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Inhibition of Dapsone-Induced Methaemoglobinaemia by Cimetidine in the Rat During Chronic Dapsone Administration

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Abstract—Dapsone undergoes *N*-acetylation to monoacetyl dapsone as well as *N*-hydroxylation to a hydroxylamine which is responsible for the haemotoxicity (i.e. methaemoglobinaemia; Met Hb) of the drug. Since dapsone is always given chronically, we have investigated the ability of cimetidine to inhibit Met Hb formation caused by repeated dapsone administration. The drug was given (i.p.) to four groups (n = 6 per group) of male Wistar rats, 300–360 g. Group I received 10 mg kg^{-1} at 1, 24, 48 and 72 h. Group II received 10 mg kg^{-1} at 1, 8, 24, 32, 48, 56, 72 and 80 h. Groups III and IV received the drug as for groups I and II, respectively, as well as cimetidine (50 mg kg^{-1}) 1 h before each dose of dapsone. Twice daily dapsone administration (Group II) resulted in a significantly greater ($P < 0.05$) Met Hb AUC (757 ± 135 vs 584 ± 115% Met Hb h), dapsone AUC (140 ± 17.5 vs 113 ± 13.0 μg h mL^{-1}) and monoacetyl dapsone AUC (48.2 ± 18.3 vs 10.8 ± 4.6 μg h mL^{-1}) compared with a single daily dapsone dose (group I). The administration of cimetidine before the once daily dose of dapsone (group III) resulted in a significant ($P < 0.05$) fall in Met Hb (302 ± 179 vs 584 ± 115% Met Hb h) and an increase in both the dapsone (151 ± 22.2 vs 113 ± 13.0 μg h mL^{-1}) and monoacetyl dapsone AUC values (33.6 ± 5.8 vs 10.8 ± 4.0 μg h mL^{-1}) compared with a single daily dose of dapsone (group I). Administration of cimetidine before the twice daily dose of dapsone (group IV) resulted in no significant change in Met Hb or monoacetyl dapsone levels, despite a marked increase in the AUC after dapsone compared with control (303 ± 53.2 vs 140 ± 17.5 μg h mL^{-1} $P < 0.05$; group II). The administration of a single dose of monoacetyl dapsone alone resulted in rapid production of methaemoglobinaemia (17.1 ± 7.2%) at 1 h; however, prior administration of cimetidine did not significantly affect methaemoglobin levels over 24 h (287.6 ± 77.9 vs 316.4 ± 120.2% Met Hb h). These studies indicate that although cimetidine may reduce Met Hb formation during chronic dapsone administration, dose reduction of dapsone is required to avoid haemotoxicity because of the increased accumulation of both parent drug and its monoacetyl metabolite.

Dapsone is an effective malarial prophylactic in combination with pyrimethamine (Bruce-Chwatt 1982). It is useful in the control of inflammatory disorders (Lang 1979), such as dermatitis herpetiformis (Swain et al 1983), and has recently found application in the treatment of *Pneumocystis carinii* pneumonia in AIDS patients in combination with trimethoprim and pentamidine (Green et al 1988). However, the adverse reactions of dapsone appear to be accentuated by concurrent administration of trimethoprim, causing a high proportion of non-compliance (Lee et al 1989).

Dapsone is metabolized extensively in man through *N*-hydroxylation to hydroxylamine derivatives (Israili et al 1973; Coleman et al 1989) and by acetylation to monacetyl dapsone (Gelber et al 1971). Monoacetyl dapsone may also undergo hydroxylation to form monoacetyl dapsone hydroxylamine. Adverse reactions to dapsone range from fatal agranulocytosis (Levine & Weintraube 1968; Ognibene 1970) to methaemoglobinaemia (Cohen et al 1968) and haemolysis (DeGowin et al 1966). All these adverse reactions are due to the *N*-hydroxylated metabolites of the drug (Cucinell et al 1972). Haemotoxicity caused by dapsone metabolites is severe in patients suffering from deficiencies in NADH-dependent methaemaglobin reductase (Ganer et al 1981), glucose-6-phosphate dehydrogenase or glutathione reductase activities (Zuidema et al 1986).

