RESEARCH ARTICLE

The Role of CYP4502E1 Genetic Polymorphism on Benzene Metabolism

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Abstract

The carcinogenic effect of benzene is associated with metabolites produced in benzene metabolism such as phenol, catechol, quinones, muconic acid (tt-MA), and phenyl mercapturic acid (s-PMA). The role of CYP4502E1 enzyme in benzene metabolism is very important, which is determined by its genetic polymorphism. This cross-sectional study is aimed to obtain the distribution of frequency of s-PMA concentration in workers who had been low exposure of benzene based on CYP4502E1 genetic polymorphism. The study was conducted between September 2007 and April 2010. Data were collected by methods of interviews, physical examinations, laboratory examinations, and direct observation on the work place. The variables studied were CYP4502E1, benzene exposure at the work place, age, type of work, history of work, length of work, body mass index (BMI), antioxidants intake, behavior and management, and s-PMA concentration in the urine. The distribution of CYP4502E1 genetic polymorphism in workers is 87.8% wild type homozygote, 11.3% heterozygote and 0.9% mutant homozygote. There was no significant difference in the proportion of s-PMA concentration based on CYP4502E1 genetic polymorphism (p=0.595; OR_{raw}=0.98; 95% CI=0.95-1.01). There were also no differences of age, type of work, length of work, BMI, antioxidants consumptions, behavior and management of subjects with s-PMA. Further study should be conducted on CYP4502E1 genetic polymorphism in various Indonesian races at different workplace with low-level benzene.
Key words: *low-level benzene exposure;CYP4502E1; s-PMA.*

Peran Polimorfisme Genetik CYP4502E1 pada Metabolisme Benzena

Abstrak:

Efek karsinogenik juga berhubungan dengan proses metabolisme benzena yang menghasilkan metabolit seperti fenol, katekol, qinon,asam mukonik (tt-MA), dan asam fenil merkapturik (s-PMA). Peran enzim CYP4502E1 dalam metabolisme benzena sangat penting, yang ditentukan oleh polimorfisme genetiknya. Penelitian dengan desain potong lintang ini bertujuan melihat distribusi konsentrasi s-PMA diantara pekerja yang terpajan benzena rendah berdasarkan polimorfisme genetik CYP4502E1. Penelitian dilakukan pada bulan September 2007 sampai April 2010. Data dikumpulkan dengan wawancara, pemeriksaan fisik, pemeriksaan laboratorium dan observasi langsung di lingkungan kerja. Variabel yang diukr adalah CYP4502E1, pajanan benzena di lingnkungan kerja, umur,jenis pekerjaan, riwayat pekerjaan, masa kerja, indeks massa tubuh, asupan antioksidan, kebiasaan dan manajemen, dan konsentrasi s-PMA dalam urin. Distribusi Polimorfisme genetik CTP4502E1 pada pekerja adalah 87.8% wild type homozygote, 11.3% heterozygote and 0.9% mutant homozygote. Tidak ada perbedaan proporsi antara konsentrasi s-PMA berdasarkan polimorfisme genetik CYP4502E1 (p=0.595; OR_{raw}=0.98; 95% CI=0.95-1.01). Tidak ada juga perbedaan dari umur, jenis pekerjaan, masa kerja, indeks massa tubuh, konsumsi antioksidan, kebiasaan dan manajemen dari s-PMA pekerja. Penelitian lanjut perlu dilakukan untuk melihat polimorfisme genetik CYP4502E1 tiap suku bangsa di Indonesian di lingkungan kerja yang terpajan benzena rendah.
Kata Kunci: *pajanan benzena rendah; CYP4502E1; s-PMA.*

Introduction

Benzene has been known as one of substances with genotoxic and carcinogenic characteristics; however, it is still widely used at the workplace. Benzene metabolism produces several metabolites such as phenol, catechol, quinones, trans-trans muconic acid (tt-MA) and s-phenyl mercapturic acid (s-PMA). Phenol, catechol and quinones metabolites subsequently produce free radicals leading to peroxidation reaction with cell, particularly the lymphocytes.[1,2]

The metabolism of benzene in the body requires CYP4502E1 enzyme and disturbance of this enzyme will have effects to its metabolism which means also influences the concentration of its metabolites. One of benzene metabolites which is recommended as a marker of low-level exposure of benzene (<1ppm) is s-PMA.[2-4] It has been known that genetic polymorphism of CYP4502E1 gives different expressions in different ethnics and such study has not been performed in Indonesia. The present study is aimed to recognize CYP4502E1 genetic polymorphism of Indonesian workers who had low-level benzene exposure and its correlation with s-PMA concentration as a marker of benzene metabolism.

