Biochemical Pharmacology, Vol. 45, No. 5, pp. 1027–1033, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00
© 1993. Pergamon Press Ltd

REDUCTION OF DAPSONE HYDROXYLAMINE TO DAPSONE DURING METHAEMOGLOBIN FORMATION IN HUMAN ERYTHROCYTES *IN VITRO*

MICHAEL D. COLEMAN* and DAVID P. JACOBUS†

Mechanisms of Drug Toxicity Group, Department of Pharmaceutical Sciences, Aston University, Aston Triangle, Birmingham B4 7ET, U.K.; and †Jacobus Pharmaceutical Co. Inc., Princeton, NJ 08540, U.S.A.

(*Received* 12 *November* 1992; *accepted* 11 *December* 1992)

Abstract—The fate of the toxic metabolite of dapsone, dapsone hydroxylamine, has been studied in the human red cell. Twice-washed red cells were incubated at 37° with dapsone hydroxylamine: at 3 and 5 min, 27.0 ± 2.2 and 33.2 ± 2.7% of the haemoglobin had been converted to methaemoglobin, leading to a maximum at 45 min (45 ± 1.8%). HPLC analysis revealed that parent amine was produced from dapsone hydroxylamine during methaemoglobin formation in the red cells. At 3 min, conversion of dapsone hydroxylamine to dapsone reached 7.0 ± 3.9% leading to a maximum at 30 min (18.1 ± 3.7%). There was a linear relationship between hydroxylamine-dependent methaemoglobin formation and conversion of hydroxylamine to dapsone ($r = 0.97$). At 4°, methaemoglobin and dapsone formation was greatly retarded, and did not exceed 10%. Co-incubation of diethyl dithiocarbamate (DDC) with dapsone hydroxylamine and red cells led to a marked increase in methaemoglobin formation (61.4 ± 3.4%) compared with hydroxylamine and red cells alone (45.0 ± 1.8%, $P < 0.001$) at 45 min, and conversion of dapsone hydroxylamine to dapsone was almost doubled at 45 min (35.7 ± 5.3%) compared with hydroxylamine and red cells (18.1 ± 2.5%). A linear relationship between methaemoglobin formation and dapsone formation ($r = 0.96$) was also shown to occur in the presence of DDC. Incubation of red cells with DDC and dapsone hydroxylamine caused a significantly greater reduction in glutathione levels (98.3 ± 1.6%) compared with red cells and dapsone hydroxylamine alone (84.8 ± 2.7%) at 5 min ($P < 0.001$), although there was no significant difference between the groups at 15 min (96.9 ± 2.6 vs 98.1 ± 2.2%). Intra-erythrocytic glutathione was then depleted by 75 ± 3.4%, by pretreatment with diethyl maleate (6 mM), and these cells in the presence of the hydroxylamine showed a significant fall in both methaemoglobin generation (29.7 ± 1.2 vs 35.0 ± 1.7%) and parent amine formation (11.1 ± 0.2 vs 16.5 ± 1.1%) compared with untreated red cells at 45 min. It is possible that a cycle exists between hepatic oxidation of dapsone to its hydroxylamine and reduction to the amine within the red cell, which may lead to re-oxidation by hepatic cytochrome P450. This process may contribute to the persistence of the drug *in vivo*.

Although dapsone is known primarily as an antileprotic, it is also used in a number of infectious diseases, such as *Pneumocystis carinii* pneumonia and malaria [1–3]. The drug is effective in inflammatory disease such as dermatitis herpetiformis [4] and is under evaluation as a steroid sparing agent in chronic asthma [5]. Although dapsone undergoes N-acetylation, its major route of clearance is N-hydroxylation [6, 7] which is mediated via hepatic cytochrome P450 isozyme CYP3A4 [8]. The resulting hydroxylamines undergo glucuronidation and are eliminated in urine [7, 9]. However, the use of dapsone is often limited by the haematological toxicity of its hydroxylamines, which result in methaemoglobinaemia, haemolysis and reduced erythrocyte life span [7, 10]. Haemolysis results in a significant fall in haematocrit [11] and may lead to anaemia. Dapsone-dependent methaemoglobinaemia may also cause acute toxicity in patients [12–14]. However, it has been demonstrated using human erythrocytes *in vitro* that methaemoglobin formation can exert a protective function, as erythrocytes will preferentially take up the toxic hydroxylamine resulting in greatly reduced toxicity in neighbouring cell types such as mononucleocytes [15]. The detoxifying capability of erythrocytes has been highlighted in a number of previous studies, where decarboxylations [16] and N-demethylation reactions were shown to be catalysed by erythrocytes [17]. In the rat, the potent methaemoglobin-forming hydroxylamine of 4-amino biphenyl, a structural analogue of dapsone, has been shown to be detoxified by erythrocytes leading to the formation of the parent amine [18]. In this study, we wish to determine whether the hydroxylamine of dapsone is reduced to the parent amine during methaemoglobin formation in human erythrocytes, and what factors may influence this process.

