Journal of Chromatography, 380 (1986) 184—189
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3122

Note

Simultaneous measurement of proguanil and cycloguanil in human plasma by high-performance liquid chromatography

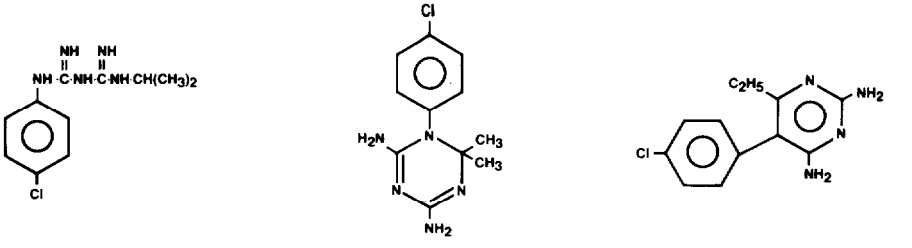
M.D. EDSTEIN

Army Malaria Research Unit, Milpo, Ingleburn, Sydney, N.S.W. 2174 (Australia)

(First received December 24th, 1985; revised manuscript received February 21st, 1986)

Proguanil (Fig. 1), an antifolate drug, has been used as a prophylactic agent for antimalarial therapy for over 30 years [1]. It has causal prophylactic activity against *Plasmodium falciparum* [2] and acts by inhibition of the plasmodial dihydrofolate reductase [3, 4]. Proguanil is well tolerated and no toxic side-effects have been reported at the recommended daily adult dose of 100 or 200 mg of proguanil base for malaria protection [4, 5]. Over the last few years there has been increasing interest in the coadministration of proguanil with a 4-aminoquinoline for protection against both falciparum and vivax malaria infections [6].

In spite of the long-standing availability of proguanil, there is very little information on its pharmacokinetics in man. There is also no data on the disposition of cycloguanil (Fig. 1), the principal active metabolite of proguanil [1]. Maegraith et al. [7] reported the absorption and excretion of Paludrine



I **II** **III**

Fig. 1. Structural formulae of proguanil (I), cycloguanil (II) and pyrimethamine (III).

0378-4347/86/$03.50

(i.e. proguanil) in human subjects using a non-selective colorimetric method [8]. A microbiological method was developed for cycloguanil but could not simultaneously measure proguanil [9]. Moody et al. [10] have reported a selective high-performance liquid chromatographic (HPLC) method capable of separating proguanil, cycloguanil and 4-chlorophenylbiguanide in serum.

This paper describes a sensitive and selective HPLC method for the simultaneous measurement of proguanil and cycloguanil in human plasma. The method was applied to the analysis of plasma samples obtained from two healthy volunteers following a single oral dose of 200 mg of proguanil base.

EXPERIMENTAL

Chemicals and standards

Proguanil, cycloguanil, pyrimethamine, mefloquine, primaquine, dapsone, sulphadoxine, quinine, chloroquine, amodiaquine and artemisinin were kindly donated by Professor W. Peters (London School of Hygiene and Tropical Medicine, U.K.). HPLC-grade acetonitrile, methanol and dichloromethane were supplied by BDH (Poole, U.K.). Pentanesulphonic acid (low UV PIC B5) was supplied by Millipore/Waters Chromatography Division (Middlesex, U.K.). Other chemicals were of the best commercially available grade. Paludrine tablets (100 mg of proguanil base per tablet) were supplied by ICI Pharmaceuticals (Alderley Edge, U.K.).

Stock solutions of proguanil hydrochloride, cycloguanil and the internal standard, pyrimethamine (Fig. 1), were prepared containing 500 μg of each compound as the base per ml of methanol. Working solutions were prepared by appropriate dilution of stock solutions with water, for spiking drug-free plasma.

Instrumentation and chromatographic conditions

The liquid chromatographic system consisted of a Kipp Analytica Model 9208 pump, a Rheodyne Model 7125 injector, a Philips PM 8251A pen recorder and an LKB 2151 variable-wavelength UV absorbance detector operated at 238 nm and 0.005 a.u.f.s. The column was a 30 cm × 3.9 mm I.D., 10 μm particle size, μBondapak C_{18} column (Millipore/Waters Chromatography Division).

