CYP2E1 POLYMORPHISMS AND SUSCEPTIBILITY TO ANTI-TUBERCULOSIS DRUG-INDUCED HEPATOTOXICITY IN INDONESIA POPULATION

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ABSTRAK

Pengobatan Tuberkulosis (TB), berdasarkan penggunaan isoniazid (INH), rifampisin (RMP) dan pirazinamid (PZA), terbukti menyebabkan hepatotoksisitas yang diinduksi oleh obat (Drug Induced Hepatotoxicity/DIH). Penelitian terbaru menunjukkan bahwa variasi genetik dapat dikaitkan dengan risiko DIH, seperti status asetilator INH, yang terkait dengan polimorfisme N-asetil transferase (NAT) 2, yang mana asetilator lambat pada umumnya lebih rentan terhadap efek samping obat. Proporsi asetilator cepat dan lambat sangat bervariasi pada populasi dengan etnis atau geografis yang berbeda yang telah dijelaskan dalam berbagai penelitian, tetapi, masih ada informasi yang terbatas dalam populasi kita. Tujuan dari penelitian ini adalah untuk menyelidiki kontribusi polimorfisme CYP2E1 terhadap DIH anti-TB pada populasi kami. Penelitian kasus kontrol ini dilakukan di Rumah Sakit Cipto Mangunkusumo, Jakarta dan Rumah Sakit Omni Alam Sutera, Tangerang, Indonesia dari Januari 2015 - Desember 2016. Kami merekrut 35 orang dengan DIH dan 34 orang tanpa DIH. Profil fungsi hati lengkap, bilirubin total serum, bilirubin tidak langsung, dan bilirubin langsung diukur. Kami melakukan genotipe polimorfisme CYP2E1 rs3813867, rs2031920 dan rs6413432. Kami menemukan bahwa polimorfisme CYP2E1 c1/c1 (tipe liar homozigot) pada 61 subjek (88,4%) dan tidak ada perbedaan yang signifikan secara statistik antara tipe liar homozigot dan varian yang jarang (mutan alel) dalam kejadian DIH (95% CI 0,403 - 8,383, P = 0,338). Kami mengusulkan bahwa polimorfisme CYP2E1 tidak dapat membantu dalam memprediksi kerentanan terhadap hepatotoksisitas yang diinduksi oleh obat anti-tuberkulosis pada populasi di Indonesia.

Kata kunci: Tuberkulosis, polimorfisme CYP2E1, hepatotoksisitas yang diinduksi oleh obat.

ABSTRACT

Tuberculosis (TB) treatment, based on the use of isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA), shown to cause drug induced hepatotoxicity (DIH). Recent studies have demonstrated that genetic variations may be associated with the risk of DIH, such as INH acetylator status, related to N-acetyl transferase (NAT) 2 polymorphism, which slow acetylators are generally more prone to side effects from drugs. The proportion of rapid and slow acetylators vary remarkably in populations of different ethnic or geographic origin which has been described in various study, but, there is still limited information in our population. The objective of this study is to investigated the contribution of CYP2E1 polymorphism to the anti-TB DIH in our population. This case control study was conducted at the Cipto Mangunkusumo Hospital, Jakarta and Omni Hospital Alam Sutera, Tangerang, Indonesia from January 2015 - December 2016. We recruited 35 individuals with DIH and 34 individuals without DIH. A complete liver function profile, serum total bilirubin, indirect bilirubin and direct bilirubin were measured. We genotype CYP2E1 polymorphism rs3813867, rs2031920 and rs6413432. We found that polymorphism CYP2E1 c1/c1 (homozygous wild type) in 61 subjects (88.4%) and no statistically significant between homozygous wild type and rare variant (alel mutant) in the incidence of DIH (95% CI 0.403 – 8.383, P = 0.338). We propose that the polymorphism of CYP2E1 could not help in predicting the susceptibility to anti-tuberculosis drug induced hepatotoxicity in Indonesian population.

