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# The Effect of Acetylation and Deacetylation on the Disposition of Dapsone and Monoacetyl Dapsone Hydroxylamines in Human Erythrocytes In-vitro†

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## Abstract

The fates of both dapsone and monoacetyl hydroxylamine have been studied in terms of acetylation and deacetylation within the human erythrocyte in-vitro.

A comparison between the two metabolites showed equipotency in methaemoglobin generation at 15 min, although the monoacetyl derivative was the more rapid haemoglobin oxidizer. Within the erythrocytes, both dapsone and monoacetyl hydroxylamines were found to undergo acetylation, deacetylation and diacetylation. Of the inhibitors of acetylation studied, folate caused an increase in methaemoglobin formation associated with both metabolites, which led to a rise in both acetylated and non-acetylated amine formation. Amethopterin was associated with a rise in hydroxylamine mediated methaemoglobin formation which coincided with a fall in acetylated products.

It is possible that the hydroxylamines undergo erythrocytic processes of acetylation and deacetylation before methaemoglobin-mediated reduction to their respective amines.

The therapeutic application of dapsone as both an antiparasitic and anti-inflammatory agent has been well documented (Vrbova et al 1992; Piscitelli et al 1993; Smith & Zone 1993; Torres et al 1993). It is known that the acetylated metabolite of dapsone can be *N*-hydroxylated as well as the parent drug (Israili et al 1973) and the usage of the drug is often limited by the potent methaemoglobin-forming effects of these hydroxylamine metabolites (Manfredi et al 1979; Coleman et al 1992). Dapsone hydroxylamine can be detoxified by reduction within intact erythrocytes to dapsone during the process of methaemoglobin formation (Coleman & Jacobus 1993) and this may contribute to the persistence of the drug in-vivo. It has also been established that erythrocytes are capable of acetylating a variety of arylamines including dapsone (Drayer et al 1974) and that this variant of *N*-acetyl transferase is monomorphic (Ohsako & Deguchi 1990).

Through the use of inhibitors of acetylation, we wished to explore to what extent the process of methaemoglobin formation might be influenced by acetylation and deacetylation and to determine the erythrocytic fate of monoacetyl and dapsone hydroxylamines.

## Materials and Methods

*Chemicals*

Monoacetyl dapsone and its hydroxylamine was synthesized, identified and purity assessed according to the methods of Vage et al (1994). Dapsone hydroxylamine was supplied by the Jacobus Pharmaceutical Co. Inc., Princeton (NJ, USA) and was analysed as 97% pure. 4-4′-Diacetyldiaminodiphenyl sulphone (diacetyl dapsone) was synthesized with minor modifications of the methods of Vage et al (1994) by exhaustive acylation of dapsone with excess acetic anhydride and pyridine yielding a 99% pure product. Dapsone and the internal standard for the HPLC assay (3,3′ diaminodiphenyl sulphone) was obtained from the Aldrich Chemical Co. (Poole Dorset, UK). All HPLC solvents were supplied by Fisons Ltd, Loughborough, UK.

*Experimental methods*

Whole human blood was drawn from normal (mean age: 27·3 ± 4·0 years, n = 4) volunteers. 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 saline, pH 7·4 with 10 mM glucose (PBGS) and resuspended to a 50% haematocrit also in PBGS. The erythrocyte incubations (0·5 mL) each contained 1·2 μmol haemoglobin and were placed on ice to preserve intracellular glutathione concentrations and equilibrated for 10 min in uncapped tubes.

The process of methaemoglobin formation due to compounds such as dapsone and monoacetyl dapsone hydroxylamine is extremely rapid and temperature dependent (Coleman & Jacobus 1993). Therefore, to avoid inaccuracies due to these processes occurring in some samples before addition of the compounds was complete, the respective hydroxylamines were added to the red cells and thoroughly mixed while they were on ice and time zero was taken to be when they were placed in the waterbath to ensure a synchronous experiment.

