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An Investigation into the Haematological Toxicity of Structural Analogues of Dapsone In-vivo and In-vitro

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Abstract—With microsomes prepared from a single human liver, 4,4′-diaminodiphenyl sulphone (DDS), 4-acetyl-4-aminodiphenyl sulphone (MADDS), 4-acetyl-4-aminodiphenyl thioether (MADDT) and 4,4′-diacetyldiphenyl thioether (DADDT) caused significantly greater methaemoglobin formation compared with control. In-vitro in the rat, the pattern of toxicity was slightly different: DADDT was not haemotoxic, whilst 3,4′-diaminodiphenyl sulphone (3,4′DDS) and 3,3′-diaminodiphenyl sulphone (3,3′DDS) as well as DDS, MADDS and MADDT were significantly greater than control. 4,4′ Acetyl diphenyl sulphone (DADDS), 4,4′ diaminodiphenyl thioether (DDT), 4,4′-diaminodiphenyl ether (DDE) and 4,4′ diamino-octofluorodiphenyl sulphone (F_8DDS) did not cause significant methaemoglobinaemia in either human or rat liver microsomes. DDS, MADDS, and MADDT were not significantly different in haemotoxicity generation in-vitro in the presence of human microsomes. In the rat in-vitro, DDS, MADDS, and 3,4′DDS did not differ significantly in red cell toxicity, and were the most potent methaemoglobin formers. The 3,3′ DDS and MADDT derivatives were both significantly less toxic compared with DDS. None of the compounds tested caused haemoglobin oxidation in the absence of NADPH in-vitro. In the whole rat, DDS, MADDS and MADDT caused significantly higher levels of methaemoglobin compared with control. None of the remaining compounds caused methaemoglobin formation which was significantly greater than control. DDS and MADDS were the most potent methaemoglobin formers tested, in-vivo and in-vitro. The 3,3′ and 3,4′DDS analogues caused no detectable haemotoxicity in-vivo. However, the plasma elimination of the 3,4′ analogue was much more rapid compared with that of DDS. Overall, there was no correlation between log k_0 and increasing haemotoxicity. The use of the two-compartment system together with in-vivo studies may be applied to the evaluation of the structural features required for bioactivation of candidate antiparasitic compounds to haemotoxic metabolites by cytochrome P450 enzymes.

Dapsone has been used as an antileprotic for over 40 years and is still effective as part of a triple drug combination including rifampicin and clofazimine (WHO 1982). The drug is also used in the therapy of dermatitis herpetiformis (Swain et al 1983) and more recently in the treatment of *Pneumocystis carinii* infections in AIDS patients (Green et al 1988; Lee et al 1989).

Dapsone is metabolized extensively in man, mainly through *N*-hydroxylation (Cucinell et al 1972; Israili et al 1973) and acetylation (Gelber et al 1971). Monoacetyl dapsone may also undergo *N*-hydroxylation to form an acetylated hydroxylamine (Uetrecht et al 1988; Grossman & Jollow 1988). Adverse reactions to dapsone may rarely include fatal agranulocytosis (1:2000; Friman et al 1983), although methaemoglobinaemia (Kramer et al 1972) and haemolysis (DeGowin et al 1966) are more common side effects. Adverse reactions to dapsone are primarily caused by the *N*-hydroxylated metabolites of the drug (Kramer et al 1972).

Previous studies into the structure-activity relationships of dapsone have concentrated on the development of more potent anti-microbial sulphones (Pieper et al 1989; Saxena et al 1989). In the present study we have investigated the chemical characteristics of the molecule which predispose it to bioactivation to a toxic metabolite by cytochrome P450 enzyme systems. Specifically, we have modified the sulphone, amine, and aromatic groups in the molecule. We have determined the influence of these different chemical substitutions on the haematological toxicity of these analogues of dapsone in the rat, both in-vivo and in-vitro. The phase I activation of the analogues was determined in-vitro using a two-compartment system as described previously (Tingle et al 1990), whereby either human or rat liver microsomes are maintained separately from human red cells by a semi-permeable membrane.

