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An investigation of the role of metabolism in dapsone-induced methaemoglobinaemia using a two compartment *in vitro* test system

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1 We have utilized a two compartment system in which two teflon chambers are separated by a semi-permeable membrane in order to investigate the role of metabolism in dapsone-induced methaemoglobinaemia. Compartment A contained a drug metabolizing system (microsomes prepared from human liver ± NADPH), whilst compartment B contained target cells (human red cells).

2 Incubation of dapsone (1–100 μM) with human liver microsomes (2 mg protein) and NADPH (1 mM) in compartment A (final volume 500 μl) led to a concentration-dependent increase in the methaemoglobinaemia (15.4–18.9% at 100 μM) compared with control (2.3 ± 0.4%) detected in the red cells within compartment B. In the absence of NADPH dapsone had no effect.

3 Of the putative dapsone metabolites investigated, only dapsone-hydroxylamine caused methaemoglobin formation in the absence of NADPH (40.6 ± 6.3% with 100 μM). However, methaemoglobin was also detected when monoacetyl-dapsone, 4-amino-4′-nitro-diphenylsulphone and 4-aminoacetyl-4′-nitro-diphenylsulphone were incubated with human liver microsomes in the presence of NADPH.

4 Dapsone-dependent methaemoglobin formation was inhibited by addition of ketoconazole (1–1000 μM) to compartment A, with IC_{50} values of 285 and 806 μM for the two liver microsomal samples studied. In contrast, methaemoglobin formation was not inhibited by cimetidine or a number of drugs pharmacologically-related to dapsone. The presence of glutathione or ascorbate (500 μM) did not alter the level of methaemoglobin observed.

Keywords dapsone metabolism methaemoglobinaemia

Introduction

Dapsone (4,4′-diamino-diphenylsulphone) has been the major component of leprosy treatment for the last 40 years (Smith, 1988). The drug has also been used for inflammatory disorders involving polymorphonucleocyte infiltration (Lang, 1979), rheumatoid arthritis (Grindulis & McConkey, 1984) and in combination with pyrimethamine for malaria prophylaxis (Bruce-Chwatt, 1982).

In man, dapsone is metabolized extensively, the major pathway involving *N*-acetylation (Gelber *et al.*, 1971). However, dapsone also

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undergoes *N*-hydroxylation to form dapsone-hydroxylamine (DDS-NOH), and this route of metabolism has been implicated in the methaemoglobinaemia and haemolysis associated with dapsone administration (Glader & Conrad, 1973; Kramer *et al.*, 1972). It is possible to reduce the formation of methaemoglobinaemia in the rat by pretreating the animals with known inhibitors of cytochrome P-450, such as cimetidine, ketoconazole and piperonyl butoxide (Coleman *et al.*, 1990), which suggests that such a strategy might reduce the haemtoxicity associated with dapsone in man. To investigate this possibility we required an *in vitro* test system in which human tissues can be utilized exclusively.

There exists a variety of *in vitro* tests which have been used to facilitate the study of toxicity observed *in vitro*. In order to examine the toxicity of metabolite(s) as well as parent compounds, these assays often include a microsomal fraction prepared from the livers of experimental animals (Ames *et al.*, 1976; Spielberg *et al.*, 1984). Recently human tissues have been used as both activating system and target cells in cytotoxicity assays (Coleman *et al.*, 1989; Riley *et al.*, 1988). However, in these simple assays it is not possible to evaluate the effect of microsomal adherence to the target cell or to determine whether the toxic metabolite(s) formed can diffuse from the site of production and lead to tissue damage at some distance away. We have therefore employed a two compartment system (Riley *et al.*, 1990) in which the activating system (human liver microsomes) is separated from the target cells (human erythrocytes, RBC) by a semi-permeable membrane.

This method has been applied to the investigation of the toxicity of dapsone and some of its putative metabolites toward human red cells in the presence of microsomes prepared from human liver. In addition, we have also screened a series of drugs structurally related to dapsone as well as known inhibitors of cytochrome P-450 for their ability to inhibit the metabolism of dapsone in an attempt to decrease the adverse events associated with administration of this drug. The test system developed for dapsone can be used to investigate the metabolism and toxicity of other drugs which are associated with methaemoglobin formation in man.