Kata kunci: *Tuberculosis, CYP2E1 polymorphism, drug-induced hepatotoxicity.*

INTRODUCTION

Drug-induced hepatotoxicity (DIH) is defined as significant abnormality in liver function test (LFT), with elevation of serum aminotransferases or bilirubin, along with clinical features such as anorexia, nausea, vomiting and jaundice during treatment (Sharma et al., 2002). It spectrum can ranges from asymptomatic elevation of serum transferases to hepatic failure requiring liver transplantation (Timbrell et al., 1985). The pathogenesis of DIH caused by these offending drugs is still enigmatic, and various mechanisms have been postulated.

Tuberculosis (TB) treatment, based on the use of isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA), shown to cause adverse drug reaction (ADRs). One of the most serious ADRs is DIH, associated with high morbidity and mortality, as well as with increased treatment costs (Wang et al., 2012). Other ADRs are gastrointestinal intolerance, kidney failure and cutaneous and hematological reaction which can lead to therapy discontinuation or more serious morbidity and mortality (Forget and Menzies, 2006).

The incidence of anti-TB DIH ranges from 1% to 36% (Forget and Menzies, 2006, Saukkonen et al., 2006, Tostmann et al., 2008). A meta-analysis by Steele et al. (Steele et al., 1991) has shown an incidence rate of liver toxicity of 2.6% with RMP and INH co-administration, but only 1.1% with RMP alone, and 1.6% with INH alone. It has been speculated that this is due to a drug-drug interaction in which induction of hepatic microsomal enzymes by RMP results in production of increased concentration of a hepatotoxic metabolic product of INH (Steele et al., 1991, Sarma et al., 1986), and on the other, increased plasma RMP levels may occur due to the displacement of the drug from plasma protein binding sites by INH. These pharmacokinetic interaction between the two drugs may explain their added toxicity (Sarma et al., 1986). The other pathogenesis of INH-RMP induced hepatotoxicity is not entirely clear, but the proposed mechanisms may include dose-related roxicity (Yew, 1998), oxidative stress (Attri et al., 2000), lipid peroxidation (Richards et al., 2004), induction of liver enzyme in the hydralase system, thus enhancing the toxicity of some of the INH toxic metabolites (Askgaard et al., 1995), and hypersensitivity to INH-RMP (Schreiber et al., 1999).

Hepatotoxicity is reported mainly in patients with risk factors such as advanced age, female gender, alcohol consumption, malnutrition or a high dosage of antituberculosis medications in relation to body weight (Singh et al., 1995, Grönhagen-Riska et al., 1978), Human Immunodeficiency Virus (HIV) co-infection, pre-existing liver disease, slow acetylation status, wild type of CYP2E1 and concomitant use of hepatotoxic drugs (Tostmann et al., 2008, Arbex et al., 2010).

Huang et al, 2014 studies have demonstrated that genetic variations may be associated with the risk of DIH (Huang, 2014). Among them, genetic polymorphism in the drug-metabolizing enzyme (DME) genes, such as slow acetylator of *N-acetylator transferase* 2 enzyme, activation of CYP2E1 status, (Gupta et al., 2013), reduced glutathione level (Chowdhury et al., 2006), *null depletion of* gluthatione S-transferase M1 (Roy et al., 2001), histocompatibility Complex Class II associated HLA-DQ alleles (Sharma et al., 2002), choline deficiency leading to lowering of phospholipids protein synthesis with alteration in cell wall configuration (Karthikeyan, 2005) also believed have a certain fraction of the population to DIH.

In the liver, INH is first metabolised into acetyl-isoniazid via N-acetyltransferase 2 (*NAT2*), followed by hydrolysis to acetyl hydrazine. Acetyl hydrazine is further oxidized into hepatotoxic intermediates by cytochrome P450 2E1 (CYP2E1) (Huang, 2007). Direct hydrolysis of INH also generates hydrazine, a potent hepatotoxin. Disposal of acetyl hydrazine also depends on further acetylation by NAT2 to form a non-toxic metabolite, diacetyl hydrazine (Mitchell et al., 1976). Glutathione S-transferase (GSTs) are detoxification enzymes that catalyze the conjugation of glutathione to several classes of molecules including toxic intermediates of the INH metabolism (Forestiero et al., 2013).

