Br. J. clin. Pharmac. (1990), **30**, 761–767

The use of cimetidine as a selective inhibitor of dapsone *N*-hydroxylation in man

M. D. COLEMAN, A. K. SCOTT, A. M. BRECKENRIDGE & B. K. PARK
Department of Pharmacology and Therapeutics, Ashton Street, Liverpool L69 3BX

1 The *N*-hydroxylation of dapsone is thought to be responsible for the methaemoglobinaemia and haemolysis associated with this drug. We wished to investigate the effect of concurrent administration of cimetidine (400 mg three times per day) on the disposition of a single dose (100 mg) of dapsone in seven healthy volunteers in order to inhibit selectively *N*-hydroxylation.

2 The AUC of dapsone (31.0 ± 7.2 μg ml^{-1} h) was significantly increased ($P < 0.001$) in the presence of cimetidine (43.3 ± 8.8 μg ml^{-1} h).

3 Peak methaemoglobin levels observed after dapsone administration (2.5 ± 0.6%) were significantly ($P < 0.05$) reduced in the presence of cimetidine (0.98 ± 0.35%).

4 The percentage of the dose excreted in urine as the glucuronide of dapsone hydroxylamine was significantly ($P < 0.05$) reduced in the presence of cimetidine (34.2 ± 9.3 *vs* 23.1 ± 4.2%).

5 Concurrent cimetidine therapy might reduce some of the haematological side-effects of dapsone.

Keywords dapsone cimetidine *N*-hydroxylation inhibition man

Introduction

Dapsone has been used in leprosy therapy since the early 1950s. Due to the emergence of resistance, the drug has been administered with rifampicin and clofazimine in a triple combination which has been introduced in a phased manner since 1982 (WHO, 1982). However dapsone monotherapy is still widely used (Teterissa & Nathin, 1988). Dapsone is also used as a malarial prophylatic with pyrimethamine (Bruce-Chwatt, 1982). It is effective in the treatment of dermatitis herpetiformis (Swain *et al.*, 1983), *Pneumocystis carinii* in AIDS patients (Green *et al.*, 1988) and has been used in rheumatoid arthritis (Grindulus & MacConkey, 1984).

In man, approximately half the dose of dapsone is acetylated to monoacetyl dapsone while the remainder undergoes *N*-hydroxylation predominantly to dapsone hydroxylamine, which in turn undergoes extensive conjugation to dapsone hydroxylamine glucuronide (Israili *et al.*, 1973). Dapsone *N*-hydroxylamine has been implicated in the haemotoxic side effects of dapsone therapy, methaemoglobinaemia (Cucinell *et al.*, 1972) and haemolysis (DeGowin *et al.*, 1966; Grossman & Jollow, 1988). Chronic dosage (100 mg day^{-1}) in normal patients may result in significant methaemoglobinaemia (Manfredi *et al.*, 1979). In addition, methaemoglobinaemia may increase significantly in cases of methaemoglobin reductase enzyme deficiencies (Ganer *et al.*, 1981). Haemolysis may be life-threatening in individuals with glucose-6-phos-

Correspondence: Dr M. D. Coleman, Department of Pharmacology and Therapeutics, Ashton Street, Liverpool L69 3BX

phate dehydrogenase deficiency or diminished glutathione reductase activity (Zuidema *et al.*, 1986).

Studies in the intact rat (Coleman *et al.*, 1990a) and the rat isolated perfused liver (Coleman *et al.*, 1990b) have indicated that cimetidine is a potent inhibitor of dapsone induced haemotoxicity. In the present study, we have co-administered cimetidine with dapsone to determine if a selective reduction in the formation of toxic hydroxylamine metabolites can be achieved without affecting detoxification pathways such as acetylation. As the efficacy of dapsone as an antibacterial lies in the action of the parent compound rather than the metabolites (Bawden & Tute, 1981; Zuidema *et al.*, 1986), an increase in the AUC of dapsone as a result of inhibition of *N*-hydroxylation might also improve the effectiveness of the drug *in vivo*.

Methods

Chemicals

4,4'diaminodiphenyl sulphone (dapsone) was obtained from Sigma Chemicals (Poole, Dorset, UK). Monoacetyl dapsone was a gift from Dr S. A. Ward, Liverpool School of Tropical Medicine. Dapsone hydroxylamine was synthesized and characterised by Dr F. Hussain (Department of Chemistry, University of Liverpool) according to the method of Uetrecht *et al.* (1988). Potassium cyanide and ferricyanide were obtained from BDH Chemicals Ltd (Poole, UK) and Sigma Chemicals respectively. All other reagents were of h.p.l.c. grade and obtained from BDH Chemicals.

