Urinary 1-hydroxypyrene in coke oven workers relative to exposure, alcohol consumption, and metabolic enzymes

J Zhang, M Ichiba, K Hara, S Zhang, T Hanaoka, G Pan, Y Yamano, K Takahashi, K Tomokuni

Department of
Community Health
Science, Saga Medical
School, Saga 849-8501,
Japan
J Zhang
M Ichiba
K Tomokuni

Institute for Science of
Labour, 2-8-14 Sugao,
Miyamae-Ku,
Kawasaki 216-8501,
Japan
K Hara

Liaoning Public Health
and Anti-epidemic
Station, Heping
District, 42-1 Jixian
Street, Shenyang
110005, China
G Pan
S Zhang

Epidemiology and
Biostatistics Division,
National Cancer
Research Institute
East, 6-5-1
Kashiwanoha, Kashiwa
277-8577, Japan
T Hanaoka

Tokyo Womens'
Medical University, 8-1
Kawada-cho,
Shinjuku-ku, Tokyo
162-8666, Japan
Y Yamano

University of
Occupational and
Environmental Health,
1-1 Iseigaoka
yahatanishi-ku,
Kitakyushu 807-8555,
Japan
K Takahashi

Correspondence to:
Jiusong Zhang
g9615@post.saga-med.ac.jp

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Abstract

Objectives—To investigate the influence of personal lifestyle—such as smoking and alcohol consumption—on urinary 1-hydroxypyrene (1-OHP) concentrations in coke oven workers exposed to polycyclic aromatic hydrocarbons (PAHs) and to evaluate the association of 1-OHP concentrations with the genetic polymorphism of several metabolic enzymes including cytochrome *P*-450 (CYP) 1A1 and glutathione *S*-transferases (GSTs).

Methods—The study population contained 162 coke oven workers and 58 controls employed at the largest iron and steel factory in China. Personal data were collected at the interview. 1-OHP in urine was measured with high performance liquid chromatography with fluorescence detection. Genetic polymorphisms were identified by the polymerase chain reaction (PCR) method.

Results—A positive association between excretion of urinary 1-OHP and the levels of exposure to PAHs was confirmed. Those people who consumed ≥ 50 g/day ethanol had significantly higher 1-OHP excretion than did other coke oven workers ($p < 0.01$). No significant difference in urinary 1-OHP was found between smokers and non-smokers, in both controls and exposed subjects. The variant homozygotes at exon 7 of the CYP1A1 gene had significantly higher urinary 1-OHP concentrations than other CYP1A1 genotypes among the exposed workers ($p = 0.03$). There was less association between the concentrations of 1-OHP and the GSTM1, GSTP1, or GSTT1 polymorphism.

Conclusions—The present study confirmed that urinary 1-OHP is a good biomarker for exposure to PAHs. Alcohol consumption affected urinary 1-OHP excretion. The variant genotypes of the CYP1A1 gene may result in the enhancement of PAH metabolites. It is helpful to understand the role of individual susceptibility on metabolism of carcinogens. These findings suggest that the modulating effect of individual lifestyle factors or genetic nature should be considered in future studies on occupational exposure to PAHs and in evaluating the health risk from harmful chemicals.

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Keywords: 1-hydroxypyrene; genetic polymorphism; alcohol drinking

Coke oven workers are regularly exposed to many types of coke oven emissions, which are comprised mainly of polycyclic aromatic hydrocarbons (PAHs). 1-Hydroxypyrene (1-OHP), a metabolite of pyrene that reflects the total uptake of PAHs, can be used as a biomarker of internal dose to assess recent exposure to PAHs.^{1,2} Increased urinary concentrations of 1-OHP have been shown in several occupational populations exposed to PAHs, including coke oven workers.^{2,3} However, a wide variation in 1-OHP excretion between people was also found. Van Rooij *et al*⁴ examined the relative contribution of different sources to daily pyrene intake in volunteers who were not occupationally exposed to PAHs. They found that cigarette smoke and foods containing PAHs only accounted for a small part of the variation in urinary 1-OHP, although most pyrene intake was related to smoking and diet. Therefore, it is interesting to investigate whether other lifestyle factors or individual genetic nature could explain some of the variation in excretion of 1-OHP.

