

Influence of acetylator phenotype on the haematological and biochemical effects associated with dapsone in leprosy patients

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Summary Methaemoglobinaemia and haemolytic anaemia were the principal side-effects observed in 30 leprosy patients undergoing long-term treatment with dapsone as a single drug or as part of multidrug therapy. Hepatic, pancreatic and renal evaluations showed no relevant clinical changes. Since N-acetylation is a major metabolic pathway for dapsone, slow acetylation phenotype may be a risk factor for the development of these reactions. To confirm this hypothesis we correlated acetylator phenotype and the haematological and biochemical effects induced by dapsone.

No excess proportion of slow acetylators was found. We conclude that slow acetylators are not at greater risk of developing haematological side-effects of dapsone than fast acetylators.

Introduction

N-acetyltransferases play an important role in the biotransformation of a number of clinically useful drugs such as isoniazid, procainamide, hydralazine, dapsone, sulphamethazine as well as some carcinogenic arylamines¹. In some cases, acetylation capacity has been shown to be related to variation in drug response, susceptibility to adverse reactions and increased incidence of certain spontaneous disorders including cancer.²⁻⁶

Dapsone is a widely used drug, extensively employed in the treatment of leprosy⁷ and the prophylaxis of malaria,⁸ and more recently in the treatment of *Pneumocystis carinii* pneumonia in AIDS patients.⁹ A number of side-effects are associated with dapsone therapy, including methaemoglobinaemia and oxidative haemolysis, haematological effects that frequently limit its clinical use.^{10,11}

Zuidema *et al.*¹² reported no difference in serum or plasma concentration or in any pharmacokinetic parameters of dapsone or monoacetyldapsone between slow and fast acetylators. Also, the therapeutic response was the same for both acetylator phenotypes.

It has been speculated that slow acetylators may have relatively more of the parent drug available for oxidative metabolism by cytochrome P-450 and thus may be at an increased risk to develop haematological side-effects in response to dapsone.¹³ Although there is preliminary evidence that patients with the slow acetylator phenotype may be disproportionately represented among patients with haematological side effects, no conclusive data are available in the literature about the possible side effects of long-term treatment.

To confirm this preliminary finding, we investigated the influence of acetylation phenotype on the haematological and biochemical effects of a group of leprosy patients who were submitted to long-term treatment with dapsone as a single drug or as part of multidrug therapy (MDT), i.e., dapsone plus clofazimine plus rifampicin.

Methods

LEPROSY PATIENTS

Thirty white Brazilian leprosy patients (14 females and 16 males) on dapsone treatment for at least 6 months as a single drug or as part of multidrug therapy, seen at the Regional Sanitary Dermatology Outpatient Clinic of Ribeirão Preto (ARE-DSRP) participated in this study. No patient had a history of sulphonamide allergy or glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. Social alcohol intake was permitted, but chronic heavy alcohol users were excluded from the study. Elderly patients (older than 65 years), patients with acquired immunodeficiency and pregnant women were also excluded. The patients were divided into three groups according to therapeutic schedule: Control Group—15 patients of both sexes who had been on MDT for at least six months previously but who had been off all medication for a period of 6 months or more at the time of the study, with clinically and bacilloscopically inactive disease. The controls did not receive any medication during the study. DDS Group—15 patients of both sexes treated with 100 mg/day dapsone. Group MDT—15 patients of both sexes given dapsone 100 mg/day dapsone plus 100 mg/day clofazimine on alternate days, and a 300 mg dose given once a month under supervision, plus rifampicin, 600 mg once a month under supervision.

ACETYLATION PHENOTYPING

A single 2.5 ml heparinized blood sample was obtained 12 hr after administration of the daily dose of dapsone (CEME, Brasília, Brazil). Plasma was separated by centrifugation and stored at -20°C pending analysis. The phenotype criterion of Reidenberg *et al.*,¹⁴ i.e., a plasma (MADDS/DDS) ratio of less than 0.30, indicated slow acetylators.

ANALYSIS

Plasma dapsone and monoacetyldapsone concentrations were measured by the HPLC method of Queiroz *et al.* (in press), as follows.

A 1-ml aliquot of plasma supplemented with the internal standard (50 µl of the phenacetin solution) was alkalized with 200 µl of a 1.5 N sodium hydroxide solution and extracted with 7 ml ethyl ether for 30 min in a mechanical shaker after the addition of 200 mg sodium chloride. The samples were centrifuged at 3000 rpm for 10 min and the

organic phases transferred to conic tubes to which 50 μl 1 N HCl in methanol were added. After extract evaporation under an air flow at room temperature the residues were reconstituted with 50 μl of the mobile phase and 50 μl of n-hexane. After shaking in a mixer for 1 min and centrifugation, 20 μl of the mobile phase were injected into the liquid chromatography apparatus.

