

Comparison of the Metabolism and Toxicity of Dapsone in Rat, Mouse and Man¹

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ABSTRACT

The metabolism and toxicity of dapsone was compared *in vitro* and *in vivo* in rat, mouse and man. Metabolism was assessed by high-pressure liquid chromatography-mass spectrometry and methemoglobin formation has been used as a toxic endpoint. The greatest toxicity *in vitro* was seen in microsomes prepared from male Wistar rats ($36.6 \pm 1.5\%$ methemoglobin), although toxicity was also seen in microsomes from the female rat ($8.2 \pm 1.3\%$), male CD1 ($4.2 \pm 1.6\%$) and human ($10.9 \pm 1.1\%$). The rank order of toxicity agreed with the formation of the hydroxylamine metabolite *in vitro*. All microsomes were also capable of catalyzing the reverse reaction, *i.e.*, reduction of the

hydroxylamine to dapsone. However, *in vivo* administration of dapsone resulted in significant ($P < 0.05$) methemoglobinemia only in male rats and humans. This species difference in the susceptibility to dapsone toxicity could not be attributed solely to the sensitivity of the target erythrocytes, because the order of sensitivity to dapsone hydroxylamine was human > mouse > rat. Analysis of bile and urine revealed the formation of dapsone hydroxylamine and its glucuronide in male rats and humans, but not in female rats or mice. This species difference in the metabolism and toxicity of dapsone has important implications in the safety evaluation of related compounds for man.

Dapsone has long been used in the treatment of a wide range of diseases such as leprosy (Vadher and Lalljee, 1992), malaria (Shanks *et al.*, 1992) and dermatitis herpetiformis (Prussick *et al.*, 1992). Dapsone has been evaluated in the prevention and treatment of *Pneumocystis carinii* and *Toxoplasma gondii* infections in HIV-positive patients (Jorde *et al.*, 1993; Girard *et al.*, 1993). The mechanism of the antibacterial action for dapsone is similar to that of the sulfonamides, in that it is an antagonist of *para*-aminobenzoic acid in folate synthesis. Its action as an anti-inflammatory agent and in a variety of skin disorders is because of inhibition of neutrophil adherence and function (Booth *et al.*, 1992; Bozeman *et al.*, 1992; Thuong-Nguyen *et al.*, 1993).

The effective clinical use of dapsone is limited because of dose-dependent adverse hematological reactions, even at the low daily dosages of 100 mg in the chemotherapy of leprosy and dermatological conditions (Coleman and Tingle, 1992). In addition, patients with a genetic deficiency of certain

enzymes involved in the process of toxicity are more susceptible to the hematological effects even at therapeutic dosage. The most common adverse reaction is methemoglobinemia, which is significant at a daily dosage of 100 mg in phenotypically normal patients and is severe in patients with a deficiency of NADH-dependent methemoglobin reductase (Ganer *et al.*, 1981). Heinz-body formation and reduced life-span of the red blood cells, which occur in patients receiving chronic dapsone therapy, and in particular those with deficiencies in either glucose-6-phosphate dehydrogenase or glutathione reductase activity, are also important (De Gowin *et al.*, 1966).

The hemotoxicity of dapsone has been shown to be a consequence of N-hydroxylation to yield the hydroxylamine (Hjelm and DeVerdier, 1965; Glader and Conrad, 1973; Grossman and Jollow, 1988). This biotransformation is catalyzed either by hepatic enzymes such as cytochrome P450 (Uehleke and Tabarelli, 1973), flavin monooxygenase and prostaglandin H synthetase in liver, or by myeloperoxidase found in peripheral polymorphonuclear leukocytes (Utrecht *et al.*, 1988).

To overcome the problem of hemotoxicity, several groups have attempted to synthesize structural analogs of dapsone that have greater pharmacological activity than dapsone. The activity of dapsone analogs has been assessed *in vivo* against *Plasmodium berghei* in the mouse (Popoff *et al.*,

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ABBREVIATIONS: NADPH, nicotinamide adenine dinucleotide phosphate; DMSO, dimethyl sulfoxide; LCMS, liquid chromatography-mass spectrometry; HPLC, high-performance liquid chromatography; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; i.p., intraperitoneal; i.v., intravenous.

1971a, b), in cell culture (Colwell *et al.*, 1974; de Benedetti *et al.*, 1987) and in cell-free systems (Wiese *et al.*, 1987). However, toxicity data for any of these analogs is limited (Coleman *et al.*, 1996). The *in vivo* toxicity of dapsone has generally been studied in the rat (Coleman *et al.*, 1990a,c), whereas liver preparations from several species have been investigated for their ability to N-hydroxylate dapsone (Hjelm and DeVerdier, 1965; Uehleke and Tabarelli, 1973; Tingle and Park, 1993).

