ABSENCE OF RELATIONSHIP BETWEEN ISONIAZID-INDUCED HEPATIC DISTURBANCES AND ACETYLATOR PHENOTYPE

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Introduction

Isoniazid (isonicotinyl hydrazide; INH) continues to be the most widely used chemotherapeutic agent for the treatment of tuberculosis (O'Brien & Stroud, 1985), and its pharmacology has been extensively investigated in man. The most important metabolic pathway of INH in man is acetylation to acetylisoniazid (Weber & Hein, 1979). Large differences have been demonstrated between individuals in the rates at which INH is acetylated and the great majority of subjects can be characterized as being either "slow" or "rapid" acetylators of isoniazid (Weber, 1987; Weber & Hein, 1979). The polymorphic acetylation of INH is genetically determined in a simple Mendelian fashion and the frequencies of the genes controlling the slow or rapid acetylation of isoniazid vary among different racial populations (Weber, 1987).

The clinical significance of acetylator status may be related to the occurrence of INH toxicity during prolonged treatment of tuberculosis, including peripheral neuropathy or the hepatic disturbances. Because induced, peripheral neuropathy was the first of the drug toxicities to be associated with the human acetylator status, the occurrence and severity of this toxicity appear to be related to the usual dose of isoniazid.
Ingested and slow acetylators are particularly prone to peripheral neuropathy (Derwadatta et al., 1960; Weber, 1987).

The relationship between nonadulterated (INH)-induced hepatitis and acetylator phenotype has been disputed. Mitchell and his co-workers (Mitchell et al., 1975) observed that the incidence of INH-induced hepatitis was higher in rapid acetylators, and further postulated that it may be due to a greater exposure to the toxic metabolite, monoacetylisoniazide. In contrast, Riechers and his colleagues (Riechers et al., 1982), based on their prospective studies, observed that the incidence of INH hepatitis toxicity was higher in slow acetylators.

This study, therefore, was aimed at re-examining these conflicting hypotheses regarding the relationship of acetylator status and the occurrence of nonadulterated hepatitis toxicity. Conducted in Javanese-Indonesian tuberculous patients who underwent prolonged treatment with INH-containing regimens. The study also explored various factors which may contribute to the occurrence of INH-induced hepatic disturbances including sex, age, treatment duration, and nutritional status.

**Methods**

**Patients**

Two hundred adult pulmonary tuberculous patients of Javanese origin were included in the study. They were undergoing treatment at the Pulmonary Disease Clinic, Yogyakarta, Indonesia. Most of them were treated as outpatients, receiving prolonged INH (400 mg daily) containing regimens. Only those who had received treatment for at least 1 month were included. The diagnosis of pulmonary tuberculosis was confirmed by chest radiography and/or sputum microscopy for acid-fast bacilli. Severely ill patients were excluded from the study for medical and ethical reasons. Since the determination of acetylator phenotype was done by means of sulfaphenazole test, those who had a past history of allergy to sulpha drugs were also excluded.

Eligible patients underwent clinical examination and anthropometric assessment. Laboratory tests were also taken for albumin, hemoglobin, and total bilirubin concentrations, as well as serum transaminases, which included the glutamic pyruvic transaminase (SGPT) and glutamic oxaloacetic transaminase (SGOT). All laboratory tests were performed by using commercially available reagents kits (Merck-Köthen).

The duration of treatment with INH containing regimen and the clinical data of patients such as age and sex were also recorded.

**Determination of acetylator phenotype**

The determination of acetylator status was performed by sulfaphenazole test (Rao et al., 1970). It is based on the ratio of N-acetylisoniazidime to the total sulfaphenazole in urine collected over 5-6 hour period after the oral ingestion of 500 mg of sulfaphenazime. Urinary sulfaphenazole concentrations were assayed spectrophotometrically (Barton & Marshall, 1937), in triplicate. Samples were kept frozen (-20°C) before analysis within one week. In order to avoid urinary contamination, all of the antimicrobial drugs were temporarily withdrawn for at least 2 days prior to the test and re-administered immediately after the completion of the study. All of the patients denied of having taken other drugs instead of the antibacterials during the last 2 weeks.

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Nutritional assessment

The assessment of nutritional status was done by anthropometry and the estimation of albumin and hemoglobin concentrations. The anthropometric measurement included the body weight and height, mid-arm circumference (MAC), and triceps skinfold thickness (TSF). All measurements were performed by the same person to eliminate inter-observer variation.