The mobile phase consisted of methanol—acetonitrile—water (20:16:64) containing 0.005 *M* pentanesulphonic acid (pH 3.80). The flow-rate was 1.5 ml/min (ca. 130 bar back-pressure) and the system was operated at ambient temperature.

Extraction procedure

To a plasma sample (1 ml) in a 15 ml-glass screw-capped tube (LIP Equipment and Services, West Yorkshire, U.K.) were added 100 μl of the internal standard (50 ng pyrimethamine per 100 μl), 1 ml of 1 *M* sodium hydroxide and 8 ml of dichloromethane. The contents of the tube were mixed for 25 min using a Baird and Tatlock (Essex, U.K.) rotator at 28 rpm. After centrifugation (750 *g* for 10 min), the aqueous phase was removed. The dichloromethane phase was transferred to a clean glass tube and evaporated to dryness at 40°C under a steady stream of nitrogen. The residue was reconstituted in the mobile

phase (100 μl) and an aliquot (50—75 μl) was injected onto the column.

All glassware used in extraction was pretreated with trimethylchlorosilane in toluene (5%, v/v) in order to minimize drug adsorption.

Calibration

Standard curves were prepared by adding known quantities of proguanil and cycloguanil to drug-free plasma containing the internal standard. The concentration range studied was 12.5—200 ng/ml for each compound. Peak-height ratios of proguanil and cycloguanil to internal standard were plotted against concentrations. Standard curves were run on each day of analysis.

Analytical recovery and assay precision

Proguanil, cycloguanil and pyrimethamine recovery were estimated by comparing peak heights of each compound extracted from plasma containing known amounts of the compounds with peak heights obtained by direct injection of an aqueous solution containing the same amount of each compound. Within-day and day-to-day precisions were determined by analysis of spiked plasma at two different concentrations.

Calculations

Coefficients of variation (C.V.) for calculation of assay precision were calculated from the ratio of the standard deviation to the mean. The elimination rate constant (β) was determined by least-squares regression analysis of the post-distributive log-linear portion of the plasma drug concentration—time curve and the elimination half-life from the ratio $0.693/\beta$. Data are presented as mean ± S.D.

Clinical study

The healthy male volunteers (27 and 31 years, weighing 60 kg and 78 kg, respectively) each ingested a single oral dose of two Paludrine tablets (200 mg of proguanil base) after an overnight fast. Venous blood samples (12 ml) were collected before and at various times after dosing, using disposable plastic syringes. Blood samples were transferred immediately to heparinized tubes. Plasma was separated by centrifugation at 750 *g* for 15 min and stored at −20° C until analysis.

RESULTS AND DISCUSSION

The column effluent was monitored at 238 nm, which corresponds to a maximum absorbance for cycloguanil. Chromatograms of extracts of drug-free plasma, spiked plasma and plasma obtained from a volunteer 16 h after ingesting two Paludrine tablets are shown in Fig. 2. Retention times for cycloguanil, internal standard and proguanil were 6.5, 13 and 25.5 min, respectively. Endogenous compounds in plasma were found not to interfere with the assay (Fig. 2a). The detection limit was 5 ng/ml for proguanil and cycloguanil as measured by a peak corresponding to three times the size of baseline noise at 0.005 a.u.f.s.

