Association of hydrazine and SGPT level two hours after drug administration at the end of intensive phase treatment of pulmonary tuberculosis patients

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ABSTRACT

Introduction: Isoniazid in the regiment treatment of pulmonary tuberculosis patients causes side effects. Hepatotoxicity is one of the isoniazid's side effects that need medical attention. Isoniazid-induced hepatotoxicity has no correlation with high level of isoniazid in plasma. However, several animal studies show it has an association with hydrazine, a metabolite of isoniazid. The role of hydrazine in isoniazid-induced hepatotoxicity among tuberculosis patients is unclear.
Objective: The aim of this study was to analyze the correlation of hydrazine and serum glutamic-pyruvic transaminase (SGPT) levels at two hours after drug administration in the end of intensive phase treatment of pulmonary tuberculosis patients.
Methods: This was an observational study with cross-sectional design. Fifty eight newly diagnosed pulmonary tuberculosis patients were enrolled in this study. Venous blood sampling was collected at two hours after drug administration in the end of intensive phase treatment. SGPT level was measured by an automatic chemical analyzer. Hydrazine level was measured by using high-performance liquid chromatography (HPLC). Statistical significance was analyzed using correlation test.
Results and Discussion: The incidence of hepatotoxicity was 3.4% and about 8.6% patients had elevated SGPT at two hours after drug administration in the end of intensive phase treatment. There was no correlation between hydrazine level and SGPT levels in this study. These results indicated that hepatotoxicity or minimal liver damage in some patients might occur in the administration of standard dose isoniazid. It might be caused by isoniazid's metabolites itself, or various other factors.
Conclusions: There was no correlation between hydrazine level and SGPT levels at 2 hours after drug administration in the end of intensive phase treatment in this study.

Keywords: hepatotoxicity, isoniazid, hydrazine, pulmonary tuberculosis

INTISARI

Pendahuluan: Pemberian isonizid dalam regimen pengobatan tuberkulosis paru dapat menyebabkan efek samping. Hepatotoksisitas merupakan salah satu efek samping yang perlu mendapat perhatian. Hidrazin merupakan metabolit isoniazid yang diduga merupakan penyebab hepatotoksisitas akibat pemberian isoniazid.

Studi sebelumnya dengan model hewan coba menunjukkan korelasi kadar hidrazin dan kadar SGPT yang merupakan parameter penanda hepatotoksisitas. Penelitian-penelitian pada penderita tuberkulosis paru menunjukkan hasil yang tidak konsisten.

Tujuan: Penelitian ini bertujuan untuk menganalisis korelasi kadar hidrazin dan kadar SGPT dua jam setelah minum obat pada akhir fase intensif pada pasien tuberkulosis paru.

Metode: Penelitian ini merupakan penelitian observasional dengan rancangan *cross-sectional* yang diikuti oleh 58 subyek yang merupakan pasien tuberkulosis paru kasus baru. Pengambilan sampel darah vena dilakukan pada dua jam setelah minum obat terakhir fase intensif pengobatan tuberkulosis. Kadar SGPT diukur dengan mesin analisis kimia otomatis, sedangkan kadar isoniazid dan metabolitnya, hidrazin diukur dengan metode HPLC. Data dianalisis dengan uji korelasi.

Hasil dan Diskusi: Persentase pasien yang mengalami hepatotoksisitas sebesar 3.4% dan sekitar 8,6% pasien mengalami kenaikan SGPT dua jam setelah minum obat pada akhir fase intensif. Tidak terdapat korelasi antara kadar hidrazin dan kadar SGPT pada penelitian ini. Hal tersebut menunjukkan bahwa pada pemberian izoniazid dosis standar masih dapat terjadi hepatotosisitas atau kerusakan hati ringan pada sebagian kecil pasien, yang disebabkan oleh metabolit isoniazid atau berbagai faktor yang lain.

Simpulan: Tidak ada korelasi kadar hidrazin dan kadar SGPT dua jam setelah minum obat pada terapi akhir fase intensif pada penelitian ini.

Kata kunci : hepatotoksisitas, isoniazid, hidrazin, tuberkulosis paru

INTRODUCTION

Tuberculosis (TB) is still a major health problem in the world. Indonesia is a TB endemic country, with the fifth-highest number of cases in the world[1,2]. To treat tuberculosis patients, combinations of antituberculosis drugs are used in Indonesia. This treatment gives the good outcome in general. However, the compliance of this treatment sometimes is not so good due to its side effects[3].

Isoniazid is one of the effective antituberculosis drugs used in the curative and preventive treatments, as monotherapy or combination with others. Unfortunately, isoniazid has many side effects. Hepatotoxicity is one of the isoniazid's side effects that need clinical attention[4-6]. Hepatotoxicity of isoniazid can be presented as a mild transient elevation of aminotransferase in 10-20% patients, to a rare case of severe hepatotoxicity. Elevated serum transaminase values are frequent during the first eight week of therapy[7-9].