Methods

The present study has utilized cross-sectional design in 115 full-time workers who worked at the production and administration division of the head office. The study has been approved by the Ethical Committee of Faculty of Medicine, Universitas Indonesia. Samples were randomly selected and were stratified based on their workplace. Of a total 210 male workers, we found 11 (9.6%) workers were exposed to benzene more than 0.1ppm and 104 (90.4%) workers were exposed to benzene either equal or less than 0.1ppm. The study site was at an oil company in East Kalimantan, Indonesia. The study was conducted between September 2007 and April 2010.

Data of subject characteristics were collected by interview techniques using questionnaire. The interviews were performed by investigator, company physicians and paramedic at the study site every workday. Observations were carried out on utilization of self-protection tools, behavior of the workers when using the self-protection tools (mask, work-suit, goggle, and gloves) during their working hour, warning sign and standard of procedure at work location exposed to benzene. The observations also included data collection on the structure of organization, production flow, facilities at the workplace and type of work for each worker.

All observations were performed by investigators aided by the company physicians and nurses. The CYP4502E1 genetic polymorphism blood examinations were performed by polymerase chain reaction (PCR) method at the Departments of Biochemistry and Molecular Biology, Faculty of Medicine Universitas Indonesia. The examination of benzene concentration at the workplace was performed by passive sampler method and biomonitoring of urin s-PMA in Australia Laboratorium Services, Kuala Lumpur, Malaysia.

Data analysis were performed to obtain overall view of subject characteristics, CYP4502E1 genetic polymorphism and the concentration of s-PMA. The proportion of s-PMA concentration was based on age group, type of work, history of work, body mass index, length of work, antioxidants intake, behavior and management, the concentration of benzene on air at the work place. CYP4502E1 genetic poymorphism was analyzed by X^2 test and odds ratio crude (OR_{crude}) and 95% confidence interval were determined.[5-7]

Results

Subject Characteristics

The median age of subjects was 30 years old; the youngest was 18 years old and the oldest was 55 years old. The median value for the length of work was 7 years with the shortest duration was 1 year and the longest was 34 years. Behavior and management aspects included a compilation of preventive measures by using mask and sanction was put in effect if the mask was not used. The behavior and management were classified as appropriate and less appropriate. Our study found that 81(70.4%) subjects had appropriate behavior and management and the other 34 (29.6%) subjects had less appropriate behavior and management.

The body mass index (BMI) in the study were grouped into obesity, overweight, normal weight and underweight. In this study, 42(36.52%) subjects had BMI of obesity, 34(29.57%) subjects had excessive BMI indicating overweight, 35(30.43%) subjects had normal BMI and 4(3.48%) subjects had BMI less than normal. Distribution of frequency for subject characteristics, benzene exposure, and urine s-PMA are presented on Table 1.

Table 1. Subject Distribution of Characteristic Subject and Benzene Exposure

Characteristic and Benzene Exposure	n	%
Age		
≤ 30 years	71	61.7
>30 years	44	38.3
Type of Occupation		
Administration	25	21.7
Production	90	78.3
Length of work		
1-10 years	87	75.6
>10 years	28	24.4
Occupation history		
No	49	42.6
Yes	66	57.4
Body mass index		
Obesity	42	36.5
Overweight	34	29.6
Normoweight	35	30.4
Underweight	4	3.5
Antioxidant resources		
High	83	72.2
Low	32	27.8
Behavior and management		
Appropiate	81	70.4
Less appropiate	34	29.6
Benzene exposure in the workplace *(passive sampler)*		
<0.1 ppm	104	90.4
≥0.1 ppm	11	9.6

