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The Effect of Exposure to Mixed Organic Solvents on Lipid Peroxidation in Ship Building Painters

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Abstract

In the last several years, studies on the association of oxidative stress damage with exposure in the work place have been conducted. Xenobiotics create an imbalance of the homeostasis between oxidant molecules and antioxidant defense. By monitoring oxidative stress biomarkers, information was obtained on damages induced by oxidative stress and the toxicity of xenobiotics. In the present study, a Job Exposure Matrix (JEM) was constructed using the data from the Working Environment Measurement (WEM) of painters in the shipyard industry from the past 3 years to assess the exposure status. Additionally, by measuring the concentration of urinary malondialdehyde (MDA), the effect of lipid peroxidation was examined. The subjects consisted of 68 workers who were exposed to mixed organic solvents in the painting process and 25 non-exposure controls. The exposure indices of the exposure groups were significantly different (sprayer: 0.83, touch-up: 0.54, assistant: 0.13, $P<0.05$). The urinary MDA concentration of the exposure group was $48.60\pm$ 39.23 µmol/mol creatinine, which was significantly higher than 18.03 ± 16.33 µmol/mol creatinine of the control group ($P<0.05$). From the multiple regression analysis of urinary MDA, the regression coefficient for exposure grade was statistically significant. In future studies, evaluation of the antioxidant levels of subjects should be performed simultaneously with quantitative exposure measurements.

Keywords: Mixed organic solvents, Malondialdehyde, Job exposure matrix, Painting workers

The Working Environment Measurement (WEM) has performed an evaluation of the occupational exposure to organic solvents. However, the result is a fragmented profile that merely reflects the exposure at a given time. Therefore, to accurately reflect the effects of exposure of corresponding occupations from the results from this type of cross-sectional measurement is difficult. The type and amount of hazardous substances generated during the painting process are greatly influenced by various factors. However, the evaluation is conducted under the condition that excludes such factors, thus there are numerous limitations to understanding the characteristic of the exposure to hazardous factors as well as its' causes.

Recently, oxidative stress is frequently mentioned as a main or secondary cause as the pathogen of diseases in almost all tissues and organs. Increasing amount of data support the hypothesis that oxidative stress determines the toxicity exerted by many xenobiotics, creating an imbalance of the homeostasis between oxidant substances and antioxidant defenses in vivo. As a consequence, the damage of biological macromolecules determines the toxicity of chemicals[1]. Therefore, by monitoring oxidative stress biomarkers, information on the toxicity of xenobiotics, including the damage by oxidative stress, could be obtained. The results of several studies have reported organic solvents may express their toxicity by reactive oxygen species (ROS)[2,3]. When the respiratory system and the skin are exposed to organic solvents, the solvents are oxidized gradually by CYP 450-dependent monogenase, and generate free radicals, which may

Table 1. General characteristics of study subjects.

Groups		n	Age (years)	Work duration (years)	Smoking (%)	Alcohol (%)
Painters	Spray	26	45.3±6.4	16.7±3.2	20 (76.9)	21 (80.8)
	Touch-up	11	45.8±8.7	15.8±4.1	5 (45.5)	9 (81.8)
	Assistant	31	48.2±7.5	17.4±4.0	26 (83.9)	28 (90.3)
Controls		25	43.8±9.5	9.3±7.9	18 (72.7)	22 (88.0)
P			0.294	0.01	0.228	0.725

Data are means±S.D. n: numbers

affect biological membranes, particularly, their lipid layer[4]. Consequently, due to the exposure to toxic substances that generate free radicals, lipid peroxidation is increased[3]. Malondialdehyde (MDA) is a stable substance that has been widely used as a non-invasive biomarker of lipid peroxidation induced by oxidative stress[5,6].

Therefore, in this study, 3 years of WEM data was utilized to construct a Job Exposure Matrix (JEM) for the assessment of the exposure status of painters in shipyards. In order to assess the effect of the exposure to mixed organic solvents on lipid peroxidation, the concentration of urinary MDA of painters was measured by using MDA of lipid peroxidation as a marker.

Study Population Demographics

The exposure group and the control group consisted of all males. The mean age of the painters was 46.8 yrs, and 43.8 yrs for the control subjects. The painters' work duration was significantly higher than the controls ($P < 0.05$). There were no significant differences in prevalence of smoking and drinking among the groups (Table 1).

