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A simple method for determining acetylator phenotype using isoniazid

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A comparison was made between the results of acetylator phenotyping by isoniazid (INH) half-life measurements based on samples taken for 6 h after a single oral dose (200 mg), and by determination of the ratio of acetylisoniazid (Ac.INH) to isoniazid in the 3 h samples. In the 32 subjects, examined, there was complete agreement about classification of the subject as a fast ($t_{1/2}$ < 130 min; Ac.INH/INH > 1.5) or slow acetylator ($t_{1/2}$ > 130 min; Ac.INH/INH < 1.5). The single sample test appears to be as reliable as the more time-consuming isoniazid half-life method.

Keywords acetylator phenotype isoniazid

Introduction

Acetylation is a phase II conjugation reaction occurring in the liver which metabolises procainamide, hydralazine, dapsone, isoniazid, phenelzine, sulphonamides, and other drugs. The rate at which an individual acetylates these compounds has been found to be genetically determined, and the trait is inherited in an autosomal dominant fashion by a single gene (Price-Evans *et al.*, 1960). Thus a bimodal distribution of acetylation capacity appears within a population enabling a subject to be classified as a slow or a fast acetylator. This has clinical importance as certain major adverse effects are more frequently observed among slow rather than fast acetylators.

A variety of substrates have been used to determine acetylator status, one of the first reliable methods being the determination of the plasma isoniazid half-life. However, this test is time consuming, involving the withdrawal of blood samples over a 6–8 h period. We have attempted to see if this test can be simplified.

Methods

Thirty-two subjects, seven of whom were healthy drug-free volunteers (and the remainder patients with a variety of concomitant diseases including breast cancer and hyperthyroidism) were studied. Each gave fully informed written consent for the study, which had been approved by the local ethics committee.

After fasting overnight, the subjects received a single oral dose of isoniazid (200 mg). Blood samples (5 ml) were withdrawn into heparinised tubes at 120, 180, 240, 300 and 360 min, centrifuged, and stored at −70° C until assayed to prevent breakdown of INH or Ac.INH (Hutchings *et al.*, 1983a). INH and Ac.INH were measured in plasma by high performance liquid chromatography (Hutchings *et al.*, 1983b) and the elimination half-life ($t_{1/2}$) of the drug was calculated by regression analysis of the log concentrations in the 180–360 min samples, and a half-life of 130 min was chosen as the antimode. These measurements were compared with acetylisoniazid/isoniazid (Ac.INH/INH) ratios measured in the 180 min samples.

Results

As shown in Figure 1, 15 of the 32 subjects had an INH half-life of less than 130 min and so were

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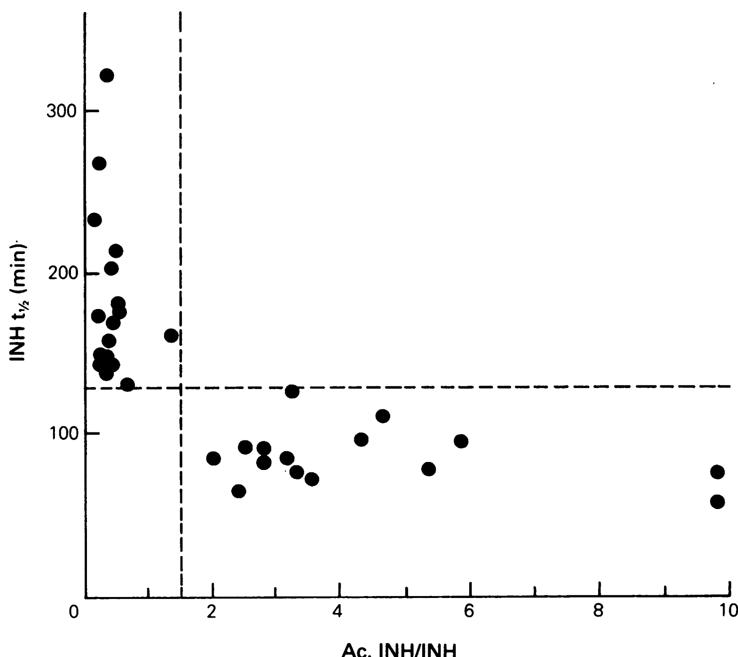

Figure 1 Relationship between the $t_{1/2}$ of elimination of isoniazid from plasma and the ratio of acetylisoniazid to isoniazid (Ac.INH/INH) in the 3 h plasma sample.

classified as rapid acetylators. All of these subjects had Ac.INH/INH ratios greater than 1.5 in their 3 h plasma sample. In two of the fast acetylators, both healthy subjects, the Ac.INH/INH ratio was much greater than in the other fast acetylators indicating a possible trimodal distribution.

The distribution of subjects in the three groups was consistent with the Hardy-Weinberg equilibrium for homozygous fast, heteroxygous fast and homozygous slow acetylator phenotypes, assuming a frequency of slow acetylators of 0.57 (Hanson *et al.*, 1981).

Discussion

Isoniazid is still a widely used substrate to determine acetylator status, although dapsone is becoming increasingly popular as an alternative. Isoniazid would be preferred in patients with glucose-6-phosphate dehydrogenase deficiency to avoid haemolysis or in those already receiving isoniazid (which interferes with dapsone elimination) (Ahmad *et al.*, 1981). Also isoniazid may more clearly distinguish slow from fast acetylators since Carr *et al.* (1978) and Clark (1985) have found dapsone to be unable to accurately phenotype subjects in 2–6% of cases (i.e. those who lie close to the antimode).

There were several disadvantages of the original isoniazid test, however. Firstly, a dose of 10 mg kg^{-1} was necessary, but improved assay methodology has enabled us to reduce the dose to 200 mg and to avoid the nausea and other adverse effects more often seen with a larger dose (Hutchings *et al.*, 1983b). Secondly, isoniazid and acetylisoniazid were unstable in plasma but storage at −70° C has been shown to prevent breakdown and avoid the necessity of immediate analysis (Hutchings *et al.*, 1983a). Thirdly multiple samples were required and this was inconvenient for subject, doctor and technician. We believe that the single sample test will overcome this last disadvantage.

Although the half-life of isoniazid is normally used to phenotype subjects it has the theoretical disadvantage of (a) being dependent on volume of distribution (*V*) which could vary in disease; and (b) being inversely related to clearance

$$(t_{1/2} = \frac{0.693V}{CL})$$

and therefore production of metabolite. Since, in fast acetylators, isoniazid undergoes extensive

presystemic elimination (Weber & Hein, 1979), half-life will be a relatively insensitive measure of acetylation rate. The same is not true of the ratio of metabolite to parent compound which will continue to rise with increasing acetylation rate because of increased production of metabolite particularly on the first pass through the liver. Thus there was only a five fold variation in half-life but a fifty-fold variation in Ac.INH/INH ratio in our subjects. The ratio of monoacetyldapsone to dapsone in 102 subjects reported by Clark (1985) varied only 13 fold and this may be responsible for the greater difficulty in separating fast from slow acetylators with dapsone.

Clark (1985) suggested that dapsone may be able to distinguish homozygous from heterozygous fast acetylator phenotypes. Our population is smaller than that of Clark but a similar distribution consistent with that predicted for each phenotype by applying the Hardy–Weinberg equilibrium was seen. Much larger studies will be necessary to confirm that the single sample isoniazid test will identify the three phenotypes. Nevertheless the single sample isoniazid test appears to be as reliable as the more laborious and time consuming isoniazid test to identify fast and slow acetylator phenotypes.

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