Methods

Ring labelled-[^{14}C]-4,4'-diaminodiphenylsulphone (dapsone; 6.8 mCi mol^{-1}, > 99% pure, Figure 1) was purchased from Amersham International (Amersham, UK). Unlabelled dapsone, reduced glutathione, ascorbic acid, potassium ferricyanide, sulphaguanidine, sulphamethoxazole, tolbutamide, quinidine and chloroquine were obtained from Sigma Chemical Co. Ltd (Poole, UK). Pyrimethamine was a gift from Wellcome and sulphadoxine and sulphadiazine were gifts from Dr S. A. Ward (School of Tropical Medicine, Unversity of Liverpool). Monoacetyl-dapsone (MADDS), diacetyl-dapsone (DADDS), dapsone-hydroxylamine (DDS-NOH), 4-amino-4'-nitro-diphenylsulphone (DDS-NO_2) and 4-aminoacetyl-4'-nitro-diphenysulphone (MADDS-NO_2) (Figure 1) were synthesized by Dr F. Hussain (Department of Chemistry, University of Liverpool) and characterized using mass spectrometry, n.m.r. and i.r. spectrometry. Reduced nicotine adenine dinucleotide phosphate (NADPH; tetrasodium salt), potassium cyanide and all other reagents were purchased from BDH Chemicals Ltd (Poole, U.K.).

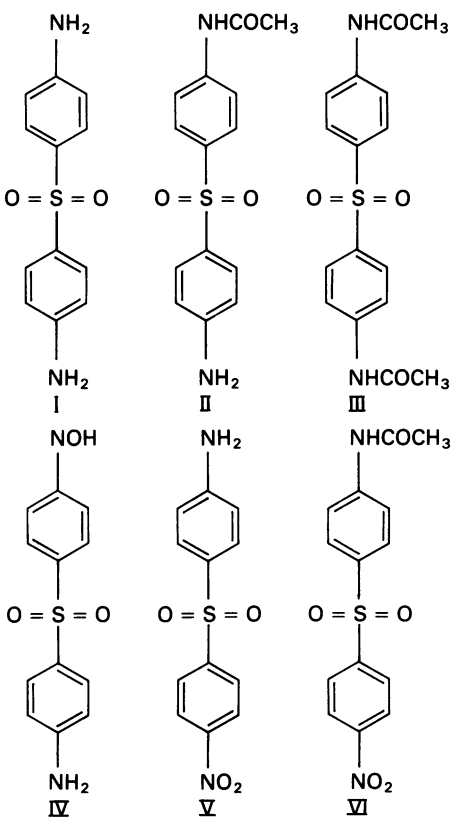

Figure 1 Structures of dapsone (I) and some putative metabolites; monoacetyl-dapsone (II), diacetyl-dapsone (III), dapsone-hydroxylamine (IV), 4-amino-4'-nitro-diphenylsulphone (V), and 4-aminoacetyl-4'-nitro-diphenylsulphone (VI).

Preparation of hepatic microsomes

Washed human microsomes were prepared from four histologically normal livers, obtained from renal transplant donors, as described previously (Purba *et al.*, 1987). Rat microsomes were prepared from the pooled livers of animals pretreated for 3 days with either phenobarbitone (PB; 60 mg kg^{-1}, i.p.) in saline (6 ml kg^{-1}), β-naphthoflavone (BNF; 75 mg kg^{-1}, i.p.) in corn oil (6 ml kg^{-1}) or the vehicles alone, as described by Riley *et al.* (1988).

Methaemoglobin formation in the presence of human liver microsomes

Dapsone (100 μM) in dimethylsulphoxide (5 μl) and microsomes (2 mg protein) prepared from liver I were placed in compartment A. The second compartment contained human red blood cells (RBC) washed twice with phosphate-buffered saline, pH 7.4, then resuspended to approximately 50% haematocrit. The two compartments were separated by a semi-permeable membrane made from cellulose with a molecular weight cut off equivalent to 10 kDa (Diachema, Munich, F.R.G.). Metabolism was initiated by the addition of NADPH (1 mM) to compartment A. The final volume in each half cell was 500 μl. After 5, 10, 15, 30 or 60 min the RBC were expelled and kept on ice until assayed for methaemoglobin content by the method of Harrison & Jollow (1986).