This study investigated the role of metabolism of dapsone in the pharmacokinetics and toxicity of the compound *in vivo* with use of the rat, mouse and man. Furthermore, we used *in vitro* techniques to compare the effects of metabolism by rat and mouse liver preparations with human liver enzymes to assess the relevance of the *in vivo* findings in animals to the situation in man.

Methods

Materials. Dapsone, 3,3'-diaminodiphenyl sulfone, potassium ferricyanide and reduced NADPH were from Sigma Chemical Co. Ltd. (Poole, UK). Potassium cyanide was obtained from BDH Chemicals Ltd. (Poole, UK). HPLC solvents and other reagents were purchased from Fischer Scientific Ltd. (Loughborough, UK). 4-Amino-4'-nitrodiphenylsulfone was prepared by the method of Raiziss *et al.* (1939) and reduced with a palladium-carbon catalyst (Entwistle *et al.*, 1978) to yield dapsone hydroxylamine, which was found to be pure by thin layer chromatography and HPLC and the structure was confirmed by mass spectrometry and nuclear magnetic resonance.

Toxicity and plasma kinetics of dapsone in the rat and mouse. Dapsone ($100 \mu\text{mol}\cdot\text{kg}^{-1}$) in DMSO ($1 \text{ ml}\cdot\text{kg}^{-1}$ i.p.) was administered to male (200–280 g, $n = 6$) and female Wistar rats (200–230 g, $n = 4$) or male CD1 mice (52–64 g, groups of $n = 4$). Blood samples ($400 \mu\text{l}$), obtained from the tail arteries of rats under diethyl ether anesthesia or from one group of mice *via* the brachial artery, were taken at 0, 1, 2, 3, 5, 8 and 24 h after dosing. Blood ($100 \mu\text{l}$) was assayed immediately for methemoglobin content (Harrison and Jollow, 1986). Plasma was prepared by centrifugation ($3000 \times g$, 5 min) and an aliquot ($100 \mu\text{l}$) was spiked with internal standard (3,3'-diaminodiphenyl sulfone, $1 \mu\text{g}$) before extraction with ethyl acetate ($2 \times 1 \text{ ml}$). The organic extracts were combined, solvent removed under a gentle stream of nitrogen and the residue dissolved in methanol ($100 \mu\text{l}$). An aliquot ($50 \mu\text{l}$) of this solution was then injected onto an octadecyl-bonded silica column (Spherisorb 5 μm ODS2, $25 \times 0.46 \text{ cm}$ internal diameter [i.d.]) and the compounds eluted with a mobile phase of water/acetonitrile/acetic acid/triethylamine (80:20:1:0.1 v/v) flowing at $1.2 \text{ ml}\cdot\text{min}^{-1}$. The eluate was monitored at 254 nm.

Metabolism and excretion of [^{14}C]dapsone in the rat. Male ($n = 8$) or female ($n = 6$) Wistar rats (200–280 g) were anesthetized with urethane ($1.4 \text{ g}\cdot\text{kg}^{-1}$) in saline ($1 \text{ ml}\cdot\text{kg}^{-1}$ i.p.). Appropriate polypropylene cannulae were inserted into the trachea, carotid artery and bile duct. For male rats, the penis was ligated. Animals were administered [^{14}C]dapsone ($100 \mu\text{mol}$, $3 \mu\text{Ci}$) in DMSO ($1 \text{ ml}\cdot\text{kg}^{-1}$) *via* the carotid artery and bile collected in 30-min fractions for 300 min. After this time, urine was collected from the bladder. Aliquots ($2 \times 50 \mu\text{l}$) of bile or urine were assayed for radioactive content after the addition of scintillant (4 ml).

Metabolism and excretion of [^{14}C]dapsone in the mouse. Male ($n = 8$) CD1 mice (20–25 g) were anesthetized with pentobarbitone ($70 \text{ mg}\cdot\text{kg}^{-1}$) in saline ($1 \text{ ml}\cdot\text{kg}^{-1}$ i.p.). Appropriate polypropylene cannulae were inserted into the trachea, carotid artery and bile duct, and the penis was ligated. Animals were administered [^{14}C]dapsone ($100 \mu\text{mol}$, $3 \mu\text{Ci}$) in DMSO ($1 \text{ ml}\cdot\text{kg}^{-1}$) *via* the carotid artery, and bile was collected in 30-min fractions for 180 min. After

this time, urine was collected from the bladder. Biological fluids were analyzed for radioactive content as described above.

