ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Pregnane-X-Receptor Genotype and Hepatotoxic Incidence on Tuberculosis Patients Receiving Antituberculosis in Bali

I Gusti Ayu Artini[1*], I Gusti Ngurah Bagus Artana[2], I Gusti Made Aman[1], Agung Wiwiek Indrayani[1]

1. *Department of Pharmacology and Therapy, Faculty of Medicine, Udayana University, Indonesia.*
2. *Department of Internal Medicine, Faculty of Medicine, Udayana University, Indonesia.*

***Corresponding Author: I Gusti Ayu Artini**

Abstract

Background: Drug-induced liver injury might lead to serious illness. One of the drugs that potentially toxic to the liver is antituberculosis. Many factors could influence antituberculosis-induced liver injury, including genetic variation. One of such genetic variation is polymorphism of pregnane-x-receptor (PXR) gene. PXR plays a crucial role in the regulation of many drug metabolizing enzymes and drug transporters. The association between PXR polymorphism and hepatotoxic incidence in several studies showed the inconsistent result. Therefore, it's very important to study the PXR genotype pattern and its relation with hepatotoxic incidence among tuberculosis patients who received antituberculosis treatment. This study aimed to investigate the incidence of hepatotoxic according to PXR genotype pattern among tuberculosis patients who received antituberculosis treatment in Bali. Methods: This study was a cross-sectional study. About 65 subjects were enrolled in this study, selected from tuberculosis patients who attended the pulmonary outpatient clinic of Sanglah Hospital, Bali Indonesia and received the antituberculosis drug. Identification of PXR genotype was performed using PCR/RFLP technique with *MboI* restriction enzyme. Results: The proportion of wild type and mutant genotype of PXR were 72.3% and 27.7%, respectively. There was no significant difference in the proportion of hepatotoxic between wild type and mutant genotype of PXR. Conclusion: There was no significant difference in the proportion of hepatotoxic between wild type and mutant genotype of PXR on tuberculosis patients who received antituberculosis in Bali.

Keywords: *Pregnane-x-receptor; Polymorphism; Hepatotoxic; Antituberculosis.*

Introduction

Drug-induced liver injury is a frequent side effect regarding antituberculosis use. This is related to the role of the liver on chemical substances or drugs metabolism in human body. Drug-induced liver injury has been proved to be the main cause of acute liver failure in western countries (about 50% of total acute liver failure cases). Approximately more than 75% of drug-induced liver injury would be fatal or required liver transplantation [1-3].

Antituberculosis is one of the drugs that potentially toxic to the liver. A study in India showed that antituberculosis was the principal cause of drug-induced liver failure in adults, as well as in children [1]. Approximately 5% of tuberculosis patients who received antituberculosis combination will experience an increased level of transaminase enzyme three to five times without significant clinical manifestation. This was reported to occur in the first two months of therapy (intensive phase of treatment) [4]. Prevalence of antituberculosis-induced liver injury (ATLI) in many countries varied. It ranged between 14-48% in Brazil, China,and India [5-9]. Genetical variation is one of the possible factors contributing to the occurrence of ATLI.

One of such gene is pregnane-x-receptor. Polymorphism in the pregnane-x-receptor gene (PXR) had been proved to be associated with the occurrence of ATLI in tuberculosis patients in several studies. Pregnane-x-receptor is also known as nuclear receptor

subfamily 1 group I member 2 (NR1I2). However, its association with ATLI is still controversial [10-12]. Normally, PXR is a transcription factor for many drug metabolizing enzymes and drug transporters that play important role in drug or xenobiotic metabolisms. Rifampicin is the potent activator of PXR. Activation of PXR by rifampicin will induce CYP3A4 expression which involved in isoniazid metabolism.

PXR also contributes to the extent of drug resistance, as well as energy metabolisms and suppression of inflammatory reaction [10]. Considering the important role of PXR in ATLI incidence and the controversy findings from previous studies regarding the association of PXR polymorphism with ATLI incidence, it is important to evaluate the association of PXR polymorphism with ATLI incidence on tuberculosis patients who received antituberculosis in Bali.

Materials and Methods

Subject Selection

Our study was an analytical cross-sectional study which conducted in the Faculty of Medicine Udayana University and Sanglah Hospital from January to December 2017. Tuberculosis patients who attended Pulmonary Outpatient Clinic of Sanglah Hospital, Baliand received a fixed-dose combination of antituberculosis category 1 were included in our study.