Equilibration of dapsone between compartments was measured by incubation of [^{14}C]-dapsone (100 μM, 0.1 μCi) for 1 h as described above. After this time, the contents of compartment A were collected into microcentrifuge tubes which contained 50 mM ascorbate (50 μl) in order to stabilize the hydroxylamine, mixed thoroughly and methanol (1 ml) added. The contents of compartment B were expelled into microcentrifuge tubes containing 50 mM ascorbate (50 μl), mixed thoroughly then centrifuged (1000 *g*, 10 min). The supernatant was decanted and methanol (1 ml) added to both the RBC fraction and supernatant. Protein was precipitated overnight (−20° C) and aliquots (50 μl) of the supernatants assayed for radioactivity content following addition of scintillant (4 ml). The metabolite profile was determined by radiometric h.p.l.c., with dapsone and its metabolite, dapsone hydroxylamine, being identified by comparison of their retention times with those of co-injected authentic unlabelled compounds. Separation was achieved on a Spherisorb 5 ODS column (25 cm × 0.46 cm i.d., HPLC Technology, Macclesfield, U.K.) by the method of Uetrecht *et al.* (1988). The solvent mobile phase consisted of water : acetonitrile : acetic acid : triethylamine (80 : 20 : 1 : 0.05 v/v) with a flow rate of 1.2 ml min^{-1}. Under these conditions, the retention times of the hydroxylamine and parent compound were 8.5 and 12.5 min respectively. Aliquots (100 μl) of supernatants were injected onto the column, and the radioactive content of the eluate was measured directly using Radiomatic A250 radiometric detector (Radiomatic Ltd, Pangbourne, U.K.). The detector had a liquid flow cell (1 ml) with a counting efficiency of > 90%.

Non-extractable material was determined after repeated washing of the precipitated protein with methanol (1 ml) until no more radioactivity was removed. The protein was then redissolved in 1 M NaOH (500 μl) and an aliquot (100 μl) assayed for radioactivity content after decolorization with hydrogen peroxide and addition of scintillant (4 ml).

The ability of microsomes prepared from four human livers to metabolize dapsone was examined by addition of dapsone (concentration in compartment A 1-100 μM) in dimethylsulphoxide (5 μl) and microsomes (2 mg protein) in 0.1 M phosphate-buffered saline, pH 7.4, to compartment A. Compartment B contained washed human red blood cells (500 μl). Metabolism was initiated by the addition of NADPH (1 mM; omitted from controls) to compartment A and conducted for 1 h at 37° C with constant rotation (8 rev min^{-1}). After this time, RBC were expelled from compartment B and methaemoglobin content was measured as described above.

The effect of reduced glutathione and ascorbate on metabolism-dependent methaemoglobin formation was investigated by incubation of dapsone (100 μM) with microsomes prepared from liver I (2 mg protein) in the presence of NADPH (1 mM) and either reduced glutathione or ascorbate (500 μM; omitted from controls) as described above.

Determination of methaemoglobin formation in the presence of dapsone metabolites

Dapsone, monoacetyl-dapsone (MADDS), diacetyl-dapsone (DADDS), 4-amino-4′-nitrodiphenylsulphone (DDS-NO_2), 4-aminoacetyl-4′-nitro-diphenylsulphone (MADDS-NO_2) or dapsone-hydroxylamine (DDS-NOH) (100 μM, Figure 1) were added to compartment A in dimethyl sulphoxide (5 μl) and incubated with human liver (IV) microsomes (2 mg protein) in the presence of NADPH (1 mM: omitted from controls) at 37° C with constant rotation (8 rev min^{-1}). Compartment B contained washed human RBC (500 μl). After 1 h the RBC were

expelled from compartment B and the methaemoglobin content determined as described previously. Methaemoglobin formation due to dapsone-hydroxylamine was investigated further by addition of the compound (1–30 μM) to compartment A in the absence of a metabolizing system.

Effect of cytochrome P-450 inhibitors on dapsone-dependent methaemoglobin formation

In compartment A, dapsone (1–100 μM) was incubated with microsomes (2 mg protein) prepared from liver I in the presence of NADPH (1 mM) and either ketoconazole (100 μM) or cimetidine (300 μM) for 1 h at 37° C with rotation. Compartment B contained washed human RBC (500 μl).

Further inhibition studies were performed by incubating dapsone (100 μM) with microsomes prepared from liver I (2 mg protein) in the presence of NADPH (1 mM) and either ketoconazole; sulphadiazine; sulphadoxine; sulphamethoxazole; sulphaguanidine; tolbutamide; chloroquine; pyrimethamine or quinidine (500 μM). Inhibition of methaemoglobin formation by ketoconazole (1–1000 μM) was confirmed using microsomes prepared from livers I and II.

Effect of enzyme induction on dapsone-dependent methaemoglobin formation

Dapsone (100 μM) was incubated with a range of microsomal protein concentrations (0.125–1 mg protein) prepared from the livers of rats pretreated with either phenobarbitone (60 mg kg^{-1}), β-naphthoflavone (75 mg kg^{-1}) or vehicle alone in compartment A. Enzyme induction was confirmed by measurement of cytochrome P-450 content by the method of Omura & Sato (1964). Compartment B contained washed human RBC (500 μl). Metabolism was initiated by the addition of NADPH (1 mM) to compartment A and incubations performed as described above.

