STUDIES ON THE EFFECTS OF PM2.5 ON INFLAMMATORY INJURY AND IMMUNE FUNCTION IN YOUNG RATS

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ABSTRACT

Purpose: *To investigate the effects of fine particulate matter (PM2.5) on inflammatory injury and immune function in young rats.*

Methods: *Young rats were randomly divided into 2 groups, with 15 rats in each group. The rats were exposed to PM2.5 or FA, using a "multifunctional aerosol concentration enrichment system" (VACES). The rats were exposed to PM2.5 or FA for 4 weeks. The control (FA) rats were exposed to the same system, except for air filters positioned at the inlet valve to remove all PM2.5 from the air flow. After the exposure, the young rats were sacrificed, and the levels of interleukin-8 (IL-8) in lung lavage fluid were determined using enzyme-linked immunosorbent assay (ELISA) and real-time quantitative PCR (qRT-PCR). Proliferation of splenic lymphocytes in model rats was assayed with methylthiazolyl tetrazolium (MTT) method. Apoptosis was determined using flow cytometry, while immunohistochemical assay was used to measure proliferation-associated protein (PCNA).*

Results: *The expression level of IL-8 in the experimental group was significantly higher than that in the control group ($p < 0.05$). Compared with the control group, PM2.5 significantly inhibited the proliferation of splenocytes and induced apoptosis ($p < 0.05$).*

Conclusion: *PM2.5 provokes persistent inflammatory injury and aggravates immune injury in young rats.*

Keywords: *$PM_{2.5}$, IL-8, Lymphocytes, Apoptosis.*

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Introduction

Studies have shown that the amount of fine particulate matter (PM2.5) in air is larger than that in coarse particulate matter. The smaller the particle size of the fine particulate matter, the deeper it enters the lungs, and the more serious the damage to humans[1-2]. When small size particulate matter enters the human respiratory tract, it damages the lung tissue and induces inflammatory reactions. By activating immune cells and enhancing the function of phagocytes, the body promotes the clearance of particles in lung tissue and repairs lung tissue injury[3-4].

Therefore, inflammatory reaction produced by lung tissue is considered to be a normal defense system of the respiratory system. Studies on mice show that particulate matter with small particle size inhibits the cellular immune function, and exerts some direct toxicity and side effects on T cells[5].

Therefore, in this study, the effect of fine particulate matter on inflammatory injury and immune function of young rats were studied using a model of continuous exposure to fine particulate matter in young rats.

Materials and Methods

Materials

Experimental rats

Sprague Dawley (SD) rats were purchased from Beijing Weitonglihua Co., Ltd., aged 6 weeks, (body weight = 120 g). The rats were raised in a clean animal room, and the experiment began after a few days of adaptation.

Reagent and instrument

Fetal bovine serum was purchased from Gibco Company (USA); Trizol reagent and re-

verse transcription kit were obtained from Promega Company (USA). Interleukin-8 (IL-8), enzyme linked immunosorbent assay (ELISA) kits and immunohistochemical kits were products of Shanghai Beyotime Biological Co., Ltd. Methylthiazolyl tetrazolium (MTT), dimethyl sulfoxide (DMSO) and apoptosis kits were obtained from Sigma Company (USA). Proliferation-associated protein (PCNA) and β-actin antibodies were purchased from Santa Crutz (USA). Particulate matter sampler was purchased from Semerfeld Company (USA).Vacuum-drying agent was obtained from Sigma Company (USA). Quantitative real-time polymerase chain reaction (qRT-PCR) instrument was product of BD Company (USA).

Modeling and identification

The young rats were randomly divided into 2 groups with 15 rats in each group[6]. The rats were exposed to PM2.5 or FA, using a "multifunctional aerosol concentration enrichment system" (VACES) developed by Sioutas and modified by Chen and Nadziejko[14]. The rats were exposed to $PM_{2.5}$ or FA for 4 weeks (26 March 2018 to 26 April 2018). The control (FA) rats in the experiment were exposed to the same system, except for air filters positioned at the inlet valve to remove all $PM_{2.5}$ from the air flow. The rats in the exposed room were fed with commercial mice feed and distilled water, at controlled temperature of 22 ± 2 °C and relative humidity of 40 - 60 %, with the 12-h light/12-h dark cycle. The rats were euthanized on the last day of exposure and the tissue samples were collected for further studies.

Determination of inflammatory factors by ELISA

Pulmonary alveolar lavage was obtained under aseptic conditions and the lavage fluid was used for assay of IL-8 with ELISA according to the kit instructions.

Determination of inflammatory factor mRNA levels by qRT-PCR

The lung tissue of young rats were washed twice in pre-cooled phosphate buffer solution (PBS). Total RNA was extracted from the lung tissues using 1mL Trizol reagent for 10 min in ice, and the RNA was reverse-transcribed into cDNA using reverse transcription reagent. The primers for IL-8 were synthesized by Shanghai Sangon. The PCR was performed using specific primers to amplify the sequences in the IL-8 gene promoter:

IL-8-Forward:

5'-CTTTGTCCATTCCCACTTCTGA-3' and IL-8-Reverse: 5'-TCCCTAACGGTTGCCTTTGTAT-3'.

The primers for β-actin were: β-actin-Forward:

5'-GGGAAATCGTGCGTGACA-3'

β-actin-Reverse:

5'-TCAGGAGGAGCAATGATC-3'.

The target gene fragment was amplified with β-actin as internal reference. The experiment was repeated three times and the mean values of the results were taken. The relative expression level of target gene was calculated with $2^{-\Delta\Delta Ct}$ method.

