**NAT2 and CYP2E1 polymorphisms and susceptibility to first-line anti-tuberculosis drug-induced hepatitis**


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**BACKGROUND:** Most cases with anti-tuberculosis drug-induced hepatotoxicity (ATDH) have been attributed to isoniazid.

**OBJECTIVE:** To evaluate whether the polymorphisms of the cytochrome P450 2E1 (CYP2E1) and N-acetyltransferase 2 (NAT2) gene are associated with ATDH.

**DESIGN:** A total of 140 tuberculosis (TB) patients without liver diseases before treatment who received anti-tuberculosis treatment were followed prospectively. Their CYP2E1 and NAT2 genotypes were determined using the TaqMan polymerase chain reaction assay.

**RESULTS:** Forty-five (32.1%) patients were diagnosed with ATDH. No significant differences were reported in age and sex between patients with and without ATDH. Slow acetylators defined by NAT2 genotypes had a higher risk of hepatotoxicity than rapid acetylators (51.2% vs. 25.2%, \(P = 0.0026\)). Odds ratio (OR) analysis showed that slow acetylator status (OR 3.15, 95%CI 1.47–6.48) was the only independent risk factor for ATDH. Pyrazinamide co-administration induced hepatitis was also associated with NAT2 acetylator status. CYP2E1 c1/c1 homozygotes are prone to developing more severe hepatotoxicity than other c1/c2 and c2/c2 genotypes.

**CONCLUSION:** The slow acetylator status of NAT2 is a significant susceptibility risk factor for ATDH. CYP2E1 is associated with the severity of ATDH.

**KEY WORDS:** NAT2; CYP2E1; anti-tuberculosis drug-induced hepatitis; ATDH

**SUMMARY**

Major adverse reactions to anti-tuberculosis drugs can cause significant morbidity and compromise treatment regimens for tuberculosis (TB). Regimens containing isoniazid (INH), rifampicin (RMP), ethambutol (EMB) and pyrazinamide (PZA) are traditionally used as first-line treatment for TB. However, acute or chronic hepatitis frequently develops in patients receiving these drugs. PZA, INH and RMP cause drug-induced hepatitis, and PZA-induced hepatotoxicity is substantially higher than other drugs.

Anti-tuberculosis drug-induced hepatotoxicity (ATDH) is commonly defined as a treatment-emergent increase in serum alanine aminotransferase (ALT) greater than three or five times the upper limit of normal (ULN), with or without symptoms of hepatitis. INH, RMP and PZA are potentially hepatotoxic drugs. Metabolism is crucial in ATDH and toxic metabolites play a central role.

N-acetyltransferase 2 (NAT2) is mainly responsible for INH metabolism and exhibits a hereditarily determined polymorphism. The individual NAT2 phenotypes can be classified as rapid, intermediate or slow acetylators according to their acetylation activity. In the liver, INH is first metabolised into acetylisoniazid via N-acetyltransferase, followed by hydrolysis to acetylhydrazine. Acetylhydrazine is then oxidised into hepatotoxic intermediaries by cytochrome P450 2E1 (CYP2E1). It has been suggested that the NAT2 genotype slow acetylators have a higher incidence of ATDH than rapid and intermediate acetylators, and study results are consistent in Taiwanese, Japanese and Korean TB patients. However, study results on the association of the CYP2E1 genotype and ATDH are inconsistent in Taiwanese and Korean TB patients. In one meta-analysis, NAT2 and CYP2E1 genotypes showed genetic association to anti-tuberculosis drug-induced liver injury.

NAT2 variants *5, *6, *7 and the CYP2E1 c1/c2 allele have been investigated for their association with ATDH in previous studies on Asian populations. In this study, the contribution of NAT2 and CYP2E1 polymorphisms to first-line ATDH were validated in TB patients at a TB centre in Taoyuan General Hospital. Furthermore, the association of NAT2 acetylator status and hepatotoxicity induced by PZA co-administration with INH, RMP and EMB were evaluated.
MATERIALS AND METHODS

Subjects
A total of 140 patients treated for active TB at the General Taoyuan Hospital, Taoyuan, Taiwan, between 2007 and 2008 were surveyed consecutively. Inclusion criteria were as follows: adult patients newly diagnosed with active TB, having evident lesions of TB by simple X-ray, computed tomography, positive results of sputum smears and cultures for detection of mycobacteria. Patients with any of the following conditions were excluded from the study: 1) positive serum hepatitis B virus surface antigen, antibody to hepatitis C virus; 2) alcoholic liver disease or habitual alcohol drinking; 3) any other hepatic or systemic diseases that may cause liver dysfunction; 4) abnormal serum ALT, aspartate aminotransferase (AST) or bilirubin levels before anti-tuberculosis treatment.

