Journal of Chromatography, 416 (1987) 394–399
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3565

Note

Determination of proguanil and its metabolites cycloguanil and 4-chlorophenylbiguanide in plasma, whole blood and urine by high-performance liquid chromatography

R.B. TAYLOR*, R.R. MOODY and N.A. OCHEKPE

School of Pharmacy, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen AB9 1FR (U.K.)

(First received November 12th, 1986; revised manuscript received December 19th, 1986)

Proguanil has been used as an antimalarial prophylactic since 1946. In spite of this, pharmacokinetic data are very limited. The major investigation usually quoted [1] was carried out using analytical methods which were incapable of distinguishing the parent drug from its metabolites, cycloguanil and 4-chlorophenylbiguanide [2]. It has been claimed that cycloguanil is the active species [3, 4]. More recently, methods have been published using reversed-phase ion-pairing chromatography with sample pretreatment involving direct injection of plasma after protein precipitation [5]. This allowed selective quantitation of proguanil but was not sensitive enough to allow detection of metabolites at the levels encountered after a single oral dose of 200 mg. This approach was extended using narrow-bore column geometry to allow detection of the parent drug in 0.2-ml blood samples obtained by thumb prick [6]. Two very recently reported methods using liquid extraction as the pretreatment step are capable of determining proguanil and cycloguanil [7, 8]. Another analytical procedure is indicated in the literature but has not yet been published in detail [9].

As part of an investigation of the pharmacokinetics of proguanil and its metabolites it was found necessary to develop an assay procedure which would allow determination of proguanil and both metabolites at the prophylactic level of the parent drug in plasma, whole blood and urine. Because of the anticipated low levels of metabolites and also the wide difference in hydrophobicity of proguanil and cycloguanil it was required to improve both the sample pretreatment and the sensitivity of the chromatographic system. The former has been achieved by employing solid-phase extraction which also has the advantage of rapid sample

0378-4347/87/$03.50

throughput [10]; the latter by use of 2 mm I.D. columns packed with 3-μm stationary phase which minimises peak volume with subsequent improvement in detection limit.

It is the purpose of the present paper to describe this procedure and to indicate its performance in terms of studying the pharmacokinetics of these compounds.

EXPERIMENTAL

Materials and equipment

A Waters Assoc. (Harrow, U.K.) liquid chromatograph was used consisting of an M6000A pump and fixed-wavelength (254 nm) ultraviolet detector fitted with a nominal 18-μl flow-cell. Injection was by a Rheodyne 7125 valve (Cotati, CA, U.S.A.), incorporating a 20-μl loop. The column was 100 × 2 mm I.D., slurry packed in the laboratory with 3-μm ODS Hypersil, HETP (Macclesfield, U.K.). Solid-phase extraction pretreatment of sample was by Bond Elut (Analytichem, Harbor City, CA, U.S.A.) C_{18} cartridge in conjunction with a Vac-Elut (Jones Chromatography, Llanbradach, U.K.) ten-cartridge manifold. Proguanil, cycloguanil, 4-chlorophenylbiguanide (CPB) and 3,4-chlorophenylbiguanide (DCPB), which was used as an internal standard, were supplied by ICI Pharmaceuticals as the hydrochlorides. Acetonitrile and methanol were obtained from Rathburn Chemicals (Walkerburn, U.K.) and sodium lauryl sulphate (SLS) from Fisons (Loughborough, U.K.). Water was purified by a Millipore Milli-Q system (Harrow, U.K.), and all other reagents were of AnalaR or equivalent grade.

Sample pretreatment

Plasma and urine were used directly as obtained. Whole blood was lysed by repeated freezing followed by ultrasonification and centrifuging before use.

To plasma (1 ml) 0.1 ml of a 1 μg/ml solution of DCPB was added as internal standard and the mixture vortexed. A 1-ml aliquot was added to the C_{18} Bond Elut cartridge which had previously been wetted with 2 ml of methanol and subsequently conditioned with 2 ml of water. After adsorption of the plasma components the cartridge was washed with 1 ml of water and 1 ml of methanol. The adsorbed compounds were eluted with 1 ml methanol containing 0.1% perchloric acid. The collected extract was evaporated in a stream of air and the residue was reconstituted in 50 μl of water immediately before chromatography.

Whole blood was analysed as above after diluting 0.5 ml with an equal volume of water. When analysing urine samples the internal standard solution concentration was 5 μg/ml. After adsorbing urine components the cartridge was washed with water only and the residue was reconstituted in 1 ml of water due to the higher levels of drugs found. These pretreatments were applied to samples and to standards prepared by spiking blank plasma or blank urine with the requisite amount of drug.

The recoveries obtained by the above treatment of spiked samples were determined by comparing the peak heights obtained after extraction with those obtained by direct injection of aqueous standards of appropriate concentration.