CYP2E1, a phase 1 enzyme, is involved in the metabolism of many carcinogens and drugs, and is associated with susceptibilities to alcoholic liver disease, nonalcoholic fatty liver disease and many cancers, such as hepatocellular carcinoma (Huang et al., 2003). This enzyme also has functional genetic variation in humans. Three restriction enzymes, *Rsa*I, *Pst*1 and *Dra*I, are commonly used to detect *CYP2E1* restriction fragment length polymorphism (RFLP) (Huang, 2014). The three genotypes of *CYP2E1* are classified as c1/c1, c1/c2 and c2/c2 by RFLP using *Rsa*I as the restriction enzyme. The wild-type allele is c1 and the variant allele is c2. According to the new nomenclature for *CYP2E1*, *1A is equivalent to c1 and *5 is equivalent to c2 (Huang, 2014).

The CYP2E1 c1/c2 allele have been investigated for their association with DIH in previous studies on Asian populations (Hiratsuka et al., 2002, Cho et al., 2007, Huang et al., 2003, Singla et al., 2014) Recent meta-analyses disclosed that the *CYP2E1 Rsa*I genetic variation was associated with the susceptibility to DIH in East Asians, although the OR was not high (OR = 1.35) (Cai et al., 2012, Deng et al., 2012). About 80% of Asians present the c1/c1 genotype (Stettner et al., 2015).

In Lee SW et al (Lee et al., 2010) study, 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.

Rat studies have shown that INH and hydrazine induced CYP2E1 activity (Singh et al., 1996). INH has an inhibiting effect on CYP1A2, 2A6, 2C19 and 3A4 activity (Jenner and Timbrell, 1994). RMP is a potent inducer of the hepatic CYP450 system in the liver and intestine, thus increasing metabolism of many other compounds (Desta et al., 2001).

Although a relationship between gene polymorphisms and anti-tuberculosis DIH has been described in various study, there is still limited information in our population, which has a heterogeneous genetic background. We have investigated the contribution of CYP2E1 variants to the anti-tuberculosis DIH.

METODE

Subjects

The present study was carried out at the Cipto Mangunkusumo Hospital, Jakarta and Omni Hospital Alam Sutera, Tangerang, Indonesia from January 2015 to December 2016. All patients presenting to the clinic who fulfilled the inclusion / exclusion criteria were recruited. From this sample, we selected 38 individuals with DIH and 37 individuals without DIH.

Study subjects were 15 – 70 years old, newly diagnosed with active TB, with lesions of TB clearly visible on chest X-ray, or positive sputum smear. Patients who developed DIH were classified as cases, while the remainder were classified as controls. Drug-induced hepatotoxicity was defined according to the criteria established by the Council for International Organizations of Medical Sciences (CIOMS) as an increase in liver biochemical parameters more than two times the upper limit of normal occuring at any time during anti-TB treatment (Benichou, 1990, Tostmann et al., 2008). The upper limit of normal used in the study was 31 IU/mL for serum alanine transferase (ALT), 32 IU/mL for serum aspartate transferase (AST) and 1.1 mg/dL for total bilirubine.

Subjects with: 1) abnormal serum ALT, AST or bilirubin levels or symptoms related to abnormal liver function, such as jaundice, before anti-TB treatment; 2) alcohol- related liver disease or habitual alcohol drinking if daily consumption is more than 40 gr for at least five years (Singh et al., 1995); 3) any other hepatic or systemic diseases that may cause liver dysfunction, such as carriers of the hepatic B or C virus, heart failure, respiratory failure, HIV infection, etc; 4) concomitant use of hepatotoxic drugs; and 5) pregnant women at the time of DIH were excluded.

Each individual agreed to participate in the study by signing an informed consent. The study protocol was approved by the Ethics Committees of the Mochtar Riady Institute of Nanotechnology.