Analysis of bile and urine by reverse-phase HPLC linked to mass spectrometry. Aliquots of bile or urine ($10 \mu\text{l}$) were eluted from a μ Bondapak C_{18} column ($30 \times 0.46 \text{ cm}$ i.d.) with acetonitrile/20 mM ammonium formate, pH 3.5. The mobile phase consisted of 7.5% acetonitrile for 10 min, followed by a linear increase up to 25% over 10 min. The flow rate was $1.2 \text{ ml}\cdot\text{min}^{-1}$. Two Jasco PU980 pumps were linked to an HG-980–30 mixing module. Eluate passed through a Jasco UV-975 absorbance detector (254 nm), and thence, *via* a stream splitter, to either a radiometric detector (A200, Canberra Packard, Reading, UK) or to the electrospray probe and interface of a Quattro II mass spectrometer (Fisons Biotech MS, Manchester, UK). The splitter and probe were connected by 1.5 m of $75\text{-}\mu\text{m}$ fused silica capillary. Nebulizing and drying gas (nitrogen) were delivered at 13 liters/h and 300 liters/h, respectively. The interface temperature was 60°C ; the capillary voltage was $4 \times 10^3 \text{ V}$. Compressed centroid spectra were acquired between m/z 100–650 with a scan duration of 4 or 5 s; the photomultiplier voltage was 530 V. Fragmentation of analyte ions was enhanced by increasing the cone voltage.

Toxicity and plasma kinetics of dapsone in humans. The pharmacokinetics and toxicity of a single dose (100 mg) of dapsone were determined in five male volunteers (65–93 kg). The study was approved by the local ethics committee, and all volunteers gave informed consent. After the dose of dapsone, blood samples were taken at 1, 2, 3, 4, 8 and 24 h. Methemoglobin levels were determined immediately and plasma was prepared by centrifugation and stored at -20°C until analyzed by HPLC. Urine was collected over ascorbate (1 g) for 24 h and stored -20°C until analyzed by HPLC. To quantify the amount of free (nonconjugated) dapsone and dapsone hydroxylamine, urine ($200 \mu\text{l}$) was spiked with internal standard (3,3'-diaminodiphenyl sulfone; $1 \mu\text{g}$) and then extracted with ethyl acetate ($2 \times 1 \text{ ml}$). The organic layers were combined and treated in the same way as for plasma samples described above. To determine the total excretion of both dapsone and the hydroxylamine metabolite, an aliquot ($200 \mu\text{l}$) of urine was incubated for 6 h with Glucuronase (500 U) in the presence of $500 \mu\text{M}$ ascorbate. Protein was precipitated with methanol ($300 \mu\text{l}$) overnight (-20°C) and centrifuged; an aliquot ($50 \mu\text{l}$) of the supernatant was injected onto the HPLC column and the compounds were eluted as described above.

Metabolism and toxicity of dapsone and dapsone hydroxylamine *in vitro*. Microsomal fractions were prepared from the pooled livers of male and female rats ($n = 6$), male mice ($n = 6$) or from six individual human livers, as described previously (Gill *et al.*, 1995). Washed erythrocytes were prepared from the blood of male and female rats, male mice and humans (Tingle and Park, 1993). Dapsone ($100 \mu\text{M}$) was incubated with microsomes (1 mg of protein) and 1 mM NADPH (omitted from controls) in the presence of washed human erythrocytes ($500 \mu\text{l}$ of a 50% suspension) in HEPES-buffered saline (1 ml) at 37°C and the methemoglobin content assessed after 1 h. For the metabolism studies, either dapsone or dapsone hydroxylamine ($100 \mu\text{M}$) were incubated with microsomes (1 mg of protein) and 1 mM NADPH in the presence of $500 \mu\text{M}$ ascorbate. After 1 h, the reaction was terminated by addition of methanol (2 ml), and protein precipitated overnight at -20°C . Protein was sedimented ($750 \times g$, 20 min), and an aliquot ($100 \mu\text{l}$) of the supernatant injected on a Spherisorb HPLC column and eluted as described above. Species-dependent sensitivity toward dapsone hydroxylamine was assessed after incubation of the cells with the compound (0–100 μM) for 1 h as described above.

Data analysis. Data shown in the text is mean \pm S.D. and values were compared by use of the Student's *t*-test for nonpaired data after tests to indicate whether the data were distributed normally. Where necessary, data were analyzed by analysis of variance with Bonferroni's correction. A difference was deemed significant when $P < .05$. Half-lives and area under the curves ($\text{AUC}_{(0-24)}$) were calculated by

the linear trapezoidal rule with the Topfit program (Schering, Germany).

Results

Toxicity and plasma kinetics of dapsone in the rat and mouse. Administration of dapsone to male Wistar rats resulted in a time-dependent increase in methemoglobinemia (fig. 1A), which reached a maximum of $29.1 \pm 9.3\%$ at 1 h. In contrast, there was no significant increase in methemoglobinemia after administration of dapsone to either the female rat or to the male mouse (fig. 1A). The area under the curve ($AUC_{(0-24)}$) for methemoglobinemia was $348\% \text{ metHb}\cdot\text{h}^{-1}$ for male rats, $74.0\% \text{ metHb}\cdot\text{h}^{-1}$ for female rats and $49.7\% \text{ metHb}\cdot\text{h}^{-1}$ for male mice.

