BIOMEDICAL AND ENVIRONMENTAL SCIENCES **17**, 452-458(2004)

Monitoring of Pollution of Air Fine Particles (PM2.5) and Study on Their Genetic Toxicity

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Objective To compare PM2.5 pollation level between the city of coal-fuel pollution (Taiyuan) and the city of pollution mixed with coal fuels and vehicle exhausts (Beijing), to analyze the concentration of B[a]p and Pb in the pollutants, and to study the DNA damage by PM2.5. **Methods** Air fine particles (PM2.5) were collected in Beijing and Taiyuan by means of the filter membrane method, the concentration of B[a]p and Pb were analyzed by high performance liquid chromatography and atomic absorption spectroscopy respectveily, and the damage of DNA by PM2.5 was detected by single cell gel-electrophoresis (SCGE) using the human lung epithelial cells (A549) as target cells. **Results** The concentration of PM2.5 in the winter of Beijing was 0.028-0.436 mg/m^3, and that in Taiyuan was 0.132-0.681 mg/m^3. The concentration of B[a]p was 0.104 and 0.156 µg/mg on PM2.5 of Beijing and Taiyuan, respectively, whereas the concentration of Pb was 1.094 and 1.137 µg/mg on PM2.5 of Beijing and Taiyuan, respectively. Exposure to PM2.5 at the concentrations of 5, 50, and 200 µg/mL for 12 h and 24 h caused DNA damage of the human alveolar epithelium, and the ratios of the tailing and length of the tail were all significantly different from those of the negative control group ($P<0.05$), and indicated a dose-response relationship. **Conclusion** PM2.5 has certain genetic toxicity.

Key words: Air pollution; Fine particles; DNA damage

INTRODUCTION

The particulate matter is an important air pollutant, and its size, shape and composition are closely related to human health. The different aerodynamic diameters of the particles and the different deposition locations in the respiratory system cause different harmful effects on human beings. The fine particles (PM2.5) indicate the particulate matter with an aerodynamic diameter of 2.5 µm or less, whose surface adsorbs a great quantity of toxic and harmful materials which deposit in alveoli and enter the blood circulation system by lung air exchange to reach the other organs, finally causing the damage of structure and function in the respiratory and other systems. Many epidemiological data suggest that increase of the fine particle concentration is closely related to the rising of the incidence and mortality of diseases, especially the diseases of respiratory and cardiovascular systems[1-5]. Based on the knowledge of PM2.5 hazard to health, the fine particles are paid widespread attention all

[1]This work was supported by National Natural Science Foundation of China (project No. 20077033).

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0895-3988/2004
CN 11-2816
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over the world. The U. S. Environmental Protection Agency (EPA), issued an air quality standard for fine particles in 1997[6], defining the yearly mean concentration as 0.015 mg/m^3 and the daily mean concentration as 0.065 mg/m^3. Since air pollution of the fine particles is serious in China, studies on its characteristics and impact on human health have been extensively developed.

This study investigated and analyzed the air pollution situation with fine particles in Beijing and Taiyuan cities, and explored the damage to DNA of human alveolar epidermic cells (A549) by PM2.5 and its genetic toxicity so as to provide scientific basis for the evaluation of biological effects of PM2.5.

MATERIALS AND METHODS

Collection and Preparation of Samples

Selection of sampling sites Taiyuan as a city characterized by coal-fume pollution and Beijing as a city characterized by mixed pollution of coal-fume and vehicle-exhaust were selected as the sampling sites, where particulate matters were consecutively collected from their respective residential areas during the cold winter. The sampling period was from November 2000 to February 2001.

Collection of samples The sampler with 80 L/min flow rate TSP/PM10/PM2.5 produced by Beijing Geological Instruments Factory was used. The glass fiber filter membrane with an effective diameter of 80 mm produced by Shanghai Hongguang Paper Mill was used. Before sampling, the filter membrane should be baked for 2 h at 550℃ in Muffle furnace, and kept for 24 h at 25℃ with a humidity of 50% after cooling and weighed. The filter membrane should be kept for 24 h under the same conditions, and weighed after sampling. The particulate mass concentration was calculated according to the mass difference of the filter before and after sampling and the sampling volume.

