Br. J. clin. Pharmac. (1980), **10**, 519–524

PHARMACOKINETICS AND PROTEIN BINDING INTERACTIONS OF DAPSONE AND PYRIMETHAMINE

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1 Seven normal volunteers received oral doses of 100 mg dapsone (DDS), 25 mg pyrimethamine (PYR) singly or in combination in random order.

2 Plasma and salivary DDS and plasma monoacetyldapsone (MADDS) and PYR were estimated simultaneously by a hitherto unpublished quantitative absorption thin layer chromatographic method. This assay was shown to be satisfactory for pharmacokinetic studies.

3 The half-life of DDS was unaltered by PYR but the apparent volume of distribution was significantly increased from a mean of 1.53 1 kg^{-1} to 1.93 1 kg^{-1} and the peak DDS plasma levels measured fell by 17%.

4 The pharmacokinetic parameters of PYR were unchanged by DDS.

5 The half-life of MADDS was unchanged by PYR and was not affected by the acetylator status of the subject.

6 Salivary DDS excretion reflects the free plasma DDS concentration. Administration of PYR with DDS significantly alters the mean saliva/plasma DDS ratio from 0.265 to 0.358 suggesting an increase in free DDS with PYR therapy.

7 *In vitro* studies of plasma protein DDS binding indicate that DDS binds to a single class of binding sites on human plasma protein and PYR competitively displaces DDS from these sites.

8 The usefulness of salivary drug measurements in detecting increases of free drug in plasma in man is demonstrated.

Introduction

Dapsone (DDS) and pyrimethamine (PYR) both inhibit folic acid metabolism by the malaria parasite and act synergistically thus forming an effective combination in suppressing malaria in those exposed to the disease (Weber, Clarke, Harwin & Shiff, 1975). This drug combination in the form of Maloprim^{R} (Wellcome) has found particular application in areas in which chloroquine-resistant malaria is endemic. Apart from an analytical paper by Jones & Ovenall (1979), no investigation has been published relating to the pharmacokinetics of this widely-used combination. The present study describes the effect of administration of the combination on the pharmacokinetics of these two drugs in normal volunteers. Since both drugs have significant binding to plasma proteins it also seemed appropriate to determine whether this binding could be a site of a possible interaction.

Methods

Human volunteer study

Seven normal subjects gave informed consent for the study which was approved by the Guy's Hospital Ethical Committee. The subjects comprised five males aged 25–36 years, weighing 54–92 kg and two females aged 24 and 26 years weighing 57 and 64 kg respectively. The fasting subjects received single oral doses of the following drugs in random order: pyrimethamine 25 mg (Daraprim^{R}, Wellcome); dapsone B.P. 100 mg (Cox Ltd, Brighton) or pyrimethamine 25 mg with dapsone 100 mg. Venous blood samples (10 ml) were taken by venepuncture at 0, 0.5, 1, 2, 4, 6, 24, 48, 72, 96 h after dosing in lithium heparin tubes, centrifuged and the separated plasma was stored at −20° pending analysis. Simultaneous unstimulated mixed saliva samples were also obtained and stored similarly. A light meal was taken at 3 h and subjects abstained from alcohol throughout the study.

Estimation of pyrimethamine, dapsone and monoacetyldapsone (MADDS) in plasma or saliva

This was performed simultaneously by high performance quantitative thin layer chromatography by a previously unpublished technique. Sodium hydroxide (0.1 ml, 8N) was added to 1 ml plasma or saliva in a

0306–5251/80/050519–06 $01.00

conical glass tube followed by 6 ml dichlorethane. The contents of the tube were then vortex mixed for 30 s and centrifuged for 5 min at 1500 rev/min. The organic layer (5 ml) was transferred to a clean conical tube and evaporated to dryness under a stream of air in a water bath to 60°C. The residue was redissolved in 50 μl acetone and 40 μl was spotted on to the thin-layer chromatographic plate using a microsyringe. The plates were fluorescent and of high performance grade (silica gel 60 F_{254} 10 cm × 20 cm, Merck, Darmstadt, supplied through BDH Ltd, Poole, Dorset). The plates were developed in glass tanks at room temperature with chloroform: ethanol: glacial acetic acid (90 : 10 : 5). This mixture should be prepared fresh on alternate days and the tanks should be equilibrated for at least 3 h before use. The plates were developed to a height of 9.8 cm and air dried for a few minutes prior to scanning using a Vitatron 100 universal densitometer (MSE Ltd, Crawley, Sussex) set for absorbance measurement using a 400 nm interference filter in the emission line. A standard curve using plasma to which varying amounts of pyrimethamine, DDS and MADDS had been added was run with each series of unknown samples.