The exact mechanism of isoniazid-induced hepatotoxicity is unknown. It has no correlation with high level of isoniazid in plasma itself and is considered idiosyncratic. Some studies proposed that toxic metabolites was responsible for it[10-12]. In the body, isoniazid will undergo acetylation to become acetyl isoniazid. Subsequently, it will be hydrolyzed into acetyl hydrazine. A small part of isoniazid is hydrolyzed directly into hydrazine and then is acetylated into acetyl hydrazine. Hydrazine is a toxic metabolites in isoniazid metabolism that could bind to hepatocytes and cause cell damage[12-14].

The studies on the role of toxic metabolite in isoniazid-induced hepatotoxicity showed inconsistent results. Previous studies showed that hydrazine level was correlated with the markers of hepatotoxicity[15-16]. Gent *et al*[17] also

demostrated the increasing of hydrazine level in isoniazid-induced hepatotoxicity. In the contrary, Donald *et al* showed no correlation between hydrazine level and SGPT level among 32 children with meningitis tuberculosis[18].

The aim of this study was to assess the correlation between hydrazine and SGPT levels at two hours after drug administration in the end of intensive phase of pulmonary tuberculosis patients.

MATERIALS AND METHODS

Participants

This study was an observational study with cross-sectional design. A total of 58 newly diagnosed pulmonary tuberculosis patients, with age more than 18 years old, were enrolled in this study. They received standard fixed dose combination of the tuberculosis treatment regimen used in Indonesia and comply with therapy. The baseline serum glutamic-oxaloacetic transaminase (SGOT), SGPT, hemoglobin, urea, and creatinine were within the normal limits. The patients have no hepatitis B and human immunodeficiency virus infection. A complete history and physical examination were performed for all participants. The demographic characteristics, history of smoking, concomitant disease and drugs, were also collected using a standard questionnaire.

Hepatotoxicity was defined as the increasing levels of SGPT at the end of intensive phase treatment of tuberculosis three times or more above the normal value. Delta SGPT was defined as difference between SGPT level at the baseline and end of the treatment. Nutritional status was assessed by measuring body mass index (BMI) and categorized according to the WHO classification[19].

Normal values of SGOT and SGPT levels were < 33 U/L and < 50 U/L in male, < 27 U/L and < 34 U/L in female respectively. The hemoglobin level 13.2-17.3 g/dL in male and 11.7-15.5 g/dL in female were considered normal. Blood urea nitrogen and creatinine level normally ranged between 13-43 mg/dL and 0.7-1.2 mg/dL in male, 17-43 mg/dL and 0.5-0.9 mg/dL in female.

This was reviewed and approved by the Ethics Committees of Faculty of Medicine, Universitas Gadjah Mada, Indonesia. This study was a part of a prospective cohort study of pharmacovigilance of antituberculosis drugs by Badan Penelitian dan Pengembangan Kesehatan (Litbangkes), Kementerian Kesehatan Republik Indonesia and Indonesia Clinical Epidemiology and Evidence-Based Medicine (ICE-EBM) Network. The participants were recruited from three Balai Pengobatan Paru-paru (BP4) and 13 Puskesmas located in Yogyakarta Province, during September 2012 to September 2013. All the participants gave their written inform consent before enrolled in this study.

Blood sampling

Blood samples were collected two hours after administration of the fixed dose combination of the treatment regimen of tuberculosis at the end of intensive phase. Plasma was separated and stored at -60p C until the analysis. The *trichloroacetic acid* (TCA) solution (10% w/v) was added into plasma, and then the admixture was centrifuged. Subs-equently, ether was added into the deproteinized sample. An aliquot was added with cinnamaldehyde 1% (in methanol) with ratio 4:1 (v/v). After incubation in room temperature for 26 hours, the sample was

injected to HPLC. The analytical conditions and parameters for HPLC used in this study were same as previously describe by Seifart *et al*[18].

We used Shimadzu HPLC system, LC-20AD series, with LC-10AT pump model, SPD-20A detector model, C18 shim-pack VP ODS column (250 mm x 4.6 mm, i.d. 5µm), and detection wavelength of 286 nm. The compositions of mobile phase were methanol and water at ratio 80:20 with flow rate one ml/minutes. Standard curves were prepared with concentration variation 0.3; 1.3; 5; 10; 15 ng/mL. Coefficient of linear correlation was > 0.99. Limit of detection was 0.40 ng/mL and limit of quantification were 1.35 ng/mL.

