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DETERMINATION OF PLASMA CONCENTRATIONS OF DAPSONE, MONOACETYL DAPSONE AND PYRIMETHAMINE IN HUMAN SUBJECTS DOSED WITH MALOPRIM

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SUMMARY

A high-performance liquid chromatographic method was developed to enable dapsone, monoacetyl dapsone and pyrimethamine to be measured simultaneously in plasma samples from volunteers in England and Malaysia who had been dosed with Maloprim. Mean half-lives of 25 and 80 h were calculated for dapsone and pyrimethamine, respectively, but there was wide individual variation. All subjects were found to be classifiable as "slow acetylators".

INTRODUCTION

Maloprim* is a product which contains pyrimethamine and dapsone; these together act synergistically in suppressing malaria in subjects who are exposed to this disease. In order to monitor a course of treatment a method was developed using high-performance liquid chromatography in which these substances were measured together with the expected monoacetylated metabolite of dapsone in the plasma of volunteers either working at these Laboratories or in Kuala Lumpur who had received Maloprim.

MATERIALS AND METHODS

The following substances were used as reference compounds in developing the analytical method. Pyrimethamine [2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine]; dapsone (DDS) (4,4′-diaminodiphenyl sulphone); monoacetyl dapsone (MADDS) (4-N-acetylamino-4′-aminodiphenyl sulphone); and metoprine [2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine].

*Maloprim is a licenced product of the Wellcome Foundation Ltd.

Volunteer studies

Three members of the staff at Wellcome Research Laboratories, Beckenham who were working with mosquito transmitted *Plasmodium knowlesi* received Maloprim as protection against malaria. Each week they received a tablet containing 12.5 mg pyrimethamine and 100 mg dapsone; blood samples were taken at 0.5, 1, 2, 4, 6, 8 and 24 h after the first dose and then at the same time of day as the original dosing each day for 7 days.

Plasma samples from nine volunteers who received a single tablet of Maloprim at the Institute for Medical Research in Kuala Lumpur were also assayed. Samples were taken at 24 h after the dose and then once daily at the same time for a further 6 days. The volunteers were all male Indians aged between 18 and 30 and living as they were in an area of continuous malaria transmission can be regarded as semi-immune.

Extraction procedure

Duplicate amounts of 2 ml from each plasma sample were diluted with an equal volume of water, made alkaline with sodium hydroxide and mechanically mixed with 2 portions of 4 ml of 1,2-dichloroethane. After centrifugation the pooled extracts were evaporated under a stream of nitrogen gas, the residue was dissolved in 50 μl of the same solvent and transferred to a small capped vial ready for high-performance liquid chromatography.

Metoprine, an analogue of pyrimethamine, was routinely added at 2 μg/ml to all the plasma samples as an internal standard. It was shown to extract identically to pyrimethamine under the conditions used, the overall recovery being close to 80%. DDS and MADDS were found to extract almost quantitatively, so the internal standard served to make allowance for any variations occasioned by, for instance, evaporation of solvent.

HPLC assay method

A Perkin Elmer Model 1210 liquid chromatograph fitted with a fixed-wavelength (254 nm) UV absorbance detector was used and the concentration of the components determined by comparison of the peak areas with that of the internal standard. A Hewlett-Packard Model 3352 data processor was used to derive the peak areas and, as the response was not linear, a further correction based upon comparison with extracts from plasma samples "spiked" with DDS, MADDS and pyrimethamine at known concentrations was made. Because the detector was somewhat insensitive and the concentrations of the measured substances were low, more than half the extract had usually to be injected.

Using di-isopropyl ether—methanol—21% aqueous ammonium hydroxide (96:4:0.1) at a flow-rate of 2 ml/min through a 100 × 4.6 mm I.D. stainless-steel column packed with 5-μm spherical silica (Spherisorb S5W; Phase Separations, Queensferry, Great Britain), DDS, pyrimethamine, metoprine and MADDS were successfully separated in about 7 min. A representative chromatogram is shown in Fig. 1.

The method proved valid over a range from 0 to 4 μg/ml; the coefficient of variation between duplicates varied with concentration and ranged from 5.0% for DDS at levels above 500 ng/ml to 19.7% for MADDS at levels below