All solvents were Analar grade and used as received from BDH Ltd (Poole, Dorset). DDS was purchased from Sigma Chemical Co. (Poole, Dorset) and MADDS was a kind gift of Dr A.S.E. Fowle of Wellcome Research (Beckenham, Kent).

Plasma protein binding

Plasma protein binding of DDS was investigated using plasma from a drug-free subject or actual samples from the pharmacokinetic study in this subject. In the former case, the plasma was incubated at 37°C for 1 h with DDS at concentrations ranging between 500 to 2000 ng ml^{-1}. These solutions were then transferred to Amicon Diaflo^R ultrafiltration cones (Amicon Ltd, Amersham, Bucks). Ultrafiltration was carried out by gentle centrifugation at 37°C until approximately 10% of the fluid in the cones had been filtered. DDS concentrations were then estimated in the plasma and filtrate as detailed above. Plasma protein does not pass through these membranes. Significant binding of DDS to the apparatus was excluded by preliminary experiments. The integrity of the cones was checked by testing each ultrafiltrate for protein with Albustix^R (Ames Co, Slough).

Data analysis

Plasma concentration-time data for DDS was found to be best fitted to a two compartment open model with first order oral absorption of the form:

$$C^tp = A\,e^{-\alpha(t-t')} + B\,e^{-\beta(t-t')} - (A + B)e^{-k_a(t-t')} \quad \ldots (1)$$

where C^tp is the plasma concentration at time t, A and B are coefficients, α and β and k_a are apparent first order distribution, disposition and absorption rate constants respectively and t' is the lag time. Pyrimethamine data was best fitted by a single compartment open model expressed by

$$C^tp = \frac{FD\,k_a}{V_d\,(k_a - k)}\left(e^{-k\,(t-t')} - e^{-k_a\,(t-t')}\right) \quad \ldots (2)$$

where k is the apparent elimination rate constant, D the dose, F the bioavailability fraction and V_d the apparent volume of distribution.

The data was fitted by non-linear optimisation using a digital computer and the simplex algorithm of Nelder & Mead (1965). Areas under the plasma concentration-time curve (AUC) were calculated by the Spline-Akima approximation (Fried & Zietz, 1973). The apparent first order rate constant of the decline of the terminal phase of MADDS plasma concentrations were determined from linear least squares regression of ln (plasma concentration) *v* time.

Whole body clearance was calculated from Cl = D/AUC and V_d from V_d = Cl/elimination rate constant. This assumes complete absorption and no loss of drug through first-pass metabolism.

Statistical comparisons were made using Student's *t*-test with the significance level at 5%.

Double reciprocal plots in the form of equation (3) were used to evaluate the plasma protein binding data:

$$\frac{1}{D_b} = \frac{1}{nKP_t} \cdot \frac{1}{D_f} + \frac{1}{nP_t} \quad \ldots (3)$$

where D_b and D_f are the molar concentrations of bound and unbound drug respectively; n is the number of binding sites on each protein molecule; P_t is the total protein concentration and K is the association constant for the drug-protein complex. This equation assumes the existence of a single class of binding sites and predicts a linear relationship when $1/D_b$ is plotted as a function of $1/D_f$. Therefore, the binding parameters (K and nP_t) can be obtained from slope and intercept values. In this way, no direct knowledge of the total protein concentration is required for the product nP_t reflects the total concentration of binding sites in the plasma sample.