MATERIALS AND METHODS

Dapsone hydroxylamine was provided by the Jacobus Pharmaceutical Company Inc. (Princeton, NJ, U.S.A.), and was found to be 97% pure by HPLC. Dapsone was obtained from the Sigma Chemical Co. (Poole, U.K.). The internal standard for the HPLC assay (3,3'-diaminodiphenyl sulphone) was obtained from the Aldrich Chemical Co. (Poole, U.K.). All HPLC solvents were supplied by Fisons

* Corresponding author. Tel. (021) 359 3611 ext. 4204; FAX (021) 359 0733.

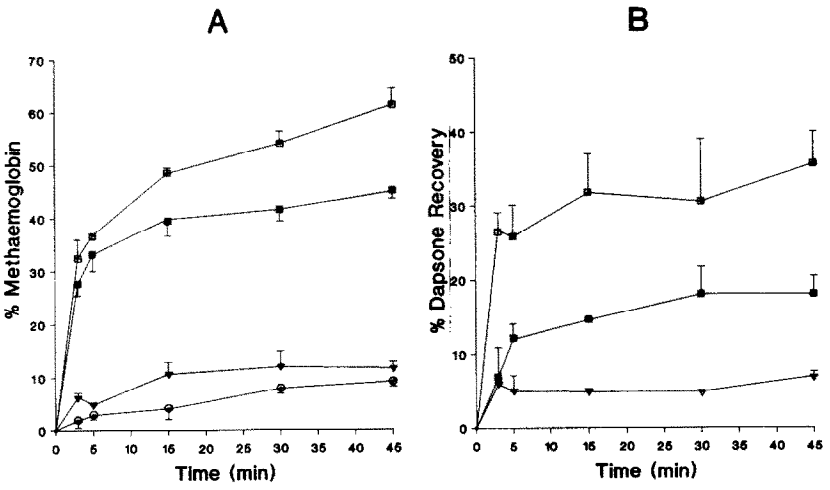

Fig. 1. (A) Methaemoglobin formed after the incubation of human erythrocytes with dapsone hydroxylamine alone at 37° (■); dapsone hydroxylamine and DDC (5 mM) at 37° (□); dapsone hydroxylamine at 4° (▽); and DDC alone (○). Each point is the mean ± SD of four determinations. (B) Percentage recovery of dapsone hydroxylamine as dapsone after the incubation of human erythrocytes with dapsone hydroxylamine alone at 37° (■); dapsone hydroxylamine and DDC at 37° (□); and dapsone hydroxylamine at 4° (▽). Each point is the mean ± SD of four determinations.

Ltd (Loughborough, U.K.). Haemoglobin concentrations were determined by the use of diagnostic kit No. 525A, which was supplied by Sigma. Whole human blood was drawn from healthy volunteers and anticoagulated with sodium heparin and placed on ice. The erythrocytes were separated from the plasma and the buffy coat and top layer of cells removed. The cells were then washed twice in equal volumes of phosphate-buffered (pH 7.4) 0.9% saline (PBS*) and resuspended to a 50% haematocrit also in PBS. The erythrocyte incubations (0.5 mL) each contained 1.2 μM of haemoglobin and were equilibrated to 37° in uncapped tubes. All incubations were carried out in quadruplicate. At time zero, dapsone hydroxylamine (0.075 μmol in 20 μL of acetone) was added to the erythrocytes to give a final concentration of 150 μM and overall ratio of hydroxylamine to haemoglobin molecules of 1:16. Incubations were carried out for durations of 3, 5, 15, 30 and 45 min. At the end of each incubation, the samples were placed on ice, and a 100 μL aliquot from each sample immediately assayed for methaemoglobin formation using the spectrophotometric method of Harrison and Jollow [19]. The remainder of the samples were frozen at −20° until assayed by the HPLC method of Grossman and Jollow [20]. Experiment 1 was carried out as described at 37°. Experiment 2 was carried out at 4°. In experiment 3, 3 min prior to the addition of dapsone hydroxylamine, diethyl dithiocarbamate (DDC) dissolved in PBS was added to the incubation mixture to produce a final concentration of 5 mM. In experiment 4, sodium azide (5 mM) was added to the erythrocytes dissolved in PBS as described above. Experiment 5 was identical to experiment 4, except that the azide was removed from the erythrocytes by three PBS washes just prior to methaemoglobin determination. Azide binds avidly to the iron III of methaemoglobin, thus forming a complex which absorbs at 586 and 526 nm [21, 22] but not at the 635 nm used in the method of Harrison and Jollow [19]. Hence in this study, an apparent reduction in methaemoglobin levels in the presence of azide (reversible after extensive washing with PBS) is assumed to indicate azide binding to methaemoglobin, and is not a true reduction in methaemoglobin levels.