Results

Incubation of dapsone (100 μM in compartment A before equilibration) with human liver microsomes in the presence of NADPH (1 mM) led to a time-dependent formation of methaemoglobin (Figure 2). Incubation of [^{14}C]-dapsone (100 μM, 0.1 μCi; added to compartment A) with microsomes showed that after 1 h approximately 36% of radioactivity was present in compartment A, of which 2.0% was not extractable from microsomal protein. In compartment B, approximately 60% of the radioactivity was associated with the RBC, of which 11.7% was not extractable from protein. In the presence of NADPH approximately 28.9% of radioactivity was in compartment A, of which 7.5% was not extractable. In compartment B approximately 72.3% of the radioactivity was associated with the RBC, with 15.3% of incubated radioactivity not extracted from protein. Analysis of the supernatants from both compartments by reversed-phase h.p.l.c. revealed the presence of a single peak which corresponded to dapsone. No hydroxylamine was detected in any incubation.

Incubation of dapsone (1–100 μM) with microsomes prepared from four human livers in the presence of NADPH (1 mM) for 1 h resulted in a concentration-dependent methaemoglobin formation (Figure 3). In the absence of NADPH, methaemoglobin content was less than 2%.

Neither glutathione nor ascorbate (500 μM) reduced the formation of methaemoglobin (14.2 ± 1.9% and 16.8 ± 2.2% respectively) in the presence of a metabolizing system compared with control (15.7 ± 2.8%).

Methaemoglobin formation as a result of activation of dapsone metabolites

Metabolism (NADPH)-dependent methaemoglobin formation was observed when either dapsone or its putative metabolites; monoacetyldapsone, 4-amino-4′-nitro-diphenylsulphone, 4-aminoacetyl-4′-nitro-diphenylsulphone and dapsone-hydroxylamine were incubated with

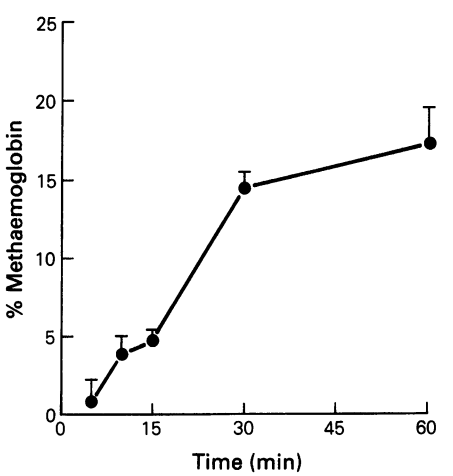

Figure 2 Effect of incubation time on methaemoglobin formation in the presence of dapsone (100 μM), human liver (LI) microsomes and NADPH. Values are mean ± s.d. (n = 3).

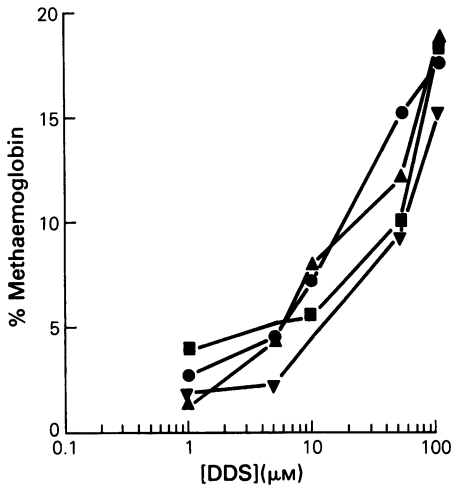

Figure 3 Methaemoglobin formation in the presence of dapsone (1–100 μM), microsomes prepared from four human livers (2 mg protein) and NADPH (1 mM). Values are mean of quadruplicate incubations.

human liver (IV) microsomes (Figure 4). No methaemoglobin was observed with diacetyl-dapsone (Figure 4). Of the metabolites studied, only the hydroxylamine caused methaemoglobin formation in the absence of NADPH. Further investigation revealed that in the absence of a metabolizing system, methaemoglobin formation increased linearly with an increase in the concentration of dapsone hydroxylamine between 1 and 30 μM up to 27.7 ± 7.1% at 30 μM.

