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Inhibition of Dapsone-induced Methaemoglobinaemia by Cimetidine in the Presence of Trimethoprim in the Rat

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Abstract—Administration of dapsone in combination with trimethoprim and cimetidine to male rats resulted in a marked decrease ($P<0.05$) in measured methaemoglobin levels (46·2±24% Met Hb h) compared with administration of dapsone alone (124·5±24·4% Met Hb h). The elimination half-life of dapsone (814±351 min) was more than doubled in the presence of trimethoprim and cimetidine compared with control (355±160 min, $P<0.05$). However, there were no significant differences in AUC and clearance when dapsone was administered in combination with trimethoprim and cimetidine compared with dapsone alone. Co-administration of trimethoprim with dapsone in the absence of cimetidine did not affect either methaemoglobin formation, AUCs, half-lives, or clearance values of dapsone compared with control. There was a threefold increase in the AUC of trimethoprim (6296±2249 μg min mL^{-1}) in the presence of dapsone compared with trimethoprim alone (2122±552 μg min mL^{-1}). There was also a corresponding decrease in the clearance of trimethoprim in the presence of dapsone compared with control (19·1±6·9 vs 60·8±21·0 mL min^{-1}). However, there was no change in the elimination half-life of trimethoprim between the two experimental groups (273±120 vs 292±54 min). The AUC of trimethoprim increased more than threefold in the presence of cimetidine (7100±1501 μg min mL^{-1}) compared with trimethoprim alone (2122±552 μg min mL^{-1}). There was also a corresponding reduction in the clearance of trimethoprim in the presence of cimetidine (61·2±21·2 vs 17·8±9·3 mL min^{-1}) compared with control. However, there was no significant change in the elimination half-life of trimethoprim after the administration of cimetidine (273±136 vs 215±109 min). Administration of either trimethoprim or cimetidine alone did not cause methaemoglobin levels to exceed control values. The administration of trimethoprim with dapsone and cimetidine resulted in a significant increase in AUC (2122±552 vs 5744±3289 μg min mL^{-1}), a fall in clearance (17·8±9·3 vs 60·8±21 mL min^{-1}), but no change in half-life (252±134 vs 273±136 h) of trimethoprim. The co-administration of cimetidine significantly reduced dapsone-mediated methaemoglobin formation in the presence of trimethoprim, whilst the AUC of trimethoprim was significantly increased in the presence of both cimetidine and dapsone.

The need for alternative regimens for *Pneumocystis carinii* pneumonia in patients suffering from acquired immunodeficiency syndrome (AIDS) has led to studies conducted with sulphamethoxazole or dapsone in combination with trimethoprim (Gordin et al 1984; Green et al 1988). The dapsone/trimethoprim régime was found to be not only as effective as trimethoprim/sulphamethoxazole, but better tolerated (Lee et al 1989; Medina et al 1990), while adverse reactions to dapsone/trimethoprim that required a switch to other therapy have been recorded at 30% compared with 57% for trimethoprim in combination with sulphamethoxazole.

Adverse reactions to dapsone in human immunodeficiency virus (HIV)-positive patients were significantly increased in those who received both dapsone and trimethoprim (Lee et al 1989) compared with studies with dapsone alone (Mills et al 1988). This interaction resulted in significantly elevated dapsone and trimethoprim plasma concentrations, and also resulted in increased dapsone-mediated methaemoglobinaemia (Lee et al 1989).

Studies in our laboratory have shown that inhibition of the *N*-hydroxylation of dapsone leads to a reduction in methaemoglobinaemia in the rat isolated perfused liver (Coleman et al 1990a), acutely and chronically in the rat in-vivo (Coleman et al 1990b, 1991), and with human tissues in-vitro (Tingle et al 1990). A study in volunteers showed that the methaemoglobinaemia caused by a single dose of dapsone (100 mg) could be substantially reduced during concurrent cimetidine administration (Coleman et al 1990c).

Therefore, we wished to determine if the metabolism-mediated toxicity of dapsone could be reduced by cimetidine in the presence of trimethoprim. In addition, we have evaluated the effect of cimetidine on the disposition of trimethoprim. We have selected the rat as an experimental model for these studies, as the metabolism of dapsone in this species is broadly similar to that in man.