Biological Monitoring of Benzene Exposure by Measuring s-PMA

The median for s-PMA, a benzene metabolite, was 9 ug/g creatinine, with minimum value was 0 and maximum value was 31ug/g creatinine. The quantity of benzene metabolites measured by urinary s-PMA was categorized into more than 25ug/g creatinine and equal/less than 25ug/g creatinine. There were 2(1.74%) subjects with urinary s-PMA concentration above 25ug/g creatinine, and 113(98.26%) subjects who had urinary s-PMA concentration equal/less than 25ug/g creatinine.

The Profile of CYP4502E1 Genetic Polymorphism

The CYP4502E1 is an enzyme that has important role in benzene metabolism which occurs in the liver. In our study, the genetic polymorphism of CYP4502E1 was grouped into wild-type *homozygote, heterozygote and mutant homozygote.* The results demonstrated that 101(87.8%) subjects had wild-type homozygote, 13(11.3%) subjects was heterozygote and 1(0.9%) subject had mutant homozygote.

From 115 total subjects, wild-type homozygote was found in 101(87.8%) subjects measured by 202 C1 alleles (dominant allele); while heterozygote was found in 13(11.3%) subjects with 13 C1 alleles (dominant allele) and 13 C2 alleles (mutant allele) and one subject (0.9%) had mutant homozygote with 2 C2 alleles (mutant allele). The total alleles are 230 with the quantity of C1 allele (dominant allele) was 215 alleles (93.48%), and C2 allele (mutant allele) was 15 alleles (5.52%). Further details can be seen on Table 2.

Table 2. Distribution of Genetic Polymorphysm CYP4502E1 Based on Genotype Quantity and Alleles Quantity

Genetic Polymorphysm	Genotype quantity	Alleles C1	Alleles C2
Homozygot wild type (C_1C_1)	101	202	-
Heterozygot (C_1C_2)	13	13	13
Homozygot mutan (C_2C_2)	1	-	2
Total	115	215	15

Proportional Comparison of S-PMA Concentration

The results of bivariate analysis between subjects and urinary s-PMA are presented on Table 3. All characteristic of subjects had no significant difference with s-PMA concentration (p>0.05)

Table 3. Correlation between Characteristic and s-PMA Benzene Metabolite

Characteristics	s-PMA ≤ 25ug/g creatinine		s-PMA > 25ug/g creatinine		OR crude	CI 95%	p
	n	%	n	%			
Age							
≤30 years	70	98.6	1	1.4	1.63	0.09-26.71	0.621
>30 years	45	97.7	1	2.3			
Type of Occupation							
Administration	25	100	0	0	1.02	0.99-1.07	0.611
Production	88	97.8	2	2.2			
Length of work							
1-10 years	86	98.9	1	1.1	3.19	0.19-52.66	0.429
>10 years	27	96.4	1	3.6			
Occupation history							
No	65	98.5	1	1.5	1.35	0.08-22.2	0.673
Yes	48	98	1	2			
Body mass index							
Obesity*	4	100	0	0	0.51	0.03-8.33	1.000
Overweight*	34	97.1	1	2.9			
Normoweight**	34	100	0	0			
Underweight**	41	97.6	1	2.4			
Antioxidant resources							
High	81	97.6	2	2.4	0.98	0.94-1.01	0.519
Low	32	100	0	0			
Behavior and management							
Appropiate	79	97.5	2	2.5	0.98	0.94-1.01	0.494
Less appropiate	34	100	0	0			

Note: *be added in the analysis, ** be added in the analysis

In order to recognize whether there was any correlation between CYP4502E1 genetic polymorphism and urinary s-PMA metabolites, a bivariate analysis was performed. The results showed that there was no significant difference between CYP4502E1 genetic polymorphism with the concentration of urinary s-PMA metabolites in all subjects (p=0.595; OR_{crude}= 0.98; 95%CI =0.95-1.01). Further details are expressed on Table 4.