Inhibition of methaemoglobin formation

Co-incubation of ketoconazole (100 μM) with dapsone (1–100 μM) and a metabolizing system in compartment A led to a decrease in methaemoglobin formation compared with control (-ketoconazole) (Figure 5). Co-incubation of ketoconazole (1–1000 μM) with dapsone (100 μM) and microsomes prepared from livers I and II, in the presence of NADPH (1 mM), gave IC_{50} values of 285 and 806 μM respectively.

Co-incubation of cimetidine (300 μM) with dapsone (1–100 μM) and microsomes prepared from liver I did not lead to inhibition of dapsone-dependent methaemoglobin formation, even

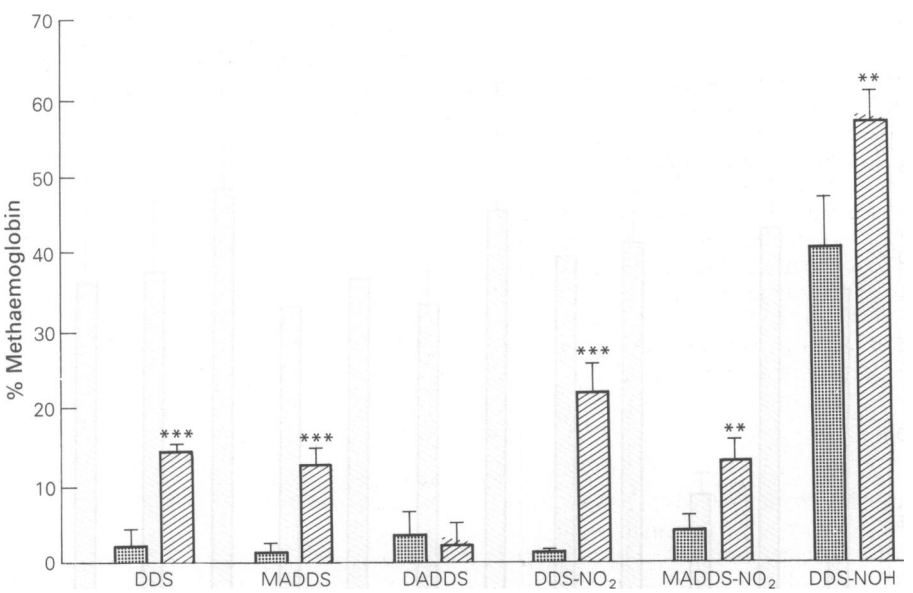

Figure 4 Methaemoglobin formation in the presence of dapsone (DDS), monoacetyl-dapsone (MADDS), diacetyl-dapsone (DADDS), 4-amino-4′-nitro-diphenylsulphone (DDS-NO_2), 4-aminoacetyl-4′-nitro-diphenylsulphone (MADDS-NO_2) or dapsone-hydroxylamine (DDS-NOH) (100 μM) and human liver (LIV) microsomes. Values are mean ± s.d. (n = 4). Significant differences from control (-NADPH) are $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's non-paired t-test). ▦ −NADPH, ▨ +NADPH.

if the microsomes were preincubated with the cimetidine for 30 min in the presence of NADPH (Figure 5). Addition of sulphadiazine, sulphadoxine, sulphaguanidine, sulphamethoxazole, chloroquine, pyrimethamine, quinidine or tolbutamide did not decrease the dapsone-dependent methaemoglobin formation (Figure 6).

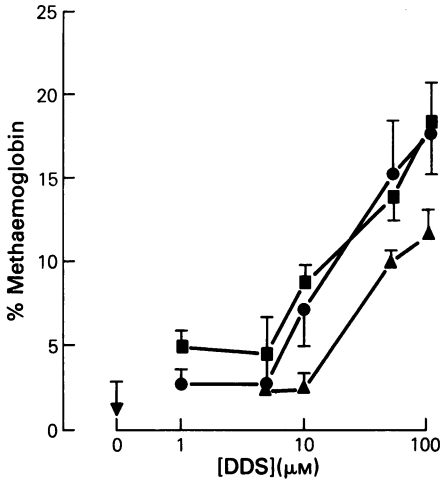

Figure 5 The effect of cimetidine (300 μM, ■) and ketoconazole (100 μM, ▲) on methaemoglobin formation in the presence of dapsone (1–100 μM), human liver (LI) microsomes and NADPH (1 mM). Values are mean ± s.d. (n = 4).