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Materials and Methods

Chemicals

NADPH (tetra-sodium salt), potassium cyanide, potassium ferricyanide and all other reagents were obtained from BDH Chemicals Ltd (Poole, UK). 4,4′-Diaminodiphenyl sulphone (DDS) was supplied by the Sigma Chemical Co. Ltd (Poole, UK). 3,3′-Diaminodiphenyl sulphone (3,3′DDS) and 3,4′-diaminodiphenyl sulphone (3,4′DDS) were synthesized according to the methods of Baker et al (1950). 4-Acetyl 4-aminodiphenyl sulphone (MADDS), 4,4′-diacetyl diphenyl sulphone (DADDS), 4,4′-diaminodiphenyl thioether, (DDT); 4-acetyl-4′aminodiphenyl thioether (MADDT) and 4,4′-diacetyl diaminodiphenyl thioether (DADDT) were synthesized according to the methods of Raiziss et al (1939). 4,4′-Diaminodiphenyl ether (DDE) was obtained from the Aldrich Chemical Co. (Poole, UK). 4,4′-Diamino-octofluorodiphenyl sulphone (F_8DDS) was synthesized according to the following method. Pentafluoronitrobenzene (2·2 g) was mixed with thiourea (3·1 g) and dimethylformamide (50 mL) was added. The reaction mixture was stirred at room temperature (21 °C) for 90 min, then poured onto water (100 mL) and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with water, dried over

anhydrous sodium sulphate and evaporated under reduced pressure to yield 4,4′-dinitro-octofluorodiphenyl sulphide (60%: 94–95°C mp, m/z 420 (Coe et al 1972)). The sulphide was then dissolved in methanol (20 mL) and 10% palladium on charcoal (5 mg) added. Sodium borohydride was then added carefully until complete reduction had occurred, which was shown by thin layer chromatography. Glacial acetic acid (20 mL) was added to destroy any excess sodium borohydride and the mixture was poured onto water (100 mL) and basified with sodium hydroxide. The product was extracted with dichloromethane, dried over anhydrous sodium sulphate, and evaporated under reduced pressure to yield the yellow crystals of 4,4′-diamino-octofluorodiphenyl sulphide (mp 180°C, m/z 360), 1 g (3 mmol) of which was suspended in trifluoroacetic acid (50 mL) and sodium perborate 4·3 g, 28 mmol) added. The reaction mixture was refluxed for 24 h after which time the trifluoroacetic acid was evaporated under reduced pressure. Water (basified with sodium hydroxide) was added to the resulting solid, which was then extracted with dichloromethane and dried over anhydrous magnesium sulphate. Removal of the solvent under reduced pressure and purification by flash column chromatography (30% ethyl acetate/petroleum ether, bp 40–60°C) yielded 4,4′-diamino-octofluorodiphenyl sulphone (0·7 g, 60%, mp 232–233°C, V_{max} 3300, 1415 and 1210: m/z 392 (M^+), 328, 308, 228 and 212: found C, 36·71: H,1·01: N,7·11. $C_{12}H_4F_8N_2O_2S$ requires C, 36·74: H, 1·03: N,7·14%). All compounds were fully characterized using mass spectrometry, NMR, and IR spectrometry.

In-vitro studies

Washed microsomes from a histologically normal human liver obtained from a renal transplant donor (male, 18 years old) and rat microsomes from the pooled livers of six male Wistar rats (250–350 g) were prepared as described previously (Purba et al 1987). The microsomes (2 mg protein), together with an analogue of dapsone (100 μM) dissolved in dimethyl sulphoxide (5 μL), were placed in compartment A. Compartment B contained washed human red blood cells (RBC) 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 10000 daltons (Diachema, Munich, Germany). Metabolism was initiated by the addition of NADPH (1 mM, omitted from the controls) to compartment A. The final volume in each half was 500 μL. The cells were maintained at 37°C at a constant rotation of 8 rev min^{-1}. After 60 min the RBC were expelled and kept on ice until assayed for methaemoglobin content as described below.

Animal studies

Ten groups of male Wistar rats (n = 4 per group, 250–350 g) were dosed with one of the following: DDS, MADDS, DADDS, 3,4′DDS, 3,3′DDS, DDT, MADDT, DADDT, DDE or F_8DDS (all at the molar equivalent of DDS, 33 mg kg^{-1}) in dimethyl sulphoxide (0·5 mL kg^{-1}). Blood samples (300 μL) were taken from the tail artery under light diethyl ether anaesthesia at 0, 1, 2, 3, and 5 h and analysed for methaemoglobin formation by the method of Harrison & Jollow (1986). The disposition of dapsone and its 3,4′-isomer was also determined by HPLC (Grossman & Jollow 1988).