Analysis of samples The concentration of B[a]p and Pb were analyzed by high performance liquid chromatography and atomic absorption spectroscopy, respectively.

Preparation of samples The filters carrying the particulates were immersed in distilled water, shaken repeatedly on an ultrasonic shaker, and filtrated through a multi-layer gauze. The filtrate was centrifuged for 20 min at 4℃, 10 000 r/min. The supernatant was drawn out for further ultrasonic shaking to collect the low layer of particulates, and finally the concentrated supernatant and all the low layers of particulates collected were pooled and lyophilized in vaccum, and kept in an ultra-low freezer. The particulate suspension of 5 mg/mL was prepared with tri-distilled water, and homogenized by ultra-shaking for 15 min before use.

Cell Culture and Contamination

The human alveolar epidermal cells (A549) were purchased from the Cell Center, Institute of Basic Medicine, Chinese Academy of Medical Sciences. The cell density was adjusted with Mcoy'5A liquid medium with 10% fetal calf serum to 2×10^5 cells/mL, plated into a 24-well plate with 1 mL of culture medium per well and the cells were cultured to log growth phase. The cells of log growth phase were rinsed twice with D-Hank's solution, into which the suspension of particulates was added to contaminate the cells, and the final concentrations of particulates were 5 μg/mL, 50 μg/mL and 200 μg/mL, respectively. At the same time, didistilled water and potassium dichromate were set up as the negative and positive control, respectively, incubated for 12-24 h at 37℃, 5% CO_2. The cells were

collected after contamination with the particulates, stained with trypan blue for observation of cell viability rate in order to evaluate the cell toxicity of the particulate samples. The concentrations without notable toxicity were selected for the comet experiment.

Comet Assay

Preparation of slides One hundred and twenty μL of 1.5% normal agarose was poured onto a glass slide as the first layer of gel, and then covered with a cover slip. After solidification of the gel, the cover slip was removed, 70 μL of 0.5% low melting agarose containing 10^5 cells was added as the second layer of gel, and the cover slip was removed after solidification at 4℃. Seventy μL of 0.5% low melting agarose was added as the third layer of gel and then a cover slip was covered until solidification of gel.

Lysis The slides were submersed in a freshly prepared precooled lysis buffer and then kept for light-avoidance lysis for 2 h in a 4℃ refrigerator, so as to disrupt the cell membrane and remove the cytoplasm, with the nuclear DNA left.

Unwinding The slides were taken out of the lysis buffer when the lysis finished. Then they were put in a newly prepared electrophoresis buffer. The buffer surface was made 2 mm higher than the gel. The slides were kept for light-avoidance unwinding for 30 min in order to unwind DNA double helix.

Electrophoresis The electric voltage and current were adjusted to 20 V and 250 mA, respectively, and then the slides were subjected to electrophoresis at 15℃ for 30 min. The damaged DNA migrated from nuclei to the anode. The level of DNA damage was assessed based on the total length of the tail formed by damaged DNA.

Neutralization, staining, and observation of the results After electrophoresis, the slides should be immersed in 0.4 mol/L Tris-HCl for 20 min, and then rinsed three times to neutralize the alkali. Twenty μL of staining solution dropped on each slide and was covered with a cover slip and then the results were observed by fluorescence microscopy. Three hundred cells were randomly counted for each slide, the percentage of the tailing cells was calculated and the cells should be photographed. The length of the tail was determined by means of special analysis software; at least 30 cells were analyzed for each group and the average length of DNA migration in each group was calculated.

Statistical Analysis

The data were analyzed by means of Excel and SPSS software, and the tailing rate and DNA migration length of each group were calculated and analyzed by χ^2 test and variance analysis. *P* value less than 0.05 was considered statistically significant.

RESULTS

Comparison of Particulate Pollution Levels Between Two Cities

The results of fine particles in two cities during the winter period are shown in Table 1. At present, we have no standard of fine particles. According to the standard of daily average value of PM2.5 published by U. S. EPA in 1997, the percentage was 100% and 90.6%, and the average value was 4.23 (2.04-10.47) and 2.62 (1.08-6.72) in Taiyuan and Beijing, respectively. Compared with the second grade of China air quality Standard, the percentage of PM10 daily average value in Taiyuan was 100%, and the average value was 2.67 (1.71-3.72), the ratio of TSP daily average value reached 100%, and the average value was

2.76 (1.76-3.60).