Those who refused to participate in the study were excluded. Subjects were selected using consecutive purposive sampling technique. This study has been approved by the Ethical Committee of Sanglah Hospital/Faculty of Medicine, Udayana University, Bali, Indonesia.

PXR Genotyping

The DNA was isolated from a blood sample using the Promega DNA Isolation Kit and following the manufacturer instruction. The isolated DNA was subsequently amplified by Polymerase Chain Reaction (PCR) technique. The sequence for forwarding primer and reverse primer of PXR was 5'-AAT GTT CAC CTG AAG ACA ACT GTG GTC ATT-3' and 5'-ATA GGT TAT GAT TTC CCC GGA TAT GAG ACA-3', respectively.

PCR was performed in denaturation step at 95ºC for 5 minutes, followed by 35 cycles of reaction (95ºC denaturation for 1 minute, 65ºC annealing for 1 minute, 72ºC elongation for 1 minute), and finally ended witha final extension at 72ºC for7 minutes. PCR products were digested using MboI restriction enzyme (New England Biolabs). Incubation for the restriction was performed at 37ºC for 1 hour. Electrophoresis of restriction product was performed using 2% agarose gel.

Examination of Serum Transaminase Concentration

The concentration of serum transaminase was examined at the end of the intensive phase of treatment by spectrophotometry technique at Clinical Pathology Laboratory of Sanglah Hospital. Serum transaminase level above ULN of AST and/or ALT was considered as hepatotoxic.

Statistical Analysis

All of the data obtained were analyzed descriptively to obtain the proportion of each variable. The significance of the difference was analyzed by Chi-Square test or Fischer Test using statistical software. The p-value below 0.05 was considered to be statistically significant.

Results

Sixty-five subjects were enrolled in our study. The mean age for all subjects was 36.37±13.626 years old. Subject characteristics were shown in Table 1. For overall subjects, the mean concentration of ALT and AST were 23.5±13.6 IU/L and 23.3±21.1 IU/L. The prevalent of hepatotoxic was 7.7%.

Table 1: Baseline characteristics of subjects

No	Subject Characteristics	n (%)
1	Sex	
	- Male	27 (41.5)
	- Female	38 (58.5)
2	Age	
	- < 30 y.o	35 (53.8)
	- ≥ 30 y.o	30 (46.2)
3	Initial BTA status	
	- Positive	41 (63.1)

	- Negative	24 (36.9)
4	Alcohol consumption	
	- Yes	2 (3.1)
	- No	63 (96.9)
5	Comorbid disease	
	- Yes	0 (0)
	- No	65 (100)
6	Other medication	
	- Yes	13 (20)
	- No	52 (80)

Genotype pattern of PXR gene was determined from the electrophoresis visualization. According to electrophoresis result, the proportion of wild type and mutant genotype of PXR were 72.3% and 27.7%, respectively. There was no significant difference in the proportion of hepatotoxic between wild type and mutant genotype of PXR.

Table 2: Proportion of hepatotoxic based on subject characteristics and PXR genotype

No.	Subject Characteristic	Hepatotoxic n (%)	Non hepatotoxic n (%)	Total	*p*
1	Age				
	- < 30 y.o	2 (7.4)	25 (92.6)	27	0.942
	- ≥ 30 y.o	3 (7.9)	35 (92.1)	38	
2	Sex				
	- Male	2 (5.7)	33 (94.3)	35	0.518
	- Female	3 (10)	27 (90)	30	
3	Initial BTA status				
	- Positive	3 (7.3)	38 (92.7)	41	0.882
	- Negative	2 (8.3)	22 (91.7)	24	
4	Alcohol consumption				
	- Yes	1 (50)	1 (50)	2	0.149
	- No	4 (6.3)	59 (93.7)	63	
5	Comorbid disease				
	- Yes	0 (0)	0 (0)	0	1.000
	- No	5 (7.7)	60 (92.3)	65	
6	Other medication				
	- Yes	2 (15.4)	11 (84.6)	13	0.245
	- No	3 (5.8)	49 (94.2)	52	
7	PXR genotype				
	- Mutant	2 (11.1)	16 (88.9)	18	0.522
	- Wild type	3 (6.4)	44 (93.6)	47	

Discussions

Pregnane-x-receptor (PXR) is expressed primarily in the liver. The structure of PXR, like another nuclear receptor, consists of N-terminal DNA binding domain (DBD) and ligand-binding domain (LBD). DBD interacts with hormone response element on DNA sequence through two zinc-finger motifs, whereas LBD is a flexible structure which binds to many components with variable shapes and structures. Human PXR is encoded by PXR (NR1I2) gene which is located on chromosome 3q13. The NR1I2 gene comprises 10 exons separated by nine intronic regions[10,13-16].

