N-acetylation phenotyping with dapsone in a mainland Chinese population

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1 The N-acetylation of dapsone (DDS) was studied in 108 unrelated Chinese subjects residing in the mainland of China.
2 The frequency of slow acetylators determined using the plasma monoacetyldapsone to DDS ratio (MADDS/DDS, slow acetylators < 0.30 and rapid acetylators > 0.35) at 3 h after an oral dose of DDS (100 mg) was 13.0% (14 of the 108 subjects) with a 95% confidence interval of 7.9 to 20.6%.
3 The mean plasma concentration of MADDS was about three times lower in the slow than in the rapid acetylators and there was a highly significant correlation ($r_s = 0.886, P < 0.001$) between plasma MADDS concentration and acetylation ratio.
4 Urinary acetylation ratios (MADDS/DDS) and cumulative urinary excretion of MADDS were significantly ($P < 0.001$) lower in slow acetylators compared with rapid acetylators. In addition, there was a significant relationship ($r_s = 0.510$ to $0.718, P < 0.001$) between plasma and urinary acetylation ratios. However, the distribution of the urinary acetylation ratio was not bimodal.
5 The urinary acetylation ratio after an oral dose of DDS is not a discriminative index for determining acetylation phenotype.

Keywords dapsone monoacetyldapsone acetylation polymorphism Chinese in the mainland of China phenotyping method

Introduction

As discussed at recent symposia, there is an increasing multidisciplinary interest in pharmacogenetics, especially in relation to the acetylation polymorphism (Weber, 1984; Weber et al., 1983) and polymorphic drug oxidation (Dengler et al., 1984). Relationships between variations in the metabolism of drugs associated with such polymorphisms and their toxic effects and the susceptibility of different phenotypes to disease are of particular clinical relevance (Clark, 1985; Weber & Hein, 1985; Evans, 1986).

Acetylation polymorphism refers to a genetically determined difference in the N-acetylation capacity of a variety of clinically useful drugs such as isoniazid, procainamide, hydralazine, dapsone, sulphamethazine, and sulphasalazine as well as some putative carcinogenic arylamines (β-naphthylamine, benzidine, 4-aminobiphenyl

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and 2-aminofluorene) (Lunde et al., 1977; Drayer & Reidenberg, 1977; Clark, 1985; Weber & Hein, 1985). Individuals may be phenotyped as either slow or rapid acetylators using a test drug such as isoniazid or sulphamethazine (Lunde et al., 1977; Clark, 1985). From family studies it appears that slow acetylation is an autosomal homozygous recessive trait, while rapid acetylation is either a heterozygous or a homozygous dominant trait (Evans et al., 1960). There are considerable interethnic differences in the frequency distribution of slow and rapid acetylators (Lunde et al., 1977; Clark, 1985). For example, populations of Caucasians and Negroes appear to show an approximately equal percentage of slow and rapid acetylators. In contrast, in Japanese (Sunahara et al., 1961; Horai et al., 1982) and Eskimo populations, the number of slow acetylators is only about 10% (Lunde et al., 1977; Clark, 1985). Thus, there are implications for rational drug treatment in different racial groups.

To date the pharmacogenetics of N-acetylation pharmacogenetics in mainland Chinese has not been described.

Dapsone (DDS), 4,4'-diaminodiphenyl sulphone, has been widely used for the treatment of leprosy, chloroquine-resistant malaria and dermatitis herpetiformis (Weber & Hein, 1985). It has also been used as a model drug for determining the acetylation phenotype (Gelber et al., 1971; Reidenberg et al., 1975; Carr et al., 1978; Philip et al., 1984). Phenotyping with this drug has been shown to give results comparable with those using the elimination half-life of isoniazid or the urinary excretion ratio of acetylsulphamethazine to sulphamethazine (Gelber et al., 1971; Hanson et al., 1981). The plasma acetylation ratio of monoacetyldapsone (MADDS) to DDS at 3 h after a single oral dose of 100 mg of DDS is commonly used for phenotyping (Reidenberg et al., 1975; Clark, 1985; Zuidema et al., 1986). However, the possibility of using non-invasive urinary measurements has not been investigated systematically.

Thus, the aim of the present study was to investigate the N-acetylation polymorphism of DDS in a mainland Chinese population and to determine the utility of a urinary acetylation ratio (MADDS/DDS) as a more convenient method of phenotyping.