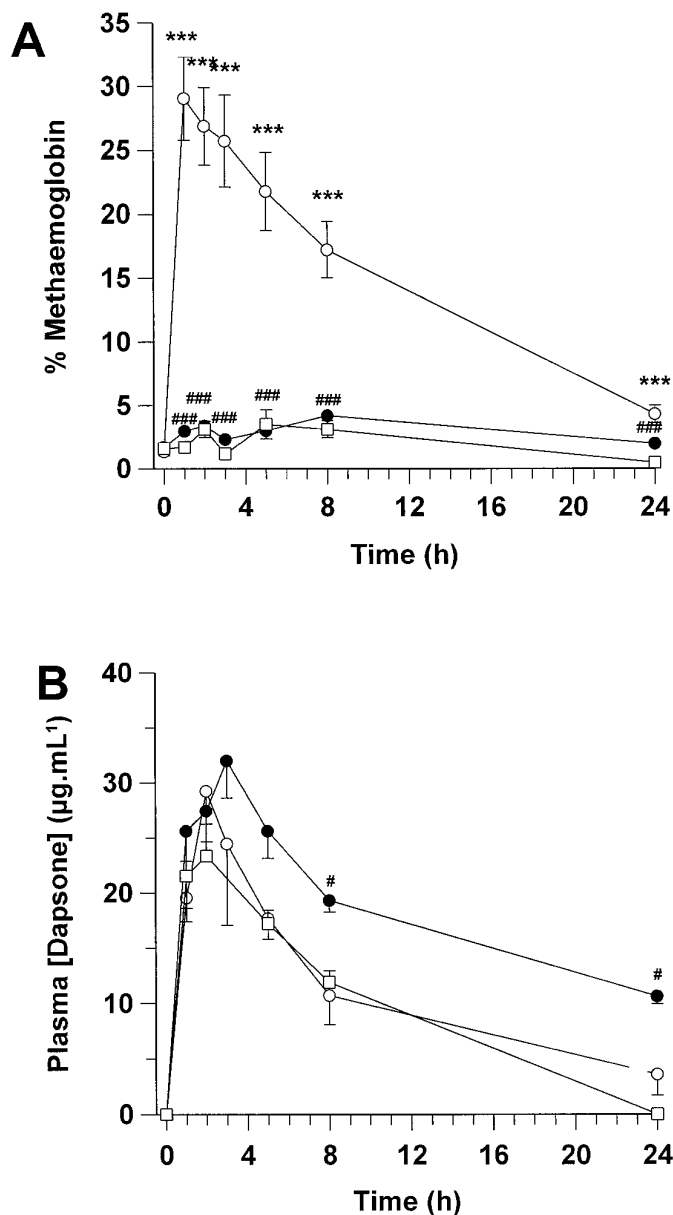


Fig. 1. Methemoglobin levels (A) and plasma levels of dapsone (B) after administration of dapsone ($100 \mu\text{mol}\cdot\text{kg}^{-1}$ i.p.) to male (○) and female (●) Wistar rats plus male CD1 mice (□). Values shown are mean \pm S.E.M. ($n = 4$). *** $P < 0.001$ compared with 0 h. # $P < 0.05$; ### $P < 0.001$ compared with male rats.

Analysis of plasma revealed that in the male rat, peak plasma concentrations of dapsone ($29.2 \pm 8.4 \mu\text{g}\cdot\text{mL}^{-1}$) were reached 2 h after the dose (fig. 1B), with a half-life of 7.7 ± 1.4 h. The area under the curve for dapsone $AUC_{(0-24)}$ was $243.0 \pm 105.6 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$. In the female rat, although there was no significant difference in the peak plasma concentrations ($32.0 \pm 6.7 \mu\text{g}\cdot\text{mL}^{-1}$), they were reached 3 h after the dose, and declined with a significantly ($P < .05$) longer half-life of 14.5 ± 1.0 h. The $AUC_{(0-24)}$ for dapsone was also significantly ($P < 0.05$) greater at $496.8 \pm 103.3 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$. In the male mouse, peak plasma concentrations were $23.4 \pm 2.6 \mu\text{g}\cdot\text{mL}^{-1}$ after 2 h, declining with a half-life of 7.6 ± 1.0 h. The $AUC_{(0-24)}$ for dapsone was $233.1 \pm 31.3 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$. No dapsone hydroxylamine could be detected in plasma at any time point (limit of detection, $10 \text{ ng}\cdot\text{mL}^{-1}$).

Excretion and metabolism of [^{14}C]dapsone in the rat.

The cumulative biliary excretion of radioactivity after administration of [^{14}C]dapsone to male and female Wistar rats is shown in figure 2. Over 300 min $46.3 \pm 13.6\%$ of the dose was excreted into the bile of males compared with $20.9 \pm 9.2\%$ in females. There was significantly ($P < .01$) greater excretion of radioactivity into bile for male rats at all time points after 30 min. There was no significant difference in the urinary excretion of radioactivity between males ($4.8 \pm 2.9\%$) and females ($3.4 \pm 3.1\%$).