Except a few special days, the daily PM2.5 average value of Taiyuan was significantly higher than that of Beijing, and the level of PM2.5 in Taiyuan was 2.09-5.90 times higher than the US Standard. Except one day, all the values on the other monitored days were 1.25-5.47 times higher than the standard, which was consistent to the above-mentioned results of the winter (Fig. 1). All the data showed that the pollution of PM2.5 was serious in Beijing and Taiyuan, and that for Taiyuan was particularly serious.

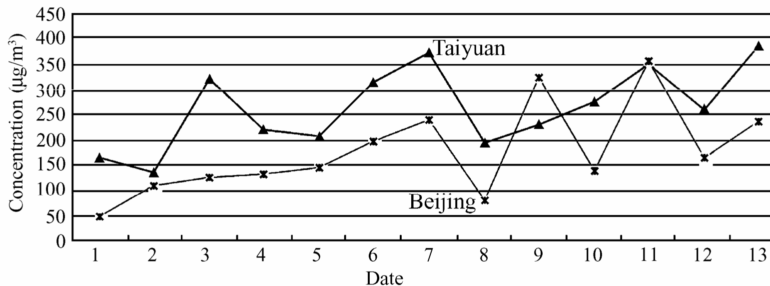

FIG. 1. Comparison of the daily PM2.5 average values in the same days of January 2001 between Beijing and Taiyuan.

TABLE 1

Pollution Levels of PM2.5, PM10, TSP in Taiyuan and PM2.5 in Beijing

City/Category of Particulates		Days of Sampling	Days of Over STD	Over STD (%)	Range (mg/m^3)	Daily Average Value(mg/m^3)	Times Over STD
Beijing	PM2.5	64	58	90.6	0.028-0.436	0.169±0.084	2.62(1.08-6.72)
Taiyuan	PM2.5	38	38	100	0.132-0.681	0.275±0.115	4.23(2.04-10.47)
Taiyuan	PM10	7	7	100	0.255-0.557	0.401±0.132	2.67(1.71-3.72)
Taiyuan	TSP	7	7	100	0.352-0.721	0.551±0.084	2.76(1.76-3.60)

Analysis of Concentration of B[a]p and Pb on Different Particulate Diameter

Table 2 shows that the smaller the particulate diameter, the higher the concentration of B[a]p and Pb was. The concentration of B[a]p and Pb on TSP, PM10, and PM2.5 in Beijing was higher than that in Taiyuan.

DNA Damage by PM2.5

The tailing cells scarcely appeared in the negative control group, however, the tailing cells and DNA migration were obviously shown in the positive control group, which illustrated DNA fragmentation occurred by the action of external chemicals. Toxicant exposure with PM2.5 at the concentrations within 5-200 μg/mL for 12 h or 24 h might cause DNA damage of human alveolar epidermic cells, the tailing rate was significantly different from the negative control group ($P<0.05$) and both the tailing rate and the length of the tail in this group displayed dose-response relationship. In the meantime, the toxicant exposure for 24 h was more serious than that for 12 h.

TABLE 2

Concentration of B[a]p and Pb on Different Particulate Diameter

City/Category of Particulates	Concentration of B[a]p (μg/mg)	Concentration of Pb (μg/mg)
Taiyuan PM2.5	0.156	1.137
Taiyuan PM10	0.092	1.054
Taiyuan TSP	0.077	1.037
Beijing PM2.5	0.104	1.094
Beijing PM10	0.072	0.948
Beijing TSP	0.046	0.606

TABLE 3

DNA Damage of Human Alveolar Epidermis Cells by Contamination of PM2.5 for 12 h

Group	Dose (μg/mL)	Normal Cells Number	Tailing Cell Number	Tailing Rate (%)	DNA Migration Length ($\bar{x}\pm s$, μm)
Neg. Contl.	0	277	23	7.67	2.35±0.99
Pos. Contl.	0.001[#]	173	127	42.33[a]	32.88±2.08[a]
Beijing	5	218	77	26.10[a,b]	26.48±1.72[a,b]
	50	175	125	41.67[a,b]	43.03±1.81[a,b]
	200	139	161	53.67[a,b]	58.52±1.72[a,b]
Taiyuan	5	207	95	31.45[a,c]	25.00±1.99[a,c]
	50	188	111	37.12[a,c]	39.61±2.97[a,c]
	200	144	157	52.16[a,c]	49.40±2.28[a,c]

Note. [#]Dose unit was mmol/L; [a]Comparison to negative control, $P<0.05$; [b, c] were the comparison within the same group, respectively, $P<0.05$.