After binding to the ligand, PXR is integrated with the retinoid X receptor, creating a heterodimerthat subsequentlywill interactwitha DNA response element on the targetgene and starting the transcription process. PXR plays an important role in the transcription of many proteins that take part in drug metabolism, drug resistance, energy metabolism, as well as on immune response. The activation of PXR by rifampicin would potentially influence the outcome of tuberculosis treatment including the efficacy and the safety [10,13-15,17,18]. There's selectivity for agonist (ligand) that could activate PXR receptor in human. This is related to the difference in the amino acid sequence of its ligand-binding domain. Rifampicin is considered as the prototype of PXR agonist in human, not in rat [13].PXR plays many important roles in the human body including regulation of drug metabolizing enzymes and drug transporters, as well as drug resistance, energy metabolism and suppression of inflammatory reaction.

Drug disposition is affected by several enzymes and transporters in hepatic clearance systems such as CYP3A4, CYP2B6, CYP2C9, GST, SULT2A1, UGT1A1, MRP2, OATP2, P-glycoprotein, MDR1 and ABCB1. Normally, if there's no specific ligand

interacts and activates PXR, genes for hepatic clearance system is expressed in the basal state. PXR is considered a master xenobiotic sensor since it could interact with many chemical substances with a distinct structure and subsequently induce the expression of drug metabolizing enzymes and transporters rapidly for detoxifying xenobiotic substances [10,13,15,17-20]. Rifampicin had been considered as the potent activator of PXR. Isoniazid, as well, could also induce the expression of PXR on a rat, but the evidence on a human had never been reported. The metabolism of rifampicin might also be influenced by the activation of PXR. PXR action could also influence isoniazid metabolism.

Particularly isoniazid was metabolized by N-acetyl transferase 2 (NAT2) enzyme. The PXR itself did not directly regulate the NAT2 enzyme. Nevertheless, PXR might induce CYP3A4 enzyme that also involved in the metabolism of isoniazid. Metabolism of other antituberculosis drugs might also be affected by rifampicin activation on PXR [10].The human PXR also plays an important role in the occurrence of drug resistance (mostly in anticancer drugs). This was associated with the enhanced expression of CYP3A4 and P-glycoprotein that present in many types of cancers. In tuberculosis cases, the resistance of antituberculosis drugs might be related to the inadequate concentration of antituberculosis in the target of action.

This could be influenced by the PXR since the PXR activation would affect drug pharmacokinetics in the site of infection. The alteration of antituberculosis pharmacokinetics was possibly influenced by the enhanced expression of p-glycoprotein that responsible for drug efflux. However, the evidence about this was still limited [10]. The action of PXR on suppressing gluconeogenesis process occured by interaction with Forkhead box protein O1 (FoxO1), cAMP response element-binding protein (CREB), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PCG1α), and hepatocyte nuclear factor 4α (HNF4α).

On lipid metabolism, PXR had been proved to inhibit the beta-oxidation process of fatty acid and ketogenesis by decreasing the carnitine palmitoyltransferase 1a (Cpt1a) and 3-hydroxy-3-methyl glutarate-CoA synthase 2 (Hmgcs2). PXR had also been proved to increase stearoyl-CoA desaturase 1 (Scd1) enzyme that was involved in the synthesis of unsaturated fatty acid and subsequently induce lipogenesis [10,21]. PXR was also showed anti-inflammatory and immunosuppressive effect. This was supported by the detection of PXR expression on immune cells in many studies. PXR eventually would inhibit the proliferation and the function of T lymphocytes. This receptor had been proved to inhibit the NFkB signaling pathways. Therefore, rifampicin (PXR ligand), through the activation of PXR, had also been proved to possess anti-inflammatory and immunosuppressive effect; but still, its contribution to the treatment response need to confirm in further research.

