Serum level of malondialdehyde, superoxide dismutases and cellular level of ras P21 and P53 in coke-oven workers

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Received November 16, 2007

Abstract

Background. Coke-oven workers exposed to coke-oven emissions (COEs) during the process of pyrolyzing coal into coke could show biofunctional damage before any clinical symptoms. The evidence is limited on the association between COE exposure and biological effects in human now. *Objective.* To explore the effect of COEs on lipid peroxidation and expression of tumor-suppressing proteins in exposed workers. *Methods.* Fifty-six workers in a coke-oven plant were randomly allocated to the exposure group and 40 healthy local people were to the control group. Serum level of malondialdehyde (MDA) and activity of total superoxide dismutases (T-SODs) were determined by spectrophotometry. Immunohistochemistry was used to measure expression of ras P21 and P53 proteins in peripheral leukocytes. *Results.* Compared with controls, coke-oven workers showed a significant serum increase of MDA level and decrease of T-SOD level. The mean concentration of MDA did not differ significantly from years of employment nor did level of T-SODs. The levels of P21 and P53 proteins were markedly higher in peripheral leukocytes of exposed workers than those of controls. Neither P21 nor P53 levels differed significantly of workers with 10 or more years from less than 10 years of employment. *Conclusions.* Occupational exposure to COEs caused lipid peroxidation biomembrane damage and increased levels of the tumor-suppressing proteins P21 and P53 in peripheral leukocytes among coke-oven workers. These biofunctional markers might be useful in screening and surveillance for occupationally derived lung cancers. [Life Science Journal. 2008; 5(1): 38 – 42] (ISSN: 1097 – 8135).

Keywords: emissions; coke-oven; malondialdehyde; superoxide dismutases; P53; P21; lung cancer

1 Introduction

Coke-oven emissions (COEs) are formed and released into the environment when coal is pyrolyzed into coke[1]. Epidemiological studies have shown coke-oven workers were with a high risk for developing lung cancers[2–6] and other respiratory diseases[7]. COEs have been classified to the known human carcinogens by the International Agency for Research on Cancer (IARC) and the U.S. Environmental Protection Agency (US EPA)[6,8]. COEs are complex mixtures containing a large amount of polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (B[a]P), which act as carcinogens in numerous animal species and are used as positive controls in skin-cancer studies. However, the detailed carcinogenic mechanisms of COEs are still not clear.

Oxidative stress is implicated in the pathogenesis induced by many environmental agents such as ionizing radiation[8] and CCl_4[9]. Level of malondialdehyde (MDA), one of the byproducts of lipid peroxidation, can reflect the degree of oxidant damage to the biomembrane to some extent[10]. Superoxide dismutase (SOD) is an antioxidant enzyme in the human body that protects cells from various toxic effects[8,9]. Considering the slow process of initiation and progression of tumorigenesis, oxidant damage and decreased level of antioxidants in coke-oven workers exposed to COEs may occur earlier than evidence of lung cancer[11].

The tumor-suppressor gene p53 is frequently mutated in patients with lung cancers[12–14]. One of the consequences of p53 gene mutation is its abnormal accumulation in

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cells. ras, the best-characterized proto-oncogene, encodes a smaller 21 kD protein, P21, and is frequently mutated in lung cancer tissues[15,16]. P53 and ras P21 may play roles in the pathogenesis of lung cancer. The abnormal protein expression of these two genes may occur before clinical symptoms of lung cancer.

We aimed to determine the alteration in serum level of MDA and SOD and expression of ras P21 and P53 in peripheral leukocytes in coke-oven workers exposed to COEs, and to assess the possible use of these indices as biomarkers in screening and surveillance for occupational cancers such as lung cancer before the appearance of clinical symptoms.