Analysis of bile from male rats by reverse-phase HPLC with radiometric detection linked to LCMS, revealed that the major metabolite ($59.7 \pm 17.8\%$ of radioactivity) was a glucuronide of dapsone with the presence of a dapsone hydroxylamine glucuronide ($27.8 \pm 19.3\%$) and unchanged dapsone ($11.6 \pm 3.6\%$). In the urine it was found that the major metabolite was the glucuronide of dapsone ($59.7 \pm 6.7\%$), whereas dapsone hydroxylamine glucuronide ($8.4 \pm 4.2\%$) and unchanged dapsone ($31.8 \pm 6.7\%$) were also detected. Analysis of bile from female rats revealed the presence of dapsone glucuronide ($77.1 \pm 5.0\%$) and dapsone ($22.9 \pm 5.0\%$), with no detectable levels of dapsone hydroxylamine glucuronide. In the urine, dapsone glucuronide ($42.3 \pm 15.3\%$) and dapsone ($57.7 \pm 15.3\%$) were present. Trace amounts ($<1\%$ of radioactivity) of N-acetyl dapsone and N-acetyl dapsone glucuronide were detected by mass spectrometry.

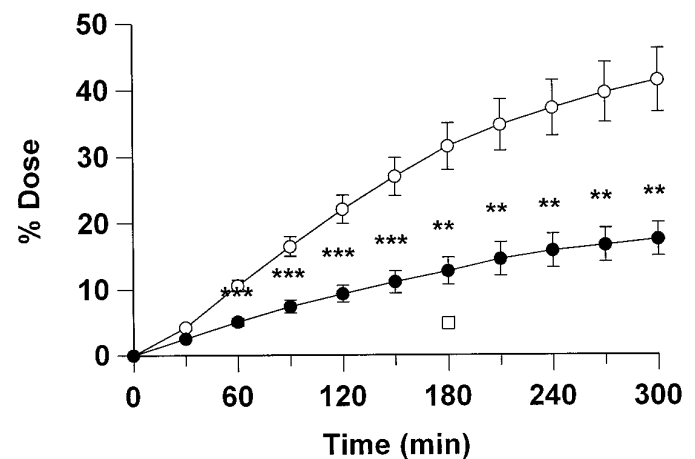


Fig. 2. Excretion of radioactivity into bile after administration of dapsone ($100 \mu\text{mol}\cdot\text{kg}^{-1}$, $3 \mu\text{Ci}$ i.v.) to male (○) and female (●) Wistar rats plus male CD1 mice (□). Values shown are mean \pm S.E.M. ($n = 4$). ** $P < 0.01$; *** $P < 0.001$ compared with male rats.

etry, but not radiometric detection, in the urine and bile of both male and female rats.

Excretion and metabolism of dapsone in the mouse. After administration of [^{14}C]dapsone to male mice, $4.1 \pm 2.3\%$ of the dose was excreted into the bile and $3.0 \pm 2.2\%$ into urine over 180 min. Analysis of bile and urine by reversed-phase HPLC/MS revealed the presence of dapsone alone, with no glucuronides of either dapsone or dapsone hydroxylamine.

Toxicity and plasma kinetics of dapsone in humans. After administration of dapsone (100 mg), it was possible to detect a significant ($P < 0.05$) increase in methemoglobin levels in four of the five volunteers. No significant elevation of methemoglobin levels were detected in one subject at any time point. The $\text{AUC}_{(0-24)}$ for methemoglobin was $37.3 \pm 11.0\%$ $\text{metHb}\cdot\text{h}^{-1}$. The mean plasma levels of dapsone are shown in figure 3. The $\text{AUC}_{(0-24)}$ for dapsone was $12.3 \pm 1.3 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$. Three control subjects were classified as rapid acetylators and two as slow acetylators based on the ratio of monoacetyl dapsone to dapsone in plasma 3 h postdose (Gelber *et al.*, 1971). There was no correlation between acetylator status and methemoglobinemia ($r = 0.3$).

Initial analysis of human urine by LCMS revealed the presence of dapsone, dapsone hydroxylamine and their respective glucuronides, plus some monoacetyl dapsone. During 24 h postdose, $5.9 \pm 2.6\%$ of the dose was excreted as free (nonconjugated) dapsone, $0.5 \pm 0.3\%$ as free hydroxylamine and $0.6 \pm 0.2\%$ as monoacetyl dapsone. After hydrolysis with β -glucuronidase, $15.3 \pm 2.4\%$ of the dose was quantified as dapsone and $13.1 \pm 2.6\%$ as dapsone hydroxylamine.

