Influence of age, sex and body weight on the dapsone acetylation phenotype

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1 The acetylation of dapsone (DDS) was determined by estimation of the ratio of monoacetyldapsone (MADDS) to DDS concentrations in plasma following a single dose of DDS in 337 white British subjects (193 female; 144 male).

2 The percentage of slow DDS acetylators in the whole group was 60.3%. There was no statistically significant difference in this proportion between 191 elderly subjects (age > 65 years) and 143 young subjects (age < 30 years).

3 Although there was a small (66.3%) but significant ($P = 0.033$) preponderance of slow acetylators in the young male group there was no difference in the distribution of acetylator phenotypes between the sexes among either the elderly group or in the whole population studied.

4 No correlation was found between absolute body weight and MADDS/DDS ratios.

Keywords dapsone acetylation phenotype age sex bodyweight

Introduction

It is well established that *N*-acetylation of a number of drugs bearing an aryl amine or hydrazino group demonstrates genetic polymorphism; slow acetylators being homozygous for an autosomal recessive gene (rr), while rapid acetylators may be either homozygous (RR) or heterozygous (Rr) for the dominant allele (Evans *et al.*, 1960). The enzyme responsible is the non-microsomal, non-inducible *N*-acetyltransferase (NAT). Drugs acetylated polymorphically include, dapsone, isoniazid, hydralazine, procainamide, phenelzine, sulphadimidine and sulphapyridine. There is wide inter-ethnic variation in the proportion of acetylator phenotypes, and approximately 50% of Caucasians are slow acetylators (Weber & Hein, 1979). Polymorphic *N*-acetylation has been linked to variation in drug response, susceptibility to adverse reaction, and to increased incidence of certain spontaneous disorders including cancer

(Cartwright *et al.*, 1982; Evans, 1984; Weber & Hein, 1985).

Epidemiological studies show that the incidence of adverse reactions to drugs increases in the elderly. This may be related, at least in part, to changes in drug metabolising activities accompanying ageing and it has been demonstrated that microsomal drug oxidizing activity is reduced in the elderly (Farah *et al.*, 1977). Although it is recognised that the activities of hepatic microsomal enzymes are modulated by factors such as age, sex, diet, disease, medication and environmental exposures (Vesell, 1982) little is known about the influence of such factors on NAT activity. Recently several studies have given contradictory results on the influence of age and sex on the acetylation phenotype (Gachalyi *et al.*, 1984; Paulsen & Nilsson, 1985; Pontiroli *et al.*, 1985).

The present paper reports an investigation

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into the influence of ageing and body weight upon the assignment of acetylation phenotype using dapsone.

Methods

Subjects

Three hundred and thirty-seven white British subjects (193 female, 144 male) took part in this study. Approval to conduct this study was granted by the local ethics committees of Bromley Health Authority and Guy's Hospital. Informed consent was obtained from each subject. All subjects were on normal diets and none was on any medication known to interact with NAT. No subject had any history of sulphonamide allergy or glucose-6-phosphate dehydrogenase deficiency. Social alcohol intake was permitted, but chronic heavy alcohol intake excluded subjects from the study. The subjects were divided into two groups: (a) *Elderly group*: 191 patients of both sexes, aged 65 years and over, attending the geriatric unit at Orpington Hospital, Kent, for various medical conditions which included urinary tract and pulmonary infections, Parkinson's disease, congestive cardiac failure, stroke and social isolation. In all cases, provided no interaction was likely with dapsone, the patient's medication was continued during the study. (b) *Young group*: 146 healthy volunteers of both sexes aged below 30 years, comprised healthy medical students and staff members at the United Medical and Dental School (Guy's Campus), London.

Acetylation phenotyping

This was ascertained employing dapsone (DDS) as described by Philip *et al.* (1984) using the phenotype criterion of Reidenberg *et al.* (1975) i.e. ratios of monoacetyldapsone/dapsone (MADDS/DDS) in plasma less than 0.30 indicated slow acetylators.

Each subject received 100 mg dapsone (Cox Pharmaceuticals, Barnstaple, England) orally. Venous blood (5 ml) was taken into a lithium heparin tube 1.5–4 h after dosing. Blood was centrifuged and plasma separated immediately to be stored at -20°C pending analysis. The MADDS/DDS ratio rapidly achieves a constant value and remains constant for a considerable period following DDS administration so that the exact time of blood sampling is immaterial (Gelber *et al.*, 1971; Ahmad, 1981).