Of many lifestyle factors contributing to the source of pyrene intake, cigarette smoke and dietary habit are thought to be relatively important, and have been most often studied. Also, many epidemiological studies reported that alcohol misuse may increase the risk of various cancers, particularly when heavy drinking is combined with other risk factors—such as smoking and exposure to PAH compounds.⁵⁻⁷ One of the prime mechanisms through which alcohol induces cancer is related to the stimulatory effect of ethanol on microsomal cytochrome *P*-450 (CYP) enzyme activity which may result in enhanced concentrations of metabolites of procarcinogens.⁸

The polymorphism of metabolising enzymes has been suggested to interpret individual differences in the rate of activation or inactivation of carcinogens related to PAHs. The phase 1 enzymes—such as CYP—are known to catalyse the formation of reactive intermediates of some carcinogens, while phase 2 enzymes—such as glutathione *S*-transferases (GSTs)—briefly participate in the detoxification of these intermediates through conjugation and excretion. Previous studies have confirmed that the pyrene is mainly oxidised by CYP enzymes—for example, CYP1A1 and CYP1B to phenols, dihydrodiols, and diphenols—which can form

such water soluble secondary metabolites as glucuronides, sulfates, or glutathione conjugates.^{9–11} Although the GST enzymes do not directly participate in the metabolism of 1-OHP, they may alter the concentrations of other pyrene derivatives, and affect the conversion of pyrene to 1-OHP. The genetic polymorphisms of CYP1A1 and GSTs have been associated with increased risk of cancer and increased PAH-DNA adducts, an indicator reflecting the actual biologically effective dose.^{12–16} Also, there are a growing number of surveys that investigate the relation of PAH metabolites such as 1-OHP with the polymorphism of CYP1A1 and GSTs.^{17–19} However, it is still limited and results are inconsistent.

The objective of this study was to investigate the influence of smoking and alcohol consumption on the excretion of urinary 1-OHP in coke oven workers and in unexposed controls. We also evaluated the association of 1-OHP concentrations with genetic polymorphism of several metabolic enzymes including CYP1A1, GSTM1, GSTP1, and GSTT1.

Subjects and methods

STUDY POPULATION AND SAMPLE COLLECTION

The study was carried out at the largest coke plant in China, which has 17 coke ovens and >1300 employees exposed to coke oven emissions. The coke plant was described in a recent paper.³ To select the subjects for study, a simple self administered questionnaire survey was performed among the coke oven workers and unexposed workers. For the exposed group, coke oven workers working at different areas (bottom, middle, and top) of the oven, which have been shown to be correlated with the concentrations of PAH exposure,³ were randomly selected. The subjects from bottom (56), middle (55), and top (51) work areas were defined as low, middle, and high exposures, respectively. The controls were recruited from workers who were employed in repair and maintenance of electronic devices in the same plant. They were known to have no work related exposure to PAHs. The final study population consisted of 162 coke oven workers and 58 controls, all men. All participants in this study were recruited on a voluntary basis. They gave their informed consent.

Coke production is continuous (24 h/day), and each work cycle consisted of 8 days and three different shifts: two consecutive morning shifts and two consecutive afternoon shifts in the first 4 days, 5th day off duty, two consecutive night shifts, and then rest on the 8th day. The workers served on one shift for 4–8 hours.

A detailed questionnaire was completed by participants. Interviews were conducted by two trained interviewers using a structured questionnaire. Each was asked to indicate age, smoking, alcohol consumption, indoor air pollution (from the use of coal for cooking or heating), and histories of occupations and diseases.

Blood sampling was performed from 162 coke oven workers and 58 controls. Venous blood samples were drawn into ethylene diamine tetraacetic acid (EDTA) tubes, and

white blood cells were collected after centrifugation. The DNA of the white blood cells was extracted with a commercial kit (Wako, Osaka, Japan) for identifying the genotypes of CYP1A1 and GSTs (GSTM1, T1, and P1). Spot urine samples were collected from each subject at the end of a 4–8 hour working period to examine urinary excretion of 1-OHP and cotinine. All samples obtained were frozen and stored at –70 °C until analyzed.