Methods

One hundred and eight unrelated healthy Chinese subjects residing in Changsha, Hunan, People’s Republic of China, participated in the study. They comprised 77 males and 31 females, who ranged in age from 19 to 32 (mean 20.5) years and in body weight from 40 to 75 (mean 54.8) kg. The study protocol was approved by the ethics committee of the National Training Center of Clinical Pharmacology, Hunan Medical College. After an overnight fast, each subject emptied their bladder and took a single oral 100 mg dose of DDS (4 tablets containing 25 mg/tablet, Protogen®, Yoshitomi Pharmaceutical Co., Ltd, Osaka, Japan). A blood sample (3 ml) was drawn into a heparinized tube at 3 h after drug ingestion. In addition, timed urine samples were collected during the periods 0–6, 6–12 and 12–24 h postdose and urine volumes were recorded. Plasma was separated immediately after the blood collection. Aliquots were frozen at −20°C until analysis. Samples from Changsha, Hunan were packed in dry ice and flown via Shanghai to Tokyo. The samples remained frozen on arrival at the National Medical Center in Tokyo.

DDS and MADDS in plasma and urine were assayed by the h.p.l.c. method of Horai & Ishizaki (1985). Slow acetylators were defined as subjects with plasma acetylation ratios (MADDS/DDS) less than 0.30 and rapid acetylators were those with a ratio greater than 0.35 (Reidenberg et al., 1975; Carr et al., 1978; Hanson et al., 1981). This standard phenotyping method using plasma sample was compared with the use of urinary MADDS/DDS ratios measured in samples collected in the postdose 0–6, 0–12 and 0–24 h periods.

Results

The frequency distribution histogram of the plasma MADDS/DDS ratio is shown in Figure 1. Fourteen (five females and nine males) of the 108 Chinese subjects had an acetylation ratio less than 0.30 and were therefore classified as slow acetylators. Ninety-one subjects were assigned as rapid acetylators and three subjects were indeterminate, having ratios of 0.321, 0.326 and 0.346. Thus, the frequency of slow acetylators was 13.0% with a confidence interval of 7.9 to 20.6% at the 95% level. Application of the Hardy Weinberg Law indicated that the frequency of the allele controlling recessive slow acetylators (q) was 0.360 and for the allele controlling dominant rapid acetylators (p) it was 0.640. The expected genotype frequencies for homozygous slow acetylators (q^2), heterozygous (2pq) and homozygous rapid acetylators (p^2) are 0.130, 0.460 and 0.410, respectively.

The mean (± s.d.) values and ranges of plasma concentrations of DDS and MADDS and the acetylation ratios are shown in Table 1. Relation-
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Figure 1  Frequency distribution of plasma monoacetyldapsone to dapsone ratio in 108 unrelated Chinese subjects. An oral dose of 100 mg of dapsone was administered to each of the subjects. Slow acetylators (n = 14) were defined as the subjects with a ratio less than 0.30 (shaded area), indeterminate acetylators (n = 3) as those with a ratio between 0.30 and 0.35 (hatched area), and rapid acetylators (n = 91) as those with a ratio greater than 0.35 (open area), according to the standardized phenotyping method (Reidenberg et al., 1975; Carr et al., 1978; Hanson et al., 1981).

Table 1  Plasma concentrations of dapsone (DDS) and monoacetyldapsone (MADDS) and plasma metabolic ratio (MADDS/DDS) at 3 h after oral dosing of 100 mg of DDS in rapid, indeterminate and slow acetylators

<table>
<thead>
<tr>
<th>Acetylator phenotype</th>
<th>DDS (μg ml⁻¹)</th>
<th>MADDS (μg ml⁻¹)</th>
<th>MADDS/DDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid acetylators</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 91)</td>
<td>1.86 ± 0.40</td>
<td>1.28 ± 0.515</td>
<td>0.686 ± 0.195</td>
</tr>
<tr>
<td>Indeterminate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetylators 1</td>
<td>1.75</td>
<td>0.570</td>
<td>0.326</td>
</tr>
<tr>
<td>2</td>
<td>1.91</td>
<td>0.660</td>
<td>0.346</td>
</tr>
<tr>
<td>3</td>
<td>1.45</td>
<td>0.466</td>
<td>0.321</td>
</tr>
<tr>
<td>Slow acetylators</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 14)</td>
<td>2.14 ± 0.32</td>
<td>0.485 ± 0.135**</td>
<td>0.227 ± 0.060**</td>
</tr>
<tr>
<td></td>
<td>(1.66 – 2.84)</td>
<td>(0.300 – 0.760)</td>
<td>(0.139 – 0.299)</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± s.d. mean and values in parentheses indicate the ranges observed. Acetylators are classified according to the reported criteria (Reidenberg et al., 1975; Carr et al., 1978; Hanson et al., 1981).