Protocol

Approval for this study was granted by the Merseyside Regional Health Authority Ethics Committee. Seven normal healthy male volunteers (23–39 years) were studied. All the volunteers had normal biochemical profiles (SMAC analysis) and gave written informed consent after a full explanation of the risks involved. The volunteers fasted for 8 h prior to taking an oral dose of 100 mg dapsone alone or 100 mg dapsone following 3 days pretreatment with cimetidine (400 mg three times daily). All subjects remained fasted for 2 h post dose. Cimetidine was continued throughout the period of blood and urine collection. Volunteers were studied on two occasions in random order. Venous blood samples (10 ml) were taken via direct venepuncture pre-dose, then at 2, 3, 4, 8, 24, 28, 32, 48 and 54 h into lithium heparin tubes. Urine was collected at time 0 then over periods 0–6 h, 6–12 h, 12–18 h, 18–24 h, 24–30 h, 30–36 h, 36–42 h and 42–48 h.

Analytical procedures

Blood samples were assayed for methaemoglobin levels relative to haemoglobin levels using the spectrophotometric method of Harrison & Jollow (1986). Methaemoglobin levels were measured in each volunteer at time zero and all subsequent measurements were corrected for the zero value.

Dapsone and monoacetyl dapsone was assayed according to the h.p.l.c. method of Grossman & Jollow (1988). Analysis of dapsone related products in urine was achieved after incubation of the urine samples (200 μl) with 500 μu (100 μl) of glucurase® (Sigma) along with 10 mM ascorbic acid (50 μl) at 37° C. A pilot study revealed that the maximum liberation of dapsone hydroxylamine occurred at 6 h incubation time. Dapsone hydroxylamine was identified in the urine hydrolysates by mass spectrometry. Ascorbate prevented the oxidation of dapsone hydroxylamine to nitrosodapsone. The method of Coleman *et al.* (1990b) was applied to the measurement of dapsone hydroxylamine in hydrolysed urine. The hydrolysates were spiked with internal standard (2.4 μg, 24 μl) and then precipitated with methanol (300 μl); prior to centrifugation (10 min, 10,000 *g*) they were briefly vortexed. The clear supernatant was removed from the sample and an aliquot (50 μl) injected onto the h.p.l.c. Standard curves were performed in drug free urine which had been hydrolysed under identical conditions to the samples. Coefficients of variation for within day (dapsone: 4 μg ml^{-1}, 2.4%, 8 μg ml^{-1}, 2.7%/dapsone hydroxylamine: 4 μg ml^{-1}, 4.2%, 8 μg ml^{-1}, 3.9%/monoacetyl dapsone: 4 μg ml^{-1}, 2.0%, 8 μg ml^{-1}, 2.2%) and day to day variation (dapsone: 4 μg ml^{-1}, 1.0%, 8 μg ml^{-1}, 1.4%/dapsone hydroxylamine: 4 μg ml^{-1}, 5.0%, 8 μg ml^{-1}, 6.2%/monoacetyl dapsone: 4 μg ml^{-1}, 4.2%, 8 μg ml^{-1}, 4.4%) indicated that the assay was reproducible in measuring these compounds in urine. Chromotographic separation was achieved using a Waters μBondapak column (Waters Assoc., Hartford, UK) and a mobile phase which consisted of water/acetonitrile/acetic acid/triethylamine (79 : 21 : 1 : 0.05 v/v) and was continually degassed with helium. A linear gradient was employed from 0–14.2 min and ending at 64.5 : 35.5 : 1.0 : 0.05 v/v. The flow rate was maintained at 1.2 ml min^{-1} throughout. The respective retention times of dapsone hydroxylamine, dapsone, monoacetyl dapsone and internal standard were 7.2, 8.6, 9.6 and 13.5 min.

Dapsone hydroxylamine was stabilised in solution in 5 mM ascorbate. This ensured > 95% preservation of this normally unstable compound through the steps of the assay.

