Note

Genotyping of the N-acetyltransferase2 Polymorphism in the Prediction of Adverse Drug Reactions to Isoniazid in Japanese Patients

Masahiro Hiratsuka1,2, Yukinaga Kishikawa1,3, Yoh Takekuma1, Masaki Matsuura1, Kaori Narahara1, Tomoko Inoue1, Samar Ismail Hamdy3, Naomi Endo3, Junichi Goto1,3 and Michinao Mizugaki1,2,3

1Department of Pharmaceutical Sciences, Tohoku University Hospital, Sendai, Japan
2Department of Clinical Pharmaceutics, Tohoku Pharmaceutical University, Sendai, Japan
3Division of Clinical Pharmacy, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan

Summary: To investigate the association between NAT2 genotypes and the incidence of isoniazid (INH)-induced adverse reactions, in the hope of identifying a pharmacogenetic approach that could be useful in the prediction and prevention of adverse reactions in Japanese patients, we retrospectively studied the genotypes of NAT2 in 102 Japanese patients treated with INH (without rifampicin co-administration). The subjects were classified into three groups according to their genotypes: rapid-type, intermediate-type, and slow-type. The clinical conditions of the patients were followed-up in order to evaluate the development of any adverse drug reactions (ADRs) and correlate them with patient genotypes. Six out of the 102 patients (5.9%) developed various ADRs following INH treatment. These reactions included nausea/vomiting, fever, visual impairment, and peripheral neuritis. We found a statistically significant difference between the incidence of ADRs and NAT2 genotype. The incidence of ADRs was significantly higher in the slow type than in the other two types, as 5 out of the 6 ADR patients were of the slow-type, and the other one was of the intermediate-type, while no patients of the rapid-type developed any ADRs. The results indicated that the genes coding for slow acetylation were associated with the incidence of serious ADRs following INH treatment. Our findings suggest that determination of NAT2 genotype might be clinically useful in the evaluation of patients at high risk of developing ADRs induced by INH.

Key words: NAT2; genetic polymorphism; isoniazid; adverse drug reaction; pharmacogenetics

Introduction

Isoniazid (INH) is an important drug that is indispensable for the prophylaxis and treatment of tuberculosis. N-acetyltransferase 2 (NAT2) is responsible mainly for INH metabolism. NAT2 exhibits a hereditarily determined polymorphism, and the individual phenotypes can be classified as rapid, intermediate, or slow acetylators (RAs, IAs, or SAs, respectively) according to their acetylation activity.1) The RAs should take larger doses of INH than SAs. Low plasma drug levels in RAs are one of the reasons for therapy failure. On the other hand, the SAs are at risk of adverse reaction. This polymorphism shows racial differences: almost 50% of Caucasian are SAs, whereas the frequency of SAs in Japanese is only 10%.2–4) The human NAT2 gene contains an 870-bp.5,6) To date, 1 allele code for fast acetylation (wild-type) and several mutated alleles codes for impaired acetylation activity have been discovered. Of all the NAT2 allelic variants that had been identified,7) 3 variants (NAT2*5, NAT2*6, and NAT2*7) have been shown to account for the majority of the SA genotype in Japanese subjects.5,8)

During INH treatment, serious adverse drug reactions (ADRs) including peripheral neuritis,9) fever,10–13) and hepatic toxicity9,14) have been recognized in some patients, despite the similarity of their doses to those taken by others. In most of these studies, the metabolic ratio of acetyl-INH (AcINH) and INH at 3 hours post-dose were an accurate assessment of RA, IA, and SA
phenotypes. Recently, \textit{NAT2} genotyping was shown to be useful in evaluating INH phenotypes. These findings suggested that the phenotyping or genotyping of \textit{NAT2} is useful to monitor and optimize therapy using this drug. A significant association has been previously observed between hepatotoxicity and \textit{NAT2} genotypes in a group of Japanese patients with pulmonary tuberculosis treated with both INH and rifampicin.\textsuperscript{15} However, it was not clear if this association could be observed without rifampicin co-administration.