* P < 0.05 vs rapid acetylators.
** P < 0.001 vs rapid acetylators.

Table 1 shows the plasma concentrations of dapsone (DDS) and monoacetyldapsone (MADDS) and plasma metabolic ratio (MADDS/DDS) at 3 h after oral dosing of 100 mg of DDS in rapid, indeterminate and slow acetylators. The table demonstrates the differences in plasma concentrations between the two acetylation phenotypes. Additionally, there is a significant correlation between the acetylation ratio and plasma concentrations of DDS and MADDS. The relationships between the acetylation ratio and plasma concentrations of DDS and MADDS are shown in Figures 2a and b, respectively. As well as a strong relationship (r_s = 0.886, P < 0.001) between the acetylation ratio and plasma MADDS concentration (Figure 2b), there was a highly significant (P < 0.001) difference in the mean value for plasma MADDS concentration between the two phenotypes (Table 1). Although plasma MADDS concentrations in the slow acetylators were concentrated at the lower end of the observed concentration range, there was no discernible bimodal distribution. The mean values for plasma DDS concentration were marginally (P < 0.05) greater in the slow than in the rapid acetylators (Table 1). However, plasma...
The 0-6 and 0 acetylators obtained individual values of incomplete variability during the collection periods (0-6 vs 0-12, 0-6 vs 0-24, and 0-12 vs 0-24 h postdose) were highly correlated (r = 0.887 - 0.949). The cumulative urinary excretion of DDS and MADDS and urinary metabolic ratios from samples collected during 0-6 and 0-24 h postdose periods are shown in Table 2. The frequency histograms for the urinary acetylation ratios derived from urine samples obtained during the three collection periods are shown in Figure 3. Although mean values of the urinary metabolic ratio and the cumulative urinary excretion of MADDS were significantly (P < 0.001) lower in slow acetylators (Table 2) and individual values of the acetylation ratio in the slow acetylators were at the lower end of the observed ranges (Figure 3), clear bimodality was not apparent in the distribution of the urinary acetylation ratios (Figure 3). Furthermore, although there were significant relationships between plasma and urinary acetylation ratios (r = 0.510 - 0.718, P < 0.001), the latter were not in complete concordance with the phenotyping derived from the plasma acetylation ratios.

Discussion

This study was confined to a sample of the population of Changsha, Hunan, approximately 920 km southwest of Shanghai. Therefore, it may not be possible to extrapolate the observed 13% frequency of slow acetylators to the rest of the rather large population of mainland China. In Chinese living outside of the mainland reported frequencies are 22.0 (Taiwan, Sunahara et al., 1963), 22.0 (Liverpool, Evans, 1963), 21.5 (Singapore, Ellard & Gammon, 1977), 21.7 (Hong Kong, Ellard & Gammon, 1977) and 34.0% (Thailand, Kukongviriyapan et al., 1984). Using the 95% confidence limits of these data and taking the sample sizes of these studies into account we have calculated that the last three of these frequencies are significantly (P < 0.05 to 0.01) higher than that observed in our study (Table 3). Reasons for this may reflect variable demographic characteristics of the subjects, the test drugs used and environmental factors. Nonetheless, isoniazid, sulphamethazine or DDS have been shown to give concordant results on individual phenotyping (Gelber et al., 1971; Reidenberg et al., 1975; Hanson et al., 1981), sex and age do not appear to have important influences on phenotyping (Evans et al., 1960) and the frequency of slow acetylators in patients with tuberculosis does not differ from that in normal healthy subjects (Evans, 1986).
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Table 2 Cumulative urinary excretion (% of dose) of dapsone (DDS) and monoacetyldapsone (MADDS) and urinary metabolic ratio (MADDS/DDS) after oral dosing of 100 mg of DDS in rapid, indeterminate and slow acetylators