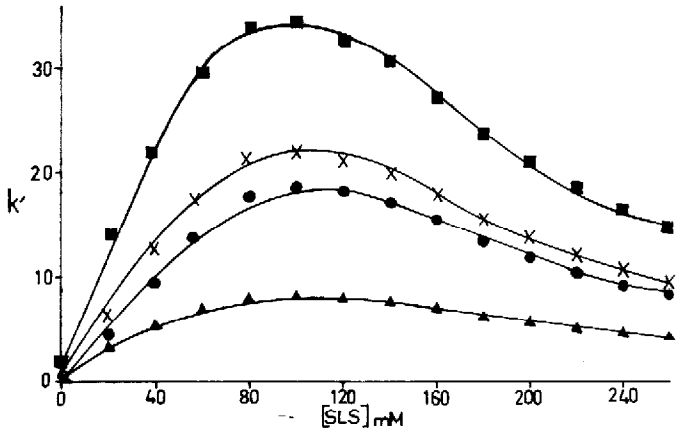

Fig. 1. Variation of capacity factor (k') with pairing ion concentration in a solvent acetonitrile–10 m*M* phosphate buffer, pH 2 (50:50). (▲) Cycloguanil; (●) 4-chlorophenylbiguanide; (■) proguanil; (×) 3,4-dichlorophenylbiguanide.

Calibration procedure

Blank samples of plasma (1 ml) were spiked with 0.1 ml of four different concentrations of the drugs to produce a range of 0–200 ng/ml for proguanil and 0–100 ng/ml for cycloguanil and CPB. Blank plasma was included in all calibration procedures. Peak-height measurements were made at appropriate absorbance ranges, usually 0.02 a.u.f.s. for the metabolites and internal standard and 0.05 a.u.f.s. for the parent drug. Blank samples of urine were similarly spiked to provide 0–10 μg/ml proguanil and 0–2 μg/ml of the metabolites. Calibration equations were obtained by linear regression of peak-height ratio on concentration in the appropriate matrix.

Mobile phase

The chromatographic solvent used for all analyses was acetonitrile–10 m*M* aqueous phosphate buffer (pH 2) at an SLS concentration of 200 m*M* (50:50).

RESULTS AND DISCUSSION

The capacity factors (k') of the biguanides proguanil, cycloguanil, CPB and DCPB were found to conform to the parabolic variation with increasing pairing ion concentration found previously for other basic drug compounds [11–13]. The variation of k' with SLS is shown in Fig. 1 for a solvent consisting of acetonitrile–10 m*M* aqueous phosphate buffer, pH 2 (50:50). The behaviour shown allowed choice of ion pairing concentration to be made such that resolution among the various compounds could be obtained in the minimum time allowing the least retained species (cycloguanil) to be eluted after any endogenous compounds or reagents appearing in the solvent front. This time was determined by the extraction efficiency of the solid-phase system used to pretreat biological samples.

Specimen chromatograms of plasma, whole blood and urine blanks, together

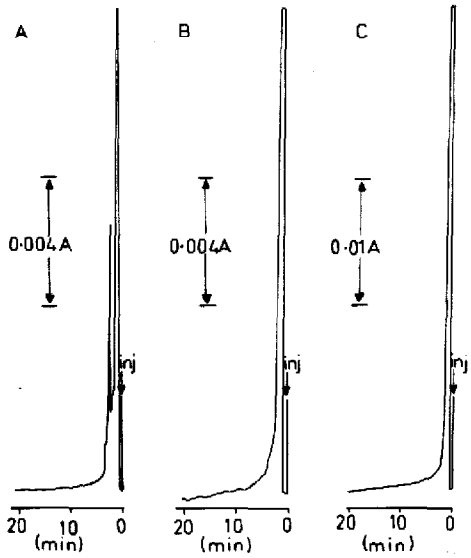

Fig. 2. Representative chromatograms obtained after blank sample pretreatment. (A) Plasma; (B) whole blood; (C) urine. Column 100×2 mm I.D., ODS silica, solvent as in Fig. 1 with 200 m*M* SLS; flow-rate 0.4 ml/min.

with chromatograms of representative biological samples are shown in Figs. 2 and 3, respectively. These show the efficiency of the extraction procedure and the chromatography employed in eliminating endogenous interference and giving high signal-to-noise ratios for the compounds. Unlike previous reports, no significant endogenous peaks were obtained at retention times greater than that of cycloguanil.

The recoveries obtained for the drugs in the different matrices are shown in Table I. These are, in general, higher than those quoted for the liquid–liquid extraction procedure with cycloguanil being the least well recovered. The reproducibilities of extraction and chromatography were found to be satisfactory as evidenced by the constancy of the calibration equations and their standard deviations on a day-to-day basis are also shown in Table I as are the detection limits estimated at the absorbance scales used.

The method described has been found to be reliable in practice. The resolution and sensitivity resulting from the use of small-particle-size stationary phase and narrow-bore column has been found necessary in a pharmacokinetic study recently undertaken. Both cycloguanil and CPB were located and quantitated in pharmacokinetic trial samples. The maximum levels encountered of the cyclo metabolite, however, were considerably less relative to the parent drug than those indicated recently [7]. The present method was found to be capable of determining the absorption, distribution and elimination stages for proguanil and the two metabolites. The pharmacokinetic information obtained from this trial will be published elsewhere.