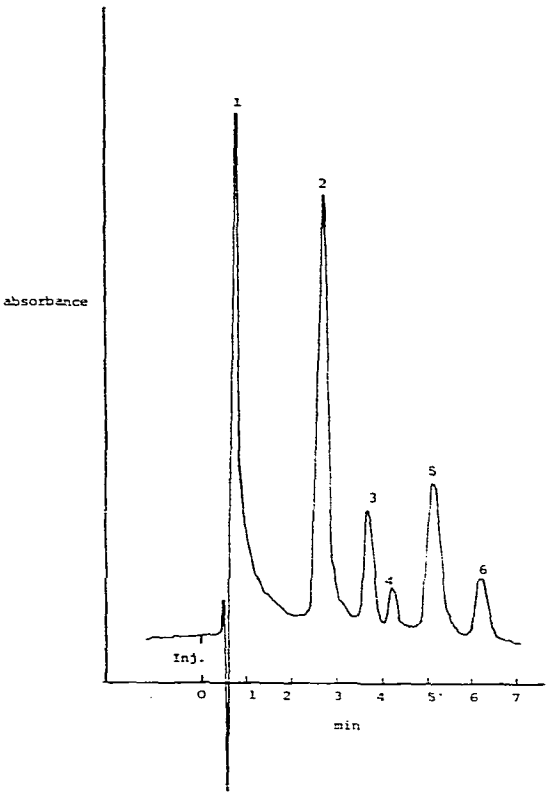

Fig. 1. Representative chromatogram of plasma extract. Extract from human plasma, spiked before extraction with internal standard. Conditions as in text. Peaks: 1, solvent front; 2, DDS; 3, pyrimethamine; 4, unknown plasma constituent; 5, internal standard; 6, MADDS.

50 ng/ml. The overall mean coefficient of variation was 11.3% and the lower limit of detection (i.e. signal-to-noise ratio = 2) was about 5 ng injected for pyrimethamine and 2.5 ng for the other substances.

Half-lives

Half-lives were calculated from semilogarithmic plots of plasma concentrations against time, the method of least squares being employed for fitting a straight line to the values.

RESULTS

Individual plasma concentrations for the Beckenham samples are shown in Table I and the mean values of the Malaysian samples in Fig. 2. Individual variation in plasma half-lives was considerable (Table II), particularly those of pyrimethamine. The mean value for all volunteers for pyrimethamine was 80 h (range 35 to 175 h) and for DDS was 25 h (range 16 to 38 h).

Highest levels as seen from the Beckenham samples were obtained at ca. 4 to 8 h for DDS and 8 to 24 h for pyrimethamine, although in some subjects both compounds showed an earlier less pronounced peak.

TABLE I

PLASMA LEVELS OF DDS, MADDS AND PYRIMETHAMINE (ng/ml) IN BECKENHAM SUBJECTS AT DIFFERENT TIMES AFTER DOSING WITH MALOPRIM

Time after 1st dose	DDS			MADDS			Pyrimethamine		
	W.H.G.R.	D.D.	M.P.	W.H.G.R.	D.D.	M.P.	W.H.G.R.	D.D.	M.P.
0.5 h	30	710	650	0	430	200	0	20	31
1 h	76	1300	1250	4	580	350	7	53	100
2 h	250	1310	1460	29	460	360	15	55	85
4 h	880	1440	1340	140	450	350	120	82	74
6 h	1150	1170	1210	170	330	280	16	69	66
8 h	1360	1140	1480	270	330	360	68	51	100
Day 1	1080	710	620	350	170	180	150	56	68
Day 2	550	440	240	120	180	77	75	64	75
Day 3	280	180	81	44	85	30	31	57	59
Day 4	110	82	32	31	61	10	24	59	69
Day 5	66	26	10	26	22	0	40	51	31
Day 6	38	21	0	15	18	0	20	52	31
Day 7	16	10	0	8	7	0	21	44	26
Second dose									
Day 8	910	910	530	130	320	190	56	100	93

TABLE II

PLASMA HALF-LIVES (h)

Subjects		DDS		Pyrimethamine	
		$t_{½}$	S.D.	$t_{½}$	S.D.
Malaysian	A	23.5	0.05	73.0	0.70
	B	21.1	0.04	67.7	0.67
	C	20.6	0.07	82.1	1.69
	D	29.5	0.04	55.4	0.05
	E	38.2	0.17	174	1.14
	F	20.9	0.05	100	0.41
	H	34.3	0.03	102	0.81
	J	22.3	0.08	54.0	0.34
	K	31.2	0.11	34.8	0.29
Beckenham	W.H.G.R.	24.0	0.02	51.6	1.43
	D.D.	22.3	0.05	N.V.*	N.V.*
	M.P.	16.3	0.01	56.9	0.97

*N.V. = not valid.