All observations were carried out in triplicate using any of the four individual donors. Incubations were terminated at 3-, 5-, 10- and 15-min intervals. Aliquots (100 μL) were

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† Dedicated to the memory of M. J. Winn, Ph.D.

withdrawn from the samples for immediate methaemoglobin analysis using an IL-482 CO-oximeter (Instrumentation Laboratory, Warrington, UK) before the freezing of the samples at −20°C until subsequent HPLC assay for dapsone, monoacetyl dapsone and diacetyl dapsone according to the method of Grossman and Jollow (1988).

Experiment 1 entailed the addition of either dapsone or monoacetyl dapsone hydroxylamines to the erythrocytes (final concentration; 150 μM). Experiments 2 and 3 were identical to experiment 1 except that they involved the washing (twice) of half the erythrocytes in PBGS containing either 3 mM folate or 3 mM amethopterin. Folate and amethopterin have been shown to inhibit erythrocytic acetylation at this concentration in previous studies (Ward et al 1992) and this was confirmed in a pilot study. In each case the other half of the erythrocytes was washed twice, also with PBGS.

*Calculations and statistical analysis*

Recovery of dapsone from the blood was measured by the HPLC assay and expressed as a percentage of the number of μmol dapsone hydroxylamine originally added to the samples (Coleman & Jacobus 1993). Statistical comparisons were made using Student's *t*-test accepting $P < 0.05$ as significant. Where more than one comparison was made with the same data, the Bonferroni correction (Elashoff 1981) was employed, where the acceptable level of significance was reduced to 0·05/k (where k is the number of tests) to compensate for the increased likelihood of reaching $P < 0.05$ during multiple testing.

## Results

*Methaemoglobin studies*

Compared with that of dapsone hydroxylamine, methaemoglobin generation mediated by monoacetyl dapsone was significantly greater at 3, 5 ($P < 0.01$) and 10 min ($P < 0.05$) but not at 15 min (Fig. 1). The presence of folate significantly increased methaemoglobin generation caused by both dapsone hydroxylamine compared with folate free erythrocytes at 3, 5 and 10 but not at 15 min. Folate caused an increase in monoacetyl dapsone hydroxylamine-mediated haemoglobin oxidation at all four time points. The presence of amethopterin also significantly increased methaemoglobin generation caused by dapsone hydroxylamine compared with amethopterin-free cells at 5 and 15 min (Fig. 2), while monoacetyl dapsone-dependent methaemoglobin was significantly increased at times 5, and 15 min. Washing of erythrocytes in PBGS containing either folate or amethopterin did not cause an increase in background methaemoglobin levels.

*HPLC analysis of dapsone, monoacetyl dapsone and diacetyl dapsone*

Analysis of the erythrocytes after administration of dapsone hydroxylamine revealed that the majority of recovered metabolite was dapsone, while monoacetyl dapsone and trace levels of diacetyl dapsone were also detected (Fig. 2). Administration of monoacetyl dapsone revealed monoacetyl dapsone to be the major recovered metabolite, while dapsone was also found as well as low levels of diacetyl dapsone.