Results

No endogenous substances from plasma or saliva were found to interfere with the assay described above. The R_F values were DDS 0.57; MADDS 0.40 and PYR 0.27. The standard curves from plasma were linear over at least the following ranges 50–1500 ng ml^{-1} (DDS); 50–1200 ng ml^{-1} (MADDS) and 25–800 ng ml^{-1} (PYR). The minimum levels of detection (duplicate readings not differing by more than 10%)

were 20 ng ml^{-1} for DDS and MADDS, and 15 ng ml^{-1} for PYR. The average recoveries from plasma were 95% for DDS, 92% for MADDS and 88% for PYR. The coefficients of variation within assays ranged between 7.5% at 100 ng ml^{-1} and 3.5% at1200 ng ml^{-1} for DDS; 8.1% at 50 ng ml^{-1} and 1.9% at 1200 ng ml^{-1} for MADDS, and 9.9% at 25 ng ml^{-1} to 1.6% at 600 ng ml^{-1} for PYR.

Table 1 shows the derived pharmacokinetic parameters for DDS when given alone or with PYR. The rate constants for absorption, distribution or disposition were not significantly changed by PYR and this was reflected by an unchanged half-life. The apparent volume of distribution of DDS was significantly increased by PYR, the mean increase being 22%. This was accompanied by a fall (mean 17%) in the peak plasma DDS concentration. Although the systemic clearance of DDS was increased by PYR in six out of the seven subjects, this did not achieve statistical significance. The derived pharmacokinetic parameters for PYR were not significantly changed by concurrent administration of DDS.

The first order elimination rate constant and half-life of MADDS when DDS was administered alone were 0.0303 (s.d. 0.0009) h^{-1} and 22.9 (s.d. 0.8) h. Similar values were obtained when DDS was given with PYR: 0.0327 (s.d. 0.0033) h^{-1} and 22.6 (s.d. 4.9) h.

Figure 1 is the double reciprocal plot for dapsone binding to plasma protein in the absence and presence of pyrimethamine at a concentration of 200 ng ml^{-1}. The linearity of this double reciprocal plot supported the assumption of a single class of binding sites for DDS in the plasma drug concentration range and samples studied and hence the appropriateness of equation (3) to describe the binding data. Linear regression analysis of the data gives values of K and nP_t of 4.4×10^8 M^{-1} and 2.9×10^{-5} M respectively for DDS binding in the presence of PYR, and 6.5×10^8 M^{-1} and 4.3×10^{-5} M in its absence. This double reciprocal plot indicates that the interactions between DDS and PYR for human plasma protein are essentially competitive.

Table 2 details the change in the mean saliva/plasma ratio of DDS concentration which occurs when DDS is given with PYR. Figure 2 shows the saliva concentration-time profile for one subject under these different conditions.

Table 2 Mean saliva/plasma ratios for dapsone following administration of dapsone alone or with pyrimethamine.

	Mean saliva/plasma	
Subject	*Dapsone alone*	*Dapsone + pyrimethamine*
1	0.269	0.331
2	0.277	0.368
3	0.271	0.371
4	0.245	0.375
5	0.263	0.347
6	0.277	0.355
7	0.254	0.356
Mean	0.265	0.358
s.d.	0.012	0.015

Significance of difference $0.0001 > P > 0.00001$

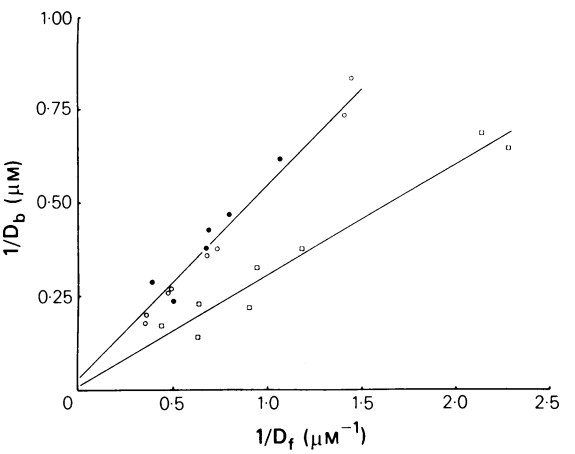

Figure 1 Relationship between the reciprocal of the bound dapsone concentration, $1/D_b$, and the reciprocal of the free drug concentration, $1/D_f$, in the presence (□) and absence (○, ●) of 200 ng ml^{-1} pyrimethamine. Points designated ● are *ex vivo* determinations.