ANALYTICAL METHODS

1-OHP in urine

Excretion of 1-OHP in urine was measured as described by Hara *et al.*²⁰ Briefly, the conjugated metabolite in a urine sample was hydrolysed with β -glucuronidase, and then the sample was measured by high performance liquid chromatography (HPLC) with fluorescence detection. Concentrations of 1-OHP were adjusted for creatinine, and expressed as $\mu\text{mol/mol}$ creatinine.

Cotinine in urine

The concentration of urinary cotinine was measured by HPLC.²¹ Cotinine values were normalised for creatinine concentration.

Identification of genotypes

The Ile-Val polymorphism in exon 7 of the CYP1A1 gene was analyzed according to the method of Oyama *et al.*²² Subjects were divided into three groups corresponding to homozygous wild (Ile/Ile), heterozygous (Ile/Val), and homozygous mutant (Val/Val) types. The GSTM1 and GSTT1 genotyping were carried out essentially as described by Groppi *et al.*,²³ and Nelson *et al.*,²⁴ respectively. The presence or absence of an amplification product results in two subgroups corresponding to GSTM1 and GSTT1 present or GSTM1 and GSTT1 absent. The A-G genotype at exon 5 of the GSTP1 gene was identified with the method proposed by Harries *et al.*²⁵ Subjects were categorised into three subgroups: homozygous wild (AA), heterozygous (AG), and homozygous mutant (GG) types. Several samples could not be genotyped due to failure to isolate DNA.

STATISTICAL ANALYSES

The 1-OHP and cotinine data were log transformed before statistical analysis. The comparison of means was carried out with Student's *t* test or analysis of variance (ANOVA). Group differences in frequency for nominal variables were tested with the χ^2 test. Correlation between 1-OHP and other continuous variables was calculated by Pearson's test. Multiple regression analysis was performed to evaluate the relation of 1-OHP concentration with exposure concentrations and some possible modulating factors. In this analysis, all of the questionnaire and examined variables were first analyzed against the 1-OHP concentrations with stepwise up regression analysis. The variables with an *F* value >1.0 were included in the final model as independent variables. Two sided *p* values are given.

Table 1 Summary data of study population

	Controls	Coke oven workers
Number	58	162
Age (y, mean (SD))	39 (8)	39 (8)
Employment (y, mean (SD))	12 (8)	10 (7)
Smokers (%)	41 (71)	108 (67)
Cigarettes/day in smokers (mean (SD))	13 (9)	17 (10)*
Drinkers (%)†	17 (29)	63 (39)
Ethanol consumption in drinkers (g/day, mean (SD))	34 (50)	42 (38)

†Drinking ≥20 days/month.

*p<0.05, compared with controls.

Figure 1 Urinary 1-OHP excretion in the controls and coke oven workers exposed to different PAH concentrations.

Results

The basic characteristics of exposed workers and controls are summarised in table 1. The distributions of age, years of employment, the prevalence of smoking, and alcohol consumption were similar between the two groups, except that number of cigarettes smoked/day was higher in coke oven workers.

The urinary concentrations of 1-OHP obtained from workers exposed to different PAH concentrations and controls are shown in figure 1. The geometric means of 1-OHP for all exposed workers and controls were 9.86 (range 0.8–89.8) and 0.54 (range 0.1–2.8) $\mu\text{mol/mol}$ creatinine, respectively. The highest concentration of urinary 1-OHP was detected in the group with high exposure. The median 1-OHP concentrations differed significantly between the four groups ($p<0.0001$).

Table 2 1-OHP concentrations ($\mu\text{mol/mol}$ creatinine) by smoking and alcohol consumption in controls and coke oven workers

	Controls			Coke oven workers*		
	n	GM (25, 75 percentile)	p Value	n	GM (25, 75 percentile)	p Value
All	58	0.54 (0.30, 0.80)		162	9.86 (5.00, 18.40)	
Smoking:						
Never	17	0.50 (0.40, 0.60)	0.58	53	10.74 (5.28, 20.43)	0.35
Ever	41	0.56 (0.30, 1.00)		109	9.51 (4.85, 17.38)	
Urinary cotinine (nmol/mol creatinine):						
<0.05	21	0.60 (0.40, 0.85)	0.48	52	8.99 (4.55, 18.15)	0.32
0.05–0.90	19	0.58 (0.40, 0.95)		53	9.55 (4.65, 16.70)	
>0.90	18	0.45 (0.30, 0.70)		57	11.17 (7.33, 21.48)	
Alcohol consumption:						
Non-drinkers	41	0.51 (0.30, 0.73)	0.37	99	9.25 (4.90, 16.80)	0.16
Drinkers†	17	0.63 (0.40, 1.02)		63	11.04 (5.15, 23.10)	
Mean consumption of ethanol (g/day):						
<50	50	0.52 (0.30, 0.80)	0.35	122	9.02 (4.90, 16.80)	<0.01
≥50	8	0.69 (0.40, 1.55)		40	13.12 (8.70, 27.80)	