Evaluation of the Exposure to Hazard Factors

The exposure index of mixed organic solvents was calculated by applying the result of the WEM from the past 3 years. The evaluated mixed organic solvents were 1,2,3-trimethyl benzene, 1,2,4-trimethyl benzene, 1,2-dichlorobenzene, 1,3,5-trimethyl benzene, 1-butanol, 1-methoxy-2-propanol, 2-hexanone, 2-methoxyethyl acetate, 2-methylcyclohexanone, acetone, butyl acetate, butyl cellosolve, cellosolve acetate, cyclohexane, dimethyl benzene, ethanol, ethyl acetate, ethyl benzene, isopropyl alcohol, methyl isobutyl ketone, m- or p-ethyl toluene, propyl benzene, propylene glycol monoethyl acetate, stylene, and toluene. The exposure index for the job type showed a significant difference ($P < 0.05$). The highest exposure index was 0.83 for the spray painters, the touch-up painter group was 0.54, and the assistant painter group was 0.13, which was the lowest. The

Table 2. Mixture exposure indices and distribution of personal protective equipment by job title.

Unit: number (%) and Mean±SD

	Spray	Touch-up	Assistant
EI[†]*	0.83±1.21	0.54±0.65	0.13±0.20
Continuous	26 (100.0)	11 (100.0)	26 (83.9)
Intermittent	−	−	1 (3.2)
No	−	−	4 (12.9)

[†]EI: exposure index (C1/T1+C2/T2+C3/T3+....+Cn/Tn) (Cn: observed atmospheric concentration, Tn: corresponding threshold limit)
*$P < 0.05$

state of wearing personal protective equipment for each job type was found to differ significantly (Table 2).

Urinary MDA of the Study Subjects

Spray, touch-up, and assistant painters were designated as the exposure group and office clerks were designated as the non-exposure group. The urinary MDA concentration according to the groups showed a statistically significant difference ($P < 0.05$). The concentration of urinary MDA in the exposure group was 48.60±39.23 μmol/mol creatinine, and the non-exposure group was 18.03±16.33 μmol/mol creatinine. As age and work duration increased, a trend of increasing urinary MDA was observed. However, this was not statistically significant. Evaluation of smoking habits show the mean concentration of urinary MDA of smokers was higher than non-smokers in both groups (Table 3). Urinary MDA concentration according to the job type showed a statistically significant difference. The order of job type was: assistant painter (54.44±42.76 μmol/mol creatinine), spray painter (44.15±37.62 μmol/mol creatinine), touch-up painter (36.54±27.22 μmol/mol creatinine), and the control group (18.03±16.33 μmol/mol creatinine) (Table 4).

Multiple Regression Analysis

The results of multiple regression analysis are shown in Table 5. In multiple regression analysis, age, smoking, alcohol, work duration, and exposure grade

Table 3. The concentration of urinary MDA by general characteristics.   Unit: μmol/mol creatinine (Mean±S.D)

Characteristics	Total	P	Exposure	P	Non-exposure	P
Age (years)						
<40	28.77±30.33	0.261	43.00±36.25	0.730	12.75±6.94	0.524
40-49	36.72±36.14		45.05±40.20		20.06±18.79	
≥50	46.80±39.45		53.47±40.85		22.36±22.02	
Work duration (years)						
≤10	25.86±24.16	0.111	31.09±33.89	0.449	22.72±18.01	0.448
11-17	36.53±39.29		47.70±42.73		12.95±13.41	
≥18	49.77±37.72		54.15±37.69		17.64±19.86	
Smoking						
yes	40.76±38.46	0.450	49.86±41.71	0.301	20.29±17.99	0.694
no	33.01±29.77		44.45±31.06		12.02±9.43	
Alcohol						
yes	38.46±36.57	0.829	48.10±39.63	0.839	18.15±16.24	0.934
no	41.18±37.68		51.42±39.75		17.27±20.60	

Table 4. The concentration of urinary MDA by job characteristic.   Unit: μmol/mol creatinine (Mean±S.D.)

	MDA	95% C.I*	P
Job title			
Spray	44.15±37.62	25.44-62.86	0.005
Touch-up	36.54±27.22	12.77-70.35	
Assistant	54.44±42.76	36.43-72.45	
Control	18.03±16.33	10.79-25.27	

*C.I: Confidence Interval for mean

Table 5. Multiple regression analysis of urinary MDA concentration.

Variables	B (SE)	t	P
Exposure grade	6.015 (2.457)	2.448	0.017
Age	0.855 (0.525)	1.628	0.109
Work duration	0.358 (0.768)	0.467	0.642
Smoking	7.355 (9.640)	0.763	0.440
Alcohol	2.851 (12.452)	0.229	0.819

Constant: −27.399
R^2: 0.193

Exposure grade=job score × exposure score
Age and Work duration: years
Smoking and Alcohol: No=0, Yes=1

were entered. The result showed that only the exposure grade was statistically significant ($P < 0.05$). Age, work duration, smoking, and alcohol showed a positive trend, however, these factors had no significant effect on the urinary MDA concentration.