The effect of folate on dapsone hydroxylamine disposition

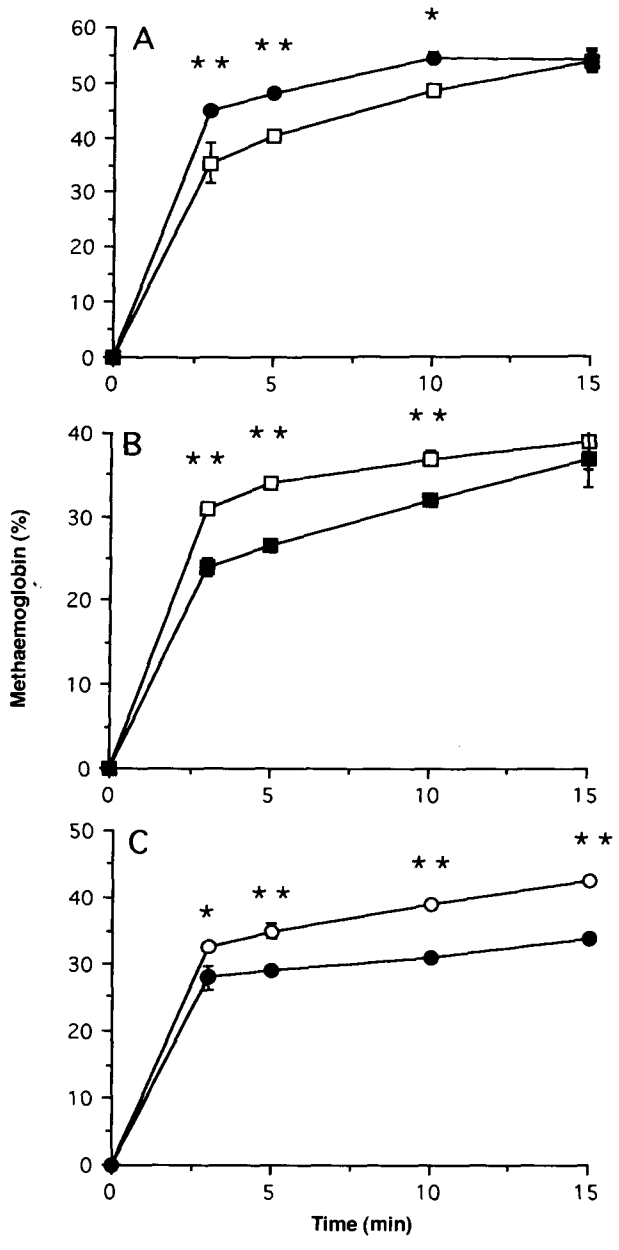


FIG. 1. Methaemoglobin formation against time after administration to human washed erythrocytes of A: dapsone hydroxylamine (□) monoacetyl dapsone hydroxylamine (●). B: dapsone hydroxylamine in the presence of folate (□) and the absence of folate (■). C: monoacetyl dapsone hydroxylamine in the presence of folate (○) and the absence of folate (●). Final concentration of hydroxylamines: 37·8 μM. Data expressed as mean ± s.d. of three determinations. *$P < 0.05$, **$P < 0.01$.

resulted in a significant reduction in dapsone recovery at 10 min (Table 1) and a reduction in monoacetyl dapsone levels at 3 min but a marked increase at 5 and 10 min, although a reduction occurred at 15 min. At all four time points, more diacetyl dapsone was recovered in the presence of folate. The presence of folate also affected dapsone recovery after the administration of monoacetyl dapsone hydroxylamine (Table 1), with a significant decrease at 15 min. Monoacetyl dapsone recovery greatly increased in the presence of folate at 10 and 15 min, while diacetyl dapsone levels increased at 10 and 15 min.