Acetylator phenotype was measured according to a standard method (Gelber *et al.*, 1971; Wright *et al.*, 1984) i.e. the ratio of the monoacetyl dapsone to dapsone plasma concentration at the 3 h time point. Fast acetylator status was accepted to be at a ratio greater than 0.30. For both methods of analysis, the h.p.l.c. system was a Spectra-Physics System, which comprised an SP 8700 solvent delivery system, an SP 8880 Autosampler and a model SP 100 Variable wavelength u.v. detector set at 254 nm. The system was controlled via Labnet® and peak areas were determined by a Spectro-Physics Integrator.

Pharmacokinetic calculations and statistical analysis

The terminal phase elimination rate constant (λ_z) was calculated from least squares regression analysis of the post-distributive plasma dapsone concentration-time data and the half-life from the ratio $0.693/\lambda_z$. The area under the curves (AUC(0,54)) for plasma concentration-time data for dapsone, and monoacetyl dapsone were each calculated from $t = 0$ to $t = 54$ h by the use of the linear trapezoidal rule (Gibaldi & Perrier, 1982). AUC from 54 h to infinity was calculated for dapsone by the ratio C_{54}/λ_z where C_{54} was the plasma concentration of dapsone at 54 h. The area under the curve from zero to infinity (AUC) was obtained from the sum of the two areas. Assuming complete systemic bioavailability from the oral dose, apparent clearance CL_o(app) was calculated by the formula

$$CL_o(app) = \frac{Dose}{AUC}$$

Statistical analysis of the disposition of dapsone and dapsone hydroxylamine was accomplished by use of Student's paired *t*-test. Analysis of monoacetyl dapsone disposition was achieved by the use of the Wilcoxon Signed rank test (two-tailed). In both tests $P < 0.05\%$ was accepted as significant. Data are tabulated as mean ± s.d. and presented graphically as mean ± s.e. mean.

Results

The plasma concentration-time data for dapsone and monoacetyl dapsone measured after the administration of dapsone 100 mg to seven volunteers are shown on Figure 1. Absorption was relatively slow with mean maximum dapsone concentrations occurring at 4 h. The drug was eliminated slowly, as at 54 h post dose the compound remained easily detectable in plasma (127 ± 30 ng ml^{-1}). Maximum methaemoglobin levels (Figure 2) 2.5 ± 0.6% (corrected for background) coincided with the time of peak plasma dapsone concentrations (4 h). At 24 h post dose, methaemoglobin levels remained above 2% (2.1 ± 0.6%).

The disposition of dapsone underwent significant change in the presence of cimetidine (Table 1). The mean dapsone AUC (31.0 ± 7.2 μg ml^{-1} h^{-1}) was increased (43.3 ± 8.8 μg ml^{-1} h^{-1}, $P <$

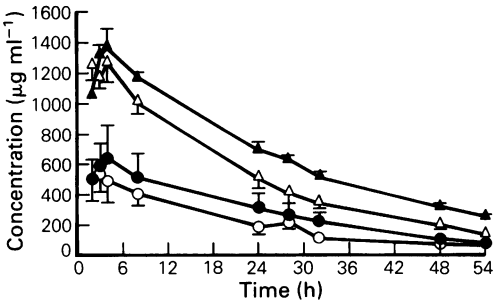

Figure 1 Plasma concentrations of dapsone and monoacetyl dapsone after oral administration of dapsone (100 mg) to seven volunteers (mean ± s.e. mean) before (dapsone, △; monoacetyl dapsone, ○) and after (dapsone, ▲; monoacetyl dapsone, ●) concurrent cimetidine (3 × 400 mg daily).

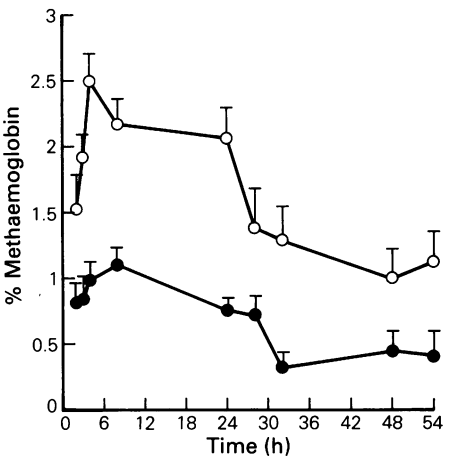

Figure 2 % Methaemoglobin against time after oral administration of dapsone (100 mg, ○) and dapsone with cimetidine (3 × 400 mg daily, ●) to seven volunteers (mean ± s.e. mean).

