Characteristic Analysis of Peripheral Blood Mononuclear Cell Apoptosis in Coke Oven Workers

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Abstract: Characteristic Analysis of Peripheral Blood Mononuclear Cell Apoptosis in Coke Oven Workers: Hong Mei ZHANG, et al. Department of Occupational and Environmental Health, Shanxi Medical University, China—Objectives: The aim of the present study was to determine the peripheral blood mononuclear cell (PBMC) apoptosis in coke oven workers so that we can take effective measures to protect coke oven workers. Methods: The subjects, 129 coke oven workers and 37 warehouse workers (controls), were investigated using a questionnaire to collect information about their age, working years, smoking and drinking habits, vocational history and other general information. The coke oven workers were divided into the oven-bottom group (34), oven-side group (48) and oven-top group (47) according to their working sites and environmental monitoring data. The concentration of benzo[a]pyrene (B[a]P) and the subjects' urinary 1-hydroxypyrene (1-OH-Py) levels were determined by HPLC. Additionally, the PBMCs were separated from blood samples, and the early and late apoptosis rates were determined by flow cytometry. Results: The airborne B[a]P concentrations were 19.5 ± 13.2, 185.9 ± 38.6 and 1,623.5 ± 435.8 ng/m³ at the bottom, side and top of the oven, respectively, and were higher than in the controls' workplaces 10.2 ± 7.6 ng/m³. Urinary 1-OH-Py, indicating the B[a]P's internal exposure level, was significantly higher in the exposed groups than in the controls (p<0.05). Compared with the controls, the coke oven workers' PBMC apoptosis rates were significantly increased and increased in association with the B[a]P level. PBMC apoptosis increased in association with the 1-OH-Py level and coking operation years and decreased in association with years of alcohol consumption. Conclusions: PBMC apoptosis in the coke oven workers was associated with the 1-OH-Py level, coking operation years and years of alcohol consumption and may be induced by B[a]P.

Key words: Alcohol, B[a]P, Coke operation years, Immunity, 1-OH-Py, PBMC apoptosis

Coke oven workers are occupationally exposed to mixtures of polycyclic aromatic hydrocarbons (PAHs) including benzo[a]pyrene (B[a]P), naphthalene, pyrene, benzo[a]anthracene, chrysene, fluoranthene and anthracene. B[a]P, a member of the PAHs, is carcinogenic and mutagenic and causes reproductive dysfunction¹⁻², immune toxicity and neurotoxicity³⁻⁴. Immune cell apoptosis is one of the hot spots in immune toxicity research, and peripheral blood mononuclear cell (PBMC) apoptosis can reflect the immune system's stability. Reduced apoptosis would result in excessive proliferation of immune cells and formation of autoimmunity, while increased apoptosis can cause immunodeficiency and formation of immune tolerance⁵. To assess the immune function of coke oven workers, we detected and analyzed the changes in PBMC apoptosis.

Subjects and Methods

Subjects

The exposed groups consisted of 129 middle-school educated, 23–49-year-old male coke oven workers employed at least 1 year in the coke plant of a large state-owned company, who were divided into the oven-bottom group (34 subjects), oven-side group (48 subjects) and oven-top group (47 subjects) according to their working sites and environmental monitoring data. A working shift lasted 8 h, and each worker's tasks were relatively constant over 1 yr. The controls were 37 warehouse workers from the raw materials plant at same company who were not occupationally exposed to PAHs from the coking operation and had not visited the coke plant in the previous 3 mo, were
matched to the exposed groups by age, gender, socioeconomic status, lifestyle and physical status. The raw materials storage house is about 4 km upwind of the coke oven plant. The subjects lived in the same area at least 2.5 km upwind of the coke plant. Moreover, the Ethics and Human Subject Committees of Shanxi Medical University approved the research protocol, and all subjects signed provided informed consent.