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Studies with single doses of dapsone in the intact rat, rat isolated perfused liver and man indicate that inhibition of the oxidative metabolism of dapsone results in marked reduction in methaemoglobin formation (Coleman et al 1990a, b, c; Tingle et al 1990). However, dapsone is administered chronically in all its therapeutic applications. Hence, we wished to investigate the inhibition of the *N*-hydroxylation of dapsone during chronic administration of both drug and an inhibitor of cytochrome P450 i.e. cimetidine (Pelkonen & Puuronen 1980). The rat is a useful model for this study, since the disposition of dapsone in this species is similar to man (Coleman et al 1990a, b).

Materials and Methods

Chemicals

4,4′-Dapsone (4,4′-diaminodiphenyl sulphone) was supplied by the Sigma Chemical Co. (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

Four groups (n = 6 per group) of male Wistar rats, 300–375 g were used. Group I received dapsone (10 mg kg^{-1}) in

dimethyl sulphoxide (DMSO, 100 μL) at 1, 24, 48 and 72 h. Group II received dapsone at the same dose at 1, 8, 24, 32, 48, 56, 72 and 80 h, i.e. twice daily. Group III was dosed as for group I, except that 1 h before each dapsone dose, cimetidine (50 mg kg^{-1}) dissolved in DMSO (200 μL) was administered i.p. Group IV animals were treated with dapsone as described for group II, except that cimetidine (50 mg kg^{-1}) was administered i.p. 1 h before each dapsone dose. Blood samples (250 μL) were withdrawn from the tails of the rats while they were under diethyl ether anaesthesia. Samples were removed pre-dose, then at 1, 8, 24, 25, 32, 48, 49, 56, 57, 72, 73 and 80 h. In a separate study, monoacetyl dapsone was administered i.p. (10 mg kg^{-1}) in DMSO (100 μL) alone (n = 6) and 1 h after cimetidine administration (50 mg kg^{-1}) in DMSO (200 μL, n = 6). Blood samples were withdrawn as above except the sample times were 1, 7, 8 and 24 h. Methaemoglobin was assayed immediately and the remainder of the samples were frozen and stored at −20°C until assayed for dapsone and monacetyl dapsone by reversed phase HPLC.

Analytical procedures

All samples were assayed for methaemoglobin levels relative to haemoglobin levels using the spectrophotometric technique of Harrison & Jollow (1986). Briefly, a sample of blood (100 μL) was haemolysed using 20 mM Na^+/K^+ phosphate buffer pH 7·8 containing 0·05% v/v Triton X-100 (5 mL). The haemolysed solution was divided into four aliquots (1·25 mL) and one drop of aqueous 10% KCN added to aliquots 2 and 4. One drop of aqueous 20% $K_3Fe(CN)$ was added to aliquots 3 and 4. The absorbance of each aliquot at 635 nm was determined (OD_1, OD_2, OD_3 and OD_4). The percentage of total haemoglobin present as methaemoglobin (Met Hb) was then calculated as:

$$\% \text{ Met Hb} = \frac{OD_1 - OD_2}{OD_3 - OD_4} \times 100$$

The background methaemoglobin levels were measured in each rat at time zero and all subsequent measurements were corrected for the zero value.