Body weight was measured to the nearest 0.1 kg on a clinical scale in light clothing without shoes. Height was measured by means of an anthropometer to the nearest centimeter (cm) without shoes. The percentage ideal body weight to height (%) IBW was calculated from the ratio of actual to ideal weight. The ideal body weight was determined from standards prepared by the World Health Organization (Jelliffe, 1968).

The measurement of mid-arm circumference was made to the nearest millimeter with an insertion tape (Deyo, 1975) with the left arm hanging relaxed. The measurement was taken midway between the tip of acromium and olecranon process. Mid-arm circumference was measured using the nearest 0.1 millimeter with a Lange skinfold caliper having a pressure of 10 gram/mm² of contact surface area. The measurement was made on the back of the left upper arm, again midway between the point of acromium and the olecranon process while the arm was hanging relaxed.

Statistical analyses

The differences between the means were evaluated with the Student's t-test, while correlations between various variables were evaluated by multiple regression analysis. The differences in the distribution between groups was analyzed by Chi-square test. A probability of less than 5% was considered significant in one-tailed test.

Result

Distribution of acetylator phenotype

The two hundred tuberculous patients consisted of 116 males and 84 females, between the ages of 15-70 years. The frequency distribution of acetylation ratio of sulphasalazine was shown in Table 1, where a bimodal pattern was clearly demonstrated with an antecedent between 0.65-0.70. Thus, individuals with values of acetylation ratio greater or less than 0.70 were classified as either rapid or slow acetylators respectively.

Out of 200 patients, 128 (64%) ± 0.006 and 0.69 ± 0.010 respectively (Table 1). The ages of patients in two groups were not significantly different, i.e., 40 ± 1.2 years in the rapid and 39.5 ± 1.9 years in the slow acetylators (Student's t-test, P > 0.05) (Table 1). The distribution of sex, however, was dissimilar where females were found in a higher proportion in the slow acetylators (Table 1).
Parameters of hepatic disturbance

None of the patients were found suffering from clinically overt hepatitis either in the rapid or slow acetylator groups, though, some may have slight increases in SGPT, SGOT or serum total bilirubin concentration. However, there were no significant differences between the two groups in the mean values of SGPT (12.39 ± 6.53 IU/L vs. 12.56 ± 6.80 IU/L), SGOT (16.47 ± 3.32 IU/L vs. 16.86 ± 4.65 IU/L), and serum total bilirubin concentration (0.84 ± 0.013 mg/dL vs. 0.84 ± 0.015 mg/dL) (Student’s t-test, P > 0.05) (Table 2).

The absence of significant differences between rapid and slow acetylator groups in the values of hepatic transaminases and serum bilirubin was further confirmed by the failure to demonstrate a significant correlation between acetylation ratio and either SGPT (r = 0.010), SGOT (r = -0.042) or serum bilirubin (r = 0.057). Neither significant correlations were found between ages and SGPT (r = -0.029), SGOT (r = 0.000) and serum bilirubin (r = 0.021) (Table 4).

The duration of treatment (months) did not appear to influence the hepatic transaminases or serum bilirubin, where no significant correlations between these variables were demonstrated (Table 4). Neither differences could be observed between males and females either of rapid or slow acetylators regarding to their SGPT, SGOT, or serum bilirubin concentrations.

Nutritional status

Nutritional status of patients of rapid acetylator and slow acetylator groups was similar. The anthropometric indices including the percentage to ideal body weight for height (BMI), triceps skinfold thickness (TSF) and mid-arm circumference (MAC) were not significantly different between both groups (Student’s t-test, P > 0.05) (Table 3). Neither significant differences could be found between both groups regarding to their albumin concentration (40.8 ± 4.8 g/L vs. 40.9 ± 5.8 g/L) and hemoglobin concentration (12.45 ± 2.33 g/dL vs. 12.57 ± 2.09 g/dL) (Table 5).

It was also found, that there were no significant correlations between either hepatic transaminases or serum bilirubin and various nutritional indices, except between albumin concentration and SGPT (r = 0.194, P < 0.01) and between hemoglobin and SGOT (r = 0.181, P < 0.01) (Table 4).

Discussion

This study clearly showed that there was no significant difference in the susceptibility to INH hepatotoxicity between the two acetylator phenotypes. Thereby, contradicting the hypothesis from previous studies that the susceptibility to INH hepatic disturbances was greater either in the rapid (Mitchell et al., 1975) or slow (Tischfieldbaum et al., 1982) acetylator. The relationship between acetylator status and INH induced hepatic toxicity has become subject of controversies for over two decades and has presented a complex problem in clinical pharmacology.