**Methods**

**Questionnaire investigation**

A self-designed questionnaire was used to collect the subjects’ general information, smoking and drinking habits (yes or no) and other personal habits, vocational history, personal and family disease histories, symptoms and medications used in the previous 2 wk, etc. Smoking was defined as currently smoking no less than 10 cigarettes per day over the last year, while drinking was defined as currently drinking wine, beer or spirits no less than 3 times a week for the last 6 mo. The durations of smoking and drinking were recorded as smoking years and drinking years, respectively. Smoking index was calculated by the amount of smoking (cigarettes/day) multiplied by the number of smoking years. Drinking index was calculated by the equivalent amount of pure alcohol (100 g/day) multiplied by the years of alcohol consumption. No subjects had a personal or family history of diseases such as hypertension, encephalopathy, cardiovascular diseases, liver or kidney diseases and autoimmune diseases. All 129 exposed workers and 37 controls completed the questionnaire and were included in the study.

**Working environment monitoring**

Samplers were arranged at the bottom, side and top of the oven and in the controls’ workplaces. Two parallel automatic air sampling pumps were also fixed at personal breathing zone height and collected air samples at a flow rate of 2 l/min for 6 continuous hours during the working day over 3 consecutive days in March 2004. Detailed air pressure and temperature data were recorded and used to calculate the standard sampling volume. After sampling, the filters were removed from the sampling pumps, sealed in a clean container, stored at 4°C and transported to the laboratory. B[a]P in the filters was extracted four times using hexamethylene and alkaline aluminum oxide and then analyzed by high performance liquid chromatography (HPLC).

**Urinary 1-OH-Py analysis**

Post-shift urine samples were collected in 50 ml polyethylene plastic tubes and stored at –80°C until 1-hydroxypyrene (1-OH-Py) was analyzed by HPLC according to a standard method.

**PBMC apoptosis detection**

A 2 ml aliquot of venous blood was drawn from each subject after fasting in the morning, the PBMCs in the blood were separated by Ficoll-Hypaque density gradient centrifugation. The PBMC purity was about 95%, while lymphocytes accounted for about 90–95% of the PBMCs. The PBMCs were washed twice with PBS (pH 7.4), adjusted to 1 × 10⁷ cells/ml density and resuspended in a buffer containing 1.0 µg/ml PI and 0.025 µg/ml AnnexinV-FITC. Double labeling was performed at room temperature (~23°C) for 15 min in the dark. Double-stained cells were immediately analyzed using FACScan flow cytometry (BD, USA) and the CellQuest program.

**Statistical analysis**

Quantitative data are presented as means ± SD. The distribution of urinary 1-OH-Py was skewed and was transformed to fit a logarithmic normal distribution. One-way ANOVA, multiple linear regression and stratification analysis were performed with the SPSS 11.5 for Windows software, and the LSD (least significant difference) test was used in comparing groups. Qualitative data are presented as percentages (%) and were analyzed by the Chi-square test. A value of $p<0.05$ (two sided) was considered statistically significant.

**Results**

**Working environment monitoring**

The concentrations of airborne B[a]P were 19.5 ± 13.2, 185.9 ± 38.6 and 1,623.5 ± 435.8 ng/m³ at the bottom, side and top of the oven, respectively, and were higher than that of the controls’ workplaces, 10.2 ± 7.6 ng/m³. Also, the mean B[a]P concentration, 1,623.5 ng/m³, at the top of the oven was over 170 times that in the controls’ workplaces, 10.2 ng/m³, and far exceeded the B[a]P maximum allowable concentration (MAC) of 0.15 µg/m³.