The goal of the present study was to investigate the relation between \textit{NAT2} genotypes and the incidence of INH-induced ADRs in a group of Japanese patients suffering from the same disease and receiving INH (without rifampicin co-administration). If the link between the \textit{NAT2} genotype and the incidence of INH-induced ADRs can be made clear, genotyping may make it possible to identify patients susceptible to ADRs prior to drug administration.

**Methods**

**Chemicals:** INH, hydrazine (Hz) and acetyl-hydrazine (AcHz) were purchased from Aldrich, Milwaukie, WI. AcINH was synthesized by the method described by Yale et al.\textsuperscript{16} All other chemicals were of reagent grades and obtained commercially.

**Blood samples:** Venous blood was obtained from in- and outpatients of the Tohoku University Hospital (Sendai, Japan). The local ethics committee approved this study and an informed consent was obtained from each blood donor.

**Patients:** One hundred-two Japanese patients (28 males and 74 females, aged 43 ± 17 years, and weighing 56.1 ± 9.2 kg, all Japanese) were eligible for this prospective investigation during 1999–2002. All patients had taken INH (300 mg/day) orally without rifampicin co-administration twice a week for more than 2 weeks, and blood samples were collected at 3 hours after INH administration. At the beginning of treatment, liver function tests showed comparatively normal findings on serum aspartate aminotransferase and alanine aminotransferase. Twenty-six patients were diagnosed with renal dysfunction, whereas the others showed normal renal function. We defined ADRs in this study as nausea/vomiting, fever (greater than 38°C), visual impairment, peripheral neuritis, and elevated serum activity of hepatic aminotransferases following the initiation of INH therapy which disappeared after its discontinuation.

**\textit{NAT2} genotyping:** DNA was isolated from peripheral blood cells anticoagulated with K2EDTA using a GFX Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) according to the manufacturer’s recommendations. \textit{NAT2*5} (T341C), \textit{NAT2*6} (G590A), and \textit{NAT2*7} (G857A) were detected by an allele-specific real-time PCR assay as previously described by Hiratsuka et al.\textsuperscript{17}

**HPLC analysis of INH, AcINH, Hz and AcHz:** Venous blood (5 mL) was collected at 3 hours after drug administration. After centrifugation plasma samples were stored at −80°C until assay. Sample preparation and HPLC analysis were performed according to the method of Seifart et al.\textsuperscript{18} with minor modifications. Fig. 1 outlines the procedures followed in the preparation of samples. The HPLC system (Tosoh SC8020, Tokyo, Japan) consisted of a pump (CCPS), an ultraviolet detector (PD8020) adjusted to 340 nm, and an auto sampler (AS8020). The stationary phase was a reverse phase Capcell Pak C18 column (4.6 mm i.d. × 150 mm, particle size 5 μm, Shiseido, Tokyo, Japan). The mobile phase was composed of a mixture of solvent A (50 mM KH₂PO₄) and solvent B (acetonitrile-isopropanol; 4:1, v/v). For the first minute of chromatography the mobile phase contained 20% of solvent B, which was then increased linearly to reach 50% after 8 min and then 70% after 11 min, after which it was maintained until completion of the run at 15 min. The flow rate was 0.8 ml/min. The retention time of INH (and post-hydrolysis of AcINH), Hz (and post-hydrolysis of AcHz), and an internal standard (berberine sulfate trihydrate in H₂O) was 6.8 min, 12.7 min, and 10.5 min, respectively. The calibration curves were linear over a concentration range of 0.1 to 5.0 μg/mL ($t^2 > 0.999$) for INH, 0.5 to 10.0 μg/mL ($t^2 > 0.999$) for AcINH, 0.01 to 0.1 μg/mL ($t^2 > 0.999$) for Hz, and 0.05 to 2.5 μg/mL ($t^2 > 0.999$) for AcHz.