2 Materials and Methods

2.1 Population studied

For the exposure group, we randomly selected 56 people working in the coke-oven branch of a steel and iron plant in northern China who were exposed to COEs. There were 51 males and 5 females in this group, aged from 23 to 53 years old, and the average age was 39.93 years. For the control group, we randomly selected 40 volunteers in the same area who had not been exposed to COEs. They were 20 to 52 years old, and the average age was 33.73 years. All exposed and control subjects were non-smokers. The Institutional Review Board at the Zhengzhou University approved the study. Informed consent was obtained from each participant prior to data collection.

2.2 Data and sample collection

A standardized questionnaire was used to collect the basic information, including sex, age, health status, and occupational history.

Blood specimens were collected from all subjects after overnight fasting. Serum was separated by centrifugation at 2000 g/min for 5 minutes and stored at – 80 ºC for measurement of MDA level and level of total SODs (T-SODs).

2.3 Assessment of COEs

The concentration of benzene-dissolved fraction (BSF) of COEs was detected from different working areas of the plant: the top of the coke oven, inside the coke-pushing and coke-receiving chambers, as well as inside the operation room. The detection method of BSF was as follows: COEs samples from the four sites were collected by an air-sampling apparatus and filtered into an erlenmeyer flask by using a negative-pressure filter filled with sand. The samples were shaken in an ultrasonic shaker, then filtered with the negative-pressure filter. This procedure was repeated twice. Benzene was added to the erlenmeyer flask to the final 30 ml. When the COEs were completely dissolved at room temperature for about 10 minutes, 5 ml of the above benzene solution was extracted to a weighing bottle with constant weight. The weighing bottle was placed in a vacuum case, and the benzene was vaporized completely at 40 ºC and 23.3 kPa (200 mmHg), then put it into a desiccator and weighed after 30 minutes. Two blank controls were created for every panel of samples. The concentration of BSF was calculated as follows:

$C = 6 \times (W_1 - W_2) / V_0$

Where C is the concentration of BSF (mg/m^3), W_1 the weight of the residual of the above benzene solution of COEs (mg), W_2 the weight of the residual of blank controls (mg), and V_0 the sampling air volume (m^3) under normal conditions.

2.4 Serum MDA level and T-SODs level

The serum levels of MDA and T-SODs were determined according to the MDA and T-SODs colorimetric assay kits (Jiancheng Bioengineering, Nanjing, China).

2.5 P21 and P53 in peripheral leukocytes

The level of P21 and P53 in peripheral leukocytes was measured by immunohistochemical assay kits (Bosto Biotechnology, Wuhan, China).

2.6 Statistical analysis

Data were expressed as means ± SD. All statistical analyses involved used SPSS 10.0 for Windows (SPSS; Chicago, IL). The Shapiro-Wilk normality test was used to examine the distribution of data. Two-independent samples *t*-test was used to compare the mean serum MDA level and T-SODs level between groups. Corrected chi-square testing was used to compare the peripheral blood cellular ras P21 and P53 levels between groups, as well as in the exposure group by years of employment. One-way ANOVA was used to compare the mean values of serum MDA level and T-SODs level in the exposure group by years of employment. $P < 0.05$ with a two-sided test was considered significant.

3 Results

The 56 members in the COE exposure group included 51 males (mean age 39.4 years, range 23 to 53 years), and the 40 members of the control group included 28 males (mean age 33.73 years, range 20 to 53 years), with no age difference between these two groups ($P > 0.05$). The mean employment time for the COE group was 15.5 years (range 2 to 32 years).

3.1 Concentration of BSF at sampling sites

Table 1 showed the concentrations of BSF of COEs at the four sampling sites. The concentrations were 8.52, 2.80, 1.23, and 0.88 mg/m^3 for the top of the coke oven, area of transporting coke, area of accepting coke, and the driving workshop, respectively. All concentrations exceeded the occupational exposure limit (OEL) for China of BSF (0.2 mg/m^3). Both the absolute and relative excess values were significantly higher than the OEL; the absolute values varied from the lowest, 0.88 mg/m^3, in the driving workshop to the highest, 8.32 mg/m^3, in the top of coke oven. Even the lowest relative values were 4.4 times of OEL in the driving workshop and the highest was, in the top of coke oven, 42.6 times of OEL. The top of the coke oven had the highest level of BSF among the four sampling sites.