There have been several additional investigations examining the role of acetylator status and INH hepatotoxicity, and found that slow acetylators were more susceptible (Sal et al., 1972; Gootenberg-Roka et al., 1978, Bailey et al., 1979, Smith et al., 1972). One of the difficulties in comparing the conflicting results by various reports.
may be related to the fact that different INH containing regimens have been involved in each study (Weber & Hein, 1988). In contrast to Mitchell and his co-workers (Mitchell et al., 1975) who observed that INH-induced hepatitis was more predominant among rapid acetylators following INH monotherapy, under the condition of multiple drug therapy with ethambutol and rifampicin, Eichelbaum and his colleagues (Eichelbaum et al., 1986) observed that the INH-induced hepaticities were found to be more frequent among the slow than the rapid acetylators. It is extremely difficult, however, to accept the explanation that the differing regimen accompanying INH have solely contributed to the different susceptibility to INH-hepatotoxicity from one phenotype to the other. Another difficulty in examining the relationship between acetylator status and INH-hepatotoxicity is that, comparisons are usually made against historical controls rather that generated on matched populations (Weber & Hein, 1985), and the number of subjects being studied is often too small for appropriate calculation of phenotype distribution.

The patients examined in the present study had all been receiving INH (400 mg daily) and streptomycin injections (1 gram twice weekly), with a sufficient number of subjects in each acetylator group from the same population. The distribution of acetylator phenotype found in this study i.e. 64% for rapid and 36% for slow acetylators, was similar to that of our previous study (Santoso, 1983) in the same Japanese population.

The hypothesis which claimed that INH might be more hepatotoxic in rapid than slow acetylators (Mitchell et al., 1975) was based on the assumption that rapid acetylators were more exposed to the toxic metabolite, monoacetyl hydrazine, because of a higher formation of the compound in these subjects. Monoacetyl hydrazine could then be converted by a cytosol P-450 dependent reaction to potent reactive electrophilic that bind to macromolecules causing hepatic necrosis (Mitchell et al., 1976). Subsequently, however, more comprehensive works based on INH pharmacokinetic data (Ellard & Gannon, 1976; Tizard et al., 1977) had disclosed that rapid acetylators are not necessarily more exposed to the toxic metabolite relative to slow acetylators. According to these later studies, the toxic metabolite monoacetyl hydrazine, also undergoes polymorphic acetylation to non-toxic diacetyl hydrazine. Thus, though rapid acetylators may form more monoacetyl hydrazine initially, subsequent polymorphic acetylation prevents monoacetyl hydrazine from accumulating in the rapid acetylators in comparison to slow acetylators due to more rapid acetylation to non-toxic diacetyl hydrazine. Therefore, both acetylator subjects are exposed to the same degree of the toxic metabolite monoacetyl hydrazine. Not very surprisingly, there were also other studies to show that there was no difference in the susceptibility to INH-hepatotoxicity between the two acetylator phenotypes (Birkha, 1976; Ellard et al., 1978). The evidence presented by our present study, could support the reconciliation of the conflicting hypotheses and that neither slow nor rapid acetylator status has any causal influence on INH-induced hepatic disturbance.

Although, age has been reported to have an association with the risk of INH-hepatotoxicity (Butley, 1981), unfortunately we failed to demonstrate this association. Neither nutritional status, treatment duration, nor sex appear to have a direct relationship with the occurrence of INH-hepatotoxicity. It should be noted, however, that...
tuberculous patients included in the present study were not severely but marginally
maltreated. The possibility of a greater risk of INH-hepatotoxicity in severely mal-
nourished patients could not, therefore, be excluded based on the present study.

The failure to demonstrate an association between treatment duration and the
hepatic disturbance was difficult to explain. If the incidence of hepatic disturbance is
proportionally related to the size of INH dose, one could expect an association with
the duration of treatment as well. However, this is probably not the case. Similarly,
Ellard and his colleagues (Ellard et al., 1978) also failed to demonstrate an association
between hepatic disturbance and either the size of INH dose or the duration of
treatment.

Acknowledgement

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1. hepatitis.


![Image](image.jpg)

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Table 1.
The distribution of age, sex, and the mean values of acetylation ratio in rapid and slow acetylators group.