Metabolism and toxicity of dapsone and dapsone hydroxylamine *in vitro*. Metabolism (NADPH)-dependent methemoglobin formation in human erythrocytes was observed when dapsone was incubated with liver microsomes prepared from all three species (table 1). However, dapsone hydroxylamine could only be detected by HPLC after incubation with rat and human liver enzymes. Microsomes prepared from the livers of all three species catalyzed the reverse reaction, *i.e.*, reduction of dapsone hydroxylamine to dapsone (table 1).

The dapsone hydroxylamine-dependent methemoglobinemia in erythrocytes, isolated from male and female Wistar

rats, CD1 mice or human blood is shown in figure 4. Of the three species investigated, human blood appeared to be the most sensitive, with $63.6 \pm 3.1\%$ methemoglobin at $100 \mu\text{M}$ hydroxylamine, compared with 28.8 ± 3.9 for the male rat, 31.3 ± 3.5 for the female rat and 45.3 ± 1.4 for the mouse.

Discussion

Administration of dapsone to male Wistar rats resulted in hemotoxicity, in the form of methemoglobinemia, in agreement with previous studies (Coleman *et al.*, 1990a,c). Methemoglobin formation is a consequence of the N-hydroxylation of dapsone to a hydroxylamine metabolite, which is well established as being toxic toward erythrocytes (Scott and Rasbridge, 1973; Glader and Conrad, 1973). In this study, we have confirmed that dapsone is N-hydroxylated by male rat liver enzymes *in vitro*, and that *in vivo* there is formation of the further metabolite, dapsone hydroxylamine glucuronide, which is excreted into bile and urine. The levels of N-oxidation products found in bile from male rats in this study ($14.2 \pm 9.9\%$ of the dose over 5 h) may be higher than reported previously ($0.1\text{--}3.2\%$ of the dose) (Israili *et al.*, 1973), because we have determined them directly by radiometric HPLC/MS, rather than after a complex chemical work-up involving hydrolysis and azoxy product formation.

Dapsone did not cause any methemoglobinemia in the female rat, in keeping with a previous study (Coleman *et al.*, 1990c), nor was any hydroxylamine or hydroxylamine glucuronide detected. Although some N-hydroxylation was detected *in vitro* with microsomes prepared from the livers of female rats, the rate of reduction for the hydroxylamine to the amine was greater. This sex difference in the bioactivation of dapsone in the rat has been ascribed to the sex-dependent expression of cytochrome P450 enzymes, and in particular to CYP2C11 and CYP3A1 (Coleman *et al.*, 1990c; Vage and Svensson, 1994), which are expressed only in male rats (Guengerich *et al.*, 1986). Clearly, there may also be sex-dependent expression of the cytochrome P450 enzyme(s) involved in the reduction of dapsone hydroxylamine. Sulfamethoxazole is N-hydroxylated by CYP2C9 in man and by CYP2C6 in rat, but the hydroxylamine is reduced by CYP3A in both species (Cribb *et al.*, 1995).

In both sexes, only trace amounts of N-acetylated metabolites of dapsone were detected in the bile and urine, whereas no monoacetyl dapsone was detected in plasma, which suggests that the Wistar rat is a "slow" acetylator of dapsone. No products of sulfation or glutathione adducts were detected by either radiometric HPLC or LCMS.

Administration of dapsone to male CD1 mice did not result in any significant methemoglobinemia, even at doses of $1 \text{ mmol}\cdot\text{kg}^{-1}$ which result in neurotoxicity (M. D. Tingle, unpublished observations). This lack of hemotoxicity is not caused by insensitivity of the target cells, because erythrocytes prepared from mouse blood were more sensitive than rat cells to the methemoglobin-forming capacity of dapsone hydroxylamine. However, the lack of toxicity can be rationalized by the fact that no N-hydroxylation could be measured *in vivo*, with no hydroxylamine or further metabolites detected. Although there was some metabolism-dependent methemoglobin formation *in vitro* with microsomes prepared from mouse livers, no hydroxylamine could be detected *in vitro*. This apparent discrepancy may be caused by the accumula-

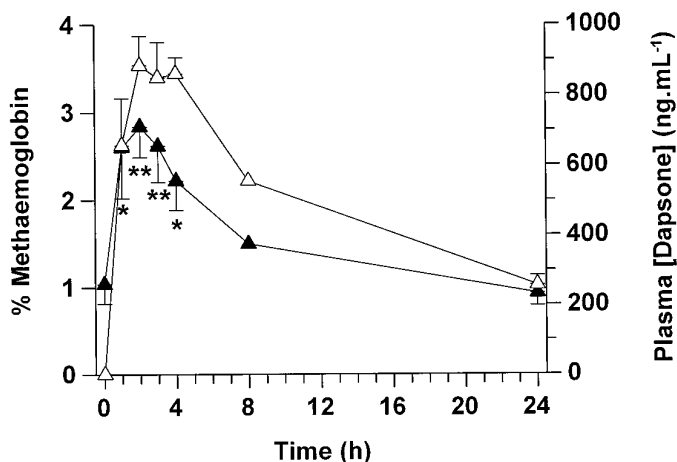


Fig. 3. Methemoglobin levels (\blacktriangle) and plasma levels of dapsone (\circ) after a single dose of dapsone (100 mg) in five human volunteers. * $P < 0.05$; ** $P < 0.01$ compared with control (0 h) methemoglobinemia.