3.2 Serum MDA level and T-SODs level

As compared with the control group, the COE group showed higher serum MDA level (5.30 ± 2.29 *vs.* 0.43 ± 0.64 nmol/ml, $P < 0.01$) and lower T-SOD level (100.04 ± 10.75 *vs.* 128.61 ± 10.00 U/ml, $P < 0.01$) (Table 2). The mean MDA concentrations of workers with 2 – 9, 10 – 19, and 20 – 36 years of employment were 5.69 ± 3.83, 4.29 ± 2.30, and 5.97 ± 2.54 nmol/ml, respectively, and T-SODs levels in those workers were 99.92 ± 4.51, 101.67 ± 10.02, and 98.98 ± 14.85 U/ml, respectively. The values of both MDA and T-SODs were not significantly increased with years of employment (Table 3).

3.3 P21 and P53 in peripheral leukocytes

The P21 levels in peripheral leukocytes for exposed group and control group were 23.21% and 0.05%, respectively, and P53 levels were 21.43% and 0.05%, respectively. Both P53 and P21 in exposure group were higher than control group (Table 4). However, the time of employment didn't affect the level of P21 and P53 in peripheral leukocytes (P21: 27.78% for > 10 years *vs.* 15% for ≤ 10 years and P53: 19.44% for > 10 years *vs.* 25% for ≤ 10 years, respectively) (Table 5).

4 Discussion

Coke-oven workers in the plant investigated are exposed to higher levels of COEs than the limitation of China, with the greatest exposure from the top of the coke oven. Since the current exposure standard in China was proposed to protect against the development of lung cancer in working conditions[17], coke-oven workers in the plant we studied might be at a higher risk. Indeed, we found significantly elevated serum level of MDA, the evidence of oxidative stress, and reduced level of T-SODs, the evidence of low antioxidation. The protein levels of the tumor-suppressors ras P21 and P53 in peripheral leukocytes were significantly higher in coke-oven workers than in controls. These alterations could portend disease activity.

COEs are a complex mixture containing a large amount of PAHs, which the IARC and US EPA classified as a known human carcinogen. Moreover, COEs contain coal tar, light oil, and other complex gases such as sulfur dioxide, nitrogen dioxide, and ammonia, which might be irritants for the respiratory system[18]. All these characteristics of COEs may subject coke-oven workers to a high risk for malignant disease such as lung cancer[2–6] and other tumors, as well as other disorders such as asthma and bronchitis[7,18].

Reactive oxygen species (ROS) are involved in a large number of biological events, including aging, carcinogenesis, inflammation, and autoimmune disorders[8,19,20]. Level of MDA, produced during lipid peroxidation, can show the severity of oxidative stress. SODs are the only enzymatic system decomposing superoxide radicals to H_2O_2 and play a significant role against the effects of oxidant stress[21]. In our study, the serum level of MDA among coke-oven workers was significantly elevated, which implies a high level of lipid peroxidation. As well, the serum level of T-SODs was markedly reduced. Comprehensively, oxidative stress participates in the pathogenesis of COEs[20]. We were puzzled by the lack of significant difference in the above biomarkers by years of employment of the coke-oven workers, which might due to the sampling size.