Determination of lymphocyte proliferation

The spleen of each young rat was taken and the cell suspension was prepared by grinding it in 200 mesh copper mesh. Lymphocyte extraction kit was used to isolate lymphocytes. After detection, lymphocytes that met the requirements of the experiment were selected for subsequent experiments. The lymphocyte density of each group was adjusted to 3×10^3. The cells were incubated in 96-well plates for 48 h. Then, 20 μL MTT was added, and incubated at 37 °C for 4 h. The resultant formazan crystals were solubilized in 200 μL of DMSO, and absorbance was read at 570 nm in a microplate reader.

Determination of lymphocyte apoptosis

According to the instructions of the apoptotic kit, the cell concentration was adjusted to 1×10^6 cells/mL and the cells were incubated for 24 h. Then, 10 μL of Annexin V-FITC and 5 μL of PI were added to each well for 15 min, then apoptosis rate was analyzed by flow cytometry.

Determination of proliferation-associated protein PCNA level

The expression of proliferation-associated protein (PCNA) in spleen lymphocytes was determined according to the instructions on immunohistochemical kits. The tissue section was observed under 400× microscope, and the average optical density of PCNA-positive cells was obtained by CMAIS system. Five views were randomly selected and the average value was taken.

Statistical Analysis

The results are expressed as mean ± standard deviation ($\bar{x}\pm s$). Single factor analysis of variance

(ANOVA) was used to compare multi-group data, while SNK-q test was used to compare data between two groups. All statistical analyses were done with SPSS 22.0 software package. Values of $p<0.05$ were considered statistically significant.

Results

Effects of $PM_{2.5}$ on expression of inflammatory factors in young rats

The results in Table 1 show that $PM_{2.5}$ significantly enhanced the expression of IL-8 in alveolar lavage solution, when compared with the control group $p<0.05$.

Group	IL-8 (pg/mL)	IL-8 mRNA
Control group	103.526 ± 15.412	1.000 ± 0.008
Experimental group	158.963 ± 15.274[a]	2.362 ± 0.251[a]
F	22.989	117.260
p	0.000	0.000

Table. 1: Effect of $PM_{2.5}$ on the expression of inflammatory factors in young rats (x ± s, n= 3).
Note: [a]p<0.05, compared with the control group.

Effect of $PM_{2.5}$ on expression of inflammatory factors in young rats

As shown in Table 2, compared with the control group, $PM_{2.5}$ significantly inhibited the proliferation of spleen lymphocytes ($p<0.05$).

Group	OD_{570nm} value
Control group	0.856 ± 0.078
Experimental group	0.701 ± 0.062[a]
F	32.920
p	0.000

Table. 2: Effect of PM2.5 on the proliferation of spleen lymphocytes in young rats (x ± s, n= 3).
Note: [a]p<0.05, compared with the control group.

Effect of PM2.5 on expression levels of proliferation-related protein PCNA in young rats

Compared with the control group, $PM_{2.5}$ significantly inhibited PCNA expression in spleen lymphocytes ($p < 0.05$; Table 3).

Discussion

The function of the respiratory system is affected by exposure to particles with small particle size, which first cause inflammatory reaction in the lungs. Fine particulate matter can also induce the immune system to produce a variety of cytokines, which can regulate each other, affect biological functions, and form a network of cytokines[7]. The expression of cytokines is related to the duration and extent of the abnormal state of the body, which can be used as a sensitive marker of immune response, and also as a biological marker of the immune toxicity of particles[8-10]. In this study, by collecting fine particulate matter from the atmosphere, $PM_{2.5}$ with different concentrations was made to interfere with young rats, and the model of continuous exposure to fine particulate matter in young rats was set up. The effect of $PM_{2.5}$ on lung inflammatory reaction, proliferation and apoptosis of lymphocytes was observed and the effects of fine particulate matter on inflammatory reaction and immune damage in young rats was investigated.

Group	Optical density value of PCNA
	1st Month
Control group	0.743 ± 0.072
Experimental group	0.628 ± 0.065[a]
F	31.685
p	0.000

Table. 3: Effect of $PM_{2.5}$ on expression levels of proliferation-related protein PCNA in young rats (x ± s, n= 3).
Note: [a]p<0.05, compared with the control group.

It is known that IL-8 is a member of CXC chemokine family (C is cysteine, X is an arbitrary amino acid) and it promotes cell directional migration. When the body is stimulated by fine particulate matter from outside, the fine particulate matter enters the respiratory system, stimulates the immune organs to produce a large number of lymphocytes, macrophages and other inflammatory factors, and inflammatory mediators, thereby inducing the body to produce immune response so as to damage the lungs[11-12].

Studies have shown that IL-8 is an important factor in inflammatory reaction which is involved in the cascade reaction of the cytokine network[13-15]. Inflammatory reaction leads to production of a large number of inflammatory cytokines that activate the coagulation system by inducing neutrophils to adhere to endothelial cells. Thus, a large number of inflammatory mediators and cytokines are released and a "waterfall" cascade reactions is formed which eventually damage multiple organs[16-17]. In this study, it was found that the expression of IL-8 in young rats increased significantly after continuous exposure to fine particulate matter.

Cell proliferation and apoptosis are the key cytological processes in life. They constitute the response of cells to signals within and outside the body, and they also represent two different ways of regulating cell populations. They are active cytological process which show the mechanism of cell life and death[18-20]. Apoptosis of immune cells plays an important role in their homeostasis and regulation. Lymphocytes are important factors in cellular immune function. The results obtained in this study showed that the proliferation and apoptosis of lymphocytes were significantly related to the action of fine particulate matter, suggesting that fine particulate matter can induce persistent inflammatory reaction and have a potential effect on immune function of the body.

Conclusion

Fine particulate matter (PM2.5) induces persistent inflammatory reaction in the lungs of young rats and regulates the proliferation and apoptosis of lymphocytes. These findings provide a theoretical basis for preventing air pollutant-induced damage to humans.

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