In experiment 6, the red cells were divided into three groups; groups A and B were pretreated at 37° with diethyl maleate (DEM) at 3 and 6 mM final concentrations for 30 min, while group C served as an untreated control. Dapsone hydroxylamine was then added to all three groups as described. Experiment 7 was set up in parallel and in identical fashion to experiment 6; however, after the 30 min pretreatment the erythrocytes were assayed for glutathione levels by the method of Anderson [23]. Control incubations containing erythrocytes and the vehicle in which dapsone hydroxylamine was dissolved (acetone; 20 μL) showed no measurable reduction in glutathione levels. Experiment 8 involved the incubation of erythrocytes with either dapsone hydroxylamine alone, DDC alone or dapsone hydroxylamine together with DDC for 5 and 15 min. Control incubations contained untreated erythrocytes. Glutathione levels were immediately measured as described above in all groups and are expressed as a percentage reduction of control. Statistical comparisons were made using Student's *t*-test accepting $P < 0.05$ as significant.

RESULTS

Incubation at 37° of erythrocytes with dapsone

* Abbreviations: PBS, phosphate-buffered (pH 7.4) 0.9% saline; DDC, diethyl dithiocarbamate; DEM, diethyl maleate.

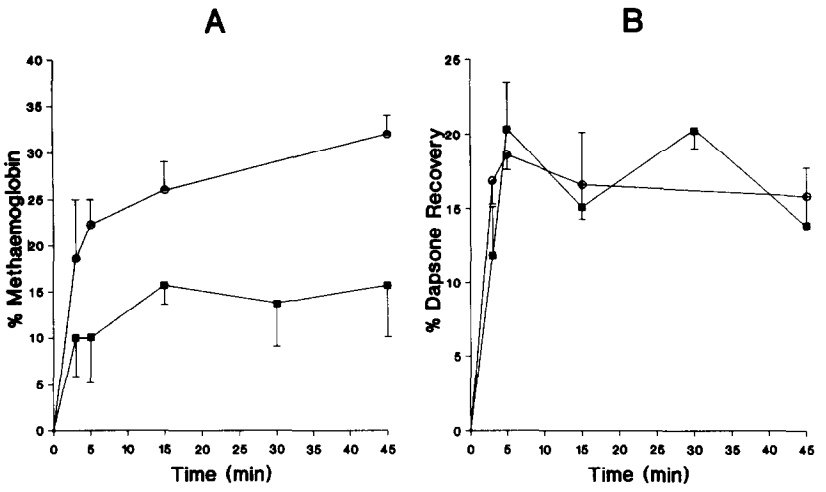

Fig. 2. (A) Measured methaemoglobin formation after the co-incubation of azide (5 mM) and dapsone hydroxylamine with human erythrocytes. Azide removed from erythrocytes by repeated washing before methaemoglobin determination (○); azide left in contact with erythrocytes during methaemoglobin determination (■). Each point is the mean ± SD of four determinations. (B) Percentage recovery of dapsone hydroxylamine as dapsone after the co-incubation of azide (5 mM) and dapsone hydroxylamine with human erythrocytes. Azide removed from erythrocytes by repeated washing before methaemoglobin determination (○); azide left in contact with erythrocytes during methaemoglobin determination (■). Each point is the mean ± SD of four determinations.

hydroxylamine resulted in rapid formation of methaemoglobin (Fig. 1A), i.e. within 3 min 27 ± 2.2% of haemoglobin had been oxidized. Methaemoglobin formation proceeded at a lower rate after 5 min (33.2 ± 2.7%) leading to a maximum at 45 min (45 ± 1.8%). Haemoglobin oxidation coincided with the formation of dapsone from dapsone hydroxylamine (Fig. 1B). Within 3 min this led to the conversion of 7.0 ± 3.9% of the dapsone hydroxylamine to dapsone. This process reached a maximum at 30 min (18.1 ± 3.7%). Linear regression of the relationship between methaemoglobin formation due to dapsone hydroxylamine and conversion of the dapsone hydroxylamine to dapsone yielded a correlation coefficient of 0.97. When the experiment was repeated at 4°, methaemoglobin formation was greatly retarded and did not exceed 10%, which was reflected in the much lower levels of dapsone formation (Fig. 1A and B).