Chromatography was performed using a Varian liquid chromatography apparatus model 5000 equipped with a Rheodyne injector model 7125 with a 20 μl sampler and a Varian ultraviolet absorbance detector model UV-100 operating at 286 nm. The chromatograms were obtained with a Varian integrator model 4290. Analysis was carried out on a reverse phase C₈ LiChrocart[®] 100 column (4 \times 125 mm, Merck) with 5 μm particles. The mobile phase used was a water:methanol mixture (70:30 v/v) with a flow of 1 ml min⁻¹.

BIOCHEMICAL AND HAEMATOLOGIC PARAMETERS

Three blood samples were collected from each patient at weekly intervals for a more judicious evaluation of haematological and biochemical data. The results obtained (means \pm SD) were compared with those obtained for the controls. The samples were obtained in the morning from fasted patients after a protocol had been filled out with patient name, age, sex, weight, scheduled medication, collection time and associated medications. The study of the adverse effects of dapsone was carried out haematologically level (blood count, reticulocytes, osmotic fragility, detection of Heinz bodies and methaemoglobinaemia) and biochemically (transaminases, bilirubins, alkaline phosphatase, gamma-glutamyltransferase, amylase, urea, creatinine, and potassium).

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Glucose-6-phosphate dehydrogenase levels were measured by the spectrophotometric method of Lohr & Waller¹⁵ using Sigma Diagnostics[®] (St Louis, MO, USA) reagents which are for quantitative, ultraviolet, kinetic determination in blood at 340 nm.

STATISTICAL METHODS

Data were analysed statistically by ANOVA and by the multiple comparisons post test (Tukey-Kramer) using the GraphPad Instant[®] and Statgraphics[®] software, with the level of significance set at $p < 0.05$.

Results

None of the 30 leprosy patients were found to have G-6-PD deficiency. The acetylation phenotype was determined on the basis of the ratio of plasma MADDS and DDS concentration. The mean values (\pm SD) for slow (13) and fast acetylators (17) were 0.28 ± 0.05 and 0.57 ± 0.01 , respectively. The percentages of slow and fast acetylators among the leprosy patients in the two groups studied were 43.3 and 56.7, respectively.

The haematologic and biochemical abnormalities observed in slow and fast acetylators are shown in Table 1 and are reported as means \pm SD of the data for the slow and fast acetylator groups compared with control. Haemolytic anaemia in addition to

Table 1 Tukey-Kramer multiple comparison test of haematology and biochemical data

Parameter	Control (n = 15) Mean ± SD	Acetylators		Slow vs Fast P value
		Slow (n = 13) mean ± SD (95% CI)	Fast (n = 17) mean ± SD (95% CI)	
Red cell count (million/mm ³)	5.19 ± 0.28	3.95 ± 0.46† (3.67 - 4.22)	4.25 ± 0.39† (4.05 - 4.46)	NS
Packed cell volume (PCV) (%)	45.80 ± 2.70	34.54 ± 4.05† (32.08 - 36.98)	36.94 ± 3.68† (35.04 - 38.83)	NS
Haemoglobin (g/dl)	14.78 ± 0.83	10.39 ± 1.62† (9.41 - 11.37)	11.17 ± 1.34† (10.48 - 11.86)	NS
Mean cell haemoglobin (MCH) (pg)	28.46 ± 0.88	26.17 ± 1.64† (26.18 - 27.17)	26.20 ± 1.16† (25.60 - 26.80)	NS
Mean cell haemoglobin concentration (g/dl)	32.32 ± 1.00	29.97 ± 1.80† (28.88 - 31.06)	29.76 ± 1.66† (28.90 - 30.62)	NS
Reticulocyte count (%)	0.90 ± 0.08	2.16 ± 0.89† (1.41 - 2.91)	2.55 ± 1.36† (1.28 - 3.81)	NS
Methaemoglobin (%)	0.99 ± 0.34	6.92 ± 1.36† (6.10 - 7.74)	5.97 ± 1.56† (5.16 - 6.78)	NS
Eosinophilis (mm ³)	246.93 ± 180.01	787.86 ± 482.43† (520.7 - 1,055.1)	775.6 ± 617.1† (433.83 - 1,117.4)	NS
Lymphocytes (mm ³)	2,285.20 ± 661.28	1,595.12 ± 470.94* (1,201.4 - 1,988.9)	1,834.28 ± 319.07* (1,539.2 - 2,129.4)	NS
Total bilirubin (mg/dl)	0.74 ± 0.12	0.83 ± 0.13* (0.75 - 0.91)	0.92 ± 0.22* (0.79 - 1.04)	NS
Creatinine (mg/dl)	0.94 ± 0.08	1.19 ± 0.12* (1.04 - 1.19)	1.24 ± 0.15* (1.16 - 1.32)	NS
Potassium (mEq/L)	3.94 ± 0.33	4.09 ± 0.23* (3.95 - 4.23)	4.22 ± 0.33* (4.05 - 4.40)	NS