**Statistical analysis:** Student’s t-test was used to compare the concentration of INH, AcINH, Hz, and AcHz, and the metabolic ratio AcINH/INH among the different genotypes. Chi-squared test and Fisher’s exact test was applied for statistical analysis of the incidence of adverse drug reaction relating to the \textit{NAT2} genotype.

**Results**

**Allele and genotype frequencies of \textit{NAT2}:** \textit{NAT2} alleles were identified in 102 patients by an allele-specific real-time PCR assay as previously described by us. As shown in Table 1, the wild-type allele \textit{NAT2*4} was expressed at a greater frequency (73.5%) than were the mutant alleles (\textit{NAT2*6}, 15.7%; \textit{NAT2*7}, 9.8%; \textit{NAT2*5}, 1.0%) in 102 patients. The homozygote of \textit{NAT2*4} was the most frequent (52.9%), followed by the heterozygote of \textit{NAT2*4} and mutant alleles (24.5% \textit{NAT2*4/6}, 14.7% \textit{NAT2*4/7}, and 2.0% \textit{NAT2*4/5}). The mutant alleles were combined in only 6 of 102 patients (5.9%).

**Genotypes and incidence of ADRs:** Fig. 2 showed the relationship between the \textit{NAT2} genotypes and INH-induced toxicity for 102 patients. Six patients (5.9%) experienced ADRs when INH was administered. Remark-
NAT2 Genotype and INH-induced ADRs

Table 1. Frequency distribution of NAT2 genotypes in the isoniazid-treated patients (N = 102)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>number</th>
<th>%</th>
<th>Acetylator status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT2*4/*4</td>
<td>54</td>
<td>52.9</td>
<td>RA-type</td>
</tr>
<tr>
<td>NAT2*4/*5</td>
<td>2</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>NAT2*4/*6</td>
<td>25</td>
<td>24.5</td>
<td>IA-type</td>
</tr>
<tr>
<td>NAT2*4/*7</td>
<td>15</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>NAT2*5/*5</td>
<td>0</td>
<td>0</td>
<td>SA-type</td>
</tr>
<tr>
<td>NAT2*5/*6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>NAT2*5/*7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>NAT2*6/*6</td>
<td>2</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>NAT2*6/*7</td>
<td>3</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>NAT2*7/*7</td>
<td>1</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

RA; rapid acetylator, IA; intermediate acetylator, SA; slow acetylator.

Fig. 1. Flow diagram of preparative procedures prior to HPLC analysis.
TCA = trichloroacetic acid; IS = internal standard (berberine sulfate trihydrate); p-HBA = p-hydroxybenzaldehyde.

Fig. 2. Distribution of frequency of genotypes in patients with and without ADRs induced by INH treatment. Hatched bar represents the occurrence of ADRs during INH treatment. Acetylator status, RA, IA and SA, was determined according to NAT2 genotyping as described in Table 1.

Figurably significant differences were seen regarding the rate of incidence of ADRs during INH-treatment when comparing RA with SA patients and IA with SA patients (Fig. 2). There were no RA patients within the group of individuals with INH-induced toxicity. Among the 42 IA patients, only one (2.4%) experienced ADRs. In con-
Table 2. Characteristics of the patients with INH-induced toxicity