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Materials and Methods

Chemicals

4,4′Diaminodiphenyl sulphone and trimethoprim were supplied by the Sigma Chemical Co. (Poole, UK). The internal standard (3-aminophenyl sulphone) was obtained from the Aldrich Chemical Company (Poole, UK). Monoacetyl dapsone was a gift from Dr S. A. Ward, Liverpool School of Tropical Medicine. Potassium cyanide and potassium ferricyanide were obtained from BDH Chemicals Ltd (Poole, UK) and Sigma Chemicals, respectively. All other reagents and solvents were of HPLC grade and obtained from Fisons Ltd (Loughborough, UK).

Protocol

Five groups (n = 5 per group) of male Wistar rats, 275–300 g, were used. Group I received dapsone (10 mg kg^{-1}, i.p.) in dimethyl sulphoxide (DMSO, 100 μL). Group II received trimethoprim alone (120 mg kg^{-1}, i.p., in 150 μL DMSO). Group III received cimetidine (100 mg kg^{-1}, in 100 μL DMSO) before the administration of trimethoprim (120 mg kg^{-1}, i.p., in 150 μL DMSO). Group IV received dapsone (10 mg kg^{-1}) and trimethoprim (120 mg kg^{-1}) both dissolved in one dose of DMSO (150 μL, i.p.). Group V received cimetidine (100 mg kg^{-1}) one hour before receiving dapsone and trimethoprim as above. Blood samples (350 μL) were obtained from the tail artery while the animals were under diethyl ether anaesthesia. Samples were obtained pre-dose and then at 1, 3, 5, 8 and 24 h. Methaemoglobin was assayed immediately and the remainder of the samples were frozen and stored at −20°C until assayed for trimethoprim, dapsone and monoactyl dapsone by reversed-phase HPLC.

Analytical procedures

All samples were assayed for methaemoglobin (Met Hb) levels relative to haemoglobin levels using the spectrophotometric technique of Harrison & Jollow (1986) and Coleman et al (1991).

Trimethoprim, dapsone and monoacetyl dapsone were assayed simultaneously according to an HPLC method based on those of of Grossman & Jollow (1988) and Coleman et al (1991). Separation was achieved using a μBondpak C_{18} stainless steel column (30 cm × 0·39 cm, 10 μm, Waters Associates, Hartford, UK). The helium degassed mobile phase consisted of 0·1 M ammonium acetate:acetonitrile (76·5:23·5). The ammonium acetate contained 0·05% triethylamine and was adjusted to pH 6·1 with phosphoric acid. The mobile phase flow-rate was 1·0 mL min^{-1}, and the detector wavelength was set at 245 nm. The retention times of trimethoprim, dapsone, monoacetyl dapsone and internal standard (3-aminophenyl sulphone) were 6·1, 7·7, 8·9 and 10·7 min, respectively. The extraction recoveries for trimethoprim, dapsone, and monoacetyl dapsone were 89·1 ± 8·1, 82·7 ± 8·0 and 88·5 ± 8·1%, respectively. Intra-assay variation studies were carried out at 1000 ng mL^{-1} (trimethoprim 9·2%; dapsone 7·0%; monoacetyl dapsone 8·8%) and 1500 ng mL^{-1} (trimethoprim 6·9%; dapsone 7·4%; monoacetyl dapsone 8·2%). In addition, interassay variation was determined at 1000 ng mL^{-1} (trimethoprim 8·5%; dapsone 8·5%; monoacetyl dapsone 9·5%) and at 1500 ng mL^{-1} (trimethoprim 7·8%; dapsone 8·0%; monoacetyl dapsone 9%). The limits of detection in a 100 μL blood sample for trimethoprim, dapsone and its monoacetyl metabolite were 340, 325 and 290 ng mL^{-1}, respectively. At 24 h, a larger volume of blood (200 μL) was extracted to facilitate drug detection.