Exempt for those patients who had developed severe ATDH, all patients received oral INH 300 mg, RMP 600 mg (or 450 mg if body weight was < 50 kg), PZA 200 mg/kg body weight and EMB 800 mg daily for the first 2 months. PZA was then discontinued, while INH, RMP and EMB were continued for another 4 months. ATDH was designated as an increase in serum ALT level of >2 × ULN after anti-tuberculosis treatment, according to the criteria of drug-induced liver injuries developed by the international consensus meeting organised by the Council for International Organizations of Medical Sciences (CIOMS).16 Thirty-seven of 45 patients with ATDH were re-challenged, beginning with INH 50 mg, 100 mg, 150 mg and 300 mg (the full dose of INH), and then started with RMP 150 mg, 300 mg, 450 mg (the full dose for patients with bodyweight < 50 kg) or 600 mg (the full dose for patients with bodyweight > 50 kg). If AST, ALT and total bilirubin levels were normal in the re-challenge process, INH, RMP and EMB combination treatment was continued for 9 months.17 PZA co-administration induced hepatitis was diagnosed based on negative INH and RMP re-challenge tests. EMB is not considered to be incriminated in ATDH.1

Written informed consent was obtained from each patient enrolled in this study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Taoyuan General Hospital.

DNA preparation
Genomic DNA was extracted from oral swabs collected from 140 TB patients using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer’s instructions. The extracted genomic DNA was analysed using agarose gel electrophoresis and quantitatively determined by spectrophotometry and stored at −80°C until use.

SNP genotyping
NAT*5, *6, *7 and CYP2E1 c1/c2 polymorphisms were selected to perform genotyping. The represen-
NAT2 slow acetylators associated with PZA-induced hepatotoxicity

Thirty-three TB patients with ATDH were susceptible to PZA co-administration with INH, RMP and EMB. The slow acetylators were at a higher risk of developing ATDH than rapid acetylators (Table 4). The OR of slow acetylators compared to rapid acetylators was 3.28 (95% CI 1.53–7.06). The slow acetylators were at a higher risk of developing hepatotoxicity induced by PZA co-administration with INH, RMP and EMB than rapid acetylators. The OR of slow acetylators compared to rapid acetylators was 3.98 (95% CI 1.72–9.25).

CYP2E1 c1/c2 polymorphism and severity of drug-induced hepatotoxicity

Among the 45 patients with ATDH, CYP2E1 c1/c1 homozygous patients had a higher mean serum AST level, but not ALT level, than patients with the c1/c2 and c2/c2 genotypes (Table 5). Furthermore, CYP2E1 c1/c1 homozygous patients were more likely to have >3× ULN of serum AST levels than did patients with the c1/c2 and c2/c2 genotypes during the first 2 months of anti-tuberculosis treatment, or to develop severe ATDH (Table 5).

**DISCUSSION**

Our study shows that NAT2 acetylator status can be regarded as an important risk factor for developing ATDH in the Taiwanese population. Previous reports in the Taiwanese population have shown that NAT2 slow acetylators are susceptible to INH-induced hepatitis. The results indicate that slow acetylators develop ATDH more frequently in current anti-tuberculosis first-line (INH+RMP+PZA+EMB) treatment, and where PZA is administered at the same time as INH, RMP and EMB. CYP2E1 c1/c2 polymorphism did not show a significant association with ATDH in this study.
Patients in this study were taking drugs concomitantly with INH. Co-administration of drugs may result in quantitative and qualitative alteration of the drug metabolism, and it is very difficult to exclude the presence of confounding factors in assessing ATDH. Rat studies have shown that INH and hydrazine induce CYP2E1 activity. INH has an inhibiting effect on CYP1A2, 2A6, 2C19 and 3A4 activity. RMP is a potent inducer of the hepatic CYP450 system in the liver and intestine, thus increasing metabolism of many other compounds. RMP is also known to reduce NAT2 activity. PZA has been shown to inhibit the activity of several CYP450 isoenzymes in a rat study, but had no inhibitory effect on CYP450 isoenzymes in a human liver microsomes study. As a combination of these drugs is known to increase the incidence of ATDH by up to 35%, it is obvious that drug-drug interactions do occur. Our data, especially on PZA co-administration, are consistent with the above observation and indicate that some interactions are still unknown. The CYP2E1 c1/c1 genotype is associated with a high CYP2E1 activity and is involved in ATDH. Although the previous results of an association study were not revealed in our study, the CYP2E1 c1/c1 genotype in this study was more likely to develop severe hepatic injury than other genotypes.

CONCLUSIONS

As birthplace in Asia is a risk factor for developing ATDH, the determination of NAT2 and CYP2E1 genotypes for anti-tuberculosis treatment should be clinically useful for the prediction and prevention of ATDH in Asian TB patients.

References


CONCLUSIÓN:
La condición de acetilador lento, definida por sus genotipos NAT2, se asocia con un mayor riesgo de hepatotoxicidad que los otros genotipos. Además, se observó predisposición a una hepatotoxicidad más grave que los otros genotipos NAT2.

CONCLUSIONS:
The status of acetylation was the only factor associated with an increased risk of hepatotoxicity (OR 1.97, 95% CI 1.47–6.48). The risk was significantly higher in patients who were slow acetylators (OR 3.15, 95% CI 1.47–6.48). The condition of acetylation was also associated with a greater tendency to develop hepatotoxicity in patients treated for latent tuberculosis. Eur J Clin Pharmacol 2006; 62: 423–429.