chromatograph consisted of a Beckman 114M pump, a Waters Intelligent Sample Processor 710B (WISP®), and a Waters 440 fixed-wavelength absorbance detector with a 340-nm filter. The chromatograph was equipped with a C_{18} reversed-phase Nucleosil (Macherey-Nagel) column (10×0.46 cm I.D.) (Société Française de Chromatographie, Gagny, France), with a particle size of 3 μm, operating at ambient temperature. The mobile phase consisted of 15% acetonitrile in a 45 m*M* potassium dihydrogenphosphate buffer (adjusted to pH 3.0 with orthophosphoric acid) at a flow-rate of 1.0 ml/min, giving a pressure of ca. 120 bar.

Extraction procedure

Red blood cells were hemolysed in distilled water (1:1, v/v). Plasma or red blood cell lysates (250–1000 μl), 250 μl of dipotassium hydrogenphosphate (pH 9.5) and 1,2-dichloroethane (8 ml) were added to a 15-ml polypropylene tube. The samples were shaken in a reciprocal shaker for 15 min and centrifuged for 5 min at 1000 *g* at room temperature. Then 7 ml of the organic layer were aspirated and transferred to a 15-ml centrifuge tube; 200 μl of 0.1 *M* hydrochloric acid were added. After shaking for 15 min and centrifuging for 5 min at 1000 *g* at room temperature, 150 μl of the aqueous phase were injected into the chromatograph.

Calibration, recovery and precision

Standard calibration curves for APQ over the range 50–1000 pmol/ml were prepared using drug-free plasma or red blood cell lysates. The calibration curves were obtained by linear regression of the peak height of APQ versus the concentration of the drug. Recovery was calculated by comparing the measured values of spiked samples of each medium with those of standard aqueous solutions at two concentrations (50 and 500 pmol/ml).

The precision of the extraction procedure and chromatography was evaluated by processing as replicates within-day (intra-assay) and day-to-day (inter-assay) aliquots of pooled plasma or red blood cell lysates containing known amounts of APQ (50 and 500 pmol/ml).

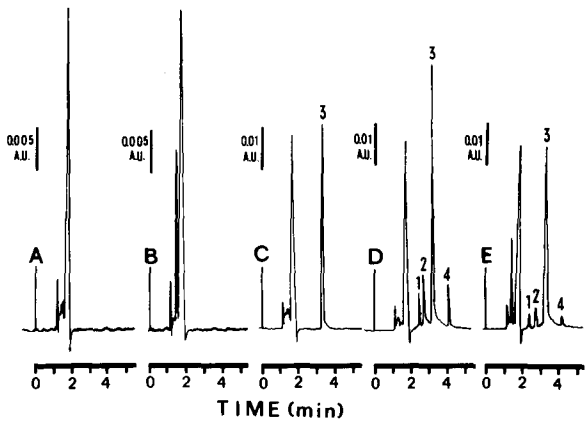

Fig. 2. Chromatograms showing (A) an extract of a blank plasma sample, (B) an extract of a blank red blood cell sample, (C) an extract from plasma spiked with APQ base (600 pmol/ml), (D) an extract of 1.0 ml of rabbit plasma containing 765 nmol/l taken 3 h after a single intramuscular injection of 10 mg/kg APQ base, and (E) an extract of 250 µl of rabbit red blood cell lysate containing 525 pmol, corresponding to 4200 nmol/l, taken 3 h after an intramuscular injection of 10 mg/kg APQ base. Peaks: 1, 2 and 4 = unidentified metabolites; 3 = APQ.

Drug disposition study

In order to test the ability of this method to measure APQ during pharmacokinetic studies, APQ base was administered to nine rabbits in a single intramuscular dose of 10 mg/kg. Blood was withdrawn via the femoral artery at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 24, 27, 32, 48, 54 and 58 h after injection, and transferred to EDTA tubes. Samples were centrifuged within 30 min at 1500 *g* for 15 min at room temperature. Plasma and red blood cells were separated and stored at -20 °C until analysis.

Plasma concentration versus time profiles were analysed with a two-compartment model using the linear regression program Graphakin® in conjunction with the Tekronix 4051 calculator.

RESULTS AND DISCUSSION

Representative chromatograms of a blank plasma and red blood cell sample and an extracted plasma spiked with 600 pmol/ml APQ are illustrated in Fig. 2A–C. Blank samples did not demonstrate any interfering peaks. The retention time (t_R) of APQ is 3.5 min.

The results of the recovery tests are outlined in Table I. The assay recovery for APQ was independent of concentration, and averaged 93.5 and 87.1% in plasma and red blood cells, respectively.