Table 1 Pharmacokinetic parameters (mean ± s.d.) for DDS and PYR when given alone and with the other drug

	DDS			*PYR*		
	Alone	*+ PYR*	*Significance of difference*	*Alone*	*+ DDS*	*Significance of difference*
k_a (h^{-1})	0.61 ± 0.42	0.48 ± 0.18	NS	1.01 ± 0.38	0.72 ± 0.25	NS
β (h^{-1})	0.026 ± 0.003	0.026 ± 0.004	NS	—	—	—
k (h^{-1})	—	—	NS	0.0086 ± 0.0016	0.0099 ± 0.0031	NS
$T_{1/2}$ (h)	27.5 ± 3.3	27.2 ± 3.9	NS	82.5 ± 13.6	83.2 ± 30.3	NS
Cl (ml $h^{-1}kg^{-1}$)	38.4 ± 10.9	47.0 ± 7.4	NS	24.8 ± 3.8	25.8 ± 7.1	NS
$V_{d\beta}$ (l kg^{-1})	1.53 ± 0.52	1.93 ± 0.34	0.05	2.93 ± 0.52	3.02 ± 0.72	NS
C_{max} (ng ml^{-1})	1875 ± 188	1550 ± 110	<0.001	234 ± 21	235 ± 15	NS

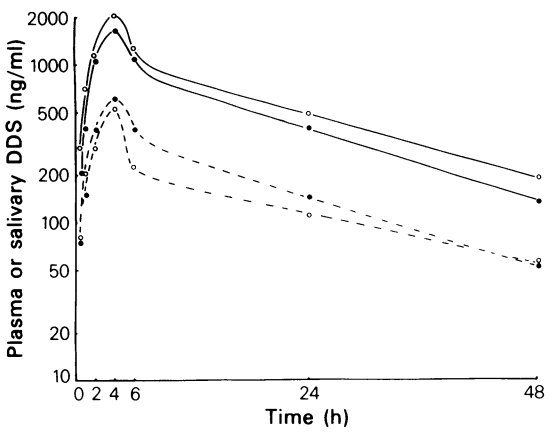

Figure 2 Plasma (solid line) and salivary (broken line) DDS concentration-time profiles for one subject after oral administration of 100 mg DDS with (●) or without (○) 25 mg pyrimethamine. Lines represent computer fitted model.

Discussion

Although fluorimetric methods exist for DDS and MADDS estimation in biological fluids (Gelber, Peters, Gordon, Glazko & Levy, 1971), such techniques cannot be extended to pyrimethamine because it is only weakly fluorescent. The ultraviolet absorption of pyrimidines was first used to quantitate these compounds after thin-layer chromatography of extracts of biological fluids by De Angelis, Simmons & Nichol (1975). The need to measure the relatively low PYR concentrations encountered during the later part of the plasma concentration, time profile requires a sensitive and specific assay. This is met by the assay described above which also has the advantage of allowing simultaneous estimation of DDS and MADDS and of being rapid in execution.

The plasma pharmacokinetics of DDS found in this study are comparable to those described in normal subjects by Glazko, Dill, Montalbo & Holmes (1968) who found the half-life to vary between 19 and 26 h, and those by Gelber *et al.* (1971) who found half-lives between 17 and 21 h. Table 1 shows that the administration of PYR with DDS does not change any of its pharmacokinetic parameters significantly.

DDS is polymorphically acetylated in man (Gelber *et al.*, 1971) initially to MADDS. Reidenberg, Drayer, De Marco & Bello (1973) have demonstrated that slow acetylators of isoniazid have plasma MADDS/DDS ratios of less than 0.30 and that this ratio exceeds 0.35 in rapid acetylators. By this criterion, two of our subjects are rapid acetylators and the others are slow acetylators. As also found by Gelber *et al.* (1971), the pharmacokinetic parameters for DDS are similar in fast and slow acetylators. The half-life of MADDS is also similar in both groups and is unaltered by concurrent PYR dosage. Our estimate of the half-life of MADDS may be compared with 20.3 ± 3.6 h in four subjects (Gelber *et al.*, 1971) and 26.2 ± 8.3 h and 33.5 ± 15.3 h for six Philippino subjects tested twice by Peters, Gordon, Ghoul, Tolentino, Walsh & Levy (1972).