Dapsone and monoacetyl dapsone were assayed according to the method of Grossman & Jollow (1988). Blood samples (100 μL) were extracted with ethyl acetate (2 × 1 mL). The organic phase was removed and evaporated to dryness, then reconstituted in methanol (40 μL). Separation was achieved using a μ Bondapak C_{18} stainless steel column (30 × 0·39 cm i.d. 10 μm, Waters Associates, Hartford, Cheshire UK). The helium degassed mobile phase consisted of 0·1 M ammonium acetate–acetonitrile-methanol (66:12:22) flowing at 1·6 mL min^{-1}. The retention times of dapsone, monoacetyl dapsone and internal standard (monopropionyl dapsone) were 4·6, 6·5 and 9·8 min, respectively. The HPLC employed was from SpectraPhysics, consisting of an SP8700 solvent delivery system, an SP8880 Autosampler and an SP100 Variable wavelength UV detector set at 254 nm. The system was controlled via Labnet and peak areas determined by a SpectraPhysics 'Chromjet' Integrator.

Pharmacokinetic calculations and statistical analysis

The area under the curves ($AUC_{0\text{-}80}$) for blood concentrations of dapsone and monoacetyl dapsone were each calculated from t = 0 to t = 80 (or in the case of the single dose of monoacetyl dapsone, t = 0 to t = 24) by the use of the trapezoidal rule (Gibaldi & Perrier 1982). The rule was also applied to calculation of $AUC_{0\text{-}80}$ for % methaemoglobinaemia (Grossman & Jollow 1988; Coleman et al 1990a). Statistical analysis was by 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

Over the 80 h period of the study, twice daily administration of dapsone (10 mg kg^{-1}) was consistent with significantly ($P < 0.05$) greater methaemoglobinaemia (757·3 ± 135% Met Hb h^{-1}) compared with the same dose (10 mg kg^{-1}) given once daily (584·0 ± 115% Met Hb h^{-1}). The AUC for dapsone was also significantly different when the drug was given twice (140·0 ± 17·5 μg h mL^{-1}) compared with once daily (113·3 ± 13·0 μg h mL^{-1}). The acetylation of dapsone to monoacetyl dapsone was more than four fold higher (48·2 ± 18·3 μg h mL^{-1}) when dapsone was administered twice daily compared with single daily dosage (10·8 ± 4·6 μg h mL^{-1} $P < 0.01$).

The administration of cimetidine (50 mg kg^{-1}) one hour before dapsone administration (Fig. 1) resulted in a significant reduction in methaemoglobinaemia with respect to dapsone alone (302 ± 179 vs 584 ± 115% Met Hb h^{-1}, $P < 0.05$). Blood concentrations of dapsone (Fig. 2) increased

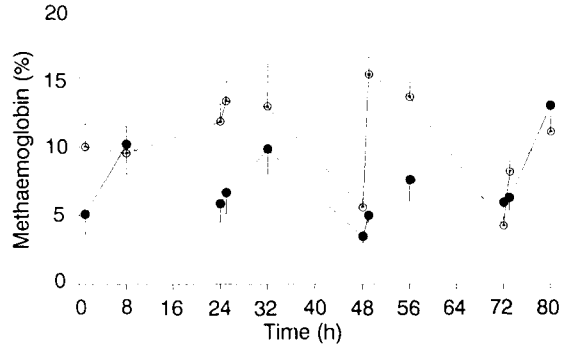

FIG. 1. % Methaemoglobin against time after once daily administration of dapsone (10 mg kg^{-1}) alone (○ n = 6) and in the presence of cimetidine (50 mg kg^{-1}, ● n = 6).

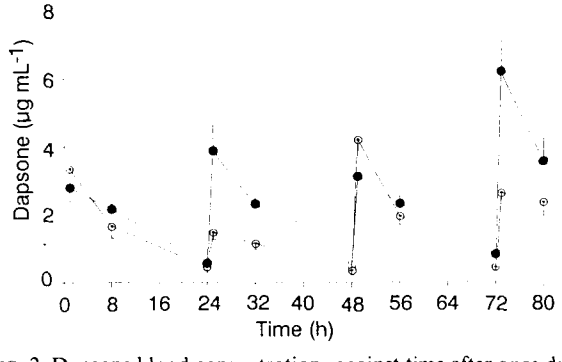

FIG. 2. Dapsone blood concentrations against time after once daily administration of dapsone (10 mg kg^{-1}) alone (○ n = 6) and in the presence of cimetidine (50 mg kg^{-1}, ● n = 6).

significantly in the presence of cimetidine (151·2 ± 22·2 vs 116·3 ± 13·0 μg h mL^{-1}, $P < 0.05$). In addition, there was a three fold increase in monoacetyl dapsone concentrations (Fig. 3) in the presence of cimetidine (33·6 ± 5·8 vs 10·82 ± 4·56 μg h mL^{-1}, $P < 0.05$).