Table 1 Pharmacokinetic parameters of dapsone (DDS) and monoacetyl dapsone (MADDS) before and after the administration of cimetidine (CIM, 3 × 400 mg daily)

Subject	AUC (μg ml^{-1} h)		$t_{1/2}$ (h)		CL_o(app) (l h^{-1})		MADDS AUC(0,54)		Acetylation ratio	
	DDS	DDS/CIM	DDS	DDS/CIM	DDS	DDS/CIM	DDS	DDS/CIM	DDS	DDS/CIM
1	27.3	46.0	15.3	24.7	3.6	2.1	3.4	3.9	0.14	0.15
2	35.5	51.2	16.8	21.3	2.8	1.9	15.4	28.6	0.56	0.66
3	27.3	39.4	17.1	18.7	3.6	2.5	2.5	2.9	0.17	0.11
4	25.9	35.5	15.0	20.8	3.8	2.8	15.2	22.0	0.8	0.81
5	21.7	29.1	16.2	19.7	4.6	3.4	11.5	15.8	0.66	0.7
6	40.7	50.6	18.3	21.4	2.4	2.0	5.5	7.0	0.17	0.16
7	38.4	51.1	13.5	19.3	2.6	1.9	21.1	33.0	0.82	0.85
Mean ± s.d.	31.0 ± 7.2	43.3 ± 8.8	16.0 ± 1.6	20.8 ± 2.0	3.4 ± 0.8	2.4 ± 0.6				
P	0.001		0.01		0.001		0.05		NS	

0.001) as was the half-life (16.0 ± 1.6 *vs* 20.8 ± 2.0 h, $P < 0.01$). Dapsone clearance was significantly reduced in the presence of cimetidine (3.4 ± 0.8 *vs* 2.4 ± 0.6 l h^{-1}, $P < 0.001$, Table 1). The AUC(0,54) for methaemoglobin (85.3 ± 25.1% Met Hb h^{-1}) fell by more than half in the presence of cimetidine (35.5 ± 11.6% Met Hb h^{-1}, $P < 0.01$, Figure 2).

A significant ($P < 0.05$) increase was seen in AUC values for monoacetyl dapsone (Table 1). In the case of the four fast acetylators 2, 4, 5 and 7, they showed an increase in the AUC of monoacetyl dapsone of 85, 44, 37 and 56%, respectively. The three slow acetylators 1, 3 and 6 showed increases of 13, 19, and 28%, respectively. However the mean acetylation ratio (concentration of monoacetyl dapsone as a fraction of dapsone concentration at 3 h in plasma) did not change after cimetidine (0.7 ± 0.1 *vs* 0.75 ± 0.1 fast acetylators; 0.16 ± 0.017 *vs* 0.14 ± 0.02 slow acetylators).

Preliminary analysis of unhydrolysed urine revealed little excretion of unconjugated dapsone hydroxylamine and dapsone. Analysis of hydrolysed urine by h.p.l.c. revealed excretion of considerable amounts of dapsone-related material

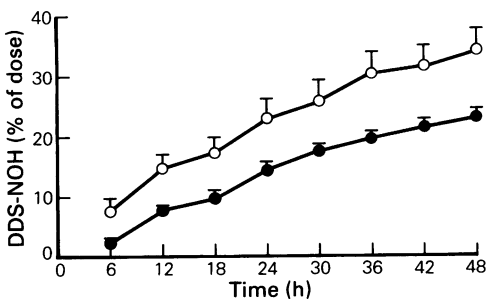

Figure 3 Cumulative excretion of dapsone hydroxylamine (DDS-NOH) in hydrolysed urine after the oral administration of dapsone (100 mg) alone (○) or in combination with cimetidine (3 × 400 mg, daily, ●) to seven volunters (mean ± s.e. mean).

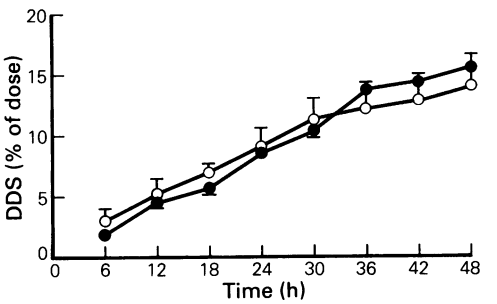

Figure 4 Cumulative excretion of dapsone (DDS) in hydrolysed urine after the oral administration of dapsone (100 mg) alone (○) or in combination with cimetidine 3 × 400 mg daily (●) to seven volunteers (mean ± s.e. mean).