Determination of methaemoglobin

All samples were assayed for methaemoglobin relative to haemoglobin using the spectrophotometric technique of Harrison & Jollow (1986). Briefly, a blood sample (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 samples (1·25 mL) and one drop of aqueous 10% KCN added to samples 2 and 4. One drop of aqueous 50% $K_3Fe(CN)_6$ was added to samples 3 and 4. The absorbance of each sample 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.

Determination of relative lipophilicity

In order to determine the relative lipophilicity of the analogues of dapsone, capacity ratios (k′) were derived from chromatographic (HPLC) analysis of each compound (Koneman et al 1979). Each compound was analysed using three mobile phase systems; 35:65, 30:70 and 25:75 methanol:phosphate buffer (pH 7·4), each flowing at 1 mL min^{-1}. The mobile phases were continually degassed with helium. Analysis was achieved using a μBondapak C_{18} stainless steel column (30 cm × 0·39 cm i.d. 10 μM, Waters Assoc., Hartford, Cheshire, UK). The capacity ratio (k′) is proportional to the octanol-water partition coefficient, and was calculated as follows:

$$k' = \frac{(V_r - V_0)}{V_0}$$

where V_r is the retention volume of the compound, V_0 is the retention volume of methanol. Hence dead volumes were corrected for by means of the retention volume of methanol. Measurements were made in duplicate. The log k_0 (i.e. the capacity ratio at pH 7·4) was calculated by extrapolation after the individual log k′ values were plotted against the decreasing methanol value. A high log k_0 is associated with a strongly lipophilic compound.

Chromatography

DDS and 3,4′DDS concentrations in rat blood were determined by the method of Grossman & Jollow (1988) using a μBondapak C_{18} column. The limit of detection was 200 ng mL^{-1} for DDS and 250 ng mL^{-1} for 3,4′ DDS from a whole blood sample (100 μL). The HPLC system employed was a SpectraPhysics Unit, 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.

Calculations and statistical analysis

The area under the curves ($AUC_{0\text{–}5}$) for blood levels DDS and for % methamoglobinaemia were each calculated from t = 0 to t = 5 h by the use of the trapezoidal rule (Gibaldi &

Perrier 1982; 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 in the text as mean ± s.d.

Results

Of the range of compounds tested (Fig. 1) in-vitro with human liver microsomes, DDS, MADDS, MADDT and DADDT caused significantly greater methaemoglobin formation compared with control. In-vitro in the rat, the pattern of toxicity differed slightly: although DADDT was not haemotoxic, 3,4′DDS and 3,3′DDS as well as DDS, MADDS and MADDT caused significantly greater methaemoglobin formation than control. DDT, DADDS and F_8DDS did not cause significant methaemoglobinaemia in either human or rat liver microsomes.

DDS, MADDS, and MADDT were not significantly different in haemotoxicity generation in-vitro with human microsomes. However, MADDS, DDS and MADDT were significantly more toxic compared with DADDS. MADDS was also significantly more toxic than DADDT. In the rat in-vitro, DDS, MADDS, and 3,4′DDS did not differ significantly in red cell toxicity, and were the most potent methaemoglobin formers. The 3,3′DDS and MADDT derivatives were both significantly less toxic compared with DDS. None of the compounds tested caused haemoglobin oxidation in the absence of NADPH in-vitro.

In the whole rat, DDS, MADDS and MADDT caused significantly higher levels of methaemoglobin compared with control. None of the remaining compounds caused methaemoglobin formation significantly greater than control. DDS and MADDS were the most potent methaemoglobin formers tested, both in-vivo and in-vitro, although their respective activities did not differ significantly.

The 3,3′- and 3,4′DDS analogues caused no detectable haemotoxicity in-vivo. However, further investigation revealed that the plasma disposition of the 3,4′-analogue contrasted sharply with that of DDS. The $AUC_{0\text{-}5}$ and $t_{\frac{1}{2}}$ for DDS was 15.2 ± 3.2 μg h mL^{-1} and 8.2 ± 2.1 h, respectively, whilst the 3,4′-analogue of DDS showed peak measured plasma concentrations at 1 h of 2.4 ± 0.5 μg mL^{-1} (compared with 9.1 ± 3.1 μg mL^{-1} for DDS) but was not detectable at subsequent time points.