Table 4. Correlation between Genetic Polymorfphysm CYP4502E1, Benzene Exposure and s-PMA Benzena Metabolite

Variables	s-PMA ≤2 ug/g creatinine		s-PMA 25 ug/g creatinine		OR crude	95% CI	p
	n	%	n	%			
Polymorfphysm CYP4502E1							
Homozygot wild type (C_1C_1)	99	98.0	2	2.0	1.00		
Heterozygot * (C_1C_2)	13	100	0	0	0.98	0.95-1.01	0.595
Homozygot mutan* (C_2C_2)	1	100	0	0			
Benzene exposure in the workplace *(passive sampler)*							
<0.1ppm	103	99.0	1	1.0	1.00	0.60-177.5	0.183
≥0.1ppm	10	90.9	1	9,1	10.3		

* addedd in the analysis

Discussion

The range of age in our study i.e. 18-55 years, was greater than the results found by Kamboj et al[9] which demonstrated 23–51 years. In contrast, the mean value of age in our study which showed 33.14 years was nearly similar to Kamboj and Sambyal[9] findings, i.e. 33.2±7.91 years. Working at the production division had higher risk of benzene exposure than working at the office. Petrol workers who work at the gasoline station had higher risk of benzene exposure compare to common population.[2,8,9] The mean value of length of work in our study was 10.04 years ranging from 1–34 years. In our study, the length of work of 1-10 years was the major findings (75.6%).

The results greater proportion in wild type homozygote C1C1 was similar to the findings in Turkey which found the proportion of genetic polymorphism for wild-type homozygote C1C1 of 96.07% and C1C2 heterozygote of 3.93%.[10] Moreover, similar results were also found in Guangzhouhans, China, with greatest proportion for wild-type homozygote C1C1 as great as 67.3%, followed by 29.3% heterozygote C1C2 and 3.4% mutant homozygote C2C2.[11]

Considering such results, the population in China had nearly similar variation on CYP4502E1 genetic polymorphism with our study, i.e. mutant homozygote (C2C2) polymorphism was found. Such findings had not been confirmed for population in Turkey. Mutant homozygote polymorphism (C2C2) was more common in China population than the population in our study. Such results indicate that there is greater quantity of mutant homozygote genetic polymorphism in China population compared to Indonesian population. The proportion of wild-type homozygote and heterozygote CYP4502E1 genetic polymorphism in Indonesia is nearly equal to those in Turkey and China.

A study in Guangzhouhans, China, found 85.3% C1 alleles and 14.7% C2 alleles; while our study found 93.48% C1 allele and 5.52% C2 allele. It is obvious that the heterozygote and mutant homozygote type of CYP4502E1 have mutant allele causing different metabolism, which lead to greater production of metabolites and consequently results in increased metabolite concentration in the urine. Cytochrome P4502E1, a liver enzyme involved in benzene metabolism, converted benzene to benzene oxide, then spontaneously metabolized to phenol.[12,13]

CYP4502E1 genetic polymorphism is determined by dominant alleles rather than the received benzene exposure. The correlation between CYP4502E1 genetic polymorphism and urinary s-PMA metabolites showed that there was no significant difference (p=0.595; OR_{crude}=0.98; 95%CI = 0.95-1.01). Such findings opposed the theory saying that CYP4502E1 genetic polymorphism influenced the benzene received in the body and converted into s-PMA metabolite in the urine. The influence of CYP4502E1 in metabolism is affected by genetic polymorphism of each individual. The genetic polymorphism is affected by individual race such as choisan, australoid, negroid, caucasoid and mongoloid.[13,14] Different race has strong influence on individual genetic. In our study, there were 80% Javanese subjects, which were included as australoid race. Most of them had CYP4502E1 genetic polymorphism with wild-type homozygote (101 subjects). From such results, it is understandable that the genetic variation was scarce and therefore the result could not be compared to other race.

