Br. J. clin. Pharmac. (1991), **32**, 289–293

N-acetylation phenotyping using dapsone in a Jordanian population

YACOUB M. IRSHAID[1], HAKAM F. AL-HADIDI[1], MUSTAFA A. ABUIRJEIE[2] & NAYEL M. RAWASHDEH[1]
[1]Department of Pharmacology, Faculty of Medicine and [2]Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan

1 The *N*-acetylation of dapsone (DDS) was studied in 160 unrelated healthy Jordanian volunteers.

2 The frequency of slow acetylators determined using the plasma monoacetyldapsone (MADDS) to DDS ratio (MADDS/DDS), was 67.5% with a 95% confidence interval of 59 to 76%. Slow acetylators had an acetylation ratio of < 0.42.

3 Applying the Hardy-Weinberg Law, the frequency of the recessive allele controlling slow acetylation was found to be 0.82 ± 0.02.

4 The frequency distribution histogram of the plasma MADDS/DDS ratio showed an apparent trimodal pattern. The number of homozygous ($n = 16$) and heterozygous ($n = 36$) rapid acetylators derived from the observed data did not agree with those predicted for the respective rapid acetylators ($n = 5$ and $n = 47$) according to the Hardy-Weinberg Law. The suggested antimode used to discriminate the two groups was 0.82.

5 The mean plasma concentration of MADDS and the mean plasma acetylation ratio were about three times lower in slow than in rapid acetylators. However, there was no difference in mean plasma DDS concentration between slow and rapid acetylators.

6 There was a significant correlation ($r = 0.853$, $P < 0.001$) between plasma MADDS concentration and the acetylation ratio. For DDS such a correlation was absent ($r = 0.059$, $P = 0.23$).

Keywords acetylation polymorphism monoacetyldapsone dapsone phenotyping Jordanians

Introduction

Interindividual variation in response to drugs, both therapeutic and toxic, is well recognised. One of the best known examples of genetic sources of variability is *N*-acetylation polymorphism (Weber & Hein, 1985). *N*-acetylation is an important conjugation reaction and is involved in the metabolism of drugs such as hydralazine, procainamide, isoniazid, sulphadimidine, DDS, phenelzine, clonazepam, acebutolol and a caffeine metabolite (Carr *et al.*, 1978; Grant *et al.*, 1983; Hardy *et al.*, 1988; Weber & Hein, 1985; Zysst & Peretti, 1986). In addition, certain aromatic amine carcinogens such as benzidine, β-naphthylamine, 4-aminobiphenyl, and 2-aminofluorene are substrates of *N*-acetyltransferase (Bicho *et al.*, 1988; Hein, 1988; Weber & Hein, 1985).

Acetylation exhibits a genetically-controlled bimodal distribution within any given population. Individuals can be phenotyped as either slow or rapid acetylators using a test drug. Slow acetylation is inherited in an autosomal recessive fashion (Evans *et al.*, 1960; Weber & Hein, 1985). There are considerable interethnic differences in the proportion of acetylator phenotype. The frequency of slow acetylation phenotype is 5% in Canadian Eskimos (Weber & Hein, 1985), 13% in Chinese (Horai *et al.*, 1988), 6.6% in Japanese (Horai & Ishizaki, 1988), 57.4% in Spanish (Ladero *et al.*, 1988), 41.6% in Portuguese (Bicho *et al.*, 1988), 60.3% in British (Philip *et al.*, 1987), 50% in German (Siegmund *et al.*, 1988), 62.2% in USA (Hein, 1988), 50.8% in French (Stoll *et al.*, 1989), and 90% in Moroccan (Weber & Hein, 1985) populations. Owing to drug accumulation it is expected that slow acetylators would have a higher incidence of toxic reactions to drugs that are mainly metabolized by acetylation. On the contrary, fast acetylators may exhibit therapeutic failure after standard

Correspondence: Dr Yacoub Irshaid, Department of Pharmacology, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

doses. These expectations are more likely to occur at the extremes of slow and fast acetylation. In addition, acetylator status may serve as a marker for certain diseases or their complications. Slow acetylation was associated with a higher incidence of peripheral neuropathy after isoniazid treatment (Devadatta *et al.*, 1960) and a strong association between slow acetylation status and hydralazine and procainamide-induced lupus erythematosus has been reported (Ladero *et al.*, 1988; Weber & Hein, 1985). In addition there appears to be a higher incidence of slow acetylators among urinary bladder cancer patients with documented occupational exposure to benzidine (Hein, 1988).

