Evidence for a Trimodal Pattern of Acetylation of Isoniazid in Uremic Subjects

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Abstract □ Isoniazid metabolic clearance values were calculated retrospectively and plotted on a frequency distribution histogram. A trimodal distribution pattern was observed. According to a two-allele codominant model, the mean clearance values of the slow and rapid acetylators closely predicted the mean clearance value for the intermediate acetylators. The potential clinical implications of identifying intermediate and rapid acetylators of isoniazid and its use in renal failure are discussed.

Keyphrases □ Isoniazid acetylation—genetic phenotypes determined using metabolic clearance values in humans □ Acetylation—isoniazid in humans, genetic phenotypes determined using metabolic clearance values □ Genetic phenotypes—of isoniazid acetylation in humans, determined using metabolic clearance values □ Metabolic clearance values—used to determine genetic phenotypes of isoniazid acetylation in humans □ Tuberculostatic antibacterials—isoniazid, genetic phenotypes of acetylation determined using metabolic clearance values in humans

Several widely used medications are metabolized by polymorphic acetylation in humans. Such drugs include isoniazid, hydralazine, procainamide, sulfamethazine, sulfapyridine, and dapsone (1–7). Genetic studies demonstrated that the acetylation polymorphism involved is controlled by two allelic genes at a single locus; slow acetylators are homozygous for the gene controlling slow acetylation, and fast acetylators are either heterozygous or homozygous for the gene controlling fast acetylation (1). Furthermore, Schloot *et al.* (8) presented evidence that the quantitative effects of the two alleles (or gene products) were simply additive.

BACKGROUND

Because the therapeutic or toxic response to polymorphically acetylated drugs may differ among the phenotypes, several methods have been developed for acetylator phenotyping in humans. Most procedures can accurately separate fast and slow acetylators, but none can adequately differentiate homozygous and heterozygous fast acetylators (9). For the sake of simplicity, heterozygous and homozygous fast acetylators will be referred to as intermediate and rapid acetylators, respectively.

The lumping of intermediate and rapid acetylators together has an obvious disadvantage, since the two phenotypes may differ with regard to therapeutic or toxic drug response. This difference may be particularly important for isoniazid; it recently was demonstrated (10) that severe hepatotoxic reactions to this drug occur predominately in fast acetylators. However, intermediate and rapid acetylators were not separated in that investigation. Consequently, demonstration of three isoniazid acetylation phenotypes could provide new insights into the nature of isoniazid toxicity.

A preliminary study suggested that metabolic clearance determination may serve as a useful technique for separating intermediate and rapid acetylators (11). Recently, Gold *et al.* (12) reported isoniazid half-lives following intravenous administration to 18 patients with chronic renal insufficiency. By using an antimodal value of 118 min for the isoniazid half-life, they classified 11 patients as apparent slow acetylators and seven as apparent fast acetylators. These isoniazid data allowed for the direct calculation of metabolic clearance.

Furthermore, since acetylation is a major pathway for isoniazid removal from plasma, differences in metabolic clearance between uremic individuals should reflect differences in acetylation capacity. Because isoniazid is not appreciably bound to plasma protein, disease-induced change in plasma proteins would not be expected to alter pharmacokinetic parameters significantly (13). Therefore, the data of Gold *et al.* (12) were evaluated to demonstrate a trimodal acetylation pattern of isoniazid in uremic subjects.

EXPERIMENTAL

Values of $t_{1/2u}$ and V_d (12) were calculated with the assumption that the data obey the kinetics of a one-compartment model. Although biexponential decline would have been observed had more data points been obtained at the early time points, the error involved in assuming monoexponential decay is minimal (14). Thus, metabolic clearance, *MC*, was calculated according to:

$$MC = \frac{0.693 V_d}{t_{1/2u}} \qquad \text{(Eq. 1)}$$

where $t_{1/2u}$ is the isoniazid half-life in uremic subjects and V_d is the volume of distribution of the drug as reported by Gold *et al.* (12). Each clearance value was corrected to body weight.

RESULTS AND DISCUSSION

When the computed metabolic clearance values were plotted on a frequency distribution histogram, a trimodal pattern became apparent, with approximate antimodes at 3.9 and 6.8 ml/min/kg (Fig. 1). The clearance method appears to classify individuals as slow, intermediate, or rapid acetylators. Table I lists the mean metabolic clearance ($\pm SD$) of isoniazid for each acetylator phenotype. In the absence of family data, definitive genotypic classification is not possible.

From the distribution histogram shown in Fig. 1, it is apparent that the clearance method allows for a clearer separation of the three phenotypes than does the half-life method where there is evident confluence of the two phenotypes. Subject 7, listed as a slow acetylator by Gold *et al.* (12), was classified as an intermediate acetylator by the clearance method.