The use of narrow-bore columns described is not new but has not been extensively applied to analytical problems. In the present work a useful sensitivity

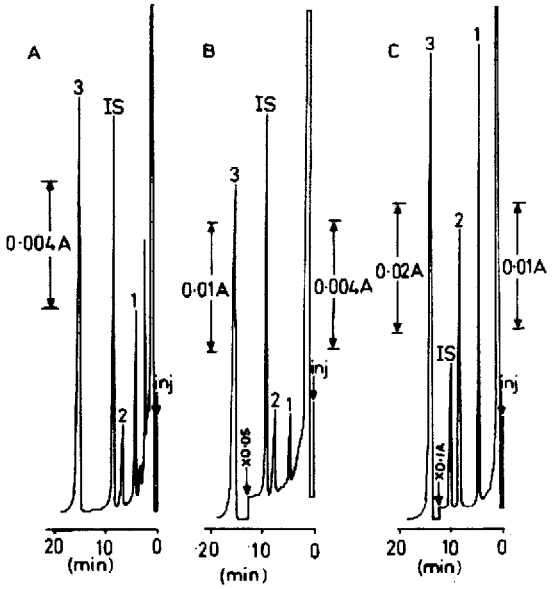

Fig. 3. Representative chromatograms obtained from trial samples 4 h after a single oral dose of 200 mg Paludrine. (A) Plasma; (B) whole blood; (C) urine. Chromatographic conditions as in Fig. 2. The concentrations of cycloguanil, 4-chlorophenylbiguanide and proguanil were: in plasma (A): 59, 14 and 164 ng/ml; in whole blood (B): 66, 49 and 992 ng/ml; in urine (C): 5.24, 1.74 and 13.9 μg/ml, respectively. Peaks: 1 = cycloguanil; 2 = 4-chlorophenylbiguanide; 3 = proguanil; IS = DCPB, internal standard.

TABLE I

RECOVERIES, PRECISION AND DETECTION LIMITS OF THE METHOD FOR CYCLOGUANIL, CPB, PROGUANIL AND DCPB

Compound	Recovery (%)		Slope of calibration line (mean ± S.D., $n=10$) ($\times 10^3$)	Correlation coefficient (mean ± S.D., $n=10$)	Detection limit★ (ng/ml)
	Plasma ($n=5$) (at 100 ng/ml)	Urine ($n=8$) (at 5 μg/ml)			
Cycloguanil	75	91	9.8 ± 0.7	0.997 ± 0.002	0.5
CPB	74	89	16.4 ± 0.81	0.998 ± 0.002	0.5
DCPB	90	90	–	–	–
Proguanil	99	89	7.1 ± 0.32	0.999 ± 0.0008	1.0

★Signal-to-noise ratio = 3.

advantage has been realised while using conventional injection and detector cell volumes [14]. A similar approach should, in principle, allow the use of smaller blood sample volumes when the level of drug in the blood is higher.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the gift of proguanil and the metabolites from ICI and the supply of whole blood from Grampian Blood Transfusion Ser-

vice. The expert technical assistance of Mrs. A.S. Low is also gratefully acknowledged.

REFERENCES

1 B.G. Maegraith, H.M. Tottey, A.R.D. Adams, W.H.H. Andrews and J.D. King, Ann.Trop. Med. Parasitol., 40 (1946) 493.
2 A. Spinks and M. Tottey, Ann. Trop. Med. Parasitol., 40 (1946) 101.
3 A.F. Crowther and A.A. Levi, Br. J. Pharmacol., 8 (1953) 93.
4 G.I. Robertson, Trans. R. Soc. Trop. Med., 51 (1957) 488.
5 R.R. Moody, A.B. Selkirk and R.B. Taylor, J. Chromatogr., 182 (1980) 359.
6 R.B. Taylor, K.E. Kendle and D.F. Walker, J. Pharm. Pharmacol., 36 (1984) 80P.
7 M.D. Edstein, J. Chromatogr, 380 (1986) 184.
8 J.A. Kelly and K.A. Fletcher, J. Chromatogr., 381 (1986) 464.
9 I.C. Bygbjerg and H. Flachs, Eur. J. Clin. Pharmacol., 30 (1986) 249.
10 R.D. McDowall, J.C. Pearce and G.S. Murkitt, J. Pharm. Biomed. Anal., 4 (1986) 3.
11 C.T. Hung and R.B. Taylor, J. Chromatogr, 209 (1981) 175.
12 R.B. Taylor, R. Reid, K.E. Kendle, C. Geddes and P.F. Curle, J. Chromatogr., 277 (1983) 101.
13 R.B. Taylor, R. Reid and C.T. Hung, J. Chromatogr., 316 (1984) 279
14 R.B. Taylor, K.E. Kendle, R.G. Reid and C.T. Hung, J. Chromatogr., 385 (1987) 383.