For all of the above mentioned reasons, identification of the acetylator status of an individual is necessary in order to ensure adequate drug therapy with minimal or no toxic effects, especially during chronic treatment with drugs known to undergo acetylation as a major metabolic pathway.

Dapsone has been used for the treatment of leprosy, chloroquine-resistant malaria and dermatitis herpetiformis (Weber & Hein, 1985). It is also used as a test drug for determination of the acetylator phenotype with results comparable with those obtained using isoniazid and sulphamethazine (sulphadimidine) (Carr *et al.*, 1978; Gelber *et al.*, 1971; Hanson *et al.*, 1981; Horai & Ishizaki, 1988; Horai *et al.*, 1988; Peters *et al.*, 1981; Philip *et al.*, 1984).

The frequency of slow acetylator status in Jordan has not been determined. Thus, the main purpose of this study was to determine this frequency in a sample of unrelated healthy Jordanian volunteers using DDS as a test drug.

Methods

One hundred and sixty (101 males and 59 females who ranged in age from 18 to 50 years) unrelated healthy Jordanian subjects, attending 'Jordan University of Science and Technology' as students or employees, participated in the study. All participants had no history of serious medical illnesses, were normal on physical examination and had normal serum creatinine, alanine aminotransferase (ALT) and normal glucose-6-phosphate dehydrogenase activity. Subjects who smoked more than 10 pack-year (that is, the number of packs of 20 cigarettes per day × the number of years of smoking) or alcohol drinkers (any person who drank alcohol on a regular basis irrespective of the amount), pregnant women, subjects with a history of allergy or those taking medications were excluded. The study protocol was approved by the local university Ethics Committee, and informed written consent was obtained from each subject.

After an overnight fast, each subject received a single oral 100 mg dose of DDS. Drinking of caffeine containing beverages was not allowed throughout the study period. A blood sample (7 ml) was obtained by venepuncture into a heparinized tube 3 h after drug intake. Plasma was separated immediately by centrifugation at 3000 rev min^{-1} and stored frozen at −20° C pending analysis.

Dapsone and monoacetyldapsone concentrations in plasma were measured by modification of the h.p.l.c method of Philip *et al.* (1984) as follows. An aliquot (500 μl) of plasma was transferred into a 1.5 ml plastic microcentrifuge tube and 1 μg sulphamethazine (SMZ) in 500 μl h.p.l.c. grade acetonitrile was added as internal standard. The samples were vortex-mixed for 30 s and centrifuged at 3000 rev min^{-1} for 15 min. An aliquot (20 μl) of the clear supernatant was injected onto the h.p.l.c column. The mobile phase comprised 0.067 M phosphate buffer pH 6 : acetonitrile : methanol (200 : 70 : 30) at a flow rate of 1 ml min^{-1} at ambient temperature. DDS, MADDS and SMZ were detected by a u.v. variable wavelength detector set at 295 nm. The concentrations of DDS and MADDS were estimated by measuring the peak height ratio of each relative to the internal standard with reference to a standard curve. The h.p.l.c. instrument consisted of a single piston pump (Model 114M, Beckman Instruments, Int, Geneva, Switzerland), a u.v. variable wavelength detector (Model 165, Beckman), a 25 μl loop injector (Beckman) and an integrator-plotter (Spectra-Physics, SP 4270, USA). Chromatographic separations were achieved using 5 μm Lichrocart C-18 reversed phase stainless steel column, 250 mm × 4 mm i.d. (Merck, Hohenbrunn, Germany).