in urine. Over 48 h, 34.2 ± 9.3% of the dose was eliminated as dapsone hydroxylamine (Figure 3). In the presence of cimetidine, significantly less dapsone hydroxylamine (23.1 ± 4.2%, $P < 0.05$) was recovered in the urine. There was no significant difference between the amounts of parent drug in the hydrolysed urine (14.1 ± 5.4 *vs* 15.5 ± 4.4%, Figure 4). Only relatively small amounts of monoacetyl dapsone were recovered from the urine (Figure 5) even after enzyme hydrolysis and the recoveries did not change significantly in the presence of cimetidine (2.2 ± 1.0 *vs* 2.7 ± 0.8%).

Discussion

Dapsone is associated with a number of adverse reactions, many of which are dose related (Smith, 1988; Weetman *et al.*, 1980). Previous studies have indicated plasma drug concentrations less than 1.9 μg ml^{-1} after a single dose of 100 mg orally (Zuidema *et al.*, 1986), similar to those found in the present study. Chronic administration (100 mg day^{-1}) results in plasma drug concentrations which are considerably higher than those seen after a single dose, (> 3.0 μg ml^{-1},

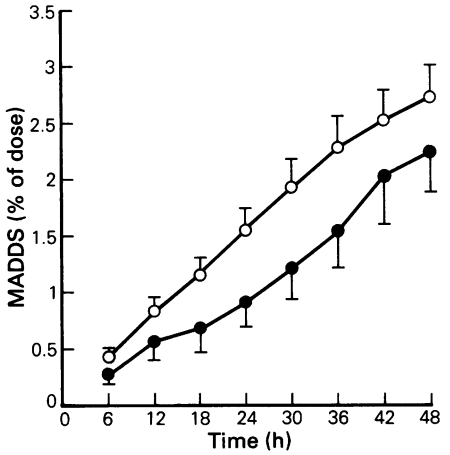

Figure 5 Cumulative excretion of monoacetyl dapsone (MADDS) in hydrolysed urine after the oral administration of dapsone (100 mg) alone (○) or in combination with cimetidine (3 × 400 mg daily, ●).

Halmekoski *et al.*, 1978) and which have been associated with methaemoglobinaemia levels of 3–6% (DeGowin *et al.*, 1966; Manfredi *et al.*, 1979). Dapsone itself does not generate methaemoglobin in human erythrocytes *in vitro* (Israili *et al.*, 1973; Tingle *et al.*, 1990). The *N*-hydroxylation of dapsone by human hepatic cytochrome P-450, results in the metabolite responsible for the toxicity of dapsone, the hydroxylamine, which is rapidly taken up by erythrocytes (Israili *et al.*, 1973; Tingle *et al.*, 1990) and is unstable in oxygen rich environments (Coleman *et al.*, 1989). Hence it has not been detected in plasma (Zuidema *et al.*, 1986). Once inside the red cell, the hydroxylamine is co-oxidised with haemoglobin to generate nitroso-dapsone and methaemoglobin (Kramer *et al.*, 1972). Methaemoglobin formation within the red cell is reduced by NADH-dependent methaemoglobin reductase. However, the oxidation of haemoglobin by the hydroxylamine of dapsone rapidly outstrips the capacity of the enzyme to reverse the oxidation of the haemoglobin. This is exacerbated by a futile oxidation-reduction cycle involving dapsone hydroxylamine and nitrosodapsone. It is thought that the major urinary metabolite of dapsone is the glucuronide of dapsone hydroxylamine (Gordon *et al.*, 1979; Israili *et al.*, 1973). Dapsone hydroxylamine is a potent former of methaemoglobin *in vivo* (Coleman *et al.*, 1990a; Grossman & Jollow, 1988) and *in vitro* (Coleman *et al.*, 1990a,b; Israili *et al.*, 1973). Dapsone hydroxylamine is also toxic to human mononucleocytes *in vitro* (Coleman *et al.*, 1989) and in granulopoiesis *in vitro* (Weetman *et al.*, 1980).

Previous studies in the rat have shown cimetidine to be a potent inhibitor of dapsone *N*-hydroxlation *in vivo* (Coleman *et al.*, 1990a), in the isolated liver (Coleman *et al.*, 1990b) and *in vitro* (Tingle *et al.*, 1990). In the present study, the co-administration of cimetidine significantly inhibited dapsone *N*-hydroxylation, as shown by the delay in the excretion of dapsone hydroxylamine glucuronide in urine in all seven volunteers. In addition, the AUC for the parent drug was increased significantly as a consequence of the partial inhibition of *N*-hydroxylation.