Data analysis

Data obtained from this study was expressed as mean values ± SD, numbers, or percentages. Categorical variables were compared using the chi-square test or Fisher's exact test. Continuous variables were analyzed using the independent sample t-test or Mann-Whitney test. Analysis of correlation between two values was carried out using Pearson or Spearman's test. All statistical tests were based on a two-tailed probability and p value < 0.05 was considered significant.

The patients' characteristic is shown in the Table 1. The age of participants ranged from 19 to 76 years with a mean of 35.9 ± 14.4 years. The 58.6% of them were males. BMI ranged from 13.78 to 26.95 with a mean of 19.01 ± 3.04. There were 41.4% subjects classified as underweight. About 39.7% subjects had history of smoking. Based on analysis of sputum, about 71.4% subjects had positive gram stain test. Almost all the patients (75.9%) received three tablets of antituberculosis drug. About 5.2% of patients had a comorbid of diabetes mellitus (DM), and about 63.8% of them received co-medication. The type of co-medication is shown in Table 2.

Table 1. Baseline Characteristics of The Patients

Characteristics (N=58)	Value
Age	35.95 ± 14.4
Sex (%)	
Male	58.6
Female	41.4
Body weight (kg, mean ± SD)	49.29 ± 9.29
Body Mass Index (kg/m^2; mean ± SD)	19.01 ± 3.04
Nutritional status (%)	
Underweight	41·4
Normalweight	58.6
Smoking history (%)	
Yes	39.7
No	60.3
Gram stain sputum (%)	
Positive	71.4
Negative	28.6
Dosing FDC [a] (%)	
2 tablet	3.4
3 tablet	75.9
4 tablet	17.2
5 tablet	3.4
Comorbidity DM[b] (%)	
Yes	5.2
No	94.8
Comedication (%)	
Yes	63.8
No	36.2
Type comedication (%)	
Curcuma and/ vitamin	24.4
Other drugs	40.5
Curcuma and/vitamin and other drugs	35.1
Haemoglobin (g/dL, mean ± SD)	13.00 ± 1.82
SGOT [c] baseline (U/L, mean ± SD)	19.94 ± 7.12
SGPT [d] baseline (U/L, mean ± SD)	16.08 ± 7.49
Ureum baseline (mg/dL, mean ±SD)	20.78 ± 6.04
Creatinine baseline (mg/dL, mean ±SD)	0.75 ± 0.14

[a]*FDC, fixed-dose combination*
[b]*DM, diabetes mellitus*
[c]*SGOT, serum glutamic-oxaloacetic transaminase*
[d]*SGPT, serum glutamic-pyruvic transaminase*

Table 2. List of co-medications

Name of drugs	Number of patients
Curcuma	10
Vitamin	16
Fortibi	3
Paracetamol	10
Theophylin	4
Salbutamol	5
Ambroxol	8
GG	9
DMP	3
Transamin	3
Mefenamic acid	2
Antalgin	1
CTM	3
Cetifizin	6
Loratadin	1
Domperidon	2

Correlation between hydrazine and SGPT levels

Mean of hydrazine level two hours after drug administration at the end of intensive phase was 15.75 ± 2.74 ng/ mL with range 0 – 125.45 ng/ mL. Five patients (8.6%) have elevated SGPT level at the end of intensive phase. Two of them were classified as having hepatotoxicity. Approximately, 51.7% subject had elevated delta SGPT. Table 3 compares the characteristics between elevated SGPT group and normal SGPT group. Among variables analyzed in this study, only sex variable showed significant difference between two groups (p = 0.009). All patients with elevated SGPT level were female. Elevated SGPT

Table 3. Comparison of elevated SGPT group and normal SGPT group.

Characteristics	SGPT level at the end of intensive phase	
	Normal n=53 (91.4%)	Elevated n=5 (8.6%)
Sex		
Male	34 (64.2)	0 (0)*
Female	19 (35.8)	5 (100)
Nutritional status		
Underweight	23 (39.7)	1 (20)
Normalweight	30 (56.6)	4 (80)
Acetylator status		
Slow	20 (37.7)	0 (0)
Fast	33 (62.3)	5 (100)
Smoking history		
Yes	23 (39.7)	0 (0)
No	30 (56.6)	5 (100)
Dosing FDC		
2-3 tablet	42 (79.2)	4 (80)
4-5 tablet	11 (20.8)	1 (20)
Comedication		
Yes	33 (62.3)	4 (80)
No	20 (37.7)	1 (20)
Receiving curcuma		
Yes	10 (18.9)	0 (0)
No	43 (81.1)	5 (100)
Receiving vitamin		
Yes	13 (24.5)	3 (60)
No	40 (75.5)	2(40)
Age (mean ± SD)	36.24 ± 14.72	33.00 ± 12.44 [a]
Dose of rifampicin mg/kgBB(mean ± SD)	9.89 ± 1.14	9.50 ± 0.62 [a]
Hydrazine level (median, min-max)	9.83 (0-125.4)	2.60 (0-6.90) [b]

*Data were analyzed by [a]independent sample t-test, [b]Mann Whitney test, and Fisher's exact test. *P value < 0.05 was considered significant.*

group had no higher level of hydrazine or dose of rifampicin.