Overall, there was no correlation between the determined log k_0 and haemotoxicity (Table 1). While the haemotoxicity generated by DDS and MADDS was similar in-vivo and in-vitro, there was a marked difference in their respective log k_0 values. However, when taken in isolation, the thioether derivatives showed that increasing lipophilicity resulted in increasing toxicity.

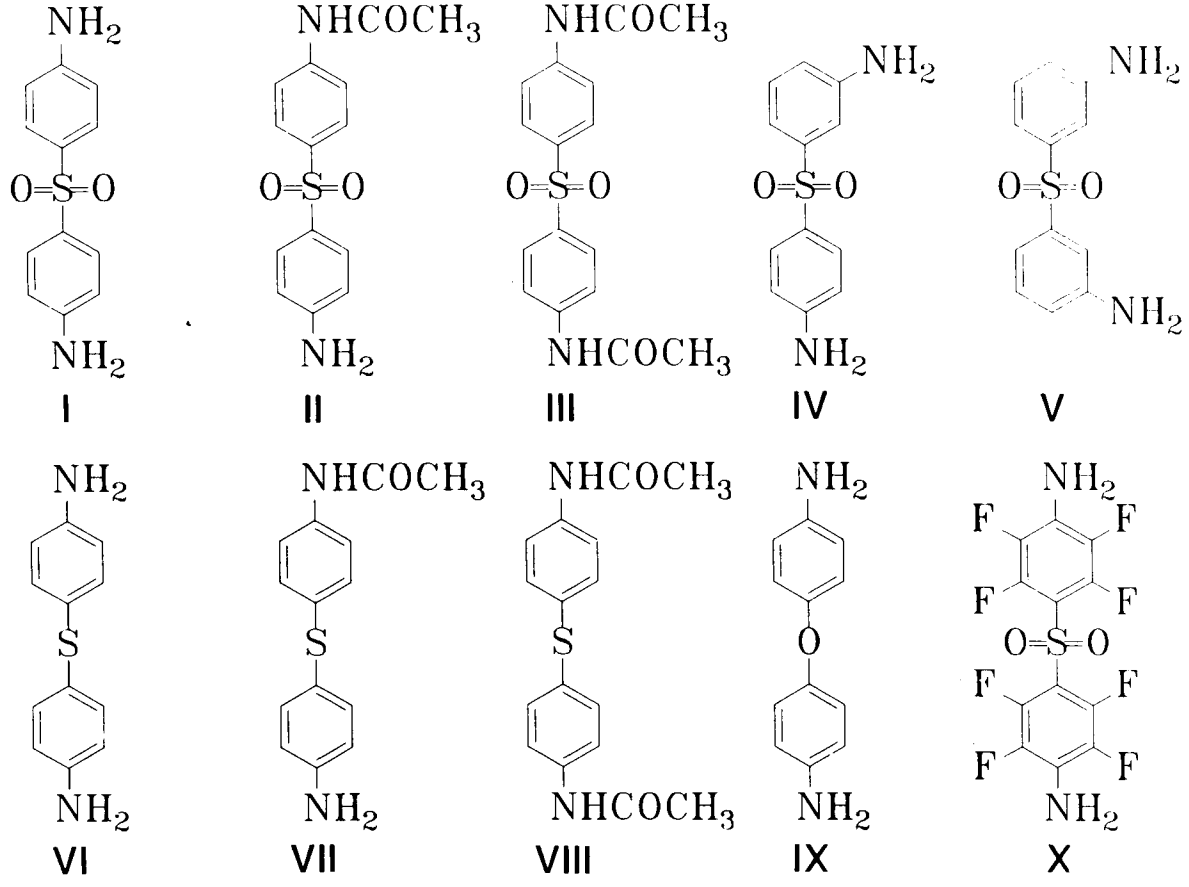

FIG. 1. Structures of 10 analogues of dapsone (DDS): I, DDS: II, MADDS: III, DADDS: IV, 3,4′DDS: V, 3,3′DDS: VI, DDT: VII, MADDT: VIII, DADDT: IX, DDE: X F_8DDS.

Table 1. Methaemoglobin (Met Hb) levels generated in-vitro with human and rat microsomal systems (n = 4 per observation) and in-vivo in the rat (n = 4 per compound) by 10 structural analogues of dapsone.