Co-incubation of DDC with dapsone hydroxylamine and erythrocytes did not lead to a significant increase with respect to control in methaemoglobin formation at 3 and 5 min. However, at 15, 30 and 45 min, there was a marked rise in methaemoglobin formation in the presence of DDC (Fig. 1A, $P < 0.001$) in comparison with control. Conversion of dapsone hydroxylamine to dapsone was more than tripled at 3 min compared with control, and overall formation of parent drug was almost doubled in the presence of DDC (Fig. 1B). Linear regression analysis of the relationship between methaemoglobin formation and dapsone formation was also close to unity ($r = 0.96$). Incubation of erythrocytes with DDC alone resulted in relatively low levels of methaemoglobin, which did not exceed 10% (9.2 ± 1.0; Fig. 1A). The presence of azide did not affect dapsone formation from dapsone hydroxylamine (Fig. 2B) although azide had become extensively bound to the methaemoglobin formed by the hydroxylamine as indicated by the reduction in measured methaemoglobin levels (Fig. 2A). The washing of the samples prior to methaemoglobin assay did not affect dapsone recovery (Fig. 2B).

Incubation of erythrocytes with DDC caused a slight reduction in glutathione levels over 15 min (10.1 ± 1.2%). Incubation of erythrocytes with DDC and dapsone hydroxylamine caused a significantly greater reduction in glutathione levels (98.3 ± 1.6%) compared with erythrocytes and dapsone hydroxylamine (84.8 ± 2.7%) at 5 min ($P < 0.001$), although there was no significant difference between the groups at 15 min (96.9 ± 2.6 vs 98.1 ± 2.2%). Erythrocytes pretreated with DEM at 3 and 6 mM showed a 71.0 ± 5.1 and 75 ± 3.4% reduction in intracellular glutathione levels, respectively. Incubation of dapsone hydroxylamine after DEM pretreatment at 3 mM showed no significant difference with respect to control in either methaemoglobin or dapsone formation (Fig. 3A and B). Incubation of erythrocytes pretreated with DEM at 6 mM led to a significant fall in methaemoglobin generation at all time points except at 30 min (Fig. 4A). There was a significant fall at all time points in dapsone formation from dapsone hydroxylamine (Fig. 4B).

DISCUSSION

Human erythrocytes contain no effective cytochrome P450 isozymes, yet are capable of considerable metabolic activity. This includes the oxidation of styrene [24] and the hydroxylation of

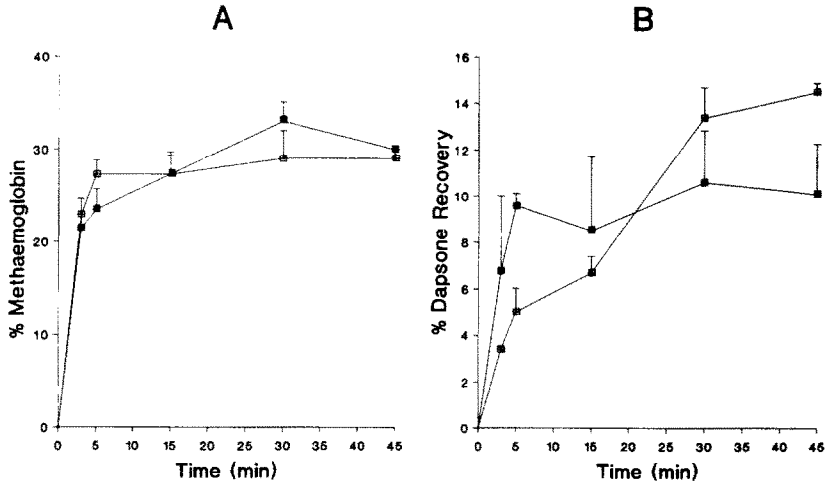

Fig. 3. (A) Methaemoglobin formed during the incubation of dapsone hydroxylamine with human erythrocytes without pretreatment with DEM (■); and with DEM (3 mM) pretreatment (□). Each point is the mean ± SD of four determinations. (B) Percentage recovery of dapsone hydroxylamine as dapsone after the incubation of dapsone hydroxylamine with human erythrocytes without pretreatment with DEM (■); and with DEM (3 mM) pretreatment (□). Each point is the mean ± SD of four determinations.