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Genotype</th>
<th>INH conc. (µg/mL)</th>
<th>Renal conditions</th>
<th>AcINH/INH ratio</th>
<th>ADR</th>
<th>Rx</th>
<th>symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>NAT2*7/*7</td>
<td>n.d.</td>
<td>normal</td>
<td>n.d.</td>
<td>nausea, vomiting</td>
<td>withdrawal</td>
<td>improved</td>
</tr>
<tr>
<td>44</td>
<td>NAT2*6/*7</td>
<td>n.d.</td>
<td>normal</td>
<td>n.d.</td>
<td>fever</td>
<td>other medicine</td>
<td>improved</td>
</tr>
<tr>
<td>74</td>
<td>NAT2*6/*6</td>
<td>1.860</td>
<td>normal</td>
<td>0.732</td>
<td>visual impairment</td>
<td>dose decreased</td>
<td>improved</td>
</tr>
<tr>
<td>84</td>
<td>NAT2*7/*7</td>
<td>2.604</td>
<td>impairment</td>
<td>2.274</td>
<td>peripheral neuritis</td>
<td>withdrawal</td>
<td>improved</td>
</tr>
<tr>
<td>95</td>
<td>NAT2*4/*6</td>
<td>1.046</td>
<td>normal</td>
<td>1.145</td>
<td>fever</td>
<td>withdrawal</td>
<td>improved</td>
</tr>
<tr>
<td>138</td>
<td>NAT2*6/*6</td>
<td>1.453</td>
<td>impairment</td>
<td>0.014</td>
<td>0.078</td>
<td>0.938</td>
<td>8.477</td>
</tr>
</tbody>
</table>

n.d.; not determined.

Table 3. The plasma concentration of INH, AcINH, Hz and AcHz after administration at 3 hour

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>INH (µg/mL)</th>
<th>AcINH (µg/mL)</th>
<th>Hz (µg/mL)</th>
<th>AcHz (µg/mL)</th>
<th>AcINH/INH</th>
<th>AcHz/Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT2*4/*4 (N = 22)</td>
<td>0.905 ± 0.721</td>
<td>6.232 ± 2.567</td>
<td>0.014 ± 0.007</td>
<td>0.634 ± 0.350</td>
<td>9.181 ± 4.129</td>
<td>58.617 ± 34.206</td>
</tr>
<tr>
<td>NAT2*4/*5, *4/*6, or *4/*7</td>
<td>1.895* ± 0.902</td>
<td>5.563 ± 2.077</td>
<td>0.018 ± 0.009</td>
<td>0.572 ± 0.395</td>
<td>3.465* ± 1.389</td>
<td>34.821 ± 17.689</td>
</tr>
<tr>
<td>NAT2*6/*7 and *6/*6 (N = 2)</td>
<td>1.453</td>
<td>1.279</td>
<td>0.014</td>
<td>0.078</td>
<td>0.938</td>
<td>8.477</td>
</tr>
</tbody>
</table>

*; P<0.005: Significantly different from the homozygotes for the wild-type allele (*4/*4).

Fig. 3. The plasma concentration of INH (A) and the AcINH/INH ratio (B) at 3 hour after oral administration (300 mg INH) in Japanese patients.
Open circle: non-ADR patients; Closed circle: ADR patients.

In contrast, of the 6 SA patients, five (83.3%) experienced ADRs. The ADRs observed in the prospective study included nausea/vomiting, fever, visual impairment, and peripheral neuritis (Table 2).

INH acetylator phenotypes and genotype-phenotype relationship: In 45 Japanese patients treated with INH, plasma concentrations of INH, AcINH, Hz, and AcHz at 3 hours after INH administration were measured by HPLC. As shown in Table 3, there were no significant differences in plasma concentrations of AcINH, Hz, and AcHz, and AcHz/Hz ratio at 3 hour post-dose between the wild-type (*4/*4) and heterozygotes. In contrast, carriers of the NAT2*4/*4 genotype showed lower INH concentrations and higher AcINH/INH ratios at 3 hour than those in subjects with other NAT2 genotypes.

Phenotypes and incidence of ADRs: As shown in Fig. 3, there was no significant relationship between plasma concentrations of INH (A) at 3 hour post dose and INH-induced ADRs. In contrast there was a good
relationship between the low value of AcINH/INH ratio and the incidence of ADRs (B).