GM=geometric mean.

*GM data were adjusted for levels of exposure.

†Drinking ≥20 days/month.

Figure 2 Comparison of urinary 1-OHP concentrations and the amount of ethanol consumed by different exposure groups.

Table 2 shows the results of urinary 1-OHP relative to cigarette smoke and alcohol consumption in exposed workers, after adjustment for exposure concentration, and controls. There were similar 1-OHP concentrations between smokers and non-smokers in both exposed subjects and controls. The subjects were then divided into three subgroups according to the concentrations of urinary cotinine: non-smokers, light smokers, and heavy smokers with <0.05, 0.05–0.9, and >0.9 nmol cotinine/mol creatinine, respectively. In the coke oven workers, 1-OHP concentrations tended to be highest among the heavy-smokers, but this was not significant. Alcohol drinkers had slightly higher 1-OHP excretion than non-drinkers among the coke oven workers ($p=0.16$). However, when the subjects were categorised with a cut off value of the daily average amount of ethanol ingested, people who consumed ≥50 g/day of ethanol had a significantly higher 1-OHP concentration than the people who consumed <50 g/day ethanol ($p<0.01$). This effect was significant in the group with low exposure after stratification by exposure concentrations ($p<0.01$), as shown in figure 2. The subjects consuming ≥50 g/day ethanol also had higher 1-OHP excretion than the subjects without such consumption in the high exposure group, although not significant

Table 3 Urinary 1-OHP concentrations ($\mu\text{mol/mol creatinine}$) by polymorphism of metabolic enzymes in the controls and exposed workers

	Controls			Coke oven workers†		
	n	GM (25, 75 percentile)	p Value	n	GM (25, 75 percentile)	p Value
CYP1A1:						
Ile/Ile	29	0.55 (0.30, 1.03)	0.86‡	76	9.91 (5.83, 20.23)	0.03‡
Ile/Val	14	0.63 (0.50, 0.80)		56	10.38 (5.08, 18.23)	
Val/Val	4	0.63 (0.35, 1.45)		7	24.77 (10.63, 25.78)**	
GSTM1:						
Present	18	0.71 (0.50, 1.20)	0.14	58	10.52 (5.00, 17.50)	0.91
Null	30	0.52 (0.30, 0.80)		82	10.67 (5.65, 22.30)	
GSTT1:						
Present	18	0.54 (0.30, 1.00)	0.54	61	10.07 (5.53, 20.35)	0.31
Null	30	0.62 (0.40, 0.90)		78	11.46 (6.45, 19.60)	
GSTP1:						
AA	31	0.54 (0.30, 0.95)	0.57‡	92	10.28 (5.53, 20.68)	0.62‡
AG	15	0.69 (0.50, 0.98)		41	11.32 (6.10, 19.20)	
GG	2	0.58 (0.50, 0.70)		6	8.47 (4.10, 13.10)	

** $p < 0.01$ v Ile/Ile type; $p = 0.01$ v Ile/Val type.

†Data were adjusted for levels of exposure.

‡ANOVA.

GM=geometric mean.

($p = 0.11$). Most drinkers were also tobacco smokers. The same analysis was also carried out with simultaneous adjustment for smoking habit or urinary cotinine concentrations, and the results were similar (data not shown). The influence of excess alcohol consumption on 1-OHP concentrations was not found in the controls. No significant relation between the urinary 1-OHP and indoor air pollution (coal use for cooking or heating) was found (data not shown).