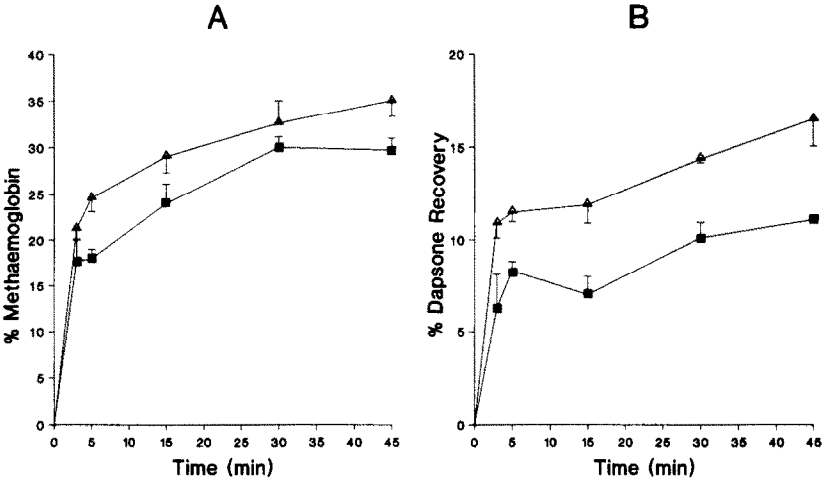

Fig. 4. (A) Methaemoglobin formed during the incubation of dapsone hydroxylamine with human erythrocytes without pretreatment with DEM (△); and with DEM (6 mM) pretreatment (■). Each point is the mean ± SD of four determinations. (B) Percentage recovery of dapsone hydroxylamine as dapsone after the incubation of dapsone hydroxylamine with human erythrocytes without pretreatment with DEM (△); and with DEM (6 mM) pretreatment (■). Each point is the mean ± SD of four determinations.

aniline [25]. Haemoglobin in its oxygenated form appears to catalyse these reactions, due to its propensity to undergo one electron oxidations and reductions [26]. During the course of these reactions *in vivo*, methaemoglobin is often formed, which cannot carry oxygen. Consequently, methaemoglobin may cause cyanosis, dyspnoea, tachycardia nausea and, in some cases, marked respiratory distress [1, 12]. However, this is probably not a truly "toxic" effect, as erythrocytes contain two electron transport systems which may convert methaemoglobin to haemoglobin. The major pathway involves an NADH-dependent reductase system associated with cytochrome *b5* [27] and a secondary system involving an NADPH-dependent pathway which is of little importance under normal conditions, but can be activated by the presence of exogenous electron carriers such as methylene blue [28].

In the present study, in the process of dapsone hydroxylamine-dependent methaemoglobin generation, approximately a fifth of the metabolite was recovered as parent drug from the erythrocytes. A linear relationship was found to exist between methaemoglobin formation and reduction of dapsone hydroxylamine to dapsone. This process was extremely rapid and temperature dependent. DDC may cause both methaemoglobin formation in erythrocytes and glutathione depletion [29], as well as superoxide dismutase inhibition [30]. In this report, DDC was a relatively weak haemoglobin oxidant alone, but was capable of significantly accelerating dapsone hydroxylamine-dependent methaemoglobin formation. This also resulted in a similar increase in parent amine formation from the hydroxylamine. The apparent synergy between dapsone hydroxylamine and DDC in the erythrocyte may be explained in terms of the instability of the hydroxylamine, as its half-life at physiological pH and temperature is only 37 min [31]. The hydroxylamine is thought to auto-oxidize to a nitroso intermediate ultimately forming a nitro derivative [32] which does not react directly with haemoglobin [33]. It is conceivable that the thiol-containing DDC stabilizes the hydroxylamine in the aqueous phase allowing a greater proportion to penetrate the erythrocytes to react with haemoglobin compared with hydroxylamine alone. This apparently synergistic effect may be of some clinical relevance, as DDC is a metabolite of disulfiram [34].

Methaemoglobin itself has been shown to react with menadione and superoxide [35, 36]. If methaemoglobin was involved in the process of hydroxylamine reduction, then the avid binding of azide to the iron III would hinder hydroxylamine reduction to dapsone. This was not the case, and it is therefore unlikely that reaction with methaemoglobin itself was responsible for dapsone formation in the erythrocyte.

Dapsone formation from dapsone hydroxylamine within the erythrocyte clearly involves the process of methaemoglobin formation. Previous studies with dapsone and the related compound 4-amino biphenyl have indicated that one mole of either of the amines may oxidize several moles of haemoglobin [18, 37, 38]. In the present study, from a starting ratio of dapsone hydroxylamine to haemoglobin of 1:16, over 30% of the haemoglobin had been oxidized within 5 min, i.e. one molecule of the hydroxylamine may have reacted with up to five molecules of haemoglobin. This has been explained in terms of a form of redox cycling, where the hydroxylamine reacts with haemoglobin to form methaemoglobin and the nitrosoarene, which is in turn reduced to the hydroxylamine by either NADPH methaemoglobin reductases or glutathione [37, 39]. The hydroxylamine then reacts with another molecule of haemoglobin thus continuing the cycle (Fig. 5).