Effect of enzyme induction on methaemoglobin formation

After incubation of dapsone (100 μM) with a range of microsomal protein concentrations (0.125–1 mg protein) prepared from the livers of rats pretreated with either phenobarbitone (60 mg kg^{-1}) or β-naphthoflavone (75 mg kg^{-1}) the methaemoglobin values ranged from 5.8–45.1% and 6.5–41.4% respectively. These values are not significantly different from the levels of methaemoglobin measured after incubation of dapsone with microsomes prepared from control (vehicle-pretreated) rat livers which ranged from 7.3% (0.125 mg protein) to 50.5% (1 mg protein).

Discussion

Chronic dapsone therapy leads to a decrease in red blood cell survival time and a resultant fall in haematocrit (DeGowin *et al.*, 1966). The major route of metabolism in man involves *N*-acetylation, although an alternative route of metabolism

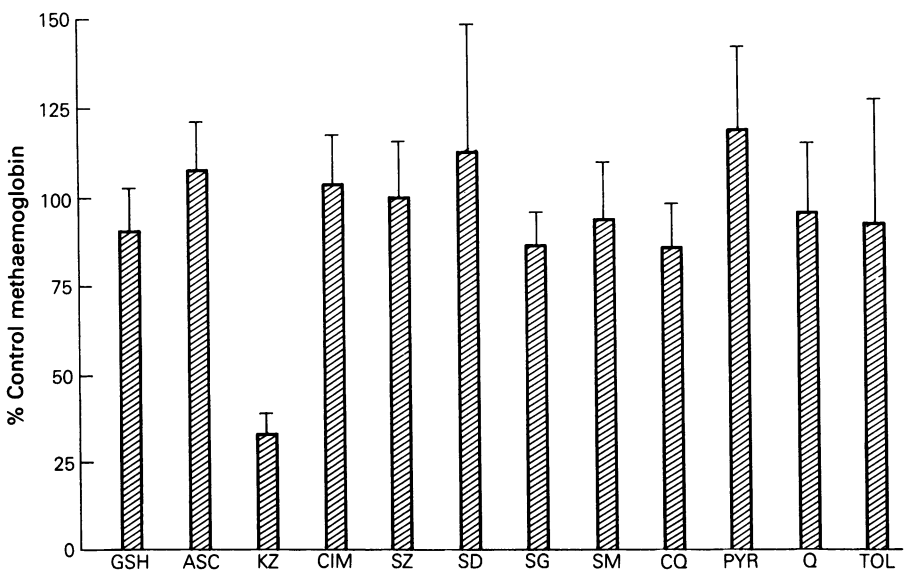

Figure 6 Methaemoglobin formation after co-incubation of dapsone (100 μM) with glutathione (GSH), ascorbate (ASC), ketoconazole (KZ), cimetidine (CIM), sulphadiazine (SZ), sulphadoxine (SD), sulphaguanidine (SG), sulphamethoxazole (SM), chloroquine (CQ), pyrimethamine (PYR), quinidine (Q), or tolbutamide (TOL) (500 μM) in the presence of microsomes prepared from human liver (LI) and NADPH (1 mM). Values are mean ± s.d. (n = 4).

involving *N*-hydroxylation mediated by cytochrome P-450, may account for 30–50% of the dose (Israili *et al.*, 1973; Uehleke & Tabarelli, 1973). The products of *N*-hydroxylation, dapsone-hydroxylamine (DDS-NOH) and the monoacetyl derivative (MADDS-NOH) have been implicated as the metabolites responsible for the haematological disorders observed with dapsone therapy (Glader & Conrad, 1973; Grossman & Jollow, 1988).

The adverse reactions to dapsone are not as frequent as would be expected considering the fact that the human livers activate the drug to such a toxic metabolite (Coleman *et al.*, 1989). Indeed, although the liver is the major site of dapsone metabolism, hepatotoxicity has been observed only when the dose exceeded 300 mg day^{-1} (Barnes & Barnes, 1951). One reason for this may be the high concentration of glutathione in the liver (> 6 mM) which may prevent further oxidation of dapsone-hydroxylamine to the nitroso derivative. Uetrecht *et al.* (1988) have demonstrated that dapsone may be oxidized *in vitro* by activated polymorphonuclear leucocytes to 4-amino-4′-nitro-diphenylsulphone via the toxic hydroxylamine and nitroso intermediates, which might account for the cell-specific toxicity observed. However, the incidence of agranulocytosis due to dapsone administration is idiosyncratic and relatively rare (< 1 in 2000), whilst methaemoglobin formation and reduction in erythrocyte life-span is dose-dependent and occurs to some extent in all subjects who take the drug (Zuidema *et al.*, 1986).