In our study, there is no correlation between the extent of benzene exposure at the workplace and the concentration of urinary s-PMA (p>0.05). The greater benzene exposure at the workplace, the greater concentration of urinary s-PMA will occur. Benzene exposure concentration received at the work place of less than 0.1ppm, measured by passive sampler and was found in most subjects (90.4%). Such concentration was below the threshold limit values of benzene exposure as recommended by ACGIH 2008 (0.5ppm) and NIOSH (0.1ppm).[3] Therefore, it can be understood that a very small amount of benzene exposure at the workplace will give little results in urinary s-PMA.

Most subjects had low-exposure of benzene. It indicates that the work-environmental control conducted by the company was good enough, which is proven by <0.1ppm benzene concentration at the workplace, i.e. has been below the threshold limit values recommended by ACGIH.[15] With a good control, there is lesser benzene exposure into the body. The lesser benzene exposure, the lesser amount of s-PMA metabolites produced. Although there was no correlation, there was 10 times greater risk to produce urinary s-PMA concentration above 25ug/g creatinine in subjects who had benzene exposure above 0.1ppm. This was appropriate with the presumption that increased benzene exposure at the workplace will increase the concentration of urinary s-PMA.

Other factors that also had roles in benzene exposure quantity into the body were utilization of self-protection tool and mask. The utilized mask should be appropriate with the concentration of benzene exposure at the workplace. Appropriate

type of mask and cartridge will reduce benzene exposure for the subjects. A good mask utilization and appropriate cartridge removal will also reduce benzene exposure into the body.[2-4]

There were two subjects who had urinary s-PMA concentration above 25ug/g creatinine, which possibly caused by inappropriate/less appropriate mask utilization and inappropriate use of cartridge or inappropriate type of mask. In this case, the most possible cause was inappropriate cartridge use, i.e. the cartridge had not been removed although it had become saturated. As a result, the benzene fume was inhaled into the body which caused increased metabolites in the body leading to increased urinary s-PMA. All process of benzene metabolism in the body is affected by CYP4502E1 enzyme.[1-4] The greater domination of wild-type homozygote causes the more likely metabolism to produce lesser S-PMA metabolites. In contrast, when there is mutant homozygote, the greater result of s-PMA metabolism will occur. The genetic polymorphism of CYP4502E1 is distributed according to alleles, segregated and transmitted to the next generation in keeping with the Mendel Law and could not have rapid alteration, which requires a very long evolution time (10^{-7} per years per cell cycle).[15]

There is no correlation between age and the concentration of urinary s-PMA ($p>0.05$), despite the greater age will increase the risk of elevated concentration of urinary s-PMA (OR_{crude}=2,3; 95%CI: 0.09-26.71). Moreover, the type of work, length of work, history of work, body mass index, antioxidants intake, behavior and management had no correlation with the concentration of urinary s-PMA. Such things could be understood since the measurement of urinary s-PMA was performed on the same day with the impact of benzene exposure. After being exposed to benzene for 8-9 hours, the benzene will be metabolized in the liver and will be excreted immediately in the urine in the form of s-PMA.[2] Working at the production division will have risk of benzene exposure causing possible increase of urinary s-PMA concentration; however, the concentration of benzene in our study was very small (<0.1ppm). Therefore, it could be said that the benzene exposure at the production division was nearly similar to the administration desk.

The accumulation of benzene exposure in the body is affected by body surface area, which is identical to the body mass index (BMI).[8] In our study, 67% subjects had excessive BMI and obesity; therefore, it can be said that all subjects got almost the same benzene exposure both in production and administration division.

Conclusion

The distribution of CYP4502E1 genetic polymorphism in workers with low-level benzene exposure (<1ppm) is relatively homogenous and most subjects (87.8%) had wild-type. A relatively homogenous condition of CYP4502E1 genetic polymorphism may cause no significant difference of urinary s-PMA concentration. There were 1.74% subjects with urinary s-PMA concentration above 25ug/g creatinine and 98.26% subjects had urinary s-PMA concentration less than 25ug/g creatinine. There is no correlation between CYP4502E1 genetic polymorphism and the concentration of urinary s-PMA metabolites ($p=0.595$; OR_{crude}= 0.98; 95% CI=0.95-1.01). Further studies are necessary involving a more various extent of ethnicity/race of the workers and the correlation with low-level benzene exposure.

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