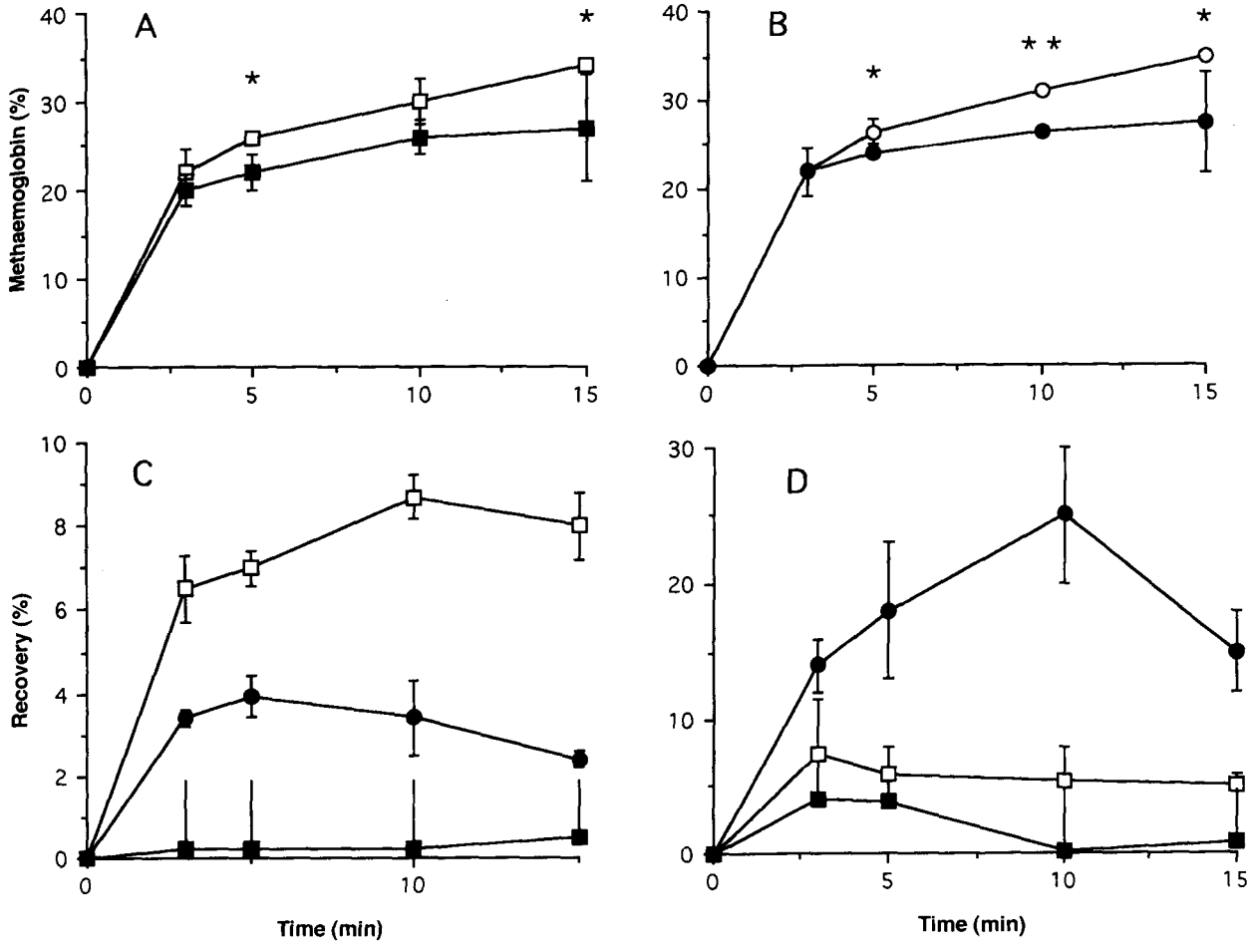


FIG. 2. Methaemoglobin formation against time after administration to human washed erythrocytes of A: dapsone hydroxylamine in the presence of amethopterin (□) and the absence of amethopterin (■). B: monoacetyl dapsone hydroxylamine in the presence of amethopterin (○) and the absence of amethopterin (●). Recovery (%) of dapsone (□), monoacetyl dapsone (●) and diacetyldapsone (■) after the administration of C: dapsone hydroxylamine D: monoacetyl dapsone hydroxylamine to human washed erythrocytes. Final concentration of hydroxylamines: 150 $\mu$M. Data expressed as mean ± s.d. of three determinations. *$P < 0.05$, **$P < 0.001$.

Table 1. Effect of washing erythrocytes in folate (3 mM) and glucose (10 mM) compared with control (10 mM glucose alone) before the addition of either dapsone hydroxylamine (150 $\mu$mol) or monoacetyl dapsone (150 $\mu$mol) on the recovery of dapsone, monoacetyl dapsone and diacetyl dapsone (DADDS). Mean ± s.d., n = 4 per incubation.