	Microsomal NADPH-dependent Met Hb formation				
	Human (%)	Rat (%)	Rat In-vivo Met Hb ($AUC_{0\ 5h}$)	Log k_0	Rank of Log k_0
Control	0·74 ± 1·2	1·0 ± 0·5	16·1 ± 7·1		
DDS	6·8 ± 1·2**	27·1 ± 5·2*	127·0 ± 13·1†	1·16	10
MADDS	9·9 ± 2·6**	32·2 ± 4·2*	143·2 ± 37·1†	2·47	5
DADDS	2·0 ± 2·0	1·2 ± 1·4	28·7 ± 8·5	2·18	6
3'4 DDS	2·8 ± 0·9	23·2 ± 4·2*	28·7 ± 11·3	1·69	9
3'3 DDS	1·2 ± 0·9	15·1 ± 3·9*	13·0 ± 1·2	1·78	8
DDT	2·1 ± 1·4	3·8 ± 2·9	27·1 ± 11·1	2·5	4
MADDT	6·8 ± 1·9**	10·8 ± 2·9*	52·5 ± 14·3†	3·7	1
DADDT	4·2 ± 1·6**	3·0 ± 5·5	31·8 ± 4·9	3·46	2
DDE	0·6 ± 0·9	1·7 ± 3·2	19·7 ± 10·3	1·88	7
F_8DDS	0·9 ± 0·64	0·1 ± 0·05	15·3 ± 3·8	2·6	3

**,*,† denote significant ($P < 0·05$) difference compared with the respective control.

Discussion

Although DDS remains effective in the treatment of a number of bacterial and protozoan infections its clinical use can be limited by its haemotoxicity. This is ultimately caused by phase I *N*-hydroxylation of the drug, which may account for up to half its metabolism (Israili et al 1973). The hydroxylamine thus formed is a potent generator of methaemoglobin (Tingle et al 1990), is cytotoxic (Coleman et al 1989) and toxicity from a clinical dose would be very severe without extensive phase II glucuronidation (Gordon et al 1979). In order to study potentially haemotoxic compounds such as DDS, we have adopted a two stage procedure. Firstly, methaemoglobin formation was monitored in-vitro using a two compartment system to determine the effect of chemical modification on the oxidative bioactivation of these compounds. Secondly, methaemoglobin formation was measured in-vivo to assess the effect of chemical modification on the balance between drug bioactivation and drug clearance through excretion and metabolism.

We found the monoacetylated derivative of DDS to be non-toxic in the absence of metabolism, but in-vivo and in the presence of an activating system, it is equitoxic with the parent compound. This is in agreement with previous studies (Coleman et al 1990b; Tingle et al 1990), as MADDS could be considered a protoxin since the remaining aromatic amino group may be rapidly *N*-hydroxylated to MADDS hydroxylamine (Grossman & Jollow 1988). In contrast, acetylation of both amine groups (DADDS) results in a lack of haemotoxicity; however, DADDS is only formed in small quantities in man (Zuidema et al 1986). Both MADDS and DADDS are considerably less potent than DDS as antibacterials (Bawden & Tute 1981; Venkatesan 1989).

The position of the amine group is an important determinate of both activity and toxicity. Amino groups in the 4 and 4' positions are thought to be optimal for maximum activity in in-vitro test systems using *Mycobacteria* (Bawden & Tute 1981). The 3,4' analogue of dapsone has less than 1/6th of the activity of DDS (Koehler & Hopfinger 1988), while the 3, 3' analogue is without activity (Colwell et al 1974). In our test system, the 3,3' and 3,4' analogues were not bioactivated by microsomes from a single human liver but were activated in-vitro by rat microsomes to haemotoxic species which were stable enough to cross a membrane and cause methaemoglobinaemia at some distance away from the site of formation. This differential bioactivation of the isomers of DDS between the rat and man may reflect selective metabolism by cytochrome P450 isozymes which are expressed in the two species. Studies in the rat in-vivo have shown that cimetidine inhibits the *N*-hydroxylation of DDS (Coleman et al 1990a) but not of MADDS (Coleman et al 1991). Hence, it is possible that in the rat, compounds as closely related as MADDS and DDS are metabolized to hydroxylamine derivatives by different cytochrome P450 isozymes.