<table>
<thead>
<tr>
<th>Acetylator phenotype</th>
<th>DDS</th>
<th>0-6 h MADDS/DDS</th>
<th>DDS</th>
<th>0-24 h MADDS/DDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid acetylators</td>
<td>0.865 ± 0.756</td>
<td>0.085 ± 0.068</td>
<td>0.106 ± 0.043</td>
<td>2.82 ± 1.95</td>
</tr>
<tr>
<td>(n = 90)</td>
<td>(0.118 - 4.65)</td>
<td>(0.015 - 0.320)</td>
<td>(0.044 - 0.217)</td>
<td>(0.812 - 14.44)</td>
</tr>
<tr>
<td>Indeterminate acetylators</td>
<td>0.362</td>
<td>0.013</td>
<td>0.035</td>
<td>2.31</td>
</tr>
<tr>
<td>(n = 13)</td>
<td>(0.310 - 1.94)</td>
<td>(0.009 - 0.060)</td>
<td>(0.025 - 0.065)</td>
<td>(0.528 - 5.36)</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± s.d. mean and values in parentheses indicate the ranges observed. The data in 3 (one each of the rapid, indeterminate and slow acetylators) of the 108 subjects could not be obtained owing to incomplete urine collection. *P < 0.001 vs rapid acetylators.

Dapsone has been used as an adequate test probe for determining acetylator phenotype (Redenberg et al., 1981; Philip et al., 1984; Clark, 1985). The plasma metabolic ratio of MADDS to DDS at 3 h after a single oral dose of 100 mg of DDS is commonly used and is considered to be clinically acceptable (Redenberg et al., 1975; Carr et al., 1978). The plasma metabolic ratio of MADDS to DDS is given by: MADDS/DDS = (MADDS/DDS) * DDS/DDS, where DDS is the dose of DDS given.

Similarly, Carr et al. (1978) were unable to phenotype three out of the 108 subjects for this reason. Accordingly, we were unable to phenotype three out of the 108 subjects for this reason. Additionally, the phenotypes of individuals with a possible disadvantage of the DDS method is that in a small number of individuals the metabolic ratio falls between 0.30 and 0.35 and is considered to be indeterminate by the DDS method. (Redenberg et al., 1975; Clark, 1985) stated that a possible disadvantage of the DDS method is that it is not sensitive enough to detect all acetylators. However, in this study, the plasma MADDS/DDS ratios of DDS were used to classify slow, indeterminate and rapid acetylators classified using their plasma MADDS/DDS ratios, as shown in Figure 1.
phenotype three out of their 50 subjects and Clark (1985) found two subjects in over 100 individuals whose metabolic ratio would not allow accurate phenotyping. Clark (1985) also suggested the existence of a trimodal pattern of MADDS/DDS with two possible antimodes at 0.35 and 0.85. In our study, however, there was no evidence of a second antimode at 0.85. Nonetheless, whether extrapolating the acetylation antimode from one population to another is valid remains to be determined, as has recently been suggested with respect to oxidation polymorphisms by us (Ishizaki et al., 1987) and others (Iyun et al., 1986).

Mass screening tests to define acetylator status in a selected population become more feasible and convenient if an adequate noninvasive procedure can be developed. Saliva samples have been shown to be unsuitable because of the extremely low concentrations of MADDS in saliva (Ahmad & Rogers, 1980; Peters et al., 1981). However, the utility of urine samples does not appear to have been evaluated. According to Gelber et al. (1971), urinary MADDS and acid-labile MADDS represent only a small fraction of the administered dose of DDS and they doubted the value of urine sampling for phenotyping with DDS. Our findings tend to support this view.

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Table 3  A summary of literature reporting the frequency of slow acetylators in Chinese populations

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location studied</th>
<th>Text drug</th>
<th>Slow acetylators (%)</th>
<th>Incidence of slow acetylators (%)</th>
<th>Location studied</th>
<th>Text drug</th>
<th>Slow acetylators (%)</th>
<th>Incidence of slow acetylators (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunahara et al. (1963)</td>
<td>Taiwan</td>
<td>isoniazid</td>
<td>22.0</td>
<td>28.1/17</td>
<td>Liverpool</td>
<td>isoniazid</td>
<td>22.0</td>
<td>28.1/17</td>
</tr>
<tr>
<td>Ellard &amp; Gammor (1977)</td>
<td>Hong Kong</td>
<td>sulphadimidine</td>
<td>40/184</td>
<td>22.1/48</td>
<td>Kohn Kaen (Thailand)</td>
<td>sulphadimidine</td>
<td>40/184</td>
<td>22.1/48</td>
</tr>
<tr>
<td>Kukongyiyapam et al.</td>
<td>Changsha (People's</td>
<td>dapsone</td>
<td>14/108</td>
<td>13.0</td>
<td>Republic of China</td>
<td>dapsone</td>
<td>14/108</td>
<td>13.0</td>
</tr>
</tbody>
</table>

* All studies except ours were performed outside the mainland of China.
** P < 0.05 from the 95% confidence interval of the present study.
† P < 0.01 from the 95% confidence interval of the present study.
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References


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