In rat erythrocytes, the methaemoglobin-forming hydroxylamine of 4-amino biphenyl has been shown to undergo redox cycling, ultimately leading to the formation of the parent amine [40, 41]. It is thought that the nitrosoarene eventually escapes the redox cycle by binding to glutathione and other intracellular free thiols, or the thiol groups of globin and erythrocyte cell walls, thus producing a number of adducts of varying stability. Hydrolysis of the adducts

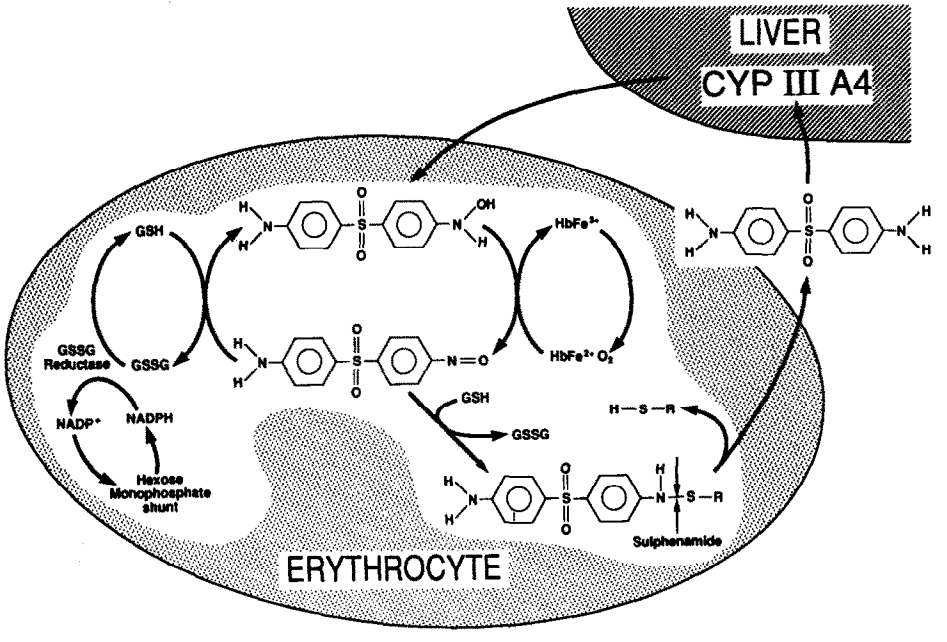

Fig. 5. Scheme showing the processes leading to dapsone hydroxylamine-dependent methaemoglobin and parent amine formation in the erythrocyte, and the possible recycling of the amine through hepatic metabolic activation.

would then release the free amine which then diffuses out of the erythrocyte [18]. The present study suggests that glutathione, rather than NADPH methaemoglobin reductases, is chiefly responsible for both the processes of methaemoglobin generation and parent amine formation from dapsone hydroxylamine in the human red cell. The steep rise in methaemoglobin and dapsone formation during the first 5 min of the incubations coincided with a precipitous fall in glutathione levels. By the time dapsone and methaemoglobin levels had reached a plateau at approximately 15 min, intra-erythrocytic glutathione levels were almost exhausted. In addition, the synergy between dapsone and DDC which resulted in a marked increase in both methaemoglobin and parent amine formation coincided with a more rapid fall in glutathione levels compared with either compound alone. Finally, prior depletion of glutathione by DEM led to a significant fall in both methaemoglobin and dapsone formation compared with untreated cells. It is likely that dapsone forms an adduct with glutathione, possibly a relatively unstable sulphenamide (Fig. 5), which may break down to form the parent amine. Glutathione conjugate instability has also been shown to occur with the nitroso derivative of the primary aromatic amine 2-aminofluorene. Glutathione reacts with 2-nitrosofluorene to form a conjugate which is sufficiently labile *in vitro* (37°; pH 7) to release the parent amine [42]. Interestingly in the present study, over 70% depletion of red cell glutathione by DEM (3 mM) caused no significant reduction in the recovery of dapsone from the erythrocytes. However, it appears that a glutathione depletion "threshold" was exceeded when the DEM concentration was increased to 6 mM, leading to a significant fall in dapsone and methaemoglobin formation.

If the process of transient thiol binding and release of dapsone occurs *in vivo*, then the parent drug is free to return to the liver to be reoxidized to the hydroxylamine, which might account for the considerable variation in the half-life measurements of the drug [1]. As only the parent drug and not its metabolites are responsible for its antiparasitic action [43], erythrocytes may contribute to the overall effectiveness of the drug. However, depending on the stability of the adducts formed, recovery of dapsone using a simple ethyl acetate extraction as carried out in the present study might not necessarily reflect the extent of actual dapsone release from the erythrocyte into the plasma *in vivo*.

Haemoglobin may react with oxidizing agents, which are reduced by the haem iron, as well as reducing agents which are oxidized by the haem-bound oxygen [26]. It is conceivable that free amine formation may also have occurred via routes involving a direct one-way reaction with oxyhaemoglobin, although it is likely that this reaction may make only a small contribution to the total methaemoglobin and amine formation.

