DAMAGE OF DIFFERENT PM 2.5 CONCENTRATIONS IN SPORTS VENUES TO THE BODY'S IMMUNE SYSTEM

XUN XU*
Department of Physical Education and Research, Huzhou Vocational & Technical College, Huzhou, 313000, China

ABSTRACT

Objective: *To investigate the damage of different PM 2.5 concentrations in sports venues to the body's immune system.*

Methods: *PM 2.5 particles were collected using a medium flow sampler and prepared into suspension. 36 male Wistar SPF (specific pathogen-free, SPF) rats were selected and classified into the control group, low dose (5 mg·kg^{-1}), medium dose (10 mg·kg^{-1}), and high dose (15 mg·kg^{-1}) groups. Low, medium and high doses of PM 2.5 (respectively 5, 10, 15 mg/kg) were given by intratracheal instillation to establish a non-exposure lung injury model in rats. After one-time intratracheal installation, perform progressive loading treadmill training, and anesthetize the animals after 3 d. Collect macrophages in bronchoalveolar lavage fluid (BALF), and detect the hypersensitive C-reactive protein (hs-CRP), IL-10, IL-1β, and rat macrophage inflammatory protein 1α (MIP-1α), Clara cell protein (CC16) and neutrophil elastase (NE) expression levels in BALF by enzyme-linked immunoassay (ELISA); detect the contents of IgA, IgM, IgG, and complements C3, C4, C5 in BALF.*

Results: *Compared with the control group, as PM 2.5 administration concentration increased, MCP-1, MIP-1α, hs-CRP, IL-10 and IL-1β increased in a dose-dependent manner, while NE and CC16 decreased in a dose-dependent manner, showing statistically significant differences (P<0.05). IgM and IgG contents in BALF were significantly higher compared with the control group, but IgA content change was not statistically significant. Complement C3, C4 and C5 contents in BALF were significantly higher compared to the control group. There was no significant difference between the low and middle dose groups and the control group (P>0.05).*

Conclusion: *PM 2.5 intratracheal instillation destroys the homeostasis of the immune system in rats. Regular exercise can maintain the immune system balance and enhance the body's resistance to PM 2.5.*

Keywords: *Exercise, PM2.5 concentrations, immune system.*

DOI: 10.19193/0393-6384_2021_5_389

Received *March 15, 2020;* ***Accepted*** *June 20, 2021*

Introduction

With the acceleration of urban development and modernization, recent years have witnessed increasingly serious air pollution. Atmospheric particles (PM2.5) seriously affect human life and health, which is directly related to the occurrence of respiratory and cardiovascular diseases and even death[1, 2]. The pathogenicity of PM depends on its size, composition, source, solubility, and its ability to generate active oxygen. It has a small diameter but a large surface area. Therefore, it can carry various toxic substances which accumulate in the pulmonary alveoli after being filtered by the nose. Through the phagocytosis of macrophages, it can pass through the respiratory mucosa and cilia from the alveolar cavity, enter the pulmonary lymphatics and reach the pulmonary lymph nodes. When alveolar macrophages are over-activated, they can release a variety of inflammatory factors and mediators, causing a series of inflammations and ultimately leading to lung injury[3, 4].

PM2.5 comes from external and internal sources. The external sources mainly include stadium doors and windows, the entrance of the wall structure gap, and the ways to bring people in and out of the stadium[5]; the internal sources mainly include particulate matter produced during abrasion of sports equipment, the staff's skin metabolites, the particulate matter carried by breath and clothes, and dust produced by the staff during exercise. The characteristics of particulate matter in the stadium are mainly manifested as complex composition, long accumulation time, difficult dispersion, small concentration fluctuation range, and slow water drawdown rate.

The particulate pollution sources inside and outside the hall together constitute the pollutant distribution network in the hall. The environmental behavior and health effects of atmospheric particles are closely related to their physical and chemical properties, such as quantity concentration, mass concentration, and particle size distribution[6]. The body's immune system is a barrier to prevent the invasion of harmful foreign substances. Therefore, studying the environmental behavior of PM2.5, the transformation behavior in the body, and the damage of different PM 2.5 