Discussion

Evaluation of the occupational exposure has been performed primarily on the concentration in the respiratory area of workers through WEM. However, the data of the WEM consists of a random selection of workers in a unit work area and a one-time measurement and therefore, the representative exposure concentration of workers is not indicative. If the exposure evaluation was linked to the systematic exposure information, and evidence of a relation between the occupational exposure and the health status could be explained, then the WEM data could be an efficient evaluation. However, to manage all risk factors of workers for the exposure evaluation, hindrance of the technical limitation and limited resources may occur, and the accuracy of the evaluation would be lowered. Because of such restrictions, the exposure was evaluated by stratifying exposure and non-exposure, or by examining the employment duration. Nonetheless, such studies are limited in that the exposure intensity and the exposure pattern cannot be evaluated together[7]. The JEM introduced initially by Reed[8] in 1941, can minimize non-discriminative erroneous information bias[9]. However, in general, the JEM that can be used is only a summarized exposure evaluation, and information on job title includes other tasks and working environments. Thus, the exposure evaluation according to job title may be inaccurate. The exposure prediction on individuals is influenced by a non-discriminative erroneous classification and therefore relative risks are evaluated to be low, and hence the significance of the studies becomes markedly low[10-13]. In the study reported by Gerin et al.[14], the process exposure matrix subdivided workers with the same duties, and workers exposed similarly were classified as a single SEG and evaluated. The results showed the exposure level was not associated with the duty, and instead, was related to the diverse combinations

of work methods pertinent to the process and which methods generated hazardous substances. Therefore, in the present study, by applying the result of the WEM assessment from the past 3 years, painting duty was subclassified according to painting methods as spray, touch-up and assist. Furthermore, a semi-quantitative JEM was constructed by multiplying the job score and the exposure score. By dividing the score into several categories, the value was used as a pattern of the exposure grade.

Numerous studies have been conducted on the correlation of oxidative stress to diseases. Nevertheless, in regard to occupational exposure, only limited studies on groups such as benzene exposed workers[15], industrial art glass workers[16], urban bus drivers[17], hexavalent chromium[18], quartz exposed workers[19], asbestos, rubber, and coke-oven workers[20] have been performed. The present study examined the relationship between the status of the exposure to mixed organic solvents of painters and the oxidative stress marker, urinary MDA. According to the job type, a significant difference among the groups was found ($P<$ 0.05). Sprayer, touch-up, and assistant painters were designated as the exposure group and office clerks were designated as the non-exposure group. The MDA concentration was compared between the groups and the results showed the urinary MDA concentration of the exposure group was 48.60 ± 39.33 µmol/mol creatinine and the non-exposure group was 18.03 ± 16.33 µmol/mol creatinine, which was significantly different. The MDA concentration of the non-exposure group showed a similar distribution compared with 19.21 ± 12.43 µmol/mol creatinine of the general population reported by Korchazhkina et al.[21].

According to the duty characteristics of the exposure group, the concentration of urinary MDA was compared, and the result showed that the assistant painte's level was 54.44 ± 42.67 µmol/mol creatinine, which was the highest, followed in decreasing concentration by 44.15 ± 37.62 µmol/mol creatinine for the spray painter, and 36.54 ± 27.22 µmol/mol creatinine for the touch-up painter. However, the results of the WEM of organic solvents in the air showed the concentration of urinary MDA for the spray painter was 0.83, 0.54 for the touch-up painter, and 0.13 for the assistant painter. Thus the results of the external exposure and the internal exposure showed a dissonant pattern. The WEM result measures the concentration in the respiratory area outside of the personal protective equipment, and the actual exposure level varies depending on the type of personal protective equipment and the wearing state. Most organic solvents that are used during painting are lipophilic substances, and thus their absorption pathway is not limited to the respiratory organs, hence, the absorption through the skin should not be ignored. The correlation analysis result between the exposure grade of JEM and urinary MDA was Pearson's correlation coefficient=0.378 ($P<0.01$, data not shown), which was a significant correlation. Through multiple regression analysis, variables that may mediate an effect on urinary MDA were adjusted, and the result showed only the exposure grade was a significant predictive variable.