In summary, dapsone hydroxylamine-dependent formation of methaemoglobin in the human erythrocyte also results in the detoxification of the hydroxylamine by reduction to the amine. This process may partly account for the ability of erythrocytes *in vitro* to protect human mononucleocytes from dapsone hydroxylamine toxicity [15] and may also contribute to the overall persistence of dapsone *in vivo*.

Acknowledgement—The authors are grateful to Ms Helen Butler for skilled technical assistance.

REFERENCES

1. Zuidema J, Hilbers-Modderman ESM and Markus FWHM, Clinical pharmacokinetics of dapsone. *Clin Pharmacokinet* **11**: 299–325, 1986.
2. Lee BL, Medina I, Benowitz NL, Jacob P, Wofsy CB and Mills J, 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, 1989.
3. Bruce-Chwatt LJ, *Essential Malariology*; pp. 181–182. Heinemann, London, 1982.
4. Zone JJ, Dermatitis herpetiformis. *Curr Probl Dermatol* **3**: 4–42, 1991.
5. Berlow BA, Liebhaber MI, Dyer Z and Spiegel TM, The effect of dapsone in steroid-dependent asthma. *J Allergy Clin Immunol* **87**: 710–715, 1991.
6. Coleman MD, Breckenridge AM and Park BK, Bioactivation of dapsone to a cytotoxic metabolite by human hepatic microsomal enzymes. *Br J Clin Pharmacol* **28**: 389–395, 1989.
7. Coleman MD, Scott AK, Breckenridge AM and Park BK, The use of cimetidine as a selective inhibitor of dapsone N-hydroxylation in man. *Br J Clin Pharmacol* **30**: 761–767, 1990.
8. Fleming CM, Branch RA, Wilkinson GR and Guergerich FP, Human liver microsomal N-hydroxylation of dapsone by cytochrome p450 IIIA4. *Mol Pharmacol* **41**: 975–980, 1992.
9. May DG, Porter JA, Uetrecht JP, Wilkinson GR and Branch RA, The contribution of N-hydroxylation and acetylation to dapsone pharmacokinetics in normal subjects. *Clin Pharmacol Ther* **48**: 619–627, 1990.
10. DeGowin RL, Eppes RB, Powell RD and Carson PE, The haemolytic effects of diphenylsulphone DDS) in normal subjects and in those with glucose 6-phosphate dehydrogenase deficiency. *Bull WHO* **35**: 165–179, 1966.
11. Blum RN, Millar LA, Gaggini LC and Cohn DL, Comparative trial of dapsone versus trimethoprim/sulfamethoxazole for primary prophylaxis of *Pneumocystis carinii* pneumonia. *J Acquired Immune Deficiency Syndrome* **5**: 341–347, 1992.
12. Gallant JE, Hoehn-Saric E and Smith MD, Respiratory insufficiency from dapsone-induced methaemoglobinaemia. *AIDS* **5**: 1392–1393, 1991.
13. Manfredi G, de Panfilis G, Zampetti M and Allegra F, Studies on dapsone induced haemolytic anaemia:methaemoglobin production and glucose-6-phosphate activity in correlation with dapsone dosage. *Br J Dermatol* **100**: 427–432, 1979.
14. Ganer A, Knobel B, Fryd CH and Rachmilewitz EA, Dapsone induced methaemoglobinaemia and haemolysis in the presence of familial hemoglobinopathy hasharon and familial methaemoglobin reductase deficiency. *Isr J Med Sci* **17**: 703–704, 1981.
15. Tingle MD, Blee MAB, Coleman MD and Park BK, The use of a novel three compartment system to investigate cell selective drug toxicity *in vitro*. *Br J Clin Pharmacol* **35**: 216P–217P, 1992.
16. Yamabe H and Lovenberg W, Decarboxylation of 3,4 dihydroxyphenylalanine by oxyhemoglobin. *Biochem Biophys Res Commun* **47**: 733–739, 1972.