Pharmacokinetic calculations and statistical analysis

The pharmacokinetic parameters for the three compounds assayed were calculated by the use of the program 'Topfit' on an Opus PCV computer. Area under the curves (AUC) for blood concentrations of dapsone and monoacetyl dapsone were each calculated from t = 0 to t = 24 by the use of the trapezoidal rule (Gibaldi & Perrier 1982). The rule was also applied to calculation of AUC_{0-24} for % methaemoglobinaemia (Grossman & Jollow 1988; Coleman et al 1990a). Statistical analysis was accomplished by the use of the Wilcoxon signed rank test accepting $P < 0·05$ as significant. Data are presented graphically as mean ± s.e.m. and in the text as mean ± s.d.

Results

The administration of dapsone in combination with trimethoprim and cimetidine caused a marked decrease ($P < 0·01$) in measured methaemoglobin levels (46·2 ± 24% Met Hb h, Fig. 1) compared with dapsone alone (124·5 ± 24·4% Met Hb h). The elimination half-life of dapsone (814 ± 351 min) was more than doubled in the presence of trimethoprim and cimetidine compared with control (355 ± 160 min, $P < 0·05$, Fig. 2). However, differences in AUC (2742 ± 1014 vs 1898 ± 695 μg min mL^{-1}, Table 1) and clearance (4·1 ± 1·6 vs 6·0 ± 2·3 mL min^{-1}) when dapsone was administered in combination with trimethoprim and cimetidine compared with dapsone alone, did not attain significance. When dapsone was administered in combination with trimethoprim in the absence of cimetidine, there were no significant differences in either the AUC, half-life, or clearance of dapsone (Table 1), compared with control. In addition, there was no significant change in the methaemoglobinaemia caused by dapsone in the presence of trimethoprim (138·2 ± 17·5% Met Hb h) compared with dapsone alone (124·5 ± 24·4% Met Hb h).

The AUC for monoacetyl dapsone was significantly increased when dapsone was administered in combination with trimethoprim and cimetidine (718 ± 453 μg min mL^{-1}) compared with dapsone alone (486 ± 166 μg min mL^{-1}, Fig. 3). However, there was a significant reduction in the AUC of monoacetyldapsone when dapsone was co-administered

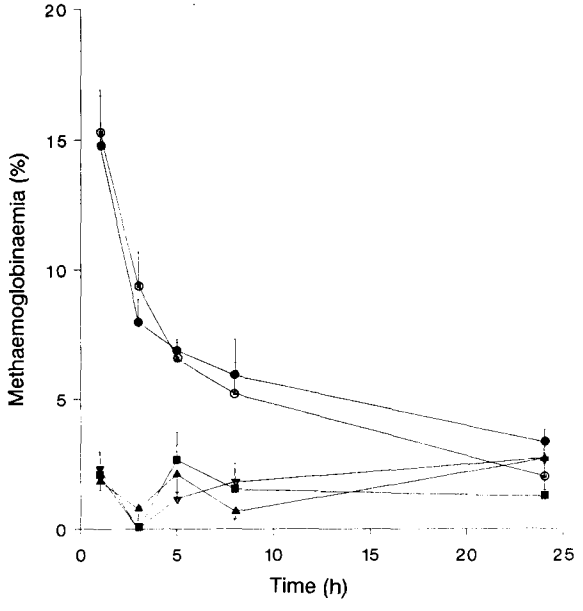

FIG. 1. % Methaemoglobinaemia after the administration of dapsone (10 mg kg^{-1}) alone (●); trimethoprim (120 mg kg^{-1}) and dapsone (10 mg kg^{-1}) (○); trimethoprim (120 mg kg^{-1}), dapsone (10 mg kg^{-1}) and cimetidine (100 mg kg^{-1}) (△); trimethoprim (120 mg kg^{-1}) alone (■); and cimetidine (100 mg kg^{-1}) and trimethoprim (120 mg kg^{-1}) (▽): n = 5 per group.