Furthermore, some simple genetic calculations lend support to the observed trimodal distribution of isoniazid metabolic clearance values. The genetics of the polymorphism for isoniazid acetylation in humans assume a two allele codominant model: two alleles, Ac^R (rapid acetylator)

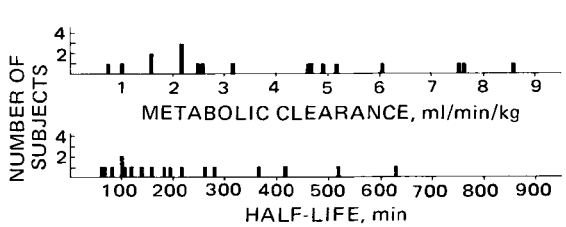

Figure 1—*Distribution of corrected metabolic clearance values and half-lives of isoniazid in 18 uremic patients.*

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Table I—Mean Isoniazid Metabolic Clearance Values as a Function of Acetylator Status

Acetylator Status	Number of Subjects	Isoniazid Clearance ± *SD*, ml/min/kg
Slow	10	2.0 ± 0.8
Intermediate	5	5.1 ± 0.6
Rapid	3	7.9 ± 0.6

and Ac^S (slow acetylator), for one autosomal gene locus (8). Slow acetylators are designated Ac^S/Ac^S, while intermediate and rapid acetylators are designated Ac^R/Ac^S and Ac^R/Ac^R, respectively.

As reported in Table I, the arithmetic mean of the acetylation capacity of the genotype Ac^S/Ac^S is 2.0 ml/min/kg. The value of the allele Ac^S is thus 2.0/2 = 1.0. Genotype Ac^R/Ac^R has the mean value of 7.9 ml/min/kg; for the allele Ac^R, 7.9/2 = 3.95 or approximately 4.0. Since the quantitative effect (or gene product) of the alleles is considered additive, the mean acetylation capacity for the group Ac^R/Ac^S should follow the relationship:

$$\frac{Ac^S/Ac^S}{2} + \frac{Ac^R/Ac^R}{2} = Ac^R/Ac^S \qquad \text{(Eq. 2)}$$

Thus, based on the calculations from the mean slow and rapid acetylator modes, the expected mean acetylation capacity of the heterozygote would be 5.0 ml/min/kg [(2.0/2) + (7.95/2)]. The observed mean value for the intermediate acetylator was 5.10 ml/min/kg, a difference of less than 3% from the expected value of 5.0 ml/min/kg. Although these retrospective data apply only to uremic patients, they do support the concept that acetylation clearance determinations may represent a useful method for identifying slow, intermediate, and rapid acetylators.

Finally, significant amounts of acetylisoniazid appear in the urine of normal subjects following oral administration of isoniazid, approximately 49 and 32% of the dose in fast and slow acetylators, respectively (10). Obviously, kidney failure should reduce significantly the contribution of the renal excretory pathway for acetylisoniazid. Thus, uremic patients receiving isoniazid might accumulate substantial levels of acetylisoniazid and, subsequently, be exposed to excessive amounts of its metabolite, acetylhydrazine. Acetylhydrazine is subsequently oxidized to hepatotoxic substances (15).

None of the published reports of isoniazid kinetics in renal failure examined the possibility of metabolite accumulation (12, 16, 17). There is a great need to study the effects of renal insufficiency on the plasma and urinary excretion kinetics of acetylisoniazid and metabolites and the potential for removal by dialysis. It is also important to investigate the effects of uremia on acetylisoniazid hydrolysis, as well as the oxidative activation of acetylhydrazine to toxic substances.

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Ketonic Oxidation Products of Cyclobarbital

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Abstract □ Chemical or biochemical oxidation of cyclobarbital yielded 5-(3-oxo-1-cyclohexen-1-yl)-5-ethyl -2,4,6- (1*H*,3*H*,5*H*)-pyrimidinetrione, and photochemical oxidation gave 5-(6-oxo-1-cyclohexen-1-yl)-5-ethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione. These compounds were isolated and purified by TLC, and their structures were determined by UV, IR, NMR, and mass spectral data; the crystalline structure was determined by X-ray diffraction.

Keyphrases □ Cyclobarbital—oxidation products isolated and identified □ Oxidation—cyclobarbital, products isolated and identified □ Depressants, central—cyclobarbital, oxidation products isolated and identified

The identification of ketonic oxidation products of cyclobarbital[1] [5-(1-cyclohexen-1-yl)-5-ethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione] (I) has been reported by several investigators. Tsukamoto *et al.* (1) isolated a ketonic derivative from rabbit urine; it was identified by UV spectrometry and chemical means as 5-(3-oxo-1-cyclohexen-1-yl)-5-ethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione (II). Later, Goldschmidt and Koss (2) extracted this same product (II) from rat urine, while Willems *et al.* (3) iden-

[1] Phanodorm, British pat. 231,150 (1924) to Farbenfabriken Bayer and Co.

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