Several recent studies on the association of the oxidative stress damage and the occupational exposure have been examined. These studies represent pioneering events for the future when oxidative stress damages and oxidative stress profiles may become endpoints for assessing the adversity of workplace exposures[22]. Despite apparent facts that oxidative stress induces adverse biological responses[23], its adversity on diseases is unclear, and remains controversial. Regardless of such uncertainty, if occupational exposure continuously induced oxidative burdens in workers, and if lipid peroxidation was increased as a result of the burden, lipid peroxidation products may be able to perform as biomarkers. Furthermore, a demonstrated correlation between exposure and oxidative damages could be used as a potential marker for monitoring biological effects.

In this study, a semi-quantitative JEM was constructed by applying the data of the WEM from the past 3 years and the exposure evaluation was performed by the exposure grade. This type of semi-quantitative evaluation has limitations by not satisfactorily assessing the quantitative association among categories. This was a retrospective evaluation applying previous data not a prospective study designed for the exposure evaluation, therefore exposure information was limited.

Materials & Methods

Subjects

Urine samples were collected from 68 painters employed in a shipyard company after obtaining informed consent. Regarding the job types, there were 26 spray painters, 11 touch-up painters and 31 paint assistants. For the control group, 25 office clerks were recruited.

The subjects were required to fill out a questionnaire regarding age, gender, work duration, work department, job type, working area, personal protective equipment, and drinking and smoking habits. Among the subjects, there was no history of liver disorder, renal dysfunction, heart disease, or diabetes mellitus.

Urine Collection

The spot urine specimens were collected at the end of the work shift. Samples were stored at $-20°C$ in a plastic bottle without any additives until analysis. Urine samples were thawed in a 40°C water bath and centrifuged at 2,000 g for 10 min.

Urine Analysis

The analysis of urinary MDA, which used solid phase extraction and subsequent high performance liquid chromatography (HPLC) with dual ultra violet detector (UVD), was based on previous work described by Korchazhkina[21].

Briefly, for the analysis of urinary MDA, 100 µL of 0.31 mM DNPH, and 100 µL of 10 mM propionaldehyde, were added to 400 µL aliquots of each urine sample present in a 2 mL glass vial with a PTFE-lined screw cap. The reaction mixture was incubated at 45°C for 30 min. The derivative solution was adsorbed to a C18 Sep-Pak cartridge preconditioned with 5 mL methanol and 5 mL DW, washed with 2 mL DW, eluted with 1 mL methanol, and analyzed by HPLC. A HPLC system (Waters, MA, USA), incorporating a 2695 Alliance separation module, a 2487 Dual λ Absorbance detector and operated by Empower Pro software, was used in this study. The separation of MDA-DNPH was carried out on Discovery C18 analytical column (Supelco, 5 µm, 4.6 × 250 mm), and the column heater temperature was set at 40°C The mobile phase used for isocratic elution of hydrazones was composed of 35% acetonitrile and 65% 1 mM boric acid. The flow rate was 1 mL/min and the run time for each sample was 30 min. Detection was performed at the maximum absorbance wavelengths. MDA-DNPH was measured at 306 nm, and propionaldehyde-DNPH was measured at 365 nm. Urinary creatinine was determined using a kit from Merck (Darmstadt, Germany) according to Jaffe's picric acid method. All samples were adjusted by urinary creatinine. The recovery rate of the derivatization and the solid phase extraction was adjusted with the recovery rate of propionaldehyde-DNPH.

Job Exposure Matrix

To effectively assess the exposure level and health risk, a JEM according to the job characteristics was constructed. The analysis of workers' task, working place, used substances, and with or without wearing personal protective equipment were assessed. Based on these factors, the working place and the job title of workers were classified and the worker groups whose exposure profile were similar were designated as the similar exposure group (SEG). The result of the WEM from the past 3 years (2002, 2003 and 2004) was applied. The job score was based on the exposure index of exposure to mixed organic solvents while performing a job. According to the method of Scarpelli et al.[24] and Astrakianakis et al.[25], no exposure was scored as 0 points, 10 % lower than mixed TLV was scored as 1 point, 10 to 25% of TLV as 2 points, 25 to 100% of TLV as 3 points, 100 to 200% as 4 points, and over 200% as 5 points. According to the state of wearing protective equipment, the exposure score was evaluated as wearing appropriate personal protective equipment continuously as 1 point, wearing protective equipment intermittently as 2 points, and wearing inappropriate personal protective equipment or not wearing equipment as 3 points. A semi-quantitative JEM was obtained by multiplying the job score and the exposure score, and the values were presented as the pattern of exposure grade.

Statistical Analysis

All data were analyzed with the SPSS statistical package for Windows (Version 12.0E, SPSS Institute Inc., Cary, NC, USA). The statistical methods included X^2 analysis, T-test, ANOVA and multiple linear regression. A level of $P < 0.05$ was considered statistically significant.

Acknowledgements

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