Statistical analysis

Results are presented as means ± s.e. mean. Differences between group means were assessed by the unpaired Student's *t*-test and considered significant when the *P* value was < 0.05. Linear correlation between the acetylation ratio and DDS or MADDS concentration was tested by simple regression analysis. The frequency of the recessive allele controlling slow acetylation (q) was estimated using the Hardy-Weinberg Law (Emery, 1976).

Materials

Dapsone powder was kindly donated by the Wellcome Foundation Ltd (London, UK), DDS tablets (50 mg) were purchased from ICI Pharmaceuticals (UK). Monoacetyldapsone was a gift from Dr J. H. Peters (Biotechnology and Biochemical Research Lab., Menlo Park, CA, USA). Disodium hydrogen phosphate and sulphamethazine were purchased from Park Scientific Ltd (Northampton, UK) and methanol from Romil Chemicals Ltd (Shepshed, Leics, UK). Acetonitrile h.p.l.c grade was purchased from May and Baker (Dagenham, UK).

Results

Figure 1 shows typical chromatograms for drug free plasma (a) and drug free plasma spiked with 400 ng DDS, 800 ng MADDS and 1000 ng SMZ (b). The retention times for SMZ, DDS and MADDS were 4.7, 5.8 and 6.9 min, respectively.

The frequency distribution histogram of the plasma MADDS/DDS ratio is shown in Figure 2. The histogram shows an apparent trimodal distribution with two antimodes at acetylation ratios of 0.42 and 0.82. One hundred and eight Jordanian subjects had an acetylation ratio $<$ 0.42 and formed a distinct group on the frequency

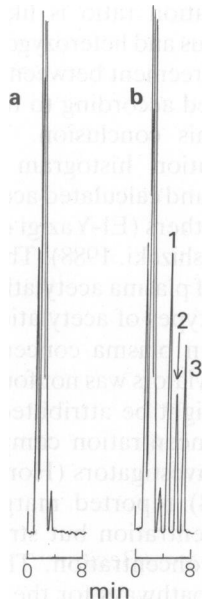

Figure 1 Representative chromatograms of drug free plasma (a); drug free plasma spiked (b) with 1000 ng sulphamethazine (peak 1), 400 ng dapsone (peak 2) and 800 ng monoacetyldapsone (peak 3) (aufs = 0.02).

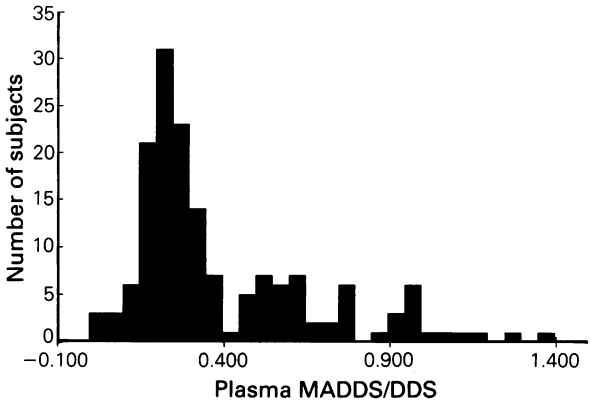

Figure 2 Frequency distribution histogram of the plasma monoacetyldapsone to dapsone ratio in 160 unrelated Jordanian subjects.

distribution histogram and were classified as slow acetylators. Thus, the frequency of slow acetylators among Jordanian subjects was found to be 67.5% with a 95% confidence interval of 59 to 76%.

Application of the Hardy-Weinberg Law indicated that the frequency of the recessive allele controlling slow acetylation (q) was 0.82 ± 0.02 and the frequency of the dominant allele controlling rapid acetylation was 0.18 (p = 1 − q). Thus, the expected genotype frequencies for homozygous slow acetylators (q^2), heterozygous (2pq) and homozygous (p^2) rapid acetylators were 0.67, 0.3 and 0.03, respectively.