The effect of genetic polymorphism on 1-OHP excretion is presented in table 3. For the CYP1A1 gene, there were significant differences in urinary 1-OHP concentrations among three genotypes in the exposed workers ($p = 0.03$). The workers carrying the Val/Val genotype had significantly higher concentrations of 1-OHP than did the Ile/Ile ($p < 0.01$) and Ile/Val ($p = 0.01$) carriers. There were slightly higher 1-OHP concentrations in controls with GSTM1 present compared with those without GSTM1 ($p = 0.14$). By contrast, the other polymorphisms of metabolic enzymes examined were not found to be significantly associated with the urinary excretion of 1-OHP.

To investigate the simultaneous effects of exposure to PAHs and possible confounding factors, multiple regression analysis on the urinary 1-OHP was applied in the exposed workers (table 4). Based on the stepwise up regression analysis, six independent variables were entered into the final model. The results

showed that exposure to PAHs was a good predictor for the concentrations of urinary 1-OHP. Duration of employment, excess alcohol intake, and CYP1A1 polymorphism also helped to explain the differences in urinary 1-OHP. There was a small effect of the GSTT1 genotype on the concentrations of 1-OHP. Urinary cotinine was not a significant predictor of 1-OHP.

Discussion

We found a significantly positive association between urinary excretion of 1-OHP and the concentrations of exposed PAHs, and confirmed additionally that the measurement of 1-OHP in urine is an available biomarker for evaluating the exposure to PAHs. To estimate individual risks from PAHs, we simultaneously examined whether personal lifestyle—such as smoking and alcohol consumption—and metabolic susceptibility genes including CYP1A1 and GSTs may affect the urinary concentrations of 1-OHP. Although pyrene itself does not show carcinogenic or mutagenic potencies, it is presumed that the conclusions obtained from the present study can be extended to other related carcinogenic PAH compounds such as benzo(a)pyrene (BaP).

The stimulatory effect of ethanol on microsomal mono-oxygenases through induction of the CYP2E1 form has been confirmed by animal experiments.^{8, 26} This effect is usually used to explain why ethanol can mediate the conversion of procarcinogens into their reactive species. A study described by Lieber *et al*²⁷ showed that treatment of rodents with ethanol produced an enhancement of the ability to metabolise the components contained in cigarette smoke—such as PAHs. Although the role of CYP2E1 enzymes in metabolism of pyrene still remains unclear, they seem to be associated with the secondary oxidation of pyrene to cause indirectly the increase of 1-OHP concentrations.⁹ The present study showed that alcohol drinking was a significant predictor of urinary 1-OHP in exposed workers, especially in heavy drinkers who consumed ≥ 50 g/day ethanol. In the present population, habitual

Table 4 Multiple regression analysis for the effect of several selected variables on urinary 1-OHP excretion in coke oven workers ($n = 127$, $R = 0.66$, $p < 0.0001$)

Variables	Standard regression coefficient	SEM	t Value	p Value
Intercept	0.72	0.25	2.87	<0.01
PAH exposure concentration	0.61	0.03	8.54	<0.0001
Employment time	0.19	<0.01	2.60	0.01
Urinary cotinine	0.07	0.03	0.96	0.34
Alcohol consumption*	0.16	0.06	2.24	0.03
CYP1A1 polymorphism†	0.17	0.12	2.34	0.02
GSTT1 polymorphism‡	0.13	0.06	1.85	0.07

*Ethanol ≥ 50 g/day v <50 g/day.

†Val/Val v Ile/Ile and Ile/Val.

‡GSTT1 present v GSTT1 null.

alcohol consumption mainly comprised drinking beer and spirits, and ingestion of ethanol from spirits accounted for a larger proportion of the total ethanol consumed by heavy drinkers. We considered that there may be no significant differences in fluid intake by drinking beer between heavy drinkers and the others, and thus the influence of urinary volume on urinary 1-OHP excretion can be excluded. Similarly, we recently reported an influence of ethanol ingestion on urinary 1-OHP in this plant but in different subjects.²⁸ Moreover, it is of interest that in the same plant we also found that increased concentrations of DNA adducts in white blood cells was related to drinking alcohol.¹² Jongeneelen *et al*²⁹ did not find an effect of alcohol consumption on the toxicokinetics of pyrene in coke workers. Two other studies also did not show any relation between alcohol intake and urinary 1-OHP.^{18, 30} However, it is important to note the differences in analyses of drinking among these studies. Previous studies did not consider consumed dose of alcohol, or used lower amounts of ethanol consumed as the cut off value. In the present population studied, the prevalence of heavy drinkers (≥ 50 g ethanol/day) was relatively higher (about 22%). This may have allowed the influence of alcohol consumption on 1-OHP excretion to be readily seen. In a cohort study of a middle aged Japanese population, Tsugane *et al*³¹ indicated that moderate alcohol consumption was associated with the lowest risk of mortality from all cancers, whereas the highest risk was found among heavy drinkers. These findings suggested that dose of alcohol is more important and should be taken into consideration when examining the effect of alcohol consumption on metabolism of carcinogens.