The administration of cimetidine with dapsone twice daily resulted in no significant reduction in methaemoglobin levels compared with control (757·3 ± 135 vs 790·0 ± 114·2% Met Hb h^{-1}, Fig. 4). However, the dapsone AUC in the presence of cimetidine (303·8 ± 53 μg h mL^{-1}, Fig. 5) was significantly greater than control (140·0 ± 17·5 μg h mL^{-1}, $P < 0.01$). Although there was a trend towards increased acetylation of dapsone (Fig. 6) with concomitant cimetidine administration, this did not attain statistical significance (76·3 ± 31·0 vs 48·1 ± 18·3 μg h mL^{-1}).

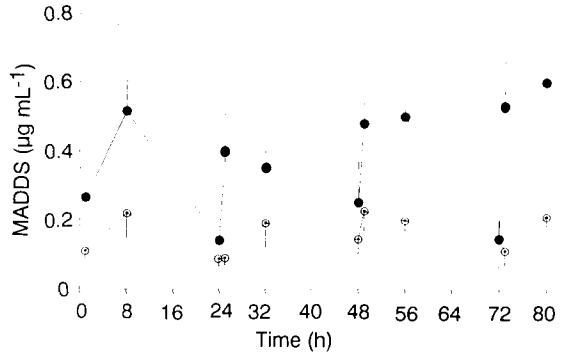

FIG. 3. Monoacetyl dapsone (MADDS) blood concentrations plotted against time after once daily administration of dapsone (10 mg kg^{-1}) alone (○ n = 6) and in the presence of cimetidine (50 mg kg^{-1}, ● n = 6).

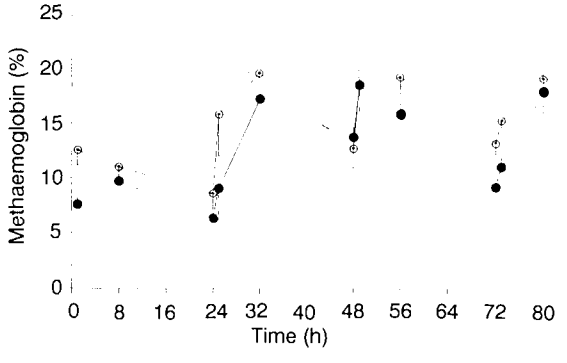

FIG. 4. % Methaemoglobin against time after twice daily administration of dapsone (10 mg kg^{-1}) alone (○ n = 6) and in the presence of cimetidine (50 mg kg^{-1}, ● n = 6).

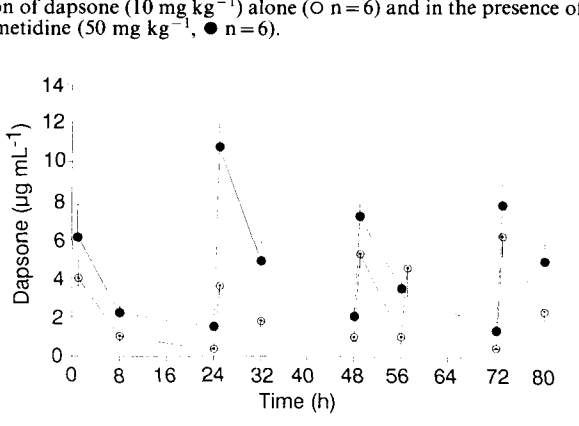

FIG. 5. Dapsone blood concentrations against time after twice daily administration of dapsone (10 mg kg^{-1}) alone (○ n = 6) and in the presence of cimetidine (50 mg kg^{-1}, ● n = 6).