<table>
<thead>
<tr>
<th></th>
<th>Rapid acetylators (n = 128)</th>
<th>Slow acetylators (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Range</td>
<td>17 - 70</td>
<td>15 - 65</td>
</tr>
<tr>
<td>- Mean ± SEM</td>
<td>40 ± 1.2</td>
<td>39.9 ± 1.9</td>
</tr>
<tr>
<td>2. Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Female</td>
<td>47</td>
<td>37</td>
</tr>
<tr>
<td>- Male</td>
<td>81</td>
<td>35</td>
</tr>
<tr>
<td>- Female/male</td>
<td>0.56</td>
<td>1.06</td>
</tr>
<tr>
<td>3. Acetylation ratio</td>
<td>0.86 ± 0.006</td>
<td>5.49 ± 0.91 **</td>
</tr>
</tbody>
</table>

SEM : Standard error of the mean
* : Chi-Square test, P<0.01
** : Student’s t-test, P<0.01

Table 2.
The mean values (± standard error of the mean, SEM) of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and serum total bilirubin in rapid and slow acetylators.

<table>
<thead>
<tr>
<th></th>
<th>Rapid acetylators (n = 128)</th>
<th>Slow acetylators (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT (IU/L)</td>
<td>12.39 ± 0.36</td>
<td>12.36 ± 0.48 *</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>16.47 ± 0.32</td>
<td>16.86 ± 0.45 *</td>
</tr>
<tr>
<td>Serum total bilirubin (mg%)</td>
<td>0.84 ± 0.013</td>
<td>1.94 ± 0.015 *</td>
</tr>
</tbody>
</table>

* Student’s t-test, P<0.05
**Table 3.**
The values of indices (mean ± standard error of the mean, SEM) for nutritional status including percentage of ideal body weight for height (IBWI), triceps skinfold thickness (TSF), mid-arm circumference (MAC), albumin, and hemoglobin concentration in rapid and slow acetylator.

<table>
<thead>
<tr>
<th></th>
<th>Rapid acetylators (n = 128)</th>
<th>Slow acetylators (n = 72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. % IBW</td>
<td>77.1 ± 0.9 %</td>
<td>79.4 ± 1.4 %</td>
</tr>
<tr>
<td>2. TSF (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>11.4 ± 0.83</td>
<td>10.6 ± 0.53</td>
</tr>
<tr>
<td>- Female</td>
<td>11.9 ± 0.53</td>
<td>12.0 ± 1.9</td>
</tr>
<tr>
<td>3. MAC (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>23.1 ± 0.3</td>
<td>23.7 ± 0.5</td>
</tr>
<tr>
<td>- Female</td>
<td>21.9 ± 0.4</td>
<td>21.8 ± 0.5</td>
</tr>
<tr>
<td>4. Serum albumin (g/L)</td>
<td>40.8 ± 4.8</td>
<td>40.9 ± 5.8</td>
</tr>
<tr>
<td>5. Hemoglobin concentration (g %)</td>
<td>12.45 ± 0.23</td>
<td>12.57 ± 0.29</td>
</tr>
</tbody>
</table>

* Student’s test, P<0.05.

**Table 4.**
The values of correlation coefficients obtained by multiple regression analysis between serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum bilirubin and the acetylation ratio, age, sex, duration of treatment, and indices for nutritional status.

<table>
<thead>
<tr>
<th></th>
<th>SGPT</th>
<th>SGOT</th>
<th>Serum bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Age</td>
<td>0.016</td>
<td>-0.042</td>
<td>0.057</td>
</tr>
<tr>
<td>2. Sex</td>
<td>0.051</td>
<td>0.110</td>
<td>0.023</td>
</tr>
<tr>
<td>3. Duration of treatment</td>
<td>-0.012</td>
<td>0.014</td>
<td>-0.077</td>
</tr>
<tr>
<td>4. Nutritional indices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- % IBW (Ideal body weight for height)</td>
<td>-0.012</td>
<td>0.014</td>
<td>-0.077</td>
</tr>
<tr>
<td>- Triceps skinfold thickness (TSF)</td>
<td>0.052</td>
<td>0.110</td>
<td>0.082</td>
</tr>
<tr>
<td>- Mid-arm circumference (MAC)</td>
<td>0.022</td>
<td>0.019</td>
<td>0.012</td>
</tr>
<tr>
<td>- Serum albumin</td>
<td>0.118 *</td>
<td>0.051</td>
<td>0.088</td>
</tr>
<tr>
<td>- Hemoglobin concentration (g %)</td>
<td>0.115</td>
<td>0.081 *</td>
<td>0.019</td>
</tr>
</tbody>
</table>

* P<0.01
Figure 1.
The frequency distribution of sulphasulphide acetylation ratio obtained in 200 tuberculous Javanesse Indonesians. A bimodal distribution with an antimode between 0.65 - 0.70 is clearly demonstrated.