TABLE 4

DNA Damage of Human Alveolar Epidermis Cells by Contamination of PM2.5 for 24 h

Group	Dose (μg/mL)	Normal Cells Number	Tailing Cell Number	Tailing Rate (%)	DNA Migration Length ($\bar{x}\pm s$, μm)
Neg. Contl.	0	271	29	9.60	3.16±0.75
Pos. Contl.	0.001[#]	19	281	93.67[a]	56.83±2.46[a]
Beijing	5	202	98	32.67[a,b]	32.48±2.19[a,b]
	50	170	130	43.33[a,b]	52.66±2.65[a,b]
	200	123	177	59.00[a,b]	68.54±2.79[a,b]
Taiyuan	5	199	101	33.67[a,c]	30.70±1.60[a,c]
	50	67	133	44.33[a,c]	44.28±2.05[a,c]
	200	122	178	59.33[a,c]	58.47±2.53[a,c]

Note. [#]Dose unit was mmol/L; [a]comparison to negative control, $P<0.05$; [b, c]were the comparison within the same group, respectively, $P<0.05$.

DISCUSSION

This investigation showed that the winter pollution of PM2.5 in Beijing and Taiyuan

was serious, and it was apparently more severe in Taiyuan than in Beijing. The coal fuel was one of the important pollution sources of particulate matters. In comparison with Beijing, Taiyuan used coal as the major fuel, so either PM2.5 mass concentration or its ratio was higher in Taiyuan than in Beijing. The fuel used in Beijing was mainly of clean type (e.g. natural gas), which was why PM2.5 mass concentration in Taiyuan was higher than that in Beijing. Since the different ratio of PM2.5 between Beijing and Taiyuan has not been clarified, further research will be necessary.

In recent years, the pollution of fine particles has been increasingly serious. Epidemiological investigations showed that fine particles could cause lung cancer. Experimental studies indicated that fine particles could adsorb many complex components[7-9], such as polycyclic aromatic hydrocarbons and heavy metals (Ni, Cd, Cr), which can directly or indirectly act on DNA and result in DNA damage, breakage or the formation of DNA adduct[10]. In addition, fine particles could also act on cells to produce radicals which indirectly induce the breakage of DNA strands[11,12]. Within the first ten causes of death among the Chinese population, the death rate of respiratory disease occupied the third place in the total urban death rate. Lung cancer is a common cause of death and shows an increasing tendency year after year. Oncogenesis is a multistage process including three stages of initiation, promotion and progression. DNA damage often occurs in the initiation stage, and causes the related gene mutation and initiates the oncogenesis process if the DNA damage is not fully repaired. SCGE test is a technique to detect DNA damage and can monitor the early stage of DNA damage caused by toxicants. This study utilized SCGE test to discuss the genetic toxicity of PM2.5.

There have been studies on the genetic toxicity of the particulates with different diameters and sources[4,11-15], indicating that the genetic toxicity of PM2.5 is higher than that of PM10. The genetic toxicity of organic components in vehicle-exhaust was confirmed by the micronuclei test in China[16]. Both the heavy metals (Pb, Ni, Cd, and Cr) and polycyclic aromatic hydrocarbons in the particulates with different diameters possessed mutagenesis[7,8], and the smaller the particulate diameter, the stronger the mutagenesis. However, these studies were concerned with only part of the particulate components, and so the toxicity of particulates could not be reflected, and the real action of the particulates on human body was very different. The results of this study indicate that fine particles in air possess potential genetic toxicity, and cause DNA damage of the alveolar epidermic cells at a certain range of concentrations and display dose-response and time-effect relationship.

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(*Received October* 10, 2003 *Accepted April* 30, 2004)