Study Design

In this case-controlled study, the following data were recorded in the beginning of the study: demographics, nutritional status (body mass index [BMI]), duration of drug-induced hepatotoxicity and data on ALT, AST and bilirubin level.

All patients enrolled in the study received a daily anti-TB regimen: INH 300 mg, RMP 600 mg (or 450 mg if body weight was < 50 kg), PZA 200 mg/kg body weight and EMT 800 mg daily for first 2 months.

Patients blood sample were collected at the beginning of the study. A complete liver function profile including serum aminotransferases, serum total bilirubin, indirect bilirubin and direct bilirubin were measured using routine laboratory methods to defined DIH.

Polymophism Analysis

Genomic DNA was extracted from 6 mL EDTA-anticoagulant peripheral whole blood sample using the DNA isolation kit from *Taqman® Sample to SNP™ kit* (*Applied Biosystems®*). Following the manufacturer's instruction and was stored at -70^{o}C until used for genotyping, which was carried out by Prodia Laboratory, Jakarta.

Genotyping for CYP2E1 variants were performed by polymerase chain reaction – rapid fragment length polymorphism (PCR-RFLP) technique. CYP2E1 PCR products were digested with RsaI (New England BioLabs, CTA: R01675) and DraI (New England BioLabs, CTA: R01295), respectively, for 4 hours at 37ºC. RFLP products were analyzed by 8.0% polyaccrylamide gel electrophoresis stained with SYBR Gold® (Molecular Probes, Invitrogen Detection Technologies, OR, USA).

Polymorphisms of CYP2E1 1053 C>T (rs2031920) by enzyme RsaI, polymorphisms of CYP2E1 7632 T>A (rs6413432) by enzyme DraI and polymorphisms of CYP2E1 1293 G>C (rs3813867) by enzyme RsaI.

Master Mix PCR for CYP2E1 rs3813867 was Taqman GTXpress Master Mix, Applied Biosystems (Foster City, CA, USA), CTA: 4403311, Lot : 1503035. Reagen PCR for CYP2E1 rs2031920 and rs6413432 were KAPA 2G*Robust PCR Kit by KAPA Biosystems, Massachusetts 01801, USA, CTA:KE5005.

PCR used was Verity Thermal Cycler, Applied Biosystem for RFLP amplification and CFX96 (Bio-Rad Labor TAories Inc., Hercules, CA, USA) for rtPCR.

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess whether data were normally or non-normally distributed. Accordingly, the mean ± Standar Deviation (SD) or median and Inter Quartile Range (IQR) was used in the descriptive statistics.

Categorical variables were compared by chi-square or Fischer's exact test as necessary, and continuous variables were compared using Student's t test or Mann-Whitney test. Odds ratio (OR) and 95% confidence intervals (95% CI) in parenthesis were calculated. All the statistical analyses were performed using SPSS v 22 (Chicago, IL, USA). Statistical significance was considered at $p < 0.05$. As no previous data were available on the prevalence of variant genotypes in Indonesia, no formal sample size calculation were performed.

RESULT

Characterisation of Subjects

Sixty nine subjects were diagnosed with TB. Among them, thirty five was diagnosed with DIH. There was no statistical difference in gender (P = 0.118) and nutrition status based on BMI (P = 0.934) between subjects with and those without anti-TB drug-induced hepatotoxicity.

Table 1. Characteristics of subjects with or without anti-TB drug-induced hepatotoxicity

Parameter	Case (n=35)	Control (n=34)	*P* value
Gender, n(%)			
Male	12 (34.3)	18 (52.9)	
Female	23 (65.7)	16 (47.1)	0.118
Age, n(%)			
< 40 years old	15 (42.9)	27 (79.4)	
≥ 40 years old	20 (57.1)	7 (20.6)	0.002*
Body Mass Index			
< 18.5 kg/m^2	11 (31.4)	11 (32.4)	0.934
≥ 18.5 kg/m^2	24 (68.6)	23 (67.6)	