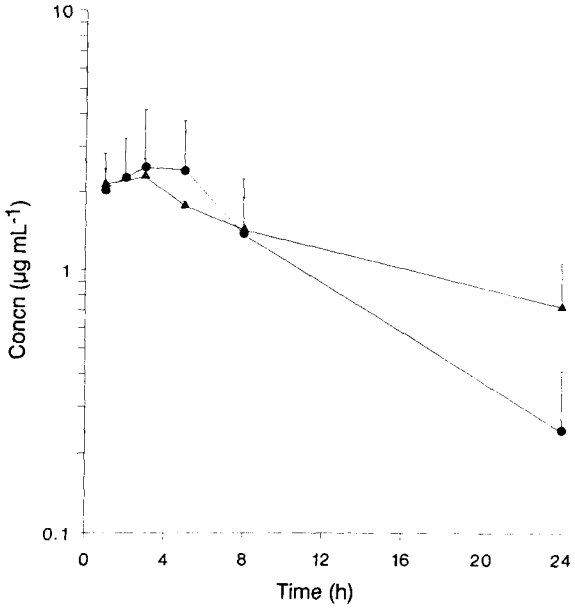

FIG. 2. Dapsone blood concentrations in the presence of trimethoprim (120 mg kg^{-1}) and cimetidine (100 mg kg^{-1}) (△), and dapsone alone (10 mg kg^{-1}) (●): n = 5 per group.

with trimethoprim (204 ± 129 μg min mL^{-1}) compared with control (486 ± 166 μg min mL^{-1}).

The administration of trimethoprim in combination with dapsone resulted in a threefold increase in the AUC of trimethoprim (6296 ± 2249 μg min mL^{-1}) compared with trimethoprim administered alone (2122 ± 552 μg min mL^{-1}, Fig. 4). There was also a corresponding decrease in the clearance of trimethoprim in the presence of dapsone compared with control (19·1 ± 7·0 vs 60·8 ± 21·0 mL min^{-1}). However, there was no change in the elimination half-life of trimethoprim between the two experimental groups (273 ± 120 vs 292 ± 54 min).

The disposition of trimethoprim after the administration of cimetidine underwent a marked change. The trimethoprim AUC increased more than threefold in the presence of cimetidine (7100 ± 1501 μg min mL^{-1}, Fig. 4) compared with trimethoprim alone (2122 ± 552 μg min mL^{-1}) with a corresponding reduction in the clearance of trimethoprim (60·8 ± 21·2 vs 17·8 ± 9·3 mL min^{-1}) compared with control. However, there was no significant change in the elimination half-life of trimethoprim after the administration of cimetidine (273 ± 136 vs 215 ± 109 min). Neither trimethoprim nor cimetidine alone caused methaemoglobin levels to exceed control values.

The administration of trimethoprim with dapsone and cimetidine resulted in significant increases in AUC (2122 ± 552 vs 5744 ± 3289 μg min mL^{-1}), a fall in clearance (17·8 ± 9·3 vs 60·8 ± 21 mL min^{-1}), but no change in half-life (252 ± 134 vs 273 ± 136 min) of trimethoprim compared with trimethoprim alone (Fig. 4, Table 1). Overall, there were no significant differences in the calculated volumes of distribution.

Discussion

The administration of cimetidine before administration of dapsone and trimethoprim produced a marked reduction in dapsone-dependent methaemoglobin formation. Although there was no significant change in the AUC and clearance of dapsone, the elimination half-life was greatly prolonged. Hence reduction in dapsone-mediated toxicity could still be achieved through cimetidine-mediated metabolic inhibition in the presence of trimethoprim. However, previous studies have shown a significant increase in the AUC of dapsone in the presence of cimetidine alone (Coleman et al 1990a). In the present study, this increase was not clear-cut, possibly due to the effect of high doses of trimethoprim (120 mg kg^{-1}) as well as cimetidine (100 mg kg^{-1}), which may have affected the hepatic uptake of dapsone, which was administered at a much lower dose (10 mg kg^{-1}). Trimethoprim is known to inhibit the oxidative metabolism of compounds such as tolbutamide (Wing & Miners 1985) and phenytoin (Hansen et al 1979) in man. In this report using the rat model, we have shown that a twelvefold higher dose of trimethoprim did not affect the disposition of dapsone, nor did it alter dapsone-dependent methaemoglobin formation, itself a hepatic metabolism-dependent process (Cucinell et al 1972).

Previous studies have shown a marked increase in the formation of monoacetyldapsone when the oxidative metabolism of dapsone was impaired (Coleman et al 1990a,b). This was also the case in the present study, where inhibition of the oxidative metabolism of dapsone resulted in increased availability of parent drug for acetylation. The net fall in the acetylation of dapsone in the presence of trimethoprim, however, was unexplained.