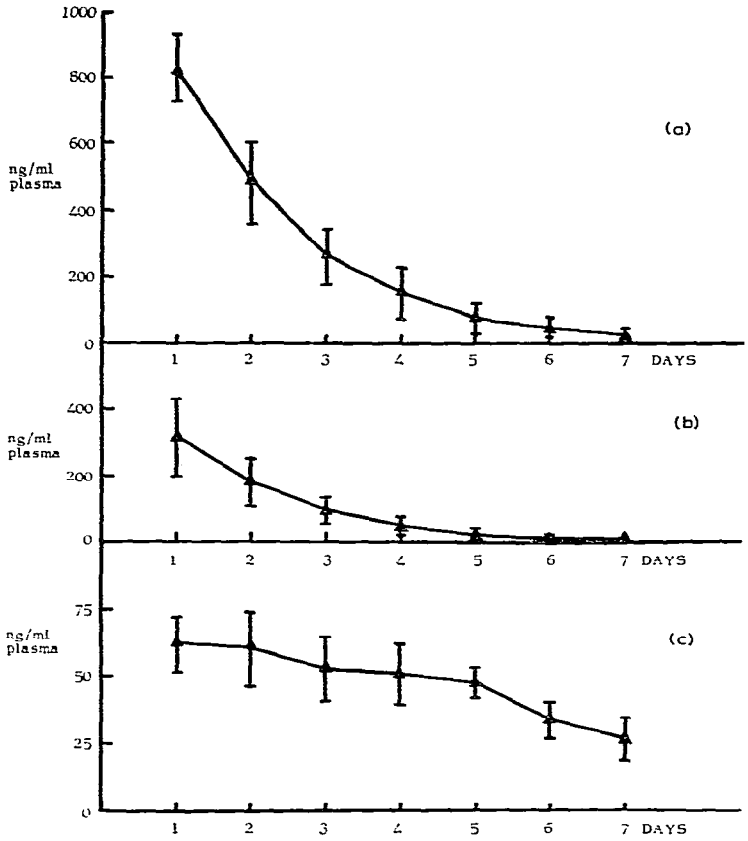

Fig. 2. Plasma concentrations measured by high-performance liquid chromatography in Malaysian subjects. Mean of 9 individuals ± 1 standard deviation. (a) Dapsone, (b) N-acetyl dapsone, (c) pyrimethamine.

By comparing the MADDS concentrations with those of DDS the acetylator status of each individual could be assessed.

DISCUSSION

Half-lives

Whereas semilogarithmic plots of plasma concentration against time gave an almost linear regression and indicated a close approximation to first order kinetics for DDS, this was not true for pyrimethamine. Whether or not the pharmacokinetics were genuinely non-linear could best be decided from a larger number of subjects. Plasma half-lives for both drugs calculated from this study, despite a wide individual variation, showed reasonable agreement with those of other workers [1—5].

It was possible to detect individual variation in rates of absorption in Beckenham volunteers (Table I). One volunteer (WHGR) apparently absorbed both constituents from the first dose more slowly than did the others, but these differences in plasma concentrations were largely gone by day 2.

The second dose resulted in a return to approximately the same plasma values at 24 h as after the first.

Acetylated metabolites of DDS

Formation of several metabolites of DDS have been reported. N-Glucuronidation [6], N-sulphation [7], N-hydroxylation [8] and N-acetylation [9] have been described. Of these, a simple extract of plasma, as used here, is only likely to show any mono- and diacetylated compounds which may be present. Peters et al. [10] stated that DADDS is not detectable in human plasma after dosing with DDS. By comparison with authentic DADDS we were able to find traces of this metabolite in the plasma of these volunteers, but the concentrations were so low as to be ignored.

Man exhibits genetic polymorphism in the metabolic acetylation of certain compounds including DDS and ratios of concentrations of MADDS to DDS in human plasma are sufficient to assign individuals to appropriate acetylator categories [9].

In this study the fastest acetylator had a MADDS value of 32% of total sulphone measured at 24 h and the mean value for all subjects was 22% (S.D. = 6.2%). Gelber et al. [9] defined fast acetylators as having a corresponding value of at least 40% with a mean above 50%. Hence all subjects in this study both from Beckenham and Malaysia can be classified as slow acetylators.

Comments on the analytical method

DDS and MADDS can be measured by fluorescence techniques [3, 4] and when a fluorescence detector is coupled to a high-performance liquid chromatograph a highly sensitive method is obtained [12, 13]. However, pyrimethamine is poorly fluorescent in solution and a method was required here which would adequately measure the plasma concentrations of all compounds simultaneously.

Levels of pyrimethamine in biological samples have long proved difficult to measure because of the small doses given and the necessity to measure the drug at long periods after dosing. Following thin-layer chromatography De Angelis et al. [11] used a photometric plate scanner to measure this compound but the possibility of naturally occurring compounds co-chromatographing with pyrimethamine is much higher for thin-layer chromatography than for high-performance liquid chromatography.

Using the liquid chromatographic method the minimum concentrations which could be reliably measured were approximately 10 ng/ml for pyrimethamine and 5 ng/ml for DDS and MADDS. Columns of higher efficiency and more sensitive detectors could further improve this technique for such assays.

A gas—liquid chromatographic method for pyrimethamine has been reported [14]. An improved method also based upon gas—liquid chromatography is under development in these laboratories to exploit the potentially greater sensitivity for measurement of pyrimethamine.

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