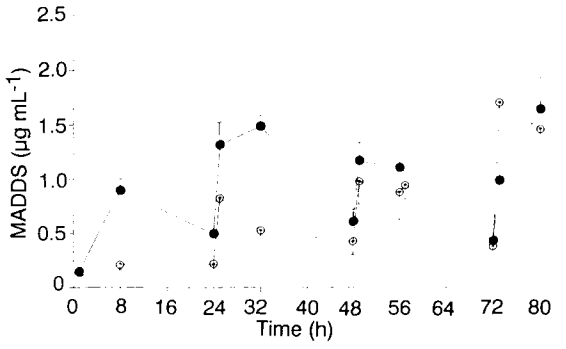

FIG. 6. Monoacetyl dapsone blood concentrations against time after twice daily administration of dapsone (10 mg kg^{-1}) alone (○ n = 6) and in the presence of cimetidine (50 mg kg^{-1}, ● n = 6).

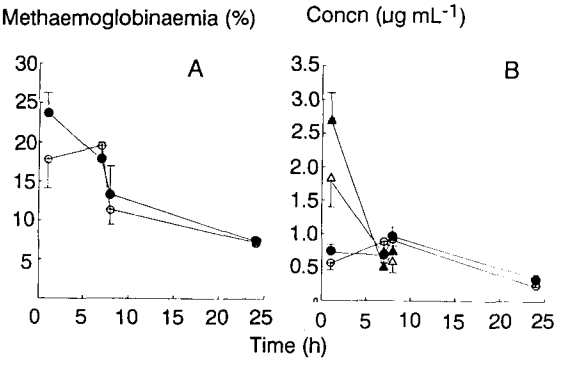

FIG. 7. (A) % Methaemoglobinaemia against time after a single dose of monoacetyl dapsone (10 mg kg^{-1}) alone (○ n = 6) and in the presence of cimetidine (50 mg kg^{-1}, ● n = 6). (B) Blood monoacetyl dapsone concentrations in the absence (△) and presence (▲) of cimetidine: blood dapsone concentrations in the absence (○) and presence (●) of cimetidine after a single dose of monoacetyl dapsone (10 mg kg^{-1}).

The administration of monacetyl dapsone alone resulted in rapid production of methaemoglobinaemia (17·1 ± 7·2%) at 1 h (Fig. 7); however, prior administration of cimetidine did not affect methaemoglobin levels significantly over 24 h (287·6 ± 77·9 vs 316·4 ± 120·2 % Met Hb h^{-1}). Within 1 h, dapsone levels were almost one third of those of monoacetyl dapsone in both the absence (0·55 ± 0·24 μg mL^{-1} vs 1·79 ± 0·96 μg mL^{-1}) and presence of cimetidine (0·72 ± 0·27 vs 2·62 ± 1·2 μg mL^{-1}). Overall, the $AUC_{0\text{-}24}$ for monoacetyl dapsone (102·4 ± 41·9 μg h mL^{-1}) did not differ significantly in the presence of cimetidine (128·1 ± 46·2 μg h mL^{-1}).

Discussion

Although in rat and man dapsone is acetylated as well as *N*-hydroxylated, previous studies in the rat and isolated perfused rat liver (Coleman et al 1990a, b) have illustrated that the major route of elimination of dapsone is via oxidative metabolism. Inhibition of this route by inhibitors of P450 enzymes caused a marked reduction in methaemoglobinaemia, the toxic manifestation of the oxidative metabolism of dapsone to dapsone hydroxylamine. In addition, plasma dapsone clearance was greatly diminished. In these single dose studies, inhibition of dapsone *N*-hydroxylation was associated with a marked rise in parent drug plasma concentrations (Coleman et al 1990a).