As a consequence of the reduction in *N*-hydroxylation, in all seven subjects monoacetyl dapsone formation increased. The fast acetylators showed greater increases in acetylation in the presence of cimetidine compared with the slow acetylators, although the percentage increase was similar. Previous studies with procainamide and hydralazine have shown that slow acetylator status is associated with a greater risk of adverse reactions, as more of the dose undergoes *N*-hydroxylation (Woosley *et al.*, 1978). However, monoacetyl dapsone may still undergo *N*-hydroxylation in man (Israili *et al.*, 1973), and is as haematologically toxic as dapsone hydroxylamine in the rat (Grossman & Jollow, 1988). Hence, with dapsone therapy, acetylator status appears to have no bearing on the frequency of toxicity, or, indeed on the half-life of the drug (Zuidema *et al.*, 1986). In the absence of *N*-hydroxylation, the acetylation of dapsone would probably retard the systemic clearance of dapsone as monoacetyl dapsone is highly protein bound and does not undergo extensive glomerular filtration. It is thought that acetylation and de-acetylation exist in equilibrium and it has also been suggested that de-acetylation must take place before drug is eliminated in urine (Zuidema *et al.*, 1986). Previous studies have indicated that monoacetyl dapsone is found in urine in very low concentrations (Gelber *et al.*, 1971) and this was reflected in the present study. It is likely that *N*-hydroxylation is the actual major route of elimination, even though more than half the dose may be acetylated in man (Gelber *et al.*, 1971). In this report, the increase in the dapsone AUC seen in the presence of cimetidine was surprisingly not accompanied by an increase in urinary unchanged dapsone concentrations. The compensatory increase in acetylation also seen in the presence of cimetidine may well have retarded dapsone clearance sufficiently to prevent the rise in urinary dapsone concentrations over the collection interval used.

Dapsone administered at 100 mg acutely has often been used as a probe drug for the assessment of acetylator phenotype (Adam *et al.*,

1984; Horai *et al.*, 1985; Hutchings *et al.*, 1984). Swain *et al.* (1983) noted that after a single dose of 150 mg no patients or volunteers suffered adverse effects. However, methaemoglobin levels were not determined in these studies. In the present work, although we employed the standard dose of 100 mg, it was still clearly possible to measure a significant elevation of methaemoglobin levels in all the volunteers. However, the observed levels of methaemoglobin were significantly less in the presence of cimetidine, which is consistent with a fall in the *N*-hydroxylation of dapsone to the hydroxylamine. Although the methaemoglobinaemia seen was not of clinical significance, these data do suggest that cimetidine might prevent the haematological toxicity which is known to be associated with chronic administration of the drug.

Dapsone is often administered in combination with rifampicin and clofazimine (Zuidema *et al.*, 1986). Clofazimine is very slowly metabolised and has a long half-life in man (Levy, 1976) and is not thought to influence appreciably plasma concentrations of dapsone (Venkatesan *et al.*, 1980). Rifampicin lowers skin and nerve concentrations of dapsone, possibly through enzyme induction (Peters *et al.*, 1977, 1978): this might promote resistance to dapsone. Cimetidine is known to be able to overcome the effect of enzyme induction by rifampicin in man (Feely *et al.*, 1984).

The co-administration of cimetidine may be of value as part of the anti-leprosy therapy of patients who are deficient in methaemoglobin reductase or glucose-6-phosphate dehydrogenase. The potentially life-threatening haematological side effects that are caused in these patients by dapsone normally dictates the use of a greatly reduced dosage of the drug, which may promote the emergence of bacterial resistance. Any increased risk in adding cimetidine to an already substantial drug burden would be outweighed by the potential benefits of reduced toxicity and more effective maintenance of curative dapsone plasma concentrations.

Cimetidine does not affect acetylation in man (Wright *et al.*, 1984) and in the present study acetylation ratio was unchanged after cimetidine administration. Rifampicin is metabolised in man via de-acetylation and it is likely that cimetidine will not affect plasma rifampicin concentrations in man.

In summary, the concurrent administration of cimetidine caused a reduction in the *N*-hydroxylation of dapsone and an increase in AUC of the drug. Concurrent cimetidine therapy might be of value in not only increasing the efficacy of dapsone, but also in reducing the haematological side effects of the drug to a tolerable level.