The deletion or inactivation of tumor suppressor genes and/or activation of proto-oncogenes play an important role in the initiation and development of malignant disease. The mutation of the tumor suppressor gene p53 and/or ras oncogene in human lung cancer has been frequently reported[12–16,22,23]. As well, P53 protein accumulation is shown in some lung cancers[24]. In the present study, the protein levels of ras P21 and P53 in peripheral leukocytes were significantly higher in coke-oven workers than in controls. However, the mechanism of COEs-induced accumulation of ras P21 and P53 is unclear. PAH metabolic activation, interacting with DNA, may be involved in ras p21 and p53 gene mutations. Earlier molecular genetic studies of the potential involvement of cancer genes in lung tumors of mice and humans have targeted a dozen regulators of growth-factor signal transduction and cell-cycle progression, mainly ras and p53[14], which was confirmed by our study to some degree.

Table 1. Concentrations of benzene soluble fraction at the four sampling sites of a coke-oven plant and levels in workers

Sampling sites	Number of sample	BSF at sites (mg/m^3)	BSF in workers (mg/m^3)	Fold difference
Top of coke oven	2	8.52	8.32[a]	42.6[b]
ATR	3	2.80	2.60[a]	14.0[b]
AAC	3	1.23	1.03[a]	6.15[b]
DRW	3	0.88	0.68[a]	4.40[b]

ATR: area of transporting coke. AAC: area of accepting coke. DRW: driving workshop. [a]: absolute difference between the measured levels and the occupational exposure limit (OEL) of BSF based on the standard in China (0.2 mg/m^3); [b]: relative difference of the measured levels above the OEL of BSF.

Table 2. Concentration of MDA and of T-SODs in serum ($\bar{X}$ ± SD)

Groups	Number	MDA (nmol/ml)	T-SOD (U/ml)
Control	40	0.43 ± 0.64	128.61 ± 10.00
Exposed	56	5. 30 ± 2.29[*]	100.04 ± 10.75[#]

vs. control, [*]: $t = 7.29$, $P < 0.01$; [#]: $t = 0.78$, $P < 0.01$.

Table 3. Concentrations of serum MDA and T-SODs in the different time of employment ($\bar{X}$ ± SD)

Years	Number	MDA (nmol/ml)	T-SOD (U/ml)
2 – 9	19	5.69 ± 3.83	99.92 ± 4.51
10 – 19	16	4.29 ± 2.30	101.67 ± 10.02
20 – 36	21	5.97 ± 2.54	98.98 ± 14.85

MDA: $F = 0.92$, $P > 0.05$; T-SOD: $F = 0.27$, $P > 0.05$.

Years of employment for the COE-exposed workers was not associated with serum levels of MDA, and T-SODs, or ras P21 and P53 levels in this study. The relatively small sample size in the subgroup analysis, the information bias for employment history, and the variation in actual exposure dosages might explain the negative findings. A longitudinal observation study is needed to examine these relations further.

5 Conclusion

The investigation of levels of oxidative stress, anti-oxidant enzymatic activity, and tumor-suppressor protein expression in workers exposed to COEs suggests that serum levels of MDA, T-SODs, ras P21 and P53 proteins in leukocytes could be used to monitor the occupational effect of coke-oven workers. This study also provides some new clues in the development of biomarkers for bio-surveillance and early prevention of unhealthy effects related to occupational exposure to COEs.

Table 4. Levels of P21 and P53 in peripheral leukocytes

Group	*n*	P21 (*n*, %)		P53 (*n*, %)	
		positive	negative	positive	negative
Exposed	56	13 (23.21)[*]	43 (76.79)	12 (21.43)[*]	44 (78.57)
Controls	40	2 (0.05)	38 (99.95)	2 (0.05)	38 (99.95)

vs. control, [*]: $P < 0.05$.

Table 5. Levels of P21 and P53 in peripheral leukocytes in the exposed group by years of employment

Group (years)	*n*	P21 (*n*, %)		P53 (*n*, %)	
		positive	negative	positive	negative
≤ 10	20	3 (15.00)	17 (85.00)	5 (25.0)	15 (75.0)
> 10	36	10 (27.78)[*]	26 (72.22)[*]	7 (19.44)[*]	29 (80.56)[*]

vs. ≤ 10 years, [*]: $P > 0.05$.

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