TABLE 1

Dapsone-dependent methemoglobin formation (corrected for control, NADPH) plus rates of hydroxylamine formation and reduction catalyzed by microsomes prepared from the pooled livers of male Wistar rats, male CD1 mice or six individual human livers^a

Species	Methemoglobin (%)	Hydroxylamine Formation (nmol·mg ⁻¹)	Hydroxylamine Reduction (nmol·mg ⁻¹)	Oxidation/Reduction Ratio
Wistar rat (male)	36.6 ± 1.5***	22.0 ± 1.5***	3.6 ± 0.2***	6.1
Wistar rat (female)	8.2 ± 1.3	1.0 ± 0.1***	1.8 ± 0.8***	0.5
CD1 mouse	4.2 ± 1.6*	N.D.	3.1 ± 0.2***	—
Human	10.9 ± 1.1	6.7 ± 0.5	6.8 ± 0.4	1.0

^a Values shown are mean ± S.D. (n = 4 determinations). N.D., not detected.

* P < .05; *** P < .001 compared with human.

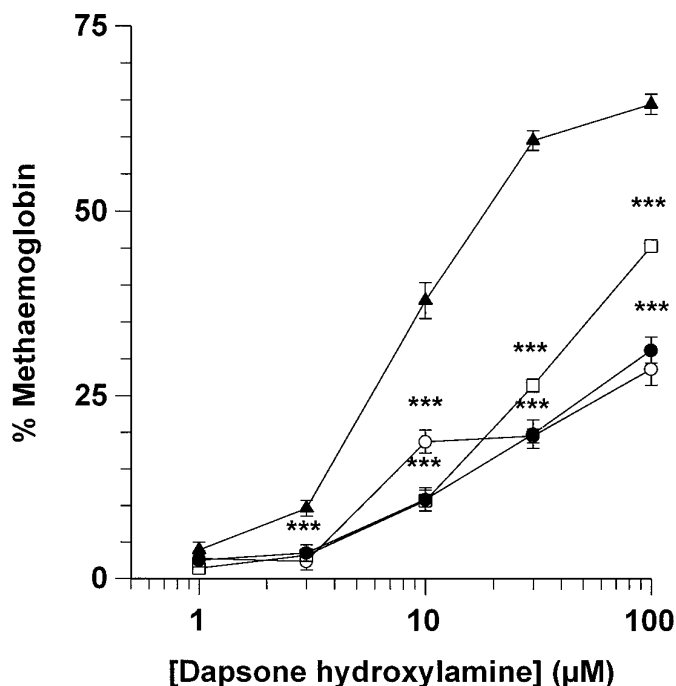


Fig. 4. Sensitivity of erythrocytes, prepared from human (▲), male rat (○), female rat (●) or male mouse (□) blood, to dapsone hydroxylamine-dependent methemoglobin formation *in vitro*. Values shown are mean ± S.E.M. (n = 4). ***P < .001 compared with human erythrocytes.

tion of the hydroxylamine into erythrocytes (Israili *et al.*, 1973; Tingle and Park, 1993) which prevents the reduction of the hydroxylamine back to dapsone by liver enzymes. As well as a lack of N-hydroxylation, no N-glucuronidation, sulfation or acetylation were detected in the mouse.

Despite the lack of metabolism plus low urinary and biliary excretion (fig. 2), the clearance of dapsone from plasma in the mouse was not significantly different from that observed in the male rat. This may suggest that there is tissue accumulation of dapsone in the mouse, perhaps into the skin (Chatterjee and Poddar, 1957) and brain, which may account for the different toxicity observed. The absence of N-hydroxylation in the mouse would suggest that this metabolic pathway is not important in the mechanism of antiparasitic activity for dapsone, because dapsone is active against *P. berghei* in the mouse model (Popoff *et al.*, 1971a).

A previous study in man has suggested a link between the rate of N-hydroxylation and clearance of dapsone (May *et al.*, 1990). This relationship between N-hydroxylation and plasma clearance is borne out in both the plasma kinetics and biliary excretion in male *versus* female rats. In the female rats, which do not N-hydroxylate dapsone, the half-life