The equations of the calibration curves are $y = 0.1850 + 0.369x$, $n = 9$, $r = 0.9997$ for plasma and $y = -0.0714 + 0.3418x$, $n = 9$, $r = 0.9992$ for red blood cells. This indicates that there is a good linearity between the detector response and amounts of APQ added in each medium in the range of tested concentrations, i.e. 50–1000 pmol/ml. The minimum detectable concentration for APQ determination in both media was 20 pmol/ml (i.e. 7.1 ng/ml) at a signal-to-noise ratio grater than 5:1

TABLE I

RECOVERY OF THE ANALYTICAL METHOD ($n=6$)

Sample	Amount added (pmol/ml)	Recovery (mean ± S.D.) (%)
Plasma	50	94.30 ± 4.3
	500	92.70 ± 3.2
Red blood cells	50	87.80 ± 5.6
	500	86.50 ± 6.3

at 0.02 a.u.f.s. Replicate analyses of samples to which known amounts of APQ had been added demonstrated that the method has acceptable precision in both biological media (Tables II and III).

Figs. 2D and E show chromatograms obtained by extracting 1 ml of plasma (765 nmol/l) and 250 μl of red blood cell lysates (4200 nmol/l) from a rabbit 3 h after an intramuscular dose of 10 mg/kg APQ base. Unchanged APQ accounts for the major circulating form of the drug, and three metabolites appear in the plasma and the red blood cells. Identification of the most polar metabolite (peak 1, $t_R = 2.6$ min in the analytical system) as bisdesethylamodiaquine is suggested by chromatographic comparison with standard bisdesethylamodiaquine. However, the other two metabolites have not been identified yet. Moreover, the con-

TABLE II

INTRA-ASSAY PRECISION OF ANALYSIS OF APQ ($n=10$)

Sample	Concentration added (pmol/ml)	Concentration found (mean ± S.D.) (pmol/ml)	Coefficient of variation (%)
Plasma	50	51.0 ± 1.8	3.5
	500	498.5 ± 13.9	2.8
Red blood cells	50	49.3 ± 2.2	4.5
	500	504.3 ± 17.1	3.4

TABLE III

INTER-ASSAY PRECISION OF ANALYSIS OF APQ

Sample	Concentration added (pmol/ml)	Concentration found (mean ± S.D.) (pmol/ml)	Coefficient of variation (%)
Plasma	50	49.6 ± 2.8	5.6
	500	497.0 ± 25.0	5.o
Red blood cells	50	49.0 ± 3.2	6.5
	500	507.0 ± 27.0	5.3

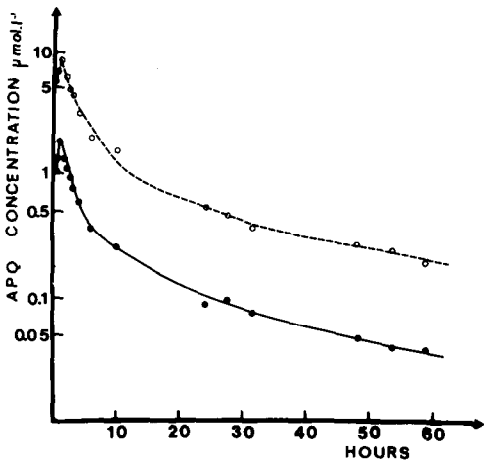

Fig. 3. Plasma and red blood cell concentration-time curves of APQ in a rabbit given a 10 mg/kg intramuscular injection of APQ base. (●) Plasma; (○) red blood cells.

centrations of these metabolites were assayed throughout the sampling and were found to decrease regularly. The terminal half-life of metabolite 1 was found to be 36 ± 15 h.

The preliminary results of a study conducted in humans administered with a single intramuscular injection of 2 mg/kg APQ base seems to show that only the most polar metabolite is detected, and only in very low concentrations (ca. 20 mmol/l) (unpublished results). APQ uptake by red blood cells (expressed by the ratio of red blood cell to plasma concentration) was greater (5.6 ± 2.9; $n=132$) than uptake of the three metabolites (3.1 ± 0.9, 3.3 ± 1.5, 0.8 ± 0.3, respectively; $n=132$). Therefore APQ represents the major form of the drug in the target cell.

Fig. 3 shows a typical concentration-time profile of APQ in plasma and red blood cells after administration of a single intramuscular dose of 10 mg/kg APQ base to a rabbit. The concentrations declined at the same rate in both media. The absorption phase of the drug was rapid, and maximum plasma concentrations of 2316 ± 1157 nmol/l ($n=9$) were achieved at 0.75 ± 0.35 h ($n=9$). The plasma levels declined with a terminal half-life value of 23.9 ± 6.7 h ($n=9$). This indicates that the elimination half-life value of APQ is shorter than that of chloroquine in the rabbit (24 and 40 h, respectively). APQ uptake by red blood cells seems to be lower than chloroquine uptake (red blood cell-to-plasma concentration ratio = 5.6 and 9.5, respectively) [8].

These results demonstrate that the method is sufficiently sensitive for determining APQ in plasma and red blood cells, thus enabling pharmacokinetic studies to be carried out in humans, and drugs to be reevaluated for use in malaria treatment.

ACKNOWLEDGEMENTS

The authors thank Jean-Marie Vallois and Jane Alix for technical assistance.

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