Calibration curves for proguanil and cycloguanil were linear with correlation

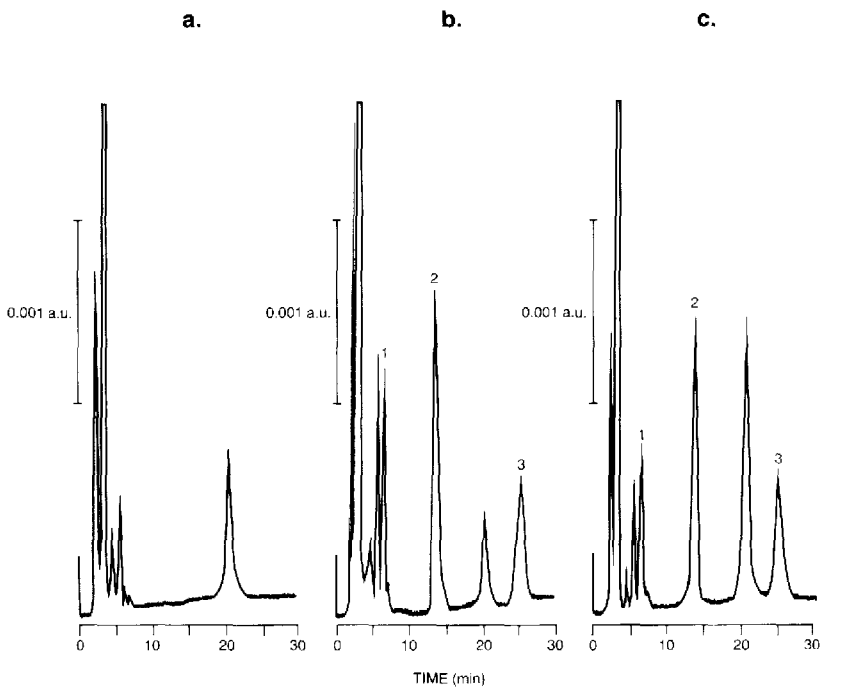


Fig. 2. Chromatograms of (a) extracted drug-free plasma, (b) extracted spiked plasma sample containing 50 ng/ml each of cycloguanil (1), internal standard, pyrimethamine (2) and proguanil (3); and (c) extracted plasma sample obtained 16 h after administration of two tablets of Paludrine to a healthy volunteer [concentrations found in this sample were: 38 ng/ml cycloguanil (1) and 57 ng/ml proguanil (2)].

TABLE I

RECOVERY OF THE HPLC METHOD FOR PROGUANIL, CYCLOGUANIL AND PYRIMETHAMINE IN PLASMA

Compound	Concentration (ng/ml)	Recovery (mean ± S.D.) (%)	Number of observations (n)
Proguanil	25	89 ± 10.1	9
	150	98 ± 6.4	6
Cycloguanil	25	65 ± 8.2	9
	150	61 ± 3.0	6
Pyrimethamine	50	82 ± 1.0	4

coefficients of 0.99 or better. Average extraction recoveries for proguanil, cycloguanil and pyrimethamine were 94, 63 and 82%, respectively (Table I). The within-day coefficients of variation and accuracy of the HPLC method are shown in Table II. The day-to-day coefficients of variation evaluated during a five-day period averaged 9.9 and 10.4% for proguanil and cycloguanil, respectively, at 25 ng/ml of plasma and 9.8% for proguanil and 5.7% for cycloguanil at 150 ng/ml. Other antimalarial drugs (i.e. dapsone, sulphadoxine, chloroquine, amodiaquine, mefloquine, primaquine, quinine and artemisinin) were found not to interfere with the assay.

TABLE II

WITHIN-DAY PRECISION AND ACCURACY DATA OF THE HPLC METHOD FOR PROGUANIL AND CYCLOGUANIL IN PLASMA

Data represent a compilation of a single experiment. Spiked plasma unknowns were bracketed by a standard curve ranging from 12.5 to 200 ng/ml for both proguanil and cycloguanil.

Compound	Amount added (ng)	Amount measured (mean ± S.D.) (ng)	C.V. (%)	*n*	Accuracy (%Δ)*
Proguanil	25	23.8 ± 1.31	5.5	9	4.5
	150	150.7 ± 2.55	1.7	6	1.1
Cycloguanil	25	23.9 ± 1.69	7.1	9	6.1
	150	147.8 ± 2.09	1.4	6	1.5

*Values represent the mean of individual determination for the absolute percentage difference of amount of drug added to plasma versus amount of drug measured.

Unlike the colorimetric [8] and microbiological [9] methods, the HPLC method described in this paper simultaneously measures proguanil and cycloguanil in human plasma. In addition, the previous methods [8, 9] involved cumbersome sample preparation and lengthy analysis time compared with the rapid and simple sample preparation of the new HPLC method. The main advantages of the present HPLC method over the previous HPLC method [10] are a twelve-fold greater sensitivity for both compounds and the use of an internal standard.