M. D. Coleman is in receipt of a Wellcome Fellowship in Toxicology. B. K. Park is a Wellcome Principal Research Fellow. The authors are grateful to Dr J. L. Maggs for his assistance with the mass spectrometer, and to Pearl Williams for typing the manuscript.

References

Adam, A. M., Rogers, H. J., Amiel, S. A. & Rubens, R. D. (1984). The effect of acetylator phenotype on the disposition of aminoglutethimide. *Br. J. clin. Pharmac.*, **18**, 495–505.

Bruce-Chwatt, L. J. (1982). *Essential malariology*, pp. 181–182. London: Heinemann.

Bawden, D. & Tute, M. S. (1981). Structure-activity relationships of antimycobacterial sulphones in a study using physicochemical constants. *Eur. J. med. Chem.*, **16**, 299–300.

Coleman, M. D., Breckenridge, A. M. & Park, B. K. (1989). Bioactivation of dapsone to a cytotoxic metabolite by human hepatic microsomal enzymes. *Br. J. clin. Pharmac.*, **28**, 389–395.

Coleman, M. D., Hoaksey, P. E., Breckenridge, A. M. & Park, B. K. (1990b). Inhibition of dapsone-induced methaemoglobinaemia in the isolated perfused rat liver. *J. Pharm. Pharmac.*, **42**, 302–307.

Coleman, M. D., Winn, M. J., Breckenridge, A. M. & Park, B. K. (1990a). Inhibition of dapsone induced methaemoglobinaemia in the rat. *Biochem. Pharmac.*, **39**, 802–805.

Cucinell, S. A., Israeli, Z. H. & Dayton, P. G. (1972). Microsomal *N*-oxidation of dapsone as a cause of methaemoglobin formation in human red cells. *Am. J. Trop. Med. Hyg.*, **21**, 322–333.

DeGowin, R. L., Eppes, R. B., Powell, R. D. & Carson, P. E. (1966). The haemolytic effects of diphenylsulphone (DDS) in normal subjects and in those with glucose 6-phosphate dehydrogenase deficiency. *Bull. W.H.O.*, **35**, 165–179.

Feely, J., Pereira, L., Guy, E. & Hockings, N. (1984). Factors affecting the response to the inhibition of drug metabolism by cimetidine-dose response and sensitivity in elderly and induced subjects. *Br. J. clin. Pharmac.*, **17**, 77–81.

Ganer, A., Knobel, B., Fryd, C. H. & Rachmilewitz, E. A. (1981). Dapsone induced methaemoglobinaemia and haemolysis in the presence of familial hemoglobinopathy hasharon and familial methaemoglobin reductase deficiency. *Is. J. med. Sci.*, **17**, 703–704.

Gelber, R., Peters, J. H., Gordon, R. G., Glazko, A. J. & Levy, L. (1971). The polymorphic acetylation of dapsone in man. *Clin. Pharmac. Ther.*, **12**, 225–238.

Gibaldi, M. & Perrier, D. (1982). In *Pharmacokinetics*, 2nd edition, p. 445. New York: Marcel Dekker.

Gordon, J. R., Murray, J. F., Peters, J. H. & Jacobson, R. R. (1979). Studies on the urinary metabolites of dapsone in man. *Int. J. Leprosy*, **47**, 681–682.

Green, S. T., Goldberg, D. J., Leach, J., Christie, P. R. & Kennedy, D. H. (1988). AIDS-related *Pneumocystis carinii* pneumonia successfully treated with dapsone and trimethoprim. *Br. J. clin. Pharmac.*, **26**, 487–488.

Grindulis, K. A. & MacConkey, B. (1984). Rheumatoid arthritis, the effects of treatment with dapsone on haemoglobin. *J. Rheumatol.*, **11**, 776–778.

Grossman, S. J. & Jollow, D. J. (1988). Role of DDS NOH in dapsone-induced haemolytic anaemia. *J. Pharmac. exp. Ther.*, **244**, 118–125.

Halmekoski, J., Matilla, M. J. & Mustakallio, K. K. (1978). Metabolism of haemolytic effect of dapsone and its metabolites in man. *Med. Biol.*, **56**, 216–221.

Harrison, J. H. & Jollow, D. J. (1986). Role of aniline metabolites in aniline-induced hemolytic anemia. *J. Pharmac. exp. Ther.*, **238**, 1045–1054.