In the present study we have utilized a two compartment system to evaluate the ability of dapsone to cause methaemoglobin formation in the presence of microsomes prepared from four human livers. The two compartments are separated from each other by a semi-permeable membrane which allows full equilibrium of dapsone between compartments. After a 1 h incubation, 60–70% of the radioactivity was associated with the red cells, which is in agreement with the results of Israili *et al.* (1973), who reported that approximately 75% of radioactivity was concentrated within the red cells after incubation of dapsone or dapsone-hydroxylamine *in vitro*. Methaemoglobin formation was found to be metabolism (NADPH)-dependent, with no apparent difference between the livers studied in their capacity to metabolize dapsone to a toxic species. This oxidation of haemoglobin to methaemoglobin was not diminished by the addition of either glutathione or ascorbic acid. This is in contrast to the toxicity of dapsone toward human mononuclear leucocytes (MNL), which is abolished completely by the presence of either glutathione or ascorbic acid (Coleman *et al.*, 1989).

This differential toxicity between MNL and red cells may be explained by a difference in the nature of the toxic agent. The ultimate toxic species responsible for MNL toxicity is thought to be nitroso-dapsone, which is reduced to the hydroxylamine by the presence of either glutathione or ascorbic acid (Uetrecht *et al.*, 1988). Addition of these reducing agents therefore leads to a decrease in the toxicity of dapsone metabolites toward MNL. However, in the red cell dapsone-hydroxylamine is co-oxidized with haemoglobin to generate nitroso-dapsone and methaemoglobin (Kramer *et al.*, 1972) (Figure 7). Addition of either glutathione or ascorbic acid, which do not penetrate the cell, serves merely to stabilize the hydroxylamine within the apparatus and does not decrease the toxicity, i.e. methaemoglobin formation. Furthermore, intracellular glutathione reduces nitroso-dapsone to dapsone-hydroxylamine in a futile cycle (Figure 7), so that glutathione depletion within the red cell actually decreases methaemoglobin formation (Glader & Conrad, 1973).

Previous studies have investigated the ability of a variety of *p*-substituted diphenylsulphones to produce methaemoglobin *in vitro* and found that only the *N*-hydroxy derivatives have any direct effect (Israili *et al.*, 1973; Kramer *et al.*, 1972). However, in this study we have shown that several of these putative metabolites of dapsone can themselves oxidize haemoglobin in the presence of a metabolizing system. Although acetylation may be considered as a detoxication pathway, we have shown that in the presence of liver microsomes and NADPH, monoacetyl-dapsone is as potent as dapsone itself in causing methaemoglobin formation *in vitro*, and it is not until both amines are acetylated that no methaemoglobin is observed. The increase in methaemoglobin formation observed in the presence of 4-amino-4′-nitro-diphenylsulphone compared with dapsone suggests that further oxidation of the hydroxylamine through to a nitro would not lead to detoxication. The level of methaemoglobin observed when 4-aminoacetyl-4′-nitro-diphenylsulphone was studied suggests that either the *N*-acetyl moiety can be oxidised to generate an arylhydroxylamine, as proposed by Lenk & Riedl (1989) or that the nitro group is reduced by cytochrome P-450 reductase, as has been implicated in chloramphenicol toxicity (Salem *et al.*, 1981).

In order to reduce the toxicity of dapsone, it is therefore necessary to inhibit *N*-hydroxylation of dapsone in the rat (Coleman *et al.*, 1990) whilst Wright *et al.* (1984) have demonstrated