Analysis

This was performed at the Department of Clinical Pharmacology, United Medical and Dental Schools, Guy's Hospital, London. DDS and MADDS were simultaneously measured in plasma using a non-extractive h.p.l.c. method described by Philip *et al.* (1984).

Statistical methods

An uncorrected chi-square test was employed to explore the distribution of rapid and slow acetylators in relation to age and sex. Non-parametric Spearman regression analysis was used to determine the degree of correlation between body weight and MADDS/DDS ratios. A level of significance was assumed to exist at 5%.

Results

Age

Table 1 gives the distribution of elderly, young and whole population at various MADDS/DDS ratios.

The proportion of slow and fast acetylators in the population as a whole was 60.3% and 39.7% respectively, which is similar to others made on Caucasian populations (Weber & Hein, 1979; Kalow, 1982).

There was no statistical difference ($\chi^2 = 2.69$, $0.50 > P > 0.100$) in the proportion of acetylator phenotypes in the young and old age groups, nor was there any significant difference by comparison of each age group with the whole population.

Sex

Table 2 shows the intersex distribution of subjects at different MADDS/DDS ratios in young, elderly and the population as a whole.

66.3% of the males and 47.9% of females below the age of 30 years were slow acetylators. This difference in proportion of slow acetylator phenotypes attained statistical significance ($\chi^2 = 4.56$, $P = 0.033$). However, there was no difference in the distribution of acetylator phenotypes among sexes in either the elderly group or population as a whole.

Body weight

Data on absolute body weight in kg were available on 95 and 118 of the elderly and young groups respectively. There was no correlation between

Table 1 Distribution of young and elderly subjects at various MADDS/DDS ratios

MADDS/DDS	Young		Elderly		Young + Elderly	
	n	%	n	%	n	%
0.01-0.09	5	3.4	7	3.7	12	3.6
0.10-0.19	63	43.2	50	26.2	113	33.5
0.20-0.29	20	13.7	41	21.5	61	18.1
0.30-0.39	15	10.3	22	11.5	37	11.0
0.40-0.49	19	13.0	25	13.1	44	13.1
0.50-0.59	10	6.8	12	6.3	22	6.5
0.60-0.69	2	1.4	14	7.3	16	4.7
0.70-0.79	1	0.7	13	6.8	14	4.2
0.80-0.89	6	4.1	2	1.0	8	2.4
0.90-0.99	2	1.4	3	1.6	5	1.5
1.00+	3	2.1	2	1.0	5	1.5
Total	146	100.0	191	100.0	337	100.0
Slow acetylators	88	60.3	98	51.3	186	55.2

Young vs Elderly $\chi^2 = 2.69$, df = 1, $0.50 > P > 0.10$ (NS)

Young vs Young + Elderly $\chi^2 = 1.46$, df = 1, $0.50 > P > 0.10$ (NS)

Elderly vs Young + Elderly $\chi^2 = 0.74$, df = 1, $0.50 > P > 0.10$ (NS).

Table 2 Percentages of MADDS/DDS ratios categorised for age and sex

MADDS/DDS	Young males n = 98	Young females n = 48	Elderly males n = 46	Elderly females n = 145	All males n = 144	All females n = 193
0.01-0.09	5.1	0.0	4.3	3.4	4.9	2.6
0.10-0.19	49.0	31.3	28.3	25.5	42.4	26.9
0.20-0.29	12.2	16.7	17.4	22.8	13.9	21.2
0.30-0.39	8.2	14.6	8.7	12.4	8.3	13.0
0.40-0.49	9.2	20.8	21.7	10.3	13.2	13.0
0.50-0.59	7.1	6.3	8.7	5.5	7.6	5.7
0.60-0.69	1.0	2.1	2.2	9.0	1.4	7.3
0.70-0.79	0.0	2.1	6.5	6.9	2.1	5.7
0.80-0.89	4.1	4.2	2.2	0.7	3.5	1.6
0.90-0.99	2.0	0.0	0.0	2.1	1.4	1.6
1.00+	2.0	2.1	0.0	1.4	1.4	1.6
Percentage slow acetylators	66.3	49.9	50.0	51.7	61.1	50.7

Young males vs young females: $\chi^2 = 4.56$, df = 1, $P = 0.033$

Elderly males vs elderly females: $\chi^2 = 0.042$, df = 1, $P > 0.50$

All males vs all females: $\chi^2 = 3.56$, df = 1, $P > 0.05$.

n = Total number.

absolute body weight and MADDS/DDS ratios ($r_s = -0.053$, $P > 0.50$) (Figure 1).