Based on Spearman's test, there was no correlation between hydrazine level and SGPT level at two hours after drug administration in the end of intensive phase (P = 0.311, Figure 1).

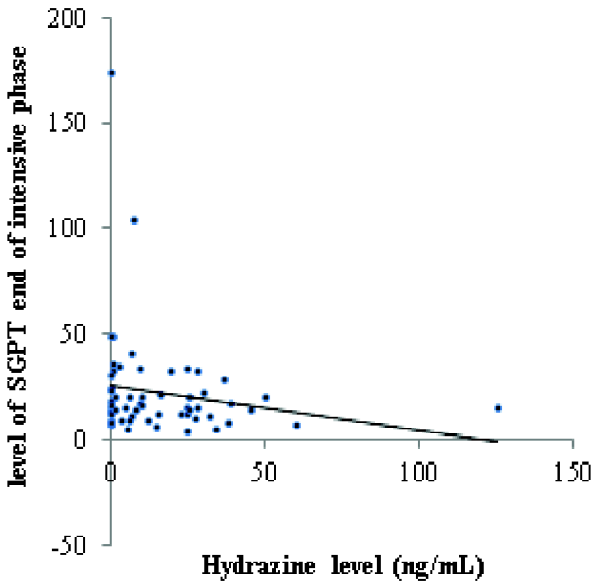

Figure 1. Correlation of hydrazine and SGPT level at the end of intensive phase in all subjects (n = 58)

The characteristic of the patients in this study was similar with the pulmonary tuberculosis patients in Indonesia[20-22]. The patients were approximately 40 years old and were dominated by male. The underweight patients were relatively common.

The rate of hepatotoxicity in this study was 3.4%, similar with previous study in Yogyakarta[20]. There was significant difference in the proportion of sex between elevated SGPT group and normal SGPT group. All participants in elevated SGPT group were female. The previous studies showed various results regarding sex as a risk factor for hepatotoxicity. Some studies showed female had higher risk to develop hepatotoxicity [6, 23], while another study showed female sex is not a risk factor[24].

There was no correlation between hydrazine and SGPT level two hours after drug administration at the end of intensive phase in this study. Previous study in rats demonstrated that hydrazine level correlated with SGPT level at one hour after administration of isoniazid[16]. Another study in rabbits showed that hydrazine level correlated with arginin succinic acid lipase (ASAL), a sensitive marker of hepatic necrosis[15]. In those study, the dose of isoniazid given to animals was higher than the dose used in the treatment of tuberculosis patients, which ranged from 50 to 100 mg/kg or approximately 10-20 times higher. In the dose of isoniazid 100 mg/kg, hydrazine level in plasma were detected around 32 ìmol/L or about 1024 µg/mL at 1 hour after administration of isoniazid. In this study, hydrazine level two hours after drug administration was very low, ranged from 0 to 125 ng/mL, possibly due to difference dose of isoniazid. Hydrazine level of 1 mM (approximately 32000 ng/mL) cause only minimal damage to hepatocyte[25].

Previous study by Donald *et al*[18]. on the isoniazid-induced hepatotoxicity in 32 children with meningitis TB, who received isoniazid 20 mg/kg/day, also showed the low levels of hydrazine and no correlation between hydrazine levels and liver damage. Preece *et al*[26]. showed that hydrazine accumulation in rat's hepatocytes reached toxic level, although hydrazine level in plasma was undetectable.

Combination with rifampicin influences the risk of isoniazid-induced hepatotoxicity. Rifampicin is known as an inducer of several cytochromes P450 (CYP) enzymes, including CYP2E1[12, 27-28]. Hydrazine levels are higher in subject with higher rifampicin levels[29]. In this study, the mean dose of rifampicin did not differ between elevated SGPT group and normal SGPT group. Further investigation is needed to determine the effect of rifampicin level in plasma on isoniazid metabolism.

This study had several limitations. Blood sampling was preformed only one time at two hours after drug administration in the end of intensive phase. It might not describe the fluctuation of drug and metabolite level in blood. We also could not control the confounding factors in this study. The data of confounding factors such as diet and environmental factors were not analyzed.

CONCLUSIONS

There was no correlation between hydrazine and SGPT level in two hours after drug administration at the end of intensive phase among 58 pulmonary tuberculosis patients. Further comprehensive investigation with larger sample size and well controlled confounding factors, such as poly-morphism in enzyme that involved in isoniazid metabolism and receptor in hepatocyte, is needed to determine the role of toxic metabolite in isoniazid-induce hepatotoxicity.

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