| Time (min) | Recovery (%) | | | | | |
|---|---|---|---|---|---|---|
| | Dapsone | | Monoacetyl dapsone | | Diacetyl dapsone | |
| | Control | Experimental | Control | Experimental | Control | Experimental |
| Dapsone hydroxylamine | | | | | | |
| 3 | 6·5 ± 0·4 | 7·5 ± 1 | 3·5 ± 0·2 | 0·2 ± 0·2* | 0·2 ± 0·1 | 1·9 ± 0·3* |
| 5 | 7·2 ± 0·3 | 9·0 ± 2·2 | 3·9 ± 0·2 | 11·0 ± 0·5*** | N/D | 2·0 ± 0·3 |
| 10 | 8·7 ± 0·4 | 5·0 ± 0·5* | 3·5 ± 1·2 | 10·5 ± 2·7*** | 0·3 ± 0·4 | 2·0 ± 1·0* |
| 15 | 8·5 ± 0·6 | 8·5 ± 0·9 | 2·2 ± 0·2 | 0·3 ± 0·3* | N/D | 2·9 ± 0·5 |
| Monoacetyl dapsone | | | | | | |
| 3 | N/D | N/D | 12·5 ± 4·2 | 11·2 ± 0·2 | 5·2 ± 3·1 | 5·9 ± 0·3 |
| 5 | N/D | N/D | 12·9 ± 4·0 | 11·0 ± 3·5 | N/D | N/D |
| 10 | 0·7 ± 0·4 | 0·3 ± 0·5 | 11·0 ± 1·2 | 18·5 ± 1·7*** | 2·5 ± 0·4 | 6·2 ± 1·0** |
| 15 | 14·5 ± 0·6 | 4·5 ± 0·9*** | 3·2 ± 0·2 | 12·5 ± 3·3*** | N/D | 2·5 ± 0·5 |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with control. N/D, not detectable.

Table 2. Effect of washing erythrocytes in amethopterin (3 mM) and glucose (10 mM) compared with control (10 mM glucose alone) before the addition of either dapsone hydroxylamine (150 μmol) or monoacetyl dapsone (150 μmol) on the recovery of dapsone, monoacetyl dapsone and diacetyl dapsone. Mean ± s.d., n = 4 per incubation.

| Time (min) | Recovery (%) | | | | | |
|---|---|---|---|---|---|---|
| | Dapsone | | Monoacetyl dapsone | | Diacetyl dapsone | |
| | Control | Experimental | Control | Experimental | Control | Experimental |
| Dapsone hydroxylamine | | | | | | |
| 3 | 6·3 ± 0·2 | 5·7 ± 1·3 | 3·5 ± 0·2 | 2·0 ± 0·3* | N/D | N/D |
| 5 | 8·2 ± 0·4 | 5·3 ± 0·2** | 8·9 ± 0·4 | 2·0 ± 0·5*** | 7·0 ± 0·4 | N/D |
| 10 | 8·0 ± 1·4 | 5·9 ± 0·6** | 2·0 ± 0·2 | 1·8 ± 0·1 | N/D | N/D |
| 15 | 8·0 ± 1·0 | 6·7 ± 1·0 | 1·8 ± 0·2 | N/D | N/D | 4·4 ± 0·2 |
| Monoacetyl dapsone | | | | | | |
| 3 | N/D | N/D | 10·5 ± 2·2 | 11·2 ± 0·2 | 5·2 ± 3·1 | 5·9 ± 0·3 |
| 5 | N/D | N/D | 12·9 ± 4·0 | 11·0 ± 3·5 | N/D | N/D |
| 10 | 1·3 ± 0·4 | N/D | 11·0 ± 1·2 | 18·5 ± 1·7*** | 2·5 ± 0·4 | 6·2 ± 1·0** |
| 15 | 14·5 ± 0·6 | 4·5 ± 0·9*** | 3·2 ± 0·2 | 12·5 ± 3·3*** | N/D | 2·5 ± 0·5 |

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with control. N/D not detectable.

Amethopterin caused a net fall in dapsone recovery after dapsone hydroxylamine administration at 5 and 10 min (Table 2) and monoacetyl dapsone levels also were reduced at 3 and 5 min and were undetectable at 15 min compared with the control (absence of amethopterin). Diacetyl dapsone was undetectable at three out of the four time points both with and without amethopterin. After the administration of monoacetyl dapsone hydroxylamine, dapsone levels were undetectable until 15 min, while in control incubations (without amethopterin) dapsone was detected at 10 and 15 min, at levels which were three fold higher than in the presence of amethopterin. There was no significant change in monoacetyl dapsone recovery in the presence of inhibitor at 3 and 5 min, although levels rose markedly at 10 and 15 min. Diacetyl dapsone levels did not differ at 3 and 5 min, although they increased at 10 and 15 min.