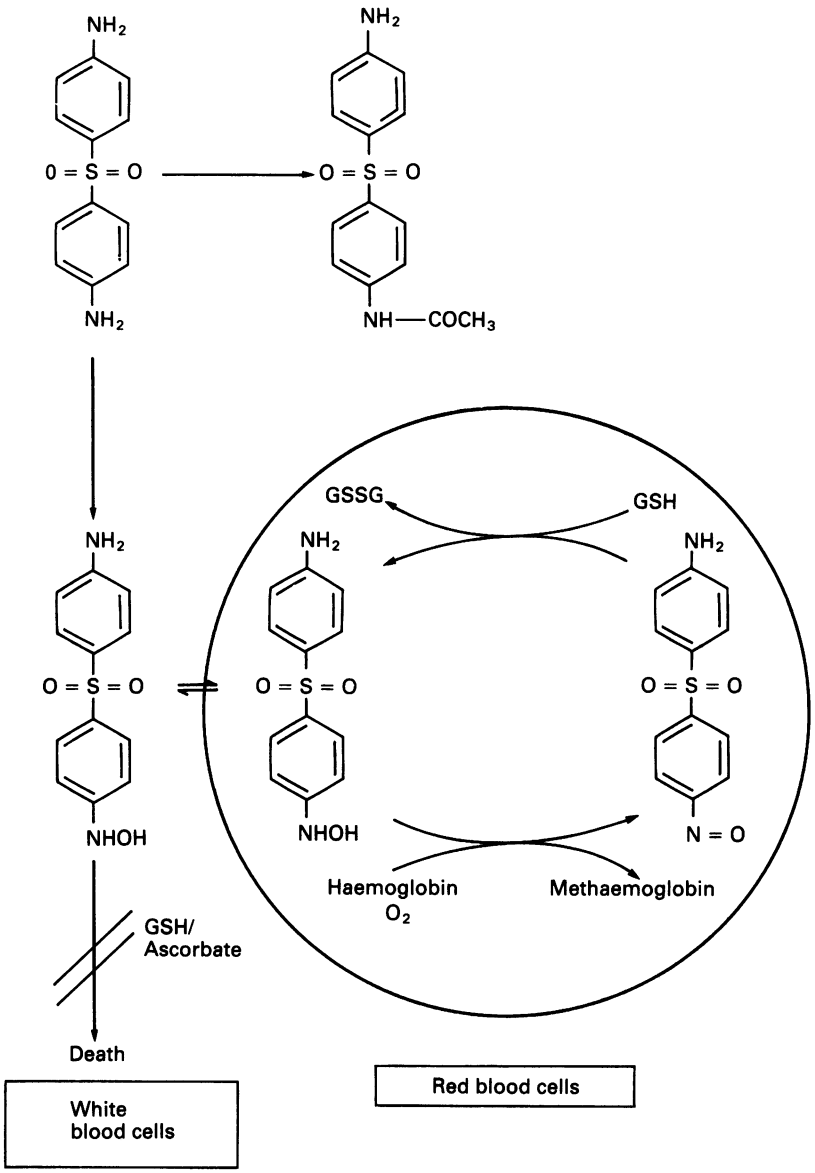

Figure 7 Toxicological consequences of the metabolism of dapsone.

the lack of effect on dapsone *N*-acetylation by cimetidine in man. Despite the inhibition of dapsone *N*-hydroxylation *in vivo*, cimetidine did not cause a reduction in dapsone-dependent methaemoglobin formation in the *in vitro* test system described in this paper. This discrepancy between *in vivo* and *in vitro* inhibition of drug metabolism has been observed previously (Mitchell *et al.*, 1981; Pelkonen & Puurunen, 1980; Reilly *et al.*, 1983) and has been attributed to either the generation of a minor metabolite which is a very potent inhibitor of cytochrome P-450, or the formation of a ligand interaction between cimetidine and cytochrome P-450 which requires the presence of NADPH (Jensen & Gugler, 1985). Although we did not detect any inhibition of dapsone metabolism in the presence of cimetidine in these experiments, it may be

possible to use the method described to investigate the inhibitory potential of putative metabolites of cimetidine in the future.

Several other drugs, which possess similar structural and/or biochemical properties were screened for a potentially beneficial pharmacokinetic interaction with dapsone. However, of the compounds tested, only ketoconazole, a potent antifungal agent which has been reported to be an inhibitor of cytochrome P-450-mediated drug metabolism (Sheets & Mason, 1984), appeared to decrease methaemoglobin formation.

It is of interest to note that the presence of the sulphonamides investigated, sulphadiazine, sulphadoxine, sulphaguanidine and sulphamethoxazole neither decreased nor increased the level of methaemoglobin measured, which indicates that they do not undergo *N*-hydroxylation as readily as dapsone *in vitro*.

Pretreatment of rats with either phenobarbitone or β-naphthoflavone appeared to have no effect on the ability of microsomes prepared from rat livers to metabolize dapsone. This finding is in agreement with *in vivo* results in the rat, which suggest that the cytochrome P-450 responsible for dapsone metabolism in the rat is a non-inducible and sex (male)-specific enzyme (Coleman *et al.*, 1990).

In conclusion, we have developed a test system in which the toxicity of dapsone has been examined using human tissues. Using this method it is possible to investigate the potential toxicity of novel compounds and to examine both the chemical nature of the toxic species generated and reasons for cell-selective toxicity. This technique may also be applied to the evaluation of various strategies to decrease adverse reactions associated with drug administration by either chemical modification or selection of multidrug regimens which will result in biochemical and pharmacological synergism.

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