The clearance of trimethoprim was markedly reduced by both the presence of dapsone and cimetidine, as well as by dapsone and cimetidine individually, although there was no apparent additive effect on trimethoprim disposition between dapsone and cimetidine. Trimethoprim is metabo-

Table 1. Pharmacokinetic parameters of dapsone (DDS), monoacetyl dapsone (MADDS) and trimethoprim (TMP) after the administration of DDS alone 10 mg kg^{-1}, TMP alone (120 mg kg^{-1}), TMP (120 mg kg^{-1}) and cimetidine (100 mg kg^{-1}), TMP (120 mg kg^{-1}) and DDS (10 mg kg^{-1}), TMP (120 mg kg^{-1}) and cimetidine (100 mg kg^{-1}) to 5 rat groups (n = 5 per group). All values are mean ± s.d.

Treatment group	DDS				TMP				MADDS
	AUC (μg min mL^{-1})	$t_{\frac{1}{2}}$ (min)	CL (mL min^{-1})	V_d (L)	AUC (μg min mL^{-1})	$t_{\frac{1}{2}}$ (min)	CL (mL min^{-1})	V_d (L)	AUC (μg min mL^{-1})
DDS	1898 ± 795	355 ± 160**	6·0 ± 2·3	3·1 ± 1·9					486 ± 166†*
TMP					2122 ± 552^{D,B}	273 ± 136	60·8 ± 21·0^{C,E}	8·4 ± 4·5	
TMP/cimetidine					7100 ± 1501^{D}	215 ± 109	17·8 ± 9·3^{E}	3·4 ± 2·0	
DDS/TMP	2028 ± 494	471 ± 127	5·2 ± 1·4	4·8 ± 1·3	6926 ± 2249^{B}	292 ± 54	19·1 ± 6·9^{C}	8·4 ± 4·5	204 ± 129†
DDS/TMP/cimetidine	2742 ± 1014	814 ± 351**	4·1 ± 1·6	4·3 ± 0·6	5744 ± 3289	252 ± 134	17·8 ± 9·3	7·8 ± 8·7	718 ± 453*

**, †, * denotes $P < 0.05$. ^{B}, ^{D}, ^{C}, ^{E} denotes $P < 0.01$.

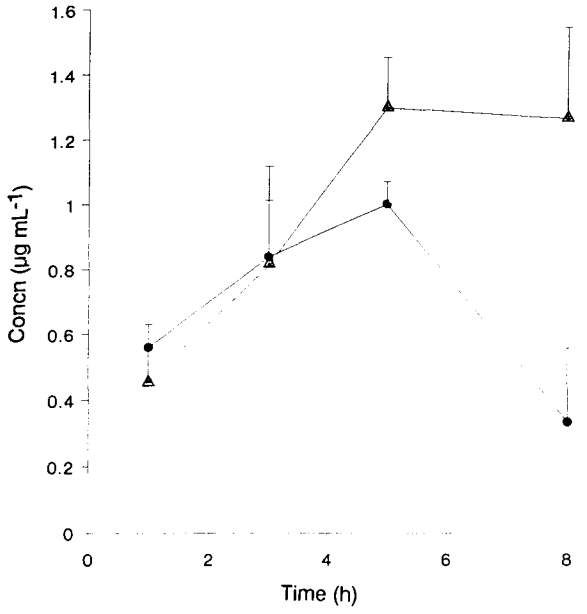

FIG. 3. Monoacetyldapsone blood concentrations after the administration of dapsone (10 mg kg^{-1}) alone (●), and in the presence of trimethoprim (120 mg kg^{-1}) and cimetidine (100 mg kg^{-1}) (△): n = 5 per group.

lized extensively by rat liver to *N*-oxides which are eliminated almost entirely as conjugates (Schwartz et al 1970). In the present study, the elimination rate of trimethoprim was unchanged, although it was apparent that the first pass of the drug was diminished considerably by the co-administered compounds. Hence it is possible that dapsone and cimetidine