Recently, the addition of anti-inflammatory agent on tuberculosis treatment had been recommended in several health centers. Regarding its action on immune response, some genetical variation on PXR gene was considered to enhance tuberculosis susceptibility [10,22]. There were two possible mechanisms of ATLI which associated with PXR, namely 1) induce the expression of drug metabolizing enzymes and drug transporters that participated in the toxic metabolites formation; 2) induce the expression of the hepatic enzyme (aminolevulinic acid synthase I) that played important role in the metabolism of endogenous toxic substances [13].

The prevalent of antituberculosis-induced liver injury was mostly related to isoniazid metabolism. However, hepatotoxic incidence regarding antituberculosis use was reported to be higher in a combination use of isoniazid and rifampicin compared to a single use of each antituberculosis. Rifampicin is stated as CYP inducer since it could induce isoniazid bioactivation and toxicity. There was a production of specific antibodies for INH, CYP2E1, CYP3A4, and CYP2C9 in isoniazid-induced liver failure patients. This did not occur in patients who received isoniazid without suffered from liver failure [10].

An in vivo study had shown no liver damage in rat that had been inserted by human PXR DNA and subsequently received rifampicin together with acetylhydrazine or hydrazine (a metabolite of isoniazid that assumed to be responsible for ATLI incidence). This supported the statement regarding the

alternative mechanism of ATLI which was not related to isoniazid, otherwise was related to PXR signaling [10]. Many studies had stated the role of PXR on ATLI, including in vitro studies (on a hepatic cell line or primary human hepatocyte), as well as in vivo studies (on rats which were already inserted by human PXR gene). Recent research had revealed that coadministration of rifampicin and isoniazid would increase the amount of protoporphyrin IX (PPIX), a heme precursor, in biliary through PXR-mediated mechanism. This would result in a higher level of ALT and ALP rats with human PXR gene compared to wild type rats (contain rats PXR gene) or PXR-null rats (without PXR gene).

The recent advances had stated that nuclear receptor, such as PXR and constitutive androstane receptor, might induce the expression of aminolevulinic acid synthase I (ALAS1), an enzyme that contributes to the production of protoporphyrin IX. The enhanced PXR-mediated ALAS1 expression was assumed as responsible for the occurrence of hepatotoxic incidence induced by rifampicin and isoniazid [13]. Studies on a human had already been conducted to evaluate the association of PXR polymorphism with ATLI incidence.

Some different sets of single nucleotide polymorphisms (SNP) on PXR gene had been reported to be associated with ATLI such as SNP on 5'-UTR (rs3814055), intron 1 (rs12488820, rs2461823, rs7643645), intron 5 (rs6785049), and 3'-UTR (rs3814057). Our research investigated the SNP on 5'-UTR (rs3814055). The previous study had represented that AA genotype of PXR (SNP rs7643645) was proved to be related to the increased risk of hepatotoxic during tuberculosis treatment in female patients; whereas AA genotype of PXR (SNP rs2461823) was proved to be associated to the decreased risk of hepatotoxic.[10] A study performed in Indonesia had shown that SNP -25385C>T (rs3814055) of PXR gene significantly enhance the risk of ATLI.[11] However, this finding was contrary to the result of a study that conducted in Taiwan.

This study represented that SNP -25385C>T of PXR gene clearly did not increase the risk of ATLI; meanwhile, SNP 69789A>G (rs7643645) and 65104G>A (rs2461823) of PXR gene had proved to significantly increase the risk of ATLI in female tuberculosis patients [12]. Our study is particularly similar to the evidence from the study that performed in Taiwan [12]. Our finding had clearly represented that there was no significant correlation between PXR polymorphism (rs3814055) and the presence of hepatotoxic incidence. On rs3814055 PXR polymorphism, there is single nucleotide polymorphism (C>T) on the 5'untranslated region -25385.

Conclusion

There was no significant difference in the proportion of hepatotoxic between wild type and mutant genotype of PXR on tuberculosis patients who received antituberculosis in Bali.