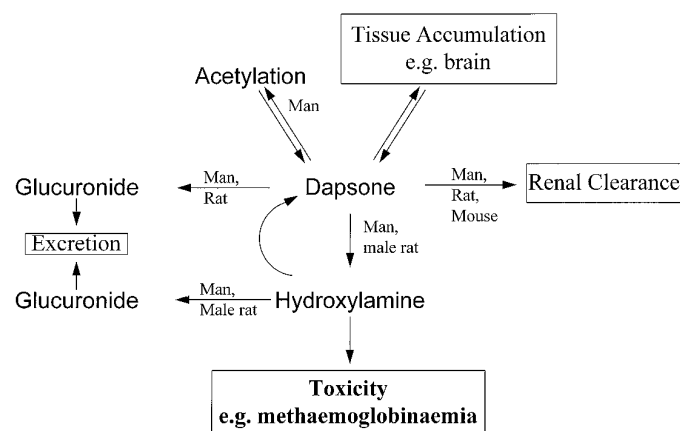


Fig. 5. Species differences in the metabolism and disposition of dapsone *in vivo*.

of dapsone in plasma is significantly ($P < 0.05$) longer after intraperitoneal administration, although peak concentrations do not differ significantly, whereas after intravenous administration, approximately half as much radioactivity (24.3 *vs.* 51.1%) is excreted into the bile and urine over 5 h in female compared with male rats. However, the similar clearance of dapsone from plasma in both the male Wistar rat and the male CD1 mouse, in which no N-hydroxylation of dapsone could be detected, would suggest that factors such as tissue accumulation are also very important.

Although human liver enzymes *in vitro* had a lower activity with respect to dapsone N-hydroxylation than rat liver microsomes, *in vivo* there was extensive N-hydroxylation in humans, with 46% of the recovered dose excreted as hydroxylamine or hydroxylamine glucuronide over 24 h, in agreement with previous studies (Coleman *et al.*, 1990b; Israili *et al.*, 1973). The apparently lower rate of N-hydroxylation *in vitro* by human microsomes compared with rat may be a function of the greater capacity for human enzymes to catalyze the reverse reaction, *i.e.*, reduction of the hydroxylamine back to the amine *in vitro*. Dapsone N-hydroxylation is known to be catalyzed by CYP2C9, CYP2E1 and CYP3A4 in man (Gill *et al.*, 1995; Mitra *et al.*, 1995) and by CYP2C11 and CYP3A1 in rat (Coleman *et al.*, 1990c; Vage and Svensson, 1994). However, it is not known which isoform(s) of cytochrome P450 catalyze the reduction pathway.

The hemotoxicity of dapsone may also be greater in man than the rat because human erythrocytes are significantly more susceptible than rodent erythrocytes to the toxic effects of the hydroxylamine. There is no significant difference in the levels of the enzyme NADH- and NADPH-methemoglobin reductase between these two species (Agar and Harley,

1972), so differences in sensitivity may reflect species differences in the ability of the cells to reduce nitroso dapsone back to the hydroxylamine in a futile cycle (Kramer *et al.*, 1972). Indeed, a single oral dose of dapsone (100 mg, approximately $5 \mu\text{mol}\cdot\text{kg}^{-1}$) in man resulted in more than 2% methemoglobin. If this is multiplied up to an equivalent dose administered to the rat ($100 \mu\text{mol}\cdot\text{kg}^{-1}$), then man would have more than 50% methemoglobin, a lethal concentration (Bodansky, 1951).

There was no correlation between the acetylation ratio and methemoglobin formation in the volunteers. The N-acetylation of dapsone is catalyzed by the polymorphic N-acetyltransferase present in the liver (*NAT2*) (Gelber *et al.*, 1971). Although generally considered as a phase II detoxication reaction, N-acetylation of dapsone does not seem to be important in the toxicity associated with the drug (Zuidema *et al.*, 1986), possibly because acetylation and deacetylation occur at a constant equilibrium in the plasma after administration of the drug (Gelber *et al.*, 1971). *In vitro* studies have shown that monoacetylated dapsone is still susceptible to metabolism by rat and human liver enzymes, to give the monoacetylated dapsone hydroxylamine, which is equitoxic with dapsone hydroxylamine (Coleman *et al.*, 1991; Vage *et al.*, 1994).

Previous studies have postulated the presence of sulfate conjugates of dapsone based on chemical stability (Zuidema *et al.*, 1986), rather than full chemical identification. However, no sulfates of either dapsone or dapsone hydroxylamine were detected in any biological fluid analyzed by LCMS in this study.

In conclusion, microsomes prepared from rat, mouse and human livers N-hydroxylate dapsone to produce a hydroxylamine which is toxic to erythrocytes from all three species *in vitro*. Microsomes from all three species also catalyzed the reduction of the hydroxylamine to dapsone. However, dapsone hydroxylamine and its glucuronide, as well as significant methemoglobinemia, were only detected in humans and male rats. The balance between oxidation, reduction and conjugation (fig. 5) is of great importance for the safety evaluation of compounds, and in the case of dapsone, the use of animal models may seriously underestimate the risk of exposure to man. This species variation in metabolism, and hence toxicity, of dapsone is important in the selection of a suitable animal model to investigate the relationship between disposition and toxicity for novel diarylsulfones, and for safety evaluation of aromatic amines in general.

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