**Discussion**

This is the first study to reveal a significant association between the NAT2 genotype and the incidence of INH-induced ADRs in Japanese patients without rifampicin co-administration. The incidence of ADRs was high in SA-type patients (83.3%) and was low in the RA-type (0%) and IA-type (2.4%). Thus, the patients with SA genotypes presented a high risk of developing INH-induced toxicity when the Japanese standard dose of INH (300 mg/day) was given. This indicates that NAT2 genotyping is useful in identifying patients susceptible to its occurrence.

Recently, Seifart et al. have reported that plasma INH concentrations and the metabolic ratio (AcINH/INH) at 3 hour post-dose were an accurate assessment of RA, IA, and SA phenotypes. In the present study, the plasma concentration of INH and AcINH/INH ratio at 3 hour after administration in the IA patients as well as the SA patients were significant different from those in the RA patients. However, the development of INH-induced ADRs in the SA patients was significantly frequent compared with RA and IA genotypes. There were several patients in RA and IA-type who did not experience the INH-induced ADRs and showed higher concentration of INH than SA-type patients. The results suggested that the mechanism developed the INH-induced ADR could not be explained by the elevated concentration of INH only. Thus, there was no correlation between the incidence of INH-induced ADR and the plasma concentration of INH at 3 hour after administration.

There was a good relationship between the low value of AcINH/INH ratio and the incidence of ADRs. However, a patient who showed the lower value of AcINH/INH ratio did not necessarily develop the INH-induced ADRs. Of IA patients, INH-induced ADRs were also observed in one IA patient (No. 95) with genotype NAT2*4/*6. Although there was no statistically significant, the concentration of INH and AcINH/INH ratio in the patient showed comparably different from the mean in the other IA patients. Other clinical observations revealed a low level of plasma albumin (3.1 g/dL) and a high level of BUN (27 mg/dL) (data not shown). Thus, renal impairment might have occurred and accounted for the decreased clearance of INH in this patient. Because there were patients who have renal impairment and did not develop any ADRs, and all SA-type patients who developed the INH-induced ADRs did not have a renal impairment (Table 2), renal impairment only might not be the risk factor to develop the INH-induced ADRs. Because of a small number of the examined, it is difficult to solve the mechanism developing the INH-induced ADRs. However, the decrease clearance of INH might be a risk factor of developing the INH-induced ADRs. Future pharmacokinetic investigation needs to explain the mechanism.

In the present study, various INH-induced ADRs were recognized in the SA patients. These reactions included nausea/vomiting, fever, visual impairment, and peripheral neuritis. Fever (approximately 39°C) was observed in two patients (No. 44 and 138). INH-induced fever has been previously described by many investigators and is thought to be a result of either toxic and/or allergic reactions; however, no reports have suggested that INH-induced fever is associated with NAT2 genotype. Since fever was observed in only two subjects (SA), it is unclear whether there is any difference in the incidence of INH-induced fever among NAT2 genotypes.

Because of a retrospective study, all in-patients were treated with INH (300 mg/day, twice a week) before genetic analysis of NAT2. After that, we have considered that all SA patients are under the higher risk of the appearance of ADR of INH, and recommended to the physician that INH be withdrawn, substituted with another drug, or its dosage reduced. After changing the prescription, all symptom improved or were lessened in SA patients occurred a INH-induced ADR (Table 2).

In conclusion, the results presented indicate that adverse effects induced by INH without rifampicin co-administration in SA-type patients are significantly frequent compared with RA and IA genotypes. The determination of the NAT2 genotypes and acetylation phenotyping in regard to INH treatment were suggested to be clinically useful for the prediction and prevention of INH-induced toxicity.

**Acknowledgement:** This work was supported by the Grant-in-Aid for Research on Health Sciences focusing on Drug Innovation (numbers 72005) from the Japan Health Sciences Foundation.

**References**

4. Deguchi, T., Mashimo, M. and Suzuki T.: Correlation...