* Analysis of variance - *P* < 0.05.
 † Analysis of variance - *P* < 0.01.
 NS - not significant (*P* > 0.05).

methaemoglobinaemia was also detected in fast and slow acetylators. Thirteen patients (6 slow and 7 fast acetylators) had anaemia with haemoglobin levels of 7.2–10.9 g/dL and packed cell volumes of 28%–37%. Reduction in total red cell number, macrocytosis, poikilocytosis and hypochromia were detected in most patients. Eleven males (5 slow and 6 fast acetylators) had normocytic, normochromic anaemia (haemoglobin levels of 10.9–12.3 g/dL and packed cell volumes of 33%–37%). The other patients had anisocytosis or poikilocytosis. Reticulocyte counts were elevated (> 1.8%) in 80% of the patients. The haemolytic action of dapsone was also evaluated by the osmotic fragility test, and a reduction in cell resistance was detected in 100% of the patients compared to controls regardless of acetylator phenotype. Heinz bodies were only detected in slow acetylators (6%).

Differential white cell counts showed significant eosinophilia for both slow (46.6%) and fast (53.3%) acetylators. Since no clinical symptoms or parasite infection that might cause the eosinophilia were detected, we postulated this as a possible allergic reaction to the medication.

Hepatic, pancreatic and renal evaluation by biochemical parameters showed occasional changes of no apparent clinical significance, although creatinine, potassium and

bilirubin values showed statistically significant differences compared to the control when the data were submitted to analysis of variance (ANOVA), Table I.

The influence of acetylator phenotype on the haematologic and biochemical effects associated with long-term dapsone treatment was evaluated by the Tukey-Kramer multiple comparison post test (Table I). Comparison of the haematological and biochemical parameters of the patients classified as slow and fast acetylators did not reveal statistically significant differences between the two groups.

Discussion

This study did not reveal a statistically significant excess of haematological side effects in slow acetylators among leprosy patients on long-term treatment with dapsone.

As also reported by Kelly & Griffiths,¹⁶ we failed to observe any association between acetylator status and severity of haemolysis or methaemoglobinaemia, side-effects, which are thought to be mediated by the hydroxylamine metabolites.¹⁷

Dapsone hydroxylamine reacts with oxyhaemoglobin (Fe^{2+}) to form methaemoglobin (Fe^{3+}) and the nitrosoarene, which is in turn reduced to the hydroxylamine by either NADPH methaemoglobin reductases or glutathione. Each hydroxylamine molecule is capable of oxidizing up to five oxyhaemoglobin molecules, and the cycle only ceases when the erythrocyte is almost totally depleted of glutathione. As methaemoglobin cannot carry oxygen, it may cause, in proportion to blood levels, lethargy, headache, cyanosis, dyspnoea, tachycardia, nausea and, in extreme cases, death. Methaemoglobin levels of under 20% are not usually associated with symptoms, although in our study four females, with 8.1–9.8% methaemoglobinaemia reported symptoms of lethargy, headache and nausea. This is consistent with literature showing that some patients cannot tolerate even low levels of methaemoglobinaemia.^{10,11}

Dapsone therapy also reduces erythrocyte survival time. Recent studies on rats have suggested that the hydroxylamine promotes the formation of disulphide-linked adducts between haemoglobin and red cell skeletal proteins. Dapsone hydroxylamine also interferes with potassium and chloride cotransport within rat red cells, and causes them to shrink and become less deformable. Overall, after exposure to dapsone hydroxylamine, erythrocytes are recognized as aged and prematurely removed from the circulation by the spleen. The presence of Heinz bodies has also been associated with reactive biotransformation products resulting from the oxidative denaturation of haemoglobin.^{10,11}

Studies reporting the occurrence of haemolysis and anaemia induced by dapsone have suggested that these clinical symptoms occur mainly in the presence of high doses (> 100 mg/day) of the medication or in G-6-PD deficient patients.¹⁷ However, in our study we showed that in patients with normal G-6-PD levels on therapeutic doses of 100 mg/day dapsone, some of them developed anaemia (44%) (Table I). And as also reported by Byrd & Gelber,¹⁸ we observed that chronic dapsone treatment results in not only haemolysis but a significant decrease in haemoglobin concentration.

Attempts have been made to counteract the haemotoxic effects of the metabolite by the use of antioxidants such as vitamins E and C.¹⁰ Recently, the coadministration of a metabolic inhibitor such as cimetidine has been shown to reduce significantly dapsone-dependent methaemoglobinaemia, without any change in drug efficacy. Such a therapeutic

strategy may be appropriate for patients who require high-dose dapsone and for those who are particularly susceptible to dapsone-induced haemotoxicity.¹⁹

This study, therefore, while supporting the view that a long-term treatment of dapsone (100 mg/day) may cause significant methaemoglobinaemia and haemolysis, also reveals that patients who are slow acetylators are not at greater risk of developing haematological side-effects of dapsone than fast acetylators.

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