In the present study, when cimetidine was administered before dapsone once daily the substantial fall in methaemoglobin levels compared with control was accompanied by a significant increase in parent drug levels. When cimetidine/dapsone administration was carried out twice daily, there was no overall reduction in methaemoglobinaemia in comparison with control. This coincided with a much greater increase in the concentrations of unchanged dapsone in blood in the presence of cimetidine, especially from 0–48 h (Fig. 5). A number of factors may have accentuated this accumulation process. When dapsone was administered alone, renal elimination of unchanged drug is relatively low (5–15%, Ellard 1966), and the drug is cleared mostly as hydroxylated conjugates (Gordon et al 1979). In addition, the monoacetyl derivative is highly protein bound and does not undergo extensive glomerular filtration. It is thought that de-acetylation and acetylation exist in equilibrium and de-acetylation must occur before drug is eliminated in urine (Zuidema et al 1986). Hence, when *N*-hydroxylation is inhibited either partially or totally, these factors may promote the accumulation of unchanged dapsone.

It has been shown that cimetidine inhibits oxidative metabolism in a competitive manner in-vitro (Reilly & Winzor 1984), although this depends on the substrate and conditions employed (Somogyi & Muirhead 1987). In the present study, it is possible that during the twice daily dapsone administration, in the presence of the inhibitor, the accumulation of unchanged dapsone may have directly overcome the enzyme inhibition via the law of mass action. However, the metabolic fate of monoacetyl dapsone may better account for the data. Accumulation of dapsone has been shown in previous studies to lead to a marked increase in the blood concentrations of monoacetyl dapsone (Coleman et al 1990a, b, c). In the present study, monoacetyl dapsone levels increased significantly in the presence of cimetidine during the once daily regimen and showed a trend towards an increase during twice daily drug administration. It has been shown that monoacetyl dapsone is equipotent with dapsone as a methaemoglobin generator in-vitro (Tingle et al 1990). Monoacetyl dapsone is also a potent former of methaemoglobin in-vivo (Fig. 7), but in marked contrast to dapsone, this effect is resistant to inhibition by cimetidine. Hence, in the rat, the methaemoglobin formed in the presence of cimetidine at the more frequent dapsone administration may have been caused by the *N*-hydroxylation of the monoacetyl dapsone, which accumulated due to inhibition of dapsone *N*-hydroxylation by cimetidine. Monoacetyl dapsone itself is found in only minute amounts in urine in man (Israili et al 1973; Coleman et al 1990c). However, conjugated derivatives of monoacetyl dapsone hydroxylamine have been found in human urine (Israili et al 1973). Thus, it is probable that the major route of elimination of monoacetyl dapsone is actually through *N*-hydroxylation.

It was apparent that in comparison with a single daily dose, acetylation increased four fold when dapsone was administered twice daily. However, in the rat, acetylation appears to occur at a slower rate compared with *N*-hydroxylation (Grossman & Jollow 1988; Coleman et al 1990a), and at lower doses most of the dapsone undergoes oxidative metabolism.

Clinically, it has been possible to inhibit the *N*-hydroxylation of a single dose (100 mg) of dapsone and reduce the formation of methaemoglobin (Coleman et al 1990c). In order to inhibit *N*-hydroxylation during chronic dapsone administration in man, it is likely that frequent divided cimetidine dosage would be necessary before dapsone administration. The accumulation of the drug due to cimetidine inhibition of *N*-hydroxylation would indicate that therapeutic antibacterial levels could be maintained at a lower dosage of dapsone, thus reducing the drug burden as well as ensuring that the inhibition is not overcome. Overall, drug toxicity would be reduced and tolerance increased.

In summary, we have shown that the toxic manifestations of dapsone *N*-hydroxylation can be inhibited by cimetidine in the rat when both compounds are given chronically; however, this effect is conditional on the frequency of dose of dapsone and prior administration of cimetidine. In addition, it is likely that in this experimental model, hydroxylation of the monoacetate of dapsone made a significant contribution to the methaemoglobinaemia caused by the parent drug.

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