The mean ± s.e. mean of the plasma concentrations of DDS and MADDS and the plasma acetylation ratio (MADDS/DDS) for slow and rapid acetylators are shown in Table 1. There was a significant difference in mean MADDS concentration and mean MADDS/DDS ratio among the two groups ($P < 0.001$). Table 2 shows the mean plasma concentrations of DDS, MADDS and the

Table 1 Plasma concentrations of dapsone (DDS) and monoacetyldapsone (MADDS) and plasma acetylation ratio (MADDS/DDS) in slow and rapid acetylators

Acetylator phenotype	*DDS* ($\mu g\ ml^{-1}$)	*MADDS* ($\mu g\ ml^{-1}$)	*MADDS/DDS*
Slow ($n = 108$)	0.75 ± 0.025	0.17 ± 0.007	0.23 ± 0.007
Rapid ($n = 52$)	0.68 ± 0.035	0.50 ± 0.034*	0.73 ± 0.032*

Values are presented as the mean ± s.e. mean.
*$P < 0.001$.

Table 2 Plasma concentrations of DDS and MADDS and plasma acetylation ratio of the two groups of rapid acetylators. Group A with an acetylation ratio between 0.42 and 0.82, and group B with an acetylation ratio above 0.82

Group	*DDS* ($\mu g\ ml^{-1}$)	*MADDS* ($\mu g\ ml^{-1}$)	*MADDS/DDS*
A ($n = 36$)	0.70 ± 0.044	0.43 ± 0.034	0.60 ± 0.017
B ($n = 16$)	0.63 ± 0.055	0.66 ± 0.068*	1.03 ± 0.034*

Values are presented as the mean ± s.e. mean.
*$P < 0.002$.

MADDS/DDS ratio for the two groups of rapid acetylators separated by an antimode of 0.82. There was a statistically significant difference between the two groups regarding MADDS concentration and MADDS/DDS ratio ($P < 0.002$).

The relationships between plasma MADDS/DDS ratio and plasma DDS and MADDS concentrations are shown in Figures 3 and 4, respectively. There was a strong correlation between plasma acetylation ratio and MADDS concentration ($r = 0.853$, $P < 0.001$), whereas such a correlation was lacking between plasma acetylation ratio and DDS concentration ($r = -0.095$, $P = 0.23$).

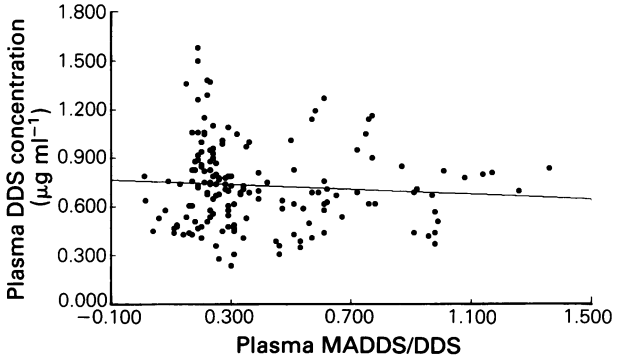

Figure 3 Relationship between plasma monoacetyldapsone to dapsone (MADDS/DDS) ratio and plasma concentration of dapsone (DDS) in 160 unrelated subjects ($r = -0.096$, $P = 0.23$).

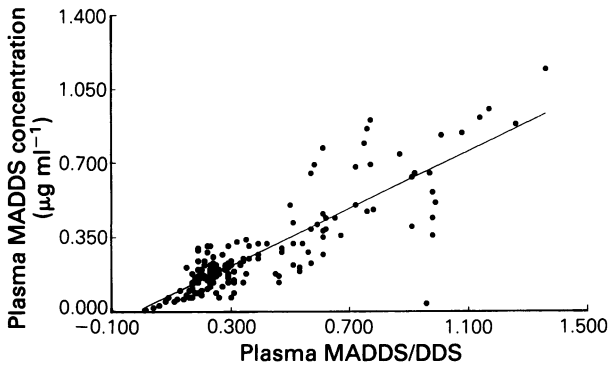

Figure 4 Relationship between plasma monoacetyldapsone to dapsone (MADDS/DDS) ratio and plasma concentration of monoacetyldapsone (MADDS) in 160 unrelated Jordanian subjects ($r = 0.85$, $P < 0.001$).