Horai, G. & Ishazaki, T. (1985). Rapid and sensitive liquid chromatographic method for the determination of dapsone and monoacetyl dapsone in plasma and urine. *J. Chromatogr.*, **345**, 447–452.

Hutchings, A., Morie, R. D., Spragg, B. & Routledge, P. A. (1984). Acetylator phenotyping: effect of ethanol on the dapsone test. *Br. J. clin. Pharmac.*, **18**, 98–100.

Israili, Z. H., Cucinell, S. A., Vaught, J., Davis, B., Zesser, J. M. & Dayton, P. G. (1973). Studies of the metabolism of DDS in man and experimental animals. Formation of *N*-hydroxy metabolites. *J. Pharmac. exp. Ther.*, **187**, 138–151.

Kramer, P. A., Gladen, B. E. & Li, T. K. (1972). Mechanism of methaemoglobin formation by diphenylsulphones. *Biochem. Pharmac.*, **21**, 1265–1274.

Levy, L. (1976). Pharmacologic studies of clofazimine. *Am. J. Trop. Med. Hyg.*, **23**, 1097–1156.

Manfredi, G., de Panfilis, G., Zampetti, M. & Allegra, F. (1979). Studies on dapsone induced haemolytic anaemia: methaemoglobin production and glucose-6-phosphate activity in correlation with dapsone dosage. *Br. J. Dermatol.*, **100**, 427–432.

Peters, J. H., Murray, J. F., Gordon, G. R., Gelber, R. M. & Laing, A. B. G. (1977). Effect of rifampicin on the disposition of dapsone in Malaysian leprosy patients. *Fed. Proc.*, **36**, 896.

Peters, J. H., Murray, J. F., Gordon, G. R. & Jacobsen, R. R. (1978). The metabolic-bacteriologic relationship in the chemotherapy of lepromatous patients with dapsone or dapsone-rifampicin. *Int. J. Leprosy*, **46**, 115–116.

Smith, W. C. S. (1988). Are hypersensitivity reactions to DDS becoming more frequent. *Leprosy Rev.*, **59**, 395–398.

Swain, A. F., Ahmad, R. A., Rogers, M. J., Leonard, J. N. & Fry, L. (1983). Pharmacokinetic observations on dapsone in dermatitis herpetiformis. *Br. J. Dermatol.*, **108**, 91–98.

Teterissa, M. R. & Nathin, M. A. (1988). Leprosy control in Indonesia. *South East Asian J. Med. Pub. Hlth*, **19**, 511–514.

Tingle, M. D., Coleman, M. D. & Park, B. K. (1990). Investigation into the role of metabolism in dapsone-induced methaemoglobinaemia using a two compartment *in vitro* test system. *Br. J. clin. Pharmac.*, **30**, (in press).

Uetrecht, J. P., Zahid, N., Shear, N. H. & Biggar, W. D. (1988). Metabolism of dapsone to a hydroxylamine by human neutrophils and mononuclear cells. *J. Pharmac. exp. Ther.*, **245**, 274–279.

Venkatesan, K., Bharadwaj, V. P., Ramu, G. & Desikan, K. V. (1980). Study on drug interactions. *Leprosy in India*, **52**, 229–236.

Weetman, R. M., Boxer, L. A., Brown, M. P., Martich, N. M. & Baehner, R. L. (1980). *In vitro* inhibition of granulopoiesis by 4-amino-4′-hydroxylaminodiphenyl sulfone. *Br. J. Haematol.*, **45**, 361–370.

Woosley, R. L., Drayer, D. E., Reidenberg, M. M., Nies, A. S., Carr, K. & Oates, J. A. (1978). Effect of acetylator phenotype on the rate at which procainamide induces antinuclear antibodies and the lupus syndrome. *New Engl. J. Med.*, **298**, 1157–1159.

World Health Organisation (WHO) (1982). *Chemotherapy of leprosy for control programmes.* Report of a WHO study group. WHO Technical Report Series No. 675.

Wright, J. T., Goodman, R. P., Bethel, A. M. M. & Lambert, C. N. (1984). Cimetidine and dapsone acetylation. *Drug Metab. Disp.*, **12**,782–783.

Zuidema, J., Hilbers-Modderman, E. S. M. & Markus, F. W. H. M. (1986). Clinical pharmacokinetics of dapsone. *Clin. Pharmacokin.*, **11**, 299–325.

(Received 27 March 1990, accepted 25 June 1990)