Discussion

Evans *et al.* (1960) demonstrated the genetic basis of polymorphic acetylation of isoniazid (INH) but failed to detect any heterogeneity in distribution of acetylator phenotypes in relation to age and sex. Their study involved 267 members of 53 white North American families. Farah *et al.* (1977) confirmed the lack of an ageing effect

on INH acetylation capacity in a small (23 young and 27 elderly subjects) population study and later we (Cartwright *et al.*, 1982) observed no sex difference in the distribution of dapsone acetylator phenotypes. However, Iselius & Evans (1983) have retrospectively detected a significant effect of age and sex upon re-analysis of the data presented in Evans' original study. Furthermore, Gachalyi *et al.* (1984) showed that slow acetylators of sulphadimidine were significantly more represented in elderly Hungarians (83 out of 125) as compared with a younger age group (64 out of 128). Conversely, Pontiroli *et*

Figure 1 Relationship between MADDs/DDS ratio and body weight.

al. (1985) failed to demonstrate any effect of age on sulphadimidine acetylator status. In both these latter studies, an age of 60 years was employed to separate the elderly from the young subjects.

In a recent retrospective study, Paulsen & Nilsson (1985) determined INH half-life in 310 Swedish patients and concluded that an excess of slow acetylation status occurred in elderly males only. They concluded that the effect of age on INH acetylation in their study was limited only to the male sex and they suggested that this might be related to concurrent treatment for disease rather than to ageing itself. If an age-dependent effect on acetylation truly exists, then present methods of detection (including the present study) would not be the ideal methods of detection since a long term longitudinal study of a cohort of subjects is required for its demonstration. Studies of two groups of subjects of different ages, although practically convenient, cannot distinguish between a change in acetylation capacity with age and a differential survival into old age by subjects with a particular acetylation capacity or some feature linked to acetylation.

Data presented in this study strongly suggest an absence of an effect of age on acetylator

status. An effect of sex is demonstrable only in the young group where more males were slow acetylators than females, 66.3% and 49.9% respectively. This difference was statistically significant ($P = 0.033$). However, no intersex difference was observed in either the elderly group or the population taken as a whole. This difference in the young group of subjects is probably not meaningful and is likely to be a spurious chance apparent difference.

Among the elderly patients, some were possibly undernourished with low body weights. However, there is evidence that undernutrition *per se* does not affect sulphadimidine acetylation capacity at least in male adults living in South India (Shastri, 1982) although Buchanan *et al.* (1979) have reported that children with kwashiorkor show impaired INH clearance which is restored by nutritional rehabilitation.

Some authors (Peters *et al.*, 1975; Carr *et al.*, 1978; Clark, 1985) have suggested that the small number of individuals with MADDs/DDS ratios falling between 0.30 and 0.35 cannot be consistently phenotyped by reference to other methods. They therefore, prefer to categorise this group as 'indeterminate' acetylators of the DDS system. If we adopt this criterion, the data base of Table 2 becomes reduced to that summarised by Table 3. None of the conclusions as set out above is changed by this exclusion: the only statistically significant contrast is that there remains an unexpected excess of slow acetylators in the young males as compared with the young female group ($\chi^2 = 5.88$; $df = 1$; $0.05 > P > 0.02$).

It should be mentioned that no attempt was made to exclude from this study patients with mild abnormalities of hepatic or kidney function nor was concurrent medication discontinued. Abnormalities of function if present however, were not so severe as to require treatment in their own right. There is little information on the possible alteration of MADDs/DDS ratios by impaired hepatic and renal function.

Evans *et al.* (1961) showed positive correlation between plasma INH concentration and absolute body weight, but found that when the INH dose was given on the basis of the metabolically active mass this correlation disappeared and the separation of fast and slow acetylator phenotypes was enhanced. Later Chapron *et al.* (1982) demonstrated close correlation between sulphamethazine clearance and absolute body weight but only in intermediate and fast acetylators. They suggest that weight-corrected acetylation clearance be used for phenotyping purposes. In the present study there is no correlation between MADDs/DDS ratios and absolute body

Table 3 Proportion of slow acetylators in populations studied after exclusion of nine 'indeterminate' acetylators with MADDs/DDS

	Young males	Young females	Elderly males	Elderly females	All males	All females
Percentage slow acetylators	69.8	48.9	51.1	56.4	63.8	54.5
Total number	96	47	45	140	141	187

weight. This lack of correlation is perhaps explained by the use of a different pharmacokinetic variable (MADDs/DDS ratio) as compared to these other studies. As there is no effect of body weight the use of DDS for rapid phenotyping of

populations where body weight is not recorded is seen to be advantageous.

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