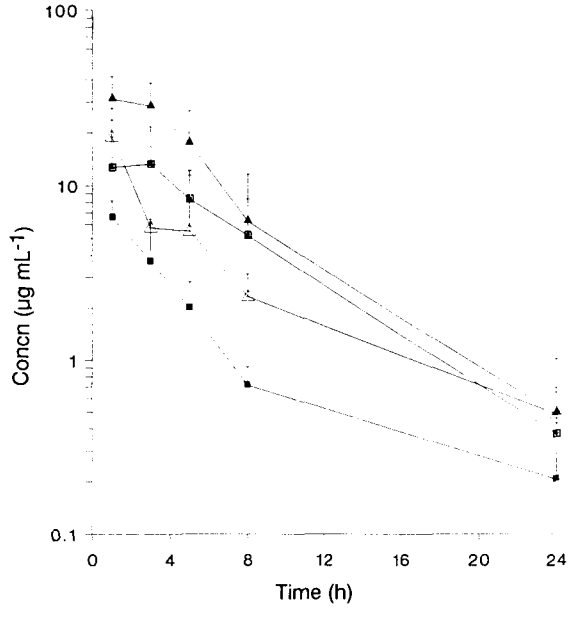

FIG. 4. Trimethoprim blood concentrations after the administration of trimethoprim (120 mg kg^{-1}) alone (■); trimethoprim (120 mg kg^{-1}) and cimetidine (100 mg kg^{-1}) (▲); trimethoprim (120 mg kg^{-1}) and dapsone (10 mg kg^{-1}) (□); trimethoprim (120 mg kg^{-1}), dapsone (10 mg kg^{-1}) and cimetidine (100 mg kg^{-1}) (△): n = 5 per group.

may have either partially inhibited the metabolism of trimethoprim or prevented its uptake from the hepatic portal system to the hepatocytes, thus significantly retarding the clearance of the drug.

Studies in man using the trimethoprim/dapsone combination indicated that the AUC values of each compound were significantly elevated in the presence of the other and that the toxicity of dapsone was increased (Lee et al 1989). Using the rat model in the present study, trimethoprim concentrations were significantly elevated in the presence of dapsone, although neither the pharmacokinetics nor the toxicity of dapsone was significantly altered; hence the clinical interaction could not be completely reproduced. However, the ability of cimetidine to reduce the toxicity of dapsone was first demonstrated in the rat (Coleman et al 1990a,b), and was subsequently found to occur in man (Coleman et al 1990c). As the site of the cimetidine/dapsone interaction is the liver (Coleman et al 1990a) and the hepatic metabolism of trimethoprim is greater in the rat than in man, the lack of effect of trimethoprim on the cimetidine-mediated inhibition of dapsone-induced toxicity in the rat suggests that trimethoprim is unlikely to affect this process in man.

The clinical observations of the mutual elevation of dapsone and trimethoprim levels can be explained in terms of partial inhibition of dapsone metabolism and competition for renal excretion (Lee et al 1989; Medina et al 1990). Since partial inhibition of the oxidative metabolism of dapsone results in a reduction in haemotoxicity in man (Coleman et al 1990c), and Lee et al (1989) actually demonstrated an increase in methaemoglobin formation, the accumulation of dapsone was probably due to a mechanism other than a metabolic interaction. It is conceivable that the oxidation of the accumulating dapsone in the presence of trimethoprim may be partially inhibited by co-administration of cimetidine, resulting in a reduction in the haemotoxicity of dapsone. Studies in man have shown that cimetidine caused an increase in trimethoprim plasma concentrations, but no overall change in the pharmacokinetics of the drug (Rogers et al 1980). Lee et al (1989) concluded that although a marked elevation of trimethoprim plasma concentrations occurred in the presence of dapsone, no side-effects could be attributed to the trimethoprim levels. Therefore, if raised trimethoprim plasma levels were to result from the co-administration of cimetidine, dapsone and trimethoprim, these may well not be associated with adverse reactions.

We have demonstrated that, in the rat, the presence of trimethoprim does not impair the cimetidine-mediated reduction in the haemotoxicity and plasma elimination rate of dapsone brought about by metabolic inhibition. This process was associated with a concomitant increase in the acetylation of dapsone. In addition, we have shown that trimethoprim does not affect the process of dapsone-mediated methaemoglobin formation and that both dapsone and cimetidine significantly retarded the elimination of trimethoprim.

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