Tabel 2. CYP2E1 polymorphism in subjects with and without anti TB drug-induced hepatotoxicity

Parameter	Case (n=35)	Control (n=34)	*P* value
Wild type (c1/c1)	32 (91.4)	29 (85.3)	0.338
Variant allele (c2/c2) & (c1/c2)	3 (8.6)	5 (14.7)	

For CYP2E1 rs2031920 and 3813867 polymorphisms, the three different genotypes are named the wild-type genotype (c1/c1), heterozygous variant allele (c1/c2) and homozygous variant allele (c2/c2) respectively (Guaoua et al., 2014). In this study, table 2 showed that 88.4% (61/69) of the subjects were wild-type compare with 11.5% (8/69) variant allele. Our results also showed that there are no statistical difference in CYP2E1 polymorphism between wild type and variant allele genotype in subjects with and without anti-TB drug-induced hepatotoxicity (95% CI 0.403 – 8.383, P = 0.338).

DISCUSSION

Among several CYP2E1 polymorphisms, the RsaI polymorphism has been evaluated mostly in association with DIH, explained by a higher wild type CYP2E1 activity and the inhibitory effect of INH. Several studies already report that wild type CYP2E1 polymorphism are at a higher risk of anti-TB DIH (Huang et al., 2003; Lee et al., 2010, Vuilleumier et al., 2006). Sun et al also found a 2.22-fold risk of developing DIH for the wild type compared to other polymorphism (Sun et al, 2008).

Our data showed that there are no statistical significance between CYP2E1 polymorphism in subject with or without drug induced hepatotoxicity which was similar with Jaramillo-Valverde et al. study in Peruvian patients (Jaramillo-Valverde et al, 2022). The statistically

nonsignificant results regarding the CYP2E1 polymophism and DIH may be due to the small sample size and low frequency of patients with DIH, so reevaluation and confirmation are needed in a large-scale population study.

Jaramillo-Valverde et al also found that the combination of intermediate NAT2 acetylators and CYP2E1 homozygous wild type polymorphism significantly protected agains the development of DIH in Peruvian population.

We also aware that this study has some limitations. Firstly, because our patients were taking a combination of anti-tuberculosis drugs, it is difficult to conclude the potential role that RMP and PZA might have had in contributing to hepatotoxicity. Rifampicin may induce hepatocellular type liver damage as INH. However, it may also interfere with the uptake and excretion of bilirubin and cause isolated direct or indirect hyperbilirubinemia, without elevation of serum aminotransferases. Pyrazinamide is known as a dose-dependent hepatotoxin and causes hepatocellular injury like INH. However, little is known about the risk factors and genetic predisposition of PZA or RMP-induced hepatotoxicity, because most studies were undertaken with the combination therapy of anti-TB agents (Huang, 2014), as shown also in this study. Measuring the plasma INH, PZA and total RMP levels, being a better predictor for subsequent development of DIH later during the course of treatment because personalized medicine has emerged as a strategy to adjust the dose regimens required to obtain optimal effectiveness in minimizing DIH. Secondly, if raw data on all subjects had been available and adjustment by other covariants, including environmental factors and other gene polymorphisms were taken into account, the results of this study would have been more accurate.

CONCLUSION

In conclusion, CYP2E1 genetic polymorphisms, including rs3813867, rs2031920, and rs6413432 variants, have no significant association with the risk of anti-TB drug-induced hepatotoxicity in the Indonesian population. Although the CYP2E1 c1/c1 polymorphism (homozygous wild type) was found in the majority of subjects (88.4%), the results of statistical analysis showed that there was no significant difference in the incidence of DIH between individuals with homozygous wild type and rare variants (mutant alleles). These findings indicate that the CYP2E1 polymorphism cannot be used as a predictive biomarker to determine susceptibility to DIH in tuberculosis treatment in this population. Therefore, further studies involving other relevant genes, as well as considering other genetic and environmental factors, are needed to understand DIH risk more comprehensively and support a more personalized treatment approach in TB patients.

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