**Biological monitoring and subjects’ general information (see Table 1)**

Table 1 shows that the urinary 1-OH-Py concentrations were significantly increased in the coke oven workers compared with the control group ($p<0.01$) and that the concentration was higher in the oven-top group than in the oven-side and oven-bottom groups. Moreover, all groups were matched by age, working years and drinking habit ($p>0.05$). Nevertheless, there was a significant difference in smoking habit among all groups ($p<0.05$), but no significant difference in smoking index and drinking index among all groups (not
PBMC apoptosis rate among groups (see Fig. 1)

The ANOVA analysis results in Fig. 1.1 show that the PBMC early and late apoptosis rates were significantly higher in the exposed groups than in the control group (* and **: p<0.05 and p<0.01, respectively). Representative flow cytometry results are shown in Fig. 1.2. A, B, C and D are representative of the control, oven-bottom, oven-side and oven-top groups, respectively. Compared with the control group, the rates of early apoptosis cells (Annexin V-/PI-) and late apoptosis cells (Annexin V-/PI+) were increased in the oven-bottom, oven-side and oven-top groups.

Effects of smoking on PBMC apoptosis rate (see Fig. 2)

Because of the variation in smoking habits among the groups, the PBMC apoptosis rate was analyzed by smoking years and smoking index stratification methods. As shown in Fig. 2.1 and Fig. 2.2, the PBMC apoptosis rate did not show significant changes with smoking years and smoking index.

Results of regression analysis (see Table 2)

The multiple linear stepwise regression method was used to find the factors that influenced the PBMC apoptosis rate. The dependent variables were the early apoptosis rate and late apoptosis rate, while the independent variables included 1-OH-Py, age, coke operation years, years of smoking, years of alcohol consumption, smoking index and drinking index. The entry and removal criteria were 0.05 and 0.10, respectively. As shown in Table 2, 1-OH-Py, coke operation years and years of alcohol consumption were included in the early apoptosis rate regression equation. In addition, 1-OH-Py and coke operation years were included in the late apoptosis rate regression equation. Moreover, 1-OH-Py and coke operation years increased the early and late apoptosis rate; on the other hand, years of alcohol consumption reduced the early apoptosis rate. The regression coefficients are given in text).
given in Table 2.

Stratification analysis results (see Fig. 3 and Fig. 4)

To further clarify the influencing factors of PBMC apoptosis, we analyzed the changes in the early apoptosis rate and late apoptosis rate by stratifying 1-OH-Py, coke operation years and years of alcohol consumption. As shown in Fig. 3, the early and late apoptosis rates significantly increased in association with the urinary 1-OH-Py level ($p<0.05$) and the coke operation years ($p<0.05$). However, the early PBMC apoptosis rate was reduced in the subgroup with 15–35 years of alcohol consumption (Fig. 4).

Discussion

PBMCs play an important role in maintaining immune function. Apoptosis is the main reason for reduction of the number of PBMCs. Excessive or reduced PBMC apoptosis disturbs the body’s immune function and results in disorders. Therefore, PBMC apoptosis is generally used as a potential biomarker of the body’s immunity.

Usually, PBMC apoptosis increases with age. Potestio et al. reported that older people’s PBMCs undergo apoptosis more easily$^8$. A previous study detected PBMC apoptosis (Annexin V/PI double stained) in 20 healthy adults (8 males and 12 females) with a mean age of $37.2 \pm 7.56$ yr and healthy elderly (12 males and 8 females) with a mean age of $78.7 \pm 9.57$ yr, and found that the in vitro apoptosis rate ($14.90 \pm 4.12\%$) in the elderly was significantly higher than that ($8.12 \pm 3.12\%$) in the adults$^9$. In the present study, the coke oven workers’ PBMC apoptosis rates were similar to those of the elderly group ($14.90 \pm 4.12\%$), and were higher than both the same age adult group ($8.12 \pm 3.12\%$) and the controls ($7.06 \pm 3.88\%$). The results indicate that the PBMC apoptosis rate was increased in the coke oven workers.

Also, smoking may interfere with PBMC apoptosis. Cigarette smoke condensation inhibits an early step in the caspase cascade to prevent apoptosis and induces necrosis$^{10}$. It has been reported that a 32-year-old female smoker presented with absolute lymphocytosis with 65% atypical lymphocytes and a total of 1% bilobulated lymphocytes$^{11}$, which was partly due to a defect in lymphocyte apoptosis signaling$^{12}$. The PBMCs of smokers exhibited a delay in cell proliferation kinetics and a decrease in replication index compared with nonsmokers$^{13}$. However, Bijl et al. reported no significant difference in PBMC apoptosis between smokers and nonsmokers$^{14}$. In this study, the PBMC apoptosis rate did not show significant changes in association with smoking years and smoking index,
although smoking habits varied among groups. It is possible that higher levels of B[a]P may obscure the effects of smoking on PBMC apoptosis.