No studies following intravenously administered DDS have been published but Israili, Cucinell, Vaught, Davis, Lesser & Dayton (1973) using radiolabelled DDS showed that 90% of the label from orally administered drug is excreted in the urine and the remainder is found in the faeces. Since there is evidence for biliary elimination of some dapsone metabolites in animals, these authors concluded that the bioavailability of DDS following oral administration is nearly complete. It may thus be permissible to estimate the total body clearance and the apparent volume of distribution of DDS after oral administration. It should be realised, however, that the estimates shown in Table 1 may be subject to error since our calculations assume complete drug absorption, and no loss through first-pass hepatic metabolism also the complete bioavailability of the commercial formulation of DDS used in these studies has not been rigorously demonstrated.

PYR absorption was found to proceed rapidly with peak levels occurring between 2 to 6 h following administration. This drug was found to have a prolonged elimination half-life of the order of 83 h which was unchanged by DDS. This is in agreement with the estimate of 82–132 h made by Smith & Ihrig (1959) using a microbiological assay, 85 h by Cavallito, Nichol, Brenckman, De Angelis, Stickney, Simmons & Sigel (1968) using florescence thin layer chromatography, and that of Jones & Ovenall (1979) who found half-lives of 35 to 175 h using a high pressure liquid chromatographic assay. These latter authors suggested that some of their subjects possibly showed non-linear pharmacokinetics: no evidence of such behaviour was found in the present study. Smith & Schmidt (1963) using radiolabelled PYR demonstrated regular and complete absorption of this drug following oral administration to monkeys. This may also be true in man and therefore estimates of systemic clearance and the apparent volume of distribution may be made following oral administration assuming that F = 1. The apparent volume of distribution is moderately large (as would be expected from the relatively low plasma concentrations) with a coefficient of variation of about 20% indicating relatively little inter-individual variation.

We have previously shown (Ahmad & Rogers, 1980) that DDS binding to human plasma protein is virtually constant over the concentration range 500–2000 ng ml^{-1}, the mean binding being 73.2%. Furthermore, salivary DDS concentration is virtually

identical to the free plasma DDS concentration although MADDS, because of its virtually complete protein binding, is not eliminated in the saliva. Salivary DDS concentration follows the free plasma DDS concentration without time or concentration dependence (see Figure 2). PYR is 87% bound to human plasma proteins (Cavallito *et al.*, 1978). We suggest that PYR competitively displaces DDS from plasma binding as evidenced by the changed slope of the double reciprocal plot (Figure 1). This increases the free DDS plasma concentration and DDS elimination into the saliva, thereby altering the saliva/plasma DDS ratio. The changed plasma DDS binding also increases the apparent volume of distribution for DDS. There is no change in DDS half-life or the MADDS/DDS ratio associated with PYR administration. Clearly changes in plasma half-life alone will not always detect changes in drug disposition, perhaps because the half-life in a two compartment system is a 'hybrid' parameter reflecting drug elimination and the distribution of drug between central and peripheral compartments. In a displacement interaction altered distribution between these compartments may offset changes in the elimination rate constant. The net change in tissue drug concentration, however, will be relatively small since the ratio of the tissue distribution volume to the plasma volume is relatively large (Coffey, Bullock & Schoenemann, 1971). These results demonstrate the usefulness of salivary drug estimations in the detection of changes in plasma protein drug binding.

The apparent volume of distribution depends upon both plasma protein and tissue binding but no simple relationship exists between these variables (Gibaldi & McNamara, 1978). The tissue binding of DDS is unknown and it would be interesting to know if PYR similarly displaces DDS from binding to tissue proteins. The concentration of DDS and PYR within human erythrocytes is similar to that in the plasma but the ratio of the red cell to plasma MADDS concentrations is 0.52 (Ahmad-unpublished observations). On the present evidence it is unlikely that protein binding displacement of DDS by PYR contributes to the synergistic effect of the combined drugs. Although our results demonstrate significant changes in DDS kinetics and indicate that displacement from protein binding could be one mechanism, it should be noted that the effect of PYR on DDS conversion to metabolites other than MADDS has not been excluded.

RAA is supported by a postgraduate research fellowship from the College of Medicine, Mosul University, Republic of Iraq.

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(*Received March 28, 1980*)