To test the clinical applicability of the present method, the concentrations of proguanil and cycloguanil in plasma were determined from two healthy

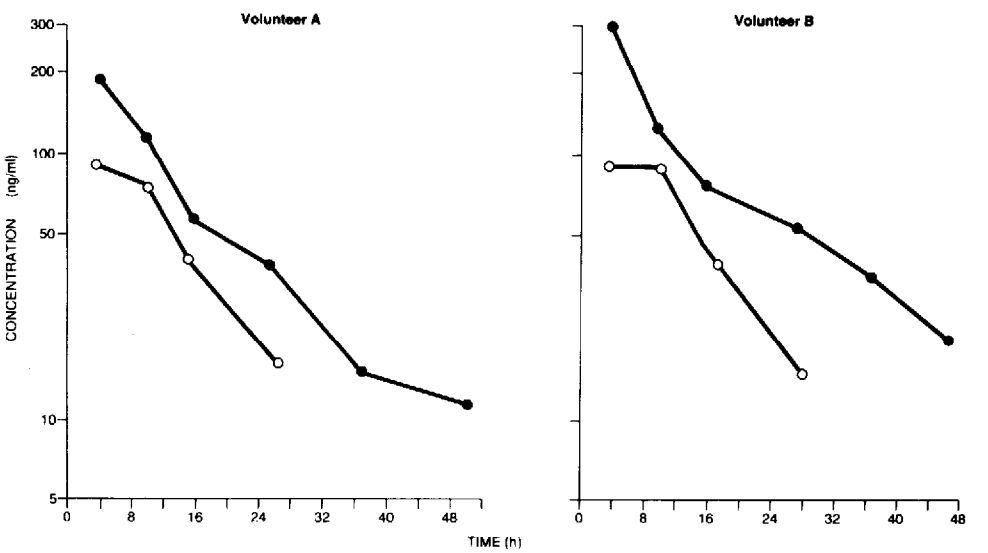


Fig. 3. Semilogarithmic plot of plasma concentrations of proguanil (•) and cycloguanil (○) versus time in two healthy volunteers after a single oral dose of two Paludrine tablets (200 mg of proguanil base).

volunteers following Paludrine administration. The plasma concentration–time curves for proguanil and cycloguanil are shown in Fig. 3. The elimination half-life ($t_{½\beta}$) of proguanil estimated from 16 to 50 h after dosing were 13.9 h (β = 0.050/h) for volunteer A and 16.9 h (β = 0.041/h) for volunteer B. These $t_{½\beta}$ values are in close agreement with the $t_{½\beta}$ of 15 h (β = 0.046/h) for proguanil estimated by Ritschel et al. [11] using the pharmacokinetic data of Maegraith et al. [7]. The $t_{½\beta}$ of cycloguanil could not be determined in this study because of the limited number of concentration points.

In summary, the HPLC method described here offers the sensitivity and selectivity for monitoring therapeutic concentrations [11] following a recommended prophylactic dosage regimen of Paludrine. The method is also suitable for studying the pharmacokinetics of proguanil and cycloguanil in man.

ACKNOWLEDGEMENTS

This paper is published with the approval of the Director General of Army Health Services, The Australian Army. The author is grateful to Lt. Col. R.C. Menzies and Mr. D. Wailling for providing the facilities and support to carry out this study at the Royal Army Medical College, Millbank, London. The author is also grateful to Mrs. J.A. Martens for typing the manuscript.

REFERENCES

1 WHO Technical Report Series, No. 711, 1984, p. 21 and p. 99.
2 V.V. Olsen, Lancet, i (1983) 649.
3 D.G. McLarty, M. Jaatinen, M. Murru, B. Aubert, R.H. Webber, C.H. Kihamia, M. Kumano and L.W. Magnuson, Lancet, ii (1984) 656.
4 F.H.N. Spracklen, S. Afr. Med. J., 65 (1984) 1037.
5 Ross Institute, Br. Med. J., 283 (1981) 214.
6 L.J. Bruce-Chwatt, Br. Med. J., 285 (1982) 674.
7 B.G. Maegraith, M.M. Tottey, A.R.D. Adams, W.H. Horner Andrews and J.D. King, Ann. Trop. Med. Parasitol., 40 (1946) 493.
8 A. Spinks and M.M. Tottey, Ann. Trop. Med. Parasitol., 40 (1946) 101.
9 C.C. Smith, J. Ihrig and R. Menne, Ann. J. Trop. Med. Hyg., 10 (1961) 694.
10 R.R. Moody, A.B. Selkirk and R.B. Taylor, J. Chromatogr., 182 (1980) 359.
11 W.A. Ritschel, G.V. Hammer and G.A. Thompson, Int. J. Clin. Pharmacol., 16 (1978) 395.