Discussion

The frequency of the slow acetylator phenotype among a sample of healthy Jordanian volunteers ($n = 160$) using DDS as a test drug was 67.5% (108/160) with a 95% confidence interval of 59 to 76%. This finding is similar to results reported from other Arab countries. The frequency of slow acetylators in a sample of Saudi Arabs was found to be 63.4% using sulphadimidine (Islam, 1982), and 72.3% in another study using a caffeine metabolite (El-Yazigi *et al.*, 1989) as test drugs. Utilizing sulphadimidine as a test drug (Karim *et al.*, 1981), it was found that 65% of Libyan Arabs were slow acetylators. The slow acetylator frequency for isoniazid among Egyptians was 82% (Hashem *et al.*, 1969). This was slightly higher than in our study and in those from Saudi Arabia and Libya. Nevertheless, taken together, these results indicate that Arabs have a similar frequency of slow acetylators, irrespective of their geographic location. Compared with Europeans, Arabs have a higher frequency of slow acetylators.

Our calculated frequency of the recessive allele controlling slow acetylation (q) of 0.82 is similar to that reported from other Arab countries. The value reported from Libya was 0.81 ± 0.05 (Karim *et al.*, 1981), that from Saudi Arabia was 0.80 ± 0.03 (Islam, 1982), while that from Egypt was 0.91 (Hashem *et al.*, 1969). Apart from Egypt, frequencies of the recessive allele controlling slow acetylation in other Arab countries are similar to each other.

The apparent trimodality noticed in the frequency distribution histogram in our study might suggest that the plasma acetylation ratio is likely to discriminate between homozygous and heterozygous rapid acetylators. However, the disagreement between the observed values and those calculated according to the Hardy/Weinberg Law is against this conclusion. Trimodality of the frequency distribution histogram and disagreement between observed and calculated acetylation ratios have been reported by others (El-Yazigi *et al.*, 1989; Grant *et al.*, 1984; Horai & Ishizaki, 1988). Thus, it seems unlikely that a trimodality of plasma acetylation ratio truly represents distinct genotypes of acetylation status.

The difference in plasma concentration of DDS in slow and rapid acetylators was not found to be statistically significant. This might be attributed to the wider variability in DDS concentration compared with that for MADDS. Other investigators (Horai & Ishizaki, 1988; Horai *et al.*, 1988) reported marginal differences in plasma DDS concentration but striking differences in plasma MADDS concentration. The possibility of the presence of other pathways for the removal of DDS in slow acetylators could be another factor contributing to the lack of difference in plasma DDS concentration in the two groups. There is indirect evidence to support the presence of other pathways of DDS metabolism such as *N*-hydroxylation (Grossman & Jollow, 1988). The finding that the mean plasma concentration of MADDS is significantly different between acetylator phenotypes and its strong correlation with the plasma acetylation ratio is in agreement with other reports (Horai & Ishizaki, 1988; Horai *et al.*, 1988). However, it is not possible to use plasma MADDS concentration alone to identify the acetylator status, because at the individual level many subjects exhibited a low MADDS concentration but a high acetylation ratio (MADDS/DDS).

In conclusion, we have studied the *N*-acetylation of dapsone in 160 unrelated healthy Jordanian subjects. It was found that 67.5% of them were slow acetylators. This percentage is similar to that observed in various other Arab countries and slightly higher than that in Europeans. Thus, Arabs have similar acetylator phenotype, irrespective of their location within the Arab World.

This study was supported by grant 11/88 from Jordan University of Science and Technology, Deanship of Research. The authors are grateful to Dr J. H. Peters, Director, Biochemical Pharmacology Program, Biotechnology and Biochemistry Research Lab., Menlo Park, CA, USA for supplying monoacetyldapsone. The authors are also grateful to the Ministry of Health represented by Mr I. El-Sa'di, chief pharmacist at Princess Basma Teaching Hospital for supplying dapsone tablets.

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(*Received 31 October 1990*
accepted 2 April 1991)