Interestingly, the 3,3'- and 3,4'DDS analogues were without toxicity in the rat in-vivo. In the case of the 3,4' analogue, this lack of toxicity may be due to the plasma clearance, which was far in excess of that of DDS. It is unlikely that these compounds underwent extensive renal clearance unchanged, as they were both more lipophilic than DDS and therefore they were probably rapidly biotransformed. As the 3,4' derivative undergoes considerable in-vitro phase I metabolism and is cleared rapidly from plasma, this would suggest that phase II metabolism was extensive. The lack of detectable systemic haemotoxicity may also suggest that the phase I derivatives of the 3,4' and 3,3' analogues are better substrates for phase II detoxication systems in the rat than DDS and MADDS hydroxylamines. It may be postulated that the assessment of the potential toxicity of novel compounds must take into account structure-activity relationships between phase I activation products and phase II detoxication pathways.

The intrinsic antimicrobial activity of sulphone compounds tends to parallel the negative charge on the oxygen moieties of the sulphone group (Pieper et al 1989). Reduction of this group to sulphite or to sulphides results in loss of activity (Popoff et al 1971). In the present study, replacement of the sulphone group with either sulphur (DDT) or oxygen (DDE) blocked methaemoglobin formation in-vitro and in-vivo, despite the fact that the greater electron density on nitrogen in these compounds should facilitate oxidation of the nitrogen group (Shusterman et al 1990). In fact, molecules which are more hydrophobic are generally more rapidly bioactivated (Shusterman et al 1990). However, with the exception of the monoacetylated derivative, the haemotoxicity of the thioethers was relatively low despite their high lipophilicity. The thioether derivative of DDS is a potent carcinogen in rats and mice (Gordon et al 1979), requiring metabolic activation to cause maximum mutagenicity; although the chemical structure of the mutagen has not been elucidated. DDS is not thought to undergo reduction to the thioether in man (Gordon et al 1979).

Both monoacetylation and diacetylation of the thioether derivatives caused significant increases in haemotoxicity; in-vitro in the case of both MADDT and DADDT with human microsomes, and in-vitro and in-vivo with MADDT in the rat. These observations, together with the increase in lipophilicity seen in DDS after it is monoacetylated, would suggest that in the case of DDS, cytosolic acetylation cannot

be considered to be a detoxication pathway. Indeed it has been suggested that *N*-acetyl groups may be oxidized by cytochrome P450 enzymes to arylhydroxylamines (Lenk & Riedl 1989) and arylhydroxamic acids (Mattano et al 1989). If this were to occur it may lead to toxicity other than methaemoglobin formation.

It is apparent that the structural features of DDS which contribute to its haemotoxicity, i.e. the free 4 and 4′ amine groups as well as the sulphone group, confer activity on the molecule against both bacteria and protozoans. Further modification of these moieties may reduce toxicity but would cause unacceptable loss of activity. However, recent studies have indicated that ring substitution in the 2′ position causes increased potency without increasing toxicity (Pieper et al 1989; Saxena et al 1989). In the present report, the octofluoro-ring-substituted derivative is without detectable haematological toxicity, either in-vitro with human or rat microsomes, or in-vivo in the rat, although its antibacterial activity is unknown.

In summary, the use of a two-compartment system which contains human tissues, in combination with animal studies, may be applied to the screening of the structural features required for bioactivation of candidate antiparasitic compounds to haemotoxic metabolites by cytochrome P450 enzymes. It would be beneficial in toxicity testing to study the phase I and II biotransformations of novel antiparasitic compounds using human tissues in-vitro as early as possible, in order to select the animal model which most closely resembles human hepatic metabolism.

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References

Baker, B. R., Kadish, A. F., Querry, M. V. (1950) Sulphones. 1. The isomeric X,X′ diaminodiphenyl sulphones. J. Org. Chem. 15: 400–401

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

Coe, P. L., Milner, N. E., Tatlow, J. C., Wragg, T. (1972) Aromatic polyfluoro-compounds LIII. Reactions of polyfluoro-arenes with thioureas. Tetrahedron 28: 105–109

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. Pharmacol. 28: 389–395

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

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

Coleman, M. D., Tingle, M. D., Park, B. K. (1991) Inhibition of dapsone-induced methaemoglobinaemia by cimetidine in the rat during chronic dapsone administration. Ibid. 43: 186–190

Colwell, W. T., Chan, G., Brown, V. H., McGraw, J. I., Peters, J. H. (1974) Potential antileprotic agents. 1. Inhibition of a model mycobacterial system by diaryl sulfones. J. Med. Chem. 17: 142–144