## Discussion

The effect of monoacetyl and dapsone hydroxylamine in erythrocytes has been recently studied (Vage et al 1994) and the derivatives were found to be equitoxic over 1 h. In this study, rather than measure the total extent of the hydroxylamine effect, the compounds were compared over a very short period in terms of rate of methaemoglobin formation, which was in keeping with previous work indicating the rapidity of the hydroxylamine-dependent methaemoglobin forming process (Coleman & Jacobus 1993). Our findings were in broad agreement with those of Vage et al (1994) as equitoxicity occurred at 15 min, although the monoacetyl derivative appeared to be the more rapid methaemoglobin former over the first 10 min of the two compounds in our system. This observation may have been due to the increased penetration of the monoacetyl hydroxylamine derivative into the erythrocyte, as monoacetyl dapsone itself has been shown to be substantially more lipophilic than the parent compound (Coleman et al 1991). However, factors such as the possible increased protein binding of the acetyl derivative which might occur in whole blood (Vage et al 1994) did not apply as washed erythrocytes were used in the present study. Hence our observations, while not directly comparable with those of Vage et al (1994) could be viewed as complementary. However, in-vivo it is possible that methaemoglobin formation might be more rapid when caused by the monoacetyl dapsone hydroxylamine and it would be very difficult to evaluate the true contribution of this metabolite to the total methaemoglobin-forming capacity of a given dapsone dose, as several processes would be occurring in-vivo, such as acetylation, deacetylation and *N*-hydroxylation as well as protein binding.

Erythrocytic conversion of dapsone hydroxylamine to dapsone is a glutathione-dependent consequence of methaemoglobin formation (Coleman & Jacobus 1993) and this process of amine formation from hydroxylamines also occurs with 4-amino-biphenyl (Heilmair et al 1991). In the present study, not only has the reduction of monoacetyl dapsone hydroxylamine to dapsone been shown for the first time, but also the reduction of both dapsone and monoacetyl dapsone hydroxylamines followed by either acetylation (in the case of dapsone hydroxylamine) or deacetylation (as dapsone was recovered after monoacetyl dapsone hydroxylamine was administered). In addition, after administration of either hydroxylamine, diacetylated parent drug was also formed after methaemoglobin-mediated reduction. Although it has long been known that dapsone itself may be acetylated by erythrocytes (Drayer et al 1974), previous studies with erythrocyte preparations have been unable to demonstrate that deacetylation occurs, although it was not ruled out (Ward et al 1992). It is of interest to determine in which order these processes, i.e. methaemoglobin-mediated reduction of metabolite to amine, or acetylation/deacetylation/diacetylation, actually occur.

If acetylation occurred as soon as the hydroxylamine metabolites entered the cell, then the use of the inhibitors might conceivably increase the rate of methaemoglobin formation as the hydroxylamines might not be delayed in the process of acetylation which had been inhibited, assuming that acetylation is indeed slower than the methaemoglobin-mediated reduction process. If the hydroxylamines underwent methaemoglobin-mediated reduction before the acety-

lation process, then it might be inferred that the inhibitors might have little or no effect. Whichever process predominantly occurs earliest might be a function of the affinity of the respective enzymes involved, i.e. oxyhaemoglobin and *N*-acetyltransferases and deacetylases, or even their actual site within the erythrocyte. Deacetylases, or arylamidases have been found in erythrocytes (Lindsay et al 1991) and it has been postulated that these enzymes may be located within erythrocyte membranes (Ward et al 1992), and might be encountered by a xenobiotic almost immediately on cell entry before reaction with oxyhaemoglobin.