The association between cigarette smoke and 1-OHP concentrations is controversial. Zhao *et al*³² reported a greater smoking related increase in urinary 1-OHP among coke workers. Also, the dose-response relation between the number of cigarettes smoked and urinary 1-OHP concentrations has been shown.⁴ However, smoking was not found to be an important source of exposure to pyrene in several studies.^{33, 34} From our results, smoking is not an important factor to explain the differences between 1-OHP excretion among the coke oven workers and unexposed controls. This finding may be partially due to the relatively higher exposure to PAHs, which may hide the effect of smoking on 1-OHP concentrations, because the mean concentrations of urinary 1-OHP measured from the present coke oven workers and controls were about fivefold higher than those previously reported.²⁹

It is well known that the CYP1A1 gene codes for the aryl hydrocarbon hydroxylase, which catalyses the oxygenation of PAH components. Jacob *et al*⁹ have examined the role of the CYP system in oxidation of pyrene using corresponding agents inducing different types of CYP enzymes in rat liver microsomes, and showed that 1-OHP was the predominating metabolite as treated with CYP1A family

inducers—for example, benzo-a-pyrene or 5, 6-benzoflavone. On the other hand, Crofts *et al*³⁵ recently showed that the variant genotypes at exon 7 of the CYP1A1 gene showed a three-fold increase in CYP1A1 enzymatic activity. This functional significance is thus supposed to cause an increase in concentrations of pyrene metabolites such as 1-OHP in the variant genotypes of CYP1A1. In the present study, a modulating effect of the CYP1A1 polymorphism on the concentrations of urinary 1-OHP was found in coke oven workers, as expected. The mean urinary 1-OHP concentration in the homozygous mutant type of CYP1A1 (Val/Val type) was twofold higher than in the other types (Ile/Ile and Ile/Val). In the other instances, a study of coke oven workers from Taiwan reported that the subjects with the homozygous variant genotype for CYP1A1 Msp I had a twofold increase in 1-OHP concentrations after the shift than the combined wild and heterozygous types.¹⁸ Similarly, the results found in aluminum smelter workers showed a slight rise in concentrations of 1-OHP in people with the CYP1A1 Val allele.¹⁹ The role of GST enzymes in biotransformation of 1-OHP is incompletely understood. GSTM1 and GSTP1 (the most abundant GST in human lung) are active towards many epoxides through glutathione conjugation, whereas GSTT1 briefly conjugates smaller compounds.³⁶ Epidemiological surveys showed that the effect of GST genetic polymorphism on 1-OHP excretion was uncertain.^{17, 30, 37} The present study indicated a slight increase in urinary 1-OHP in unexposed controls with the GSTM1 gene (table 3) and in exposed subjects with the GSTT1 gene (table 4). No systematic influence of the GSTP1 genotypes was found. Hong *et al*³⁰ recently indicated that GSTM1 deficiency was associated with relatively higher urinary 1-OHP concentrations, but conflicting reports were also published.^{17, 37} However, a usual comment in these studies is that the GST polymorphism may not or only marginally influence 1-OHP excretion. This is compatible with our results.

In summary, the present study confirmed that urinary 1-OHP is a good biomarker for exposure to PAHs. We also found an effect of CYP1A1 polymorphism on urinary 1-OHP. It is helpful to understand the role of individual susceptibility on metabolism of carcinogens. Excess alcohol consumption may result in the enhancement of PAH metabolites, but further study on the mechanism is needed. Our findings suggest that these modulating factors should be taken into consideration in future studies on occupational exposure to PAHs and in evaluating the health risk from harmful chemicals.

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