17. Stecca C, Cumps J and Duverger-Van Bogaert MD, Enzymic N-demethylation reaction catalysed by red blood cell cytosol. *Biochem Pharmacol* **43**: 207–211, 1992.
18. Heilmair R, Karreth S and Lenk W, The metabolism of 4-aminobiphenyl in the rat. II. Reaction of 4-hydroxy-4-aminobiphenyl with rat blood *in vitro*. *Xenobiotica* **21**: 805–815, 1991.
19. Harrison JH and Jollow DJ, Role of Aniline metabolites in Aniline-induced hemolytic anemia. *J Pharmacol Exp Ther* **238**: 1045–1054, 1986.
20. Grossman SJ and Jollow DJ, Role of DDS NOH in dapsone-induced haemolytic anaemia. *J Pharmacol Exp Ther* **244**: 118–125, 1988.
21. Wallace WJ, Maxwell JC and Caughey WS, The mechanisms of haemoglobin autooxidation: evidence for proton assisted nucleophilic displacement of superoxide by anions. *Biochem Biophys Res Commun* **57**: 1104–1110, 1974.
22. Wallace WH and Caughey WS, Mechanism for the autoxidation of haemoglobin by phenols, nitrite and "oxidant" drugs. Peroxide formation by one electron donation to bound dioxygen. *Biochem Biophys Res Commun* **62**: 561–567, 1975.
23. Anderson ME, Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol* **113**: 549–555, 1985.
24. Tursi F, Samaia M, Salmona M and Belvidere G, Styrene oxidation to styrene oxide in human erythrocytes catalysed by oxyhemoglobin. *Experientia* **39**: 593–594, 1983.
25. Blisard KS and Mieyal JJ, Characterisation of the aniline hydroxylase activity of erythrocytes. *J Biol Chem* **254**: 5104–5110, 1979.
26. Winterburn CC, Oxidative reactions of hemaglobin. *Methods Enzymol* **186**: 265–272, 1990.
27. Kuma F, Properties of haemoglobin reductase and kinetic study of methaemoglobin reduction. *J Biol Chem* **256**: 5518–5523, 1981.
28. Sass MD, Caruso CJ and Axelrod DR, Mechanism of the TPNH-linked reduction of methaemoglobin by methylene blue. *Clin Chim Acta* **24**: 77–85, 1969.
29. Kelner MJ and Alexander NA, Inhibition of erythrocyte superoxide dismutase by diethyldithiocarbamate also results in oxyhemoglobin-catalysed glutathione depletion and methaemoglobin production. *J Biol Chem* **261**: 1636–1641, 1986.
30. Heikkela R, Cabatt FS and Cohen G, *In vivo* inhibition of superoxide dismutase in mice by diethyldithiocarbamate *J Biol Chem* **251**: 2182–2185, 1976.
31. Pirmohamed M, Coleman MD, Hussain F, Breckenridge AM and Park BK, Direct and indirect toxicity of sulphasalazine and its principle metabolites towards human leucocytes and erythrocytes. *Br J Clin Pharmacol* **32**: 303–311, 1991.
32. Uetrecht JP, Zahid N, Shear NH and Biggar WD, Metabolism of dapsone to a hydroxylamine by human neutraphils and mononuclear cells. *J Pharmacol Exp Ther* **245**: 274–279, 1988.
33. Tingle MD, Coleman MD and Park BK, 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, 1990.
34. Brady JF, Xiao F, Wang MH, Li Y, Ning SM, Gapac JM and Yang CS, Effects of disulfiram on hepatic P450 IIE1, other microsomal enzymes, and hepatotoxicity in rats. *Toxicol Appl Pharmacol* **108**: 366–373, 1991.
35. Winterburn CC, McGrath BM and Carrell R, Reactions involving superoxide and normal and unstable haemoglobins. *Biochem J* **155**: 493–502, 1976.
36. Winterbourn CC, French JK and Claridge RFC, The reaction of menedione with haemoglobin: mechanism and effect of superoxide dismutase. *Biochem J* **179**: 665–673, 1979.
37. Kramer PA, Glader BE and Li TK, Mechanism of methaemoglobin formation by diphenylsulphones. *Biochem Pharmacol* **21**: 1265–1274, 1972.
38. Scott GL and Rasbridge MR, The *in vitro* action of dapsone and its derivatives on normal and G-6-PD deficient red cells. *Br J Haematol* **24**: 307–317, 1973.
39. Kiese M, Reinwein D and Waller HD, Kinetik der Hamiglobinbildung. IV Mitteilung. Die Hamiglobinbildung durch Phenylhydroxylamin und Nitrosobenzol in roten Zellen *in vitro*. *Naunyn Schmiedebergs Arch Exp Pathol Pharmacol* **210**: 393–398, 1950.
40. Karreth S and Lenk W, The metabolism of 4-aminobiphenyl in rat. IV. Ferrihaemoglobin formation by aminobiphenyl metabolites. *Xenobiotica* **21**: 971–977, 1991.
41. Karreth S and Lenk W, The metabolism of 4-aminobiphenyl in rat. I. Reaction of *N*-hydroxy-4-aminobiphenyl with rat blood *in vivo*. *Xenobiotica* **21**: 417–428, 1991.
42. Mulder GS, Unruh LE, Evans FE, Ketterer B and Kadlubar FF, Formation and identification of a glutathione conjugate from 2-nitrosofluorene and *N*-hydroxy-2-aminofluorene. *Chem Biol Interact* **39**: 111–127, 1982.
43. Bawden D and Tute MS, Structure–activity relationships of anti-mycobacterial sulphones in a study using physicochemical constants. *Eur J Med Chem* **16**: 299–300, 1981.