It has been reported that B[a]P augments immunity at a low exposure level and that it activates the genes involved in human PBMCs apoptosis at a high exposure level\(^\text{14}\). The results of environmental monitoring showed that the B[a]P concentration in working places sharply increased in association with the B[a]P exposure level, while 1-OH-Py did not show the same difference among the groups. This can be explained by airborne B[a]P sampling and monitoring in the most polluted sites and time. Urinary 1-OH-Py, a metabolite of pyrene that usually indicates the B[a]P internal exposure level in the body, is influenced by an individual’s metabolic enzymes and dietary factors. Moreover, the biological half-life of 1-OH-Py is 6–35 h. The coke oven workers’ PBMC apoptosis rate was significantly increased in association with the B[a]P concentration and 1-OH-Py level. Further analysis results indicated that the PBMC early and late apoptosis rates were increased in association with the urinary 1-OH-Py level and the workers’ coke operation years. The rate of PBMC apoptosis is dependent on the B[a]P exposure dose and duration. PBMCs are mainly composed of lymphocytes (about 90–95%) which consist of T lymphocytes (70–80%), B lymphocytes (15%) and killer cells (5–10%), natural killer (NK) cell and other null cells\(^\text{15}\). Karakaya et al. reported that PAHs may suppress T lymphocyte proliferation at low and high exposure levels and augment NK cell activity only at low levels of exposure in asphalt and coke oven workers\(^\text{16}\). The present study is limited by not specifying the apoptosis proportion of T- and B-lymphocyte subtypes and NK cells and not providing the numbers of WBCs, lymphocytes, granulocytes and monocytes in peripheral blood, and this imperfection should be investigated in a follow-up study. It still shows that the immune systems of coke oven workers were damaged mainly by B[a]P produced during coke operation. Immune function was suppressed in the coke oven workers by...
B[a]P\textsuperscript{27}, which may increase the body’s susceptibility to diseases\textsuperscript{18}.

The influencing factors of PBMC apoptosis in the coke oven workers were 1-OH-Py level, coke operation years and years of alcohol consumption. 1-OH-Py and coke operation years advanced PBMC apoptosis; however, years of alcohol consumption can reduce the apoptosis rate. To some extent, alcohol could prevent B[a]P-induced PBMC apoptosis or death. Schmitt et al. found that chronic ethanol consumption reduced B[a]P activation in human PBMCs\textsuperscript{19}. Laso et al. reported that alcohol or ethanol intake was associated with decreased NK cell numbers and reduced cytotoxic activity\textsuperscript{20}. Excessive alcohol intake may reduce immune system function and increase risk of lymphopenia and infectious disease susceptibility\textsuperscript{21}. Alcohol can modify immune function via alterations in immune cell numbers and functions, and lead to altered inflammatory responses\textsuperscript{22, 23} and immune suppression\textsuperscript{24}, which are also associated with the mortality of cancer and infectious diseases\textsuperscript{25}.

In short, PBMCs are in a dynamic equilibrium of constant apoptosis and proliferation. Excessive or reduced apoptosis could break down the immune system balance and increase the occurrence of disorders. Therefore, we must protect the health of coke oven workers by reducing the concentrations of B[a]P and other harmful contaminations in the workplace, implementing scientifically reasonable shift systems and other harmful contaminations in the workplace, implementing scientifically reasonable shift systems and altering unhealthy lifestyles including smoking and drinking habits.

**Conclusion**

The increased PBMC apoptosis in the coke oven workers may be caused by B[a]P exposure, which was associated with the 1-OH-Py level, coke operation years and years of alcohol consumption.

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