References

1. Devarbhavi H (2012) An update on drug-induced liver injury. Journal of Clinical and Experimental Hepatology, 2(3):247-259.
2. Lee WM (2003) Drug-induced hepatotoxicity. New England Journal of Medicine, 349:474-485.
3. Navarro VJ, Senior JR (2006) Drug-related hepatotoxicity. New England Journal of Medicine, 354:731-739.
4. Arbex MA, Varella MCL, deSiqueira HR, deMello FAF (2010) Antituberculosis drugs: drug interaction, adverse effects and use in special situations. Journal of Brasil Pneumology, 36(5):626-640.
5. Teixeira RLF, Morato RG, Cabello PH, Muniz LMK, Moreira ASR, Kritski AL, et al (2011) Genetic polymorphisms of NAT2, CYP2E1 and GST enzymes and the occurrence of antituberculosis drug-induced hepatitis in Brazilian TB patients. Mem Inst Oswaldo Cruz, 106(6):716-724.
6. Lv X, Tang S, Xia Y, Zhang Y, Wu S, Yang Z, et al (2012) NAT2 genetic polymorphisms and antituberculosis drug-induced hepatotoxicity in Chinese community population. Annals of Hepatology, 11(5):700-707.
7. Huang YS, Chern HD, Su WJ, Wu JC, Lai SL, Yang SY, et al (2002) Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for

antituberculosis drug–induced hepatitis. Hepatology, 35:883-889.

8. Lee SW, Chung LSC, Huang HH, Chuang TY, Liou YH, Wu LSH (2010) NAT2 and CYP2E1 polymorphisms and susceptibility to first line antituberculosis drug-induced hepatitis. International Journal of Tuberculosis and Lung Disease, 14(5):622-626.
9. Bose PD, Sarma MP, Medhi S, Kar PDas BC, Husain SA, (2011) Role of polymorphic *N-acetyl transferase2* and *cytochrome P4502E1* gene in antituberculosis treatment-induced hepatitis. Journal of Gastroenterology and Hepatology, 26(2):312–318.
10. Shehu AI, Li C, Xie W, Ma X (2016) The pregnane x receptor in tuberculosis therapeutics. Expert Opinion on Drug Metabolism and Toxicology, 12(1):21-30.
11. Zazuli Z, Barliana MI, Mulyani UA, Perwitasari DA, Ng H, Absulah R (2015) Polymorphism of pxr gene associated with the increased risk of drug-induced liver injury in Indonesian pulmonary tuberculosis patients. Journal of Clinical Pharmaceutical Therapy, 40(6):690-684.
12. Wang JY, Tsai CH, Lee YL, Lee LN, Hsu CL, Chang HC, et al (2015) Gender-dimorphic impact of pxr genotype and haplotype on hepatotoxicity during antituberculosis treatment. Medicine, 94(24):e982.
13. Wang Y, Chai SC, Brewer CT, Chen T (2014) Pregnane x receptor and drug-induced liver injury. Expert Opinion On Drug Metabolism and Toxicology, 10(11):1521-1532.
14. Scheer N, Ross J, Rode A, Zevnik B, Niehaves S, Faust N, et al (2008) A novel panel of mouse models to evaluate the role of human pregnane x receptor and constitutive androstane receptor in drug response. The Journal of Clinical Investigation, 118(9):3228-3239.
15. Pavek P (2016) Pregnane x receptor-mediated gene repression and cross talk of pxr with other nuclear receptors via coactivator interactions. Frontiers in Pharmacology, 7(456):1-16.
16. Wang Y, Ong SS, Chai SC, Chen T (2012) Role of car and pxr in xenobiotic sensing and metabolism. Expert Opinion on Drug Metabolism and Toxicology, 8(7):803-817.
17. Yan J and Xie W (2016) A brief history of the discovery of pxr and car as xenobiotic receptors. Acta Pharmaceutica Sinica B, 6(5):450-452.
18. Zhang L, Qiu F, Lu X, Li Y, Fang W, Zhang L, et al (2014) A functional polymorphism in the 3'utr of pxr interacts with smoking to increase lung cancer risk in southern and eastern chinese smoker. International Journal of Molecular Science, 15:17457-17468.
19. Brewer CT dan Chen T (2016) PXR variants: the impact on drug metabolism and therapeutic responses. Acta Pharmaceutia B, 6(5):441-449.
20. Lim Y and Huang J (2008) Interplay of pregnane x receptor with other nuclear receptors on gene regulation. Drug Metabolism and Pharmacokinetics, 23(1):14-21.
21. Konno Y, Neoishi M, Kodama S (2008) The role of nuclear receptors car and pxr in hepatic energy metabolism. Drug Metabolism and Pharmacokinetics, 23(1):8-13.
22. Qiu Z, Cervantes JL, Cicek BB, Mukherjee S, Venkatesh M, Maher LA, et al (2016) Pregnane x receptor regulates patogen-induced inflammation and host defense against an intracellular bacterial infection through toll-like receptor 4. Scientific Reports, 6:31936.