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. WHO 35: 165–179

Friman, G., Nystrom-Rosander, C., Jonsell, G., Bjorkman, A., Lekas, G., Sendsrup, B. N. (1983) Agranulocytosis associated with malarial prophylaxis with maloprim. Br. Med. J. 286: 1244–1245

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

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics. 2nd edn, Marcel Dekker, New York, pp 445

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

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

Grossman, S. J., Jollow, D. J. (1988) Role of DDS NOH in dapsone-induced haemolytic anaemia. J. Pharmacol. Exp. Ther. 244: 118–125

Harrison, J. H., Jollow, D. J. (1986) Role of aniline metabolites in aniline-induced hemolytic anemia. Ibid. 238: 1045–1054

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. Ibid. 187: 138–151

Koehler, M. G., Hopfinger, A. J. (1988) A comparison of QSARS proposed for the inhibition of dihydropteroate synthetase by substituted 4-aminodiphenylsulphones. J. Mol. Struct. 179: 319–332

Koneman, H., Zelle, R., Busser, F., Hammers, W. E. (1979) Determination of log Poct values of chlorosubstituted benzenes and toluenes and anilines by high-performance liquid chromatography on ODS-silica. J. Chromatogr. 178: 559–565

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

Lee, B. L., Medina, I., Benowitz, N. L., Jacob, P., Wofsy, C. B., Mills, J. (1989) Dapsone, trimethoprim and sulfamethoxazole plasma levels during treatment of pneumocystis pneumonia in patients with the acquired immunodeficiency syndrome (AIDS). Ann. Intern. Med. 110: 606–611

Lenk, W., Riedl, M. (1989) N-Hydroxy-N-arylacetamides V. Differences in the mechanism of haemoglobin oxidation in vitro by N-hydroxy-4-chloroacetoanilide and N-hydroxy-4-chloraniline. Xenobiotica 19: 453–475

Mattano, S. S., Land, S., King, C. M., Webber, W. (1989) Purification and biochemical characterization of hepatic arylamine N-acetyltransferase from rapid and slow acetylator mice: identity with arylhydroxamic acid N,O-acyltransferase and N-hydroxylamine O-acetyltransferase. Mol. Pharmacol. 35: 599–609

Pieper, H., Seydel, J. K., Kruger, G., Noll, K., Keck, J., Wiese, M. (1989) Preparation and biological activity of new substituted antimalarial diaminodiphenylsulphones. Arzneim. Forsch. 39: 1073–1080

Popoff, I. C., Singhal, G. H., Engle, A. R. (1971) Antimalarial agents and compounds related to 4,4′-bis(aminophenyl) sulfone. J. Med. Chem. 14: 550–551

Purba, H. S., Maggs, J. L., Orme, M. L'e., Back, D. J., Park, B. K. (1987) The metabolism of 17α-ethinyloestradiol by human liver microsomes: formation of catechol and chemically reactive metabolites. Br. J. Clin. Pharmacol. 23: 447–453

Raiziss, G. W., Clemance, L. W., Severac, M., Moetsch, J. C. (1939) Chemistry and chemotherapy of 4,4′ diaminodiphenyl sulphone 4-amino-4′hydroxydiphenyl sulphone and related compounds. J. Am. Chem. Soc. 61: 2763–2765

Saxena, M., Saxena, A. K., Raina, R., Chandra, S., Sen, A. B., Anand, N., Seydel, J. K., Wiese, M. (1989) Studies on 2,3, N,N′-substituted- diaminodiphenylsulphones as potential antimalarial agents. Arzneim. Forsch. 39: 1081–1084

Shusterman, A. J., Debnath, A. K., Hansch, C., Horn, G. W., Fronczek, F. R., Greene, A. C., Watkins, S. F. (1990) Mutagenicity of dimethyl heteroaromatic triazines in the Ames test: the role

of hydrophobicity and electronic effects. Mol. Pharmacol. 36: 939–944

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

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. Pharmacol. 30: 829–838

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. Pharm. Exp. Ther. 245: 274–279

Venkatesan, K. (1989) Clinical pharmacokinetic considerations in the treatment of patients with leprosy. Clin. Pharmacokin. 16: 365–386

World Health Organisation (1982) Chemotherapy of leprosy for control programmes. Report of A WHO study group. WHO Techn. Rep. Ser. No. 675

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