Folate concentrations in intact erythrocytes are often sufficient to inhibit acetylating processes (Ward et al 1992). In this study, folate concentrations in excess of background caused an acceleration of both hydroxylamine derivative-mediated methaemoglobin formation. After dapsone hydroxylamine administration, folate paradoxically was associated with increased acetylation as well as deacetylation, although it had little effect on dapsone formation. After administration of monoacetyl dapsone hydroxylamine, monoacetyl and diacetyl dapsone levels rose at the two later time points although dapsone levels did not. The effect of folate in the present work may be explained in terms of its accelerating effect on methaemoglobin levels. In a previous study, diethyldithiocarbamate was shown to accelerate both hydroxylamine-dependent methaemoglobin and dapsone formation in human erythrocytes (Coleman & Jacobus 1993). These processes are mediated by glutathione, and in combination with the hydroxylamine, diethyldithiocarbamate accelerated thiol depletion at a much quicker rate compared with either compound alone (Coleman & Jacobus 1993). It might be speculated that folate might in some way mimic the action of diethyldithiocarbamate, leading to the increase in hydroxylamine-dependent methaemoglobin formation. Alternatively, folate might conceivably influence glutathione metabolism. In any case, it is likely that the methaemoglobin-promoting action of folate is unconnected with its known effects on acetylation.

In contrast to folate, amethopterin reduced monoacetylation after dapsone hydroxylamine formation and also caused a reduction in deacetylation after monoacetyl dapsone hydroxylamine administration, although no effect was seen in diacetyl dapsone recovery. After the administration of the acetylated hydroxylamine, the fall in recovered dapsone corresponded with a rise in monoacetyl dapsone recovery, although a slight increase in diacetyl dapsone did also occur at 10 min in the presence of the inhibitor. However, these observations were accompanied by a significant increases in methaemoglobin formation mediated by both metabolites. Although the fall in monoacetyl dapsone formation after the administration of dapsone hydroxylamine was also paradoxically accompanied by a fall in dapsone recovery, which might have been expected to increase, it might be inferred that the effect of amethopterin at least partially supports the hypothesis that inhibition of acetylation allows more hydroxylamine derivative to react with the oxyhaemoglobin. As deacetylases may be associated with erythrocyte membranes (Ward et al 1992), it is conceivable that a substantial proportion of hydroxylamines entering an erythrocyte may undergo immediate acetylation or deacetylation before reaction with oxyhaemoglobin.

Polymorphic liver *N*-acetyltransferase is chiefly responsible for acetylation of dapsone in man (Blum et al 1990). The variant of the enzyme which exists in erythrocytes and leucocytes is monomorphic and more restrictive in its substrate preference (Ohsako & Deguchi 1990) and is less likely to contribute to the overall systemic disposition of dapsone. However, erythrocytes have been shown to acetylate, deacetylate and diacetylate dapsone and its related hydroxylamines in this study and erythrocytic metabolism of hydroxylamines of the drug may play a role in the aeteology of idiosyncratic conditions such as agranulocytosis (Coleman et al 1994). In addition, monoacetyl dapsone is much more lipophilic than the parent compound (Coleman et al 1991) and did show a more rapid initial methaemoglobin formation compared with dapsone hydroxylamine in this study. If the hydroxylamine of the acetylated compound does penetrate tissues more readily than dapsone hydroxylamine, then the balance of toxicity of dapsone as well as its actual therapeutic effect in skin conditions such as dermatitis herpetiformis may be in part dependent on the ability of erythrocytes to acetylate and deacetylate hydroxylamine derivatives of the drug.

The diacetylated derivative of dapsone was generally made in trace amounts by the erythrocytes, is not vulnerable to *N*-hydroxylation and thus is not toxic (Tingle et al 1990). The diacetyl derivative is even more lipophilic than the monoacetylated derivative, and bearing in mind that deacetylation may occur in many tissues, it is possible that the diacetylated metabolite also may make a small contribution to the therapeutic effect of dapsone in-vivo.

In summary, both the hydroxylamines of dapsone and monoacetyl dapsone may be acetylated and deacetylated by human erythrocytes. It is possible that acetylation reactions may occur before reduction of the hydroxylamines to their respective amines and an equilibrium may exist between acetylation, deacetylation and hydroxylamine reduction within the erythrocyte.

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## References

Blum, M., Grant, D. M., McBride, W., Heim, M., Meyer, U. A. (1990) Human arylamine *N*-acetyltransferase genes: isolation, chromosomal localisation, and functional expression. DNA Cell Biol. 9: 192–203

Coleman, M. D., Tingle, M. D., Hussain, F., Storr, R. C., Park, B. K. (1991) An investigation into the haematological toxicity of structural analogues of dapsone in-vivo and in-vitro. J. Pharm. Pharmacol. 43: 779–784

Coleman, M. D., Rhodes, L. A., Scott, A. K., Verbov, J., Friedmann, P., Breckenridge, A. M., Park, B. K. (1992) The use of cimetidine to reduce dapsone-dependent methaemoglobinaemia in dermatitis herpetiformis patients. Br. J. Clin. Pharmacol. 34: 244–249

Coleman, M. D., Jacobus, D. P. (1993) Reduction of dapsone hydroxylamine to dapsone during methaemoglobin formation in human erythrocytes in vitro. Biochem. Pharmacol. 45: 1027–1033

Coleman, M. D., Simpson, J., Jacobus, D. P. (1994) Reduction of dapsone hydroxylamine to dapsone during methaemoglobin formation in human erythrocytes in vitro IV: the role of the

erythrocyte in the development of dapsone-dependent agranulocytosis. Biochem. Pharmacol. 48: 1349–1354

Drayer, D. E., Strong, J. M., Jones, B., Sandler, A., Reidenberg, M. M. (1974) In vitro acetylation of drugs by human blood cells. Drug Metab. Dispos. 2: 499–505

Elashoff, J. D. (1981) Down with multiple 't' tests. Gastroenterology 80: 615–620

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

Heilmair, R., Karreth, S., Lenk, W. (1991) The metabolism of 4-aminobiphenyl in the rat II. Reaction of 4-hydroxy-4-aminobiphenyl with rat blood in vitro. Xenobiotica 21: 805–815

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

Lindsay, R. M., Fox, W. R., Baty, J. D., Willis, R. G. (1991) In vitro studies on the deacetylation-reacetylation of arylamides and the transacetylation of arylamides by human and rat whole blood. Biochem. Pharmacol. 41: 1671–1678

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

Ohsako, S., Deguchi, T. (1990) Cloning and expression of cDNA's for polymorphic and monomorphic arylamine *N*-acetyltransferases from human liver. J. Biol. Chem. 265: 4630–4634

Piscitelli, S. C., Occhipinti, D. J., Danziger, L. H., Hill, C., West, D. P., Fischer, J. H. (1993) Therapeutic monitoring and pharmacist intervention in a Hansens' disease clinic. Ann. Pharmacother. 27: 1526–1532

Smith, E. P., Zone, J. J. (1993) Dermatitis herpetiformis and linear IgA bullous dermatitis. Dermatol. Clin. 11: 511–583

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

Torres, R. A., Barr, M., Thorn, M., Gregory, G., Wiely, K., Chanin, E., Carlo, C., Nartin, M., Thornton, J. (1993) Randomized trial of dapsone and aerosolized pentamidine for the prophylaxis of *Pneumocystis carinii* pneumonia and toxoplasmic encephalitis. Am. J. Med. 95: 573–583

Vage, C., Saab, N., Woster, P. M., Svensson, C. K. (1994) Dapsone-induced hematologic toxicity-comparison of the methemoglobin forming ability of hydroxylamine metabolites of dapsone in rat and human blood. Toxicol. Appl. Pharmacol. 129: 309–316

Vrbova, H., Gibney, S., Gibson, F. D., Jolley, D., Heywood, P. F., Stace, J., Trenholme, F. R., Alpers, M. P. (1992) Chemoprophylaxis against malaria in Papua New Guinea: a trial of amodiaquine and a combination of dapsone and pyrimethamine. Papua New Guinea Med. J. 35: 275–284

Ward, A., Hickman, D., Gordon, J. W., Sim. E. (1992) Arylamine *N*-acetyltransferase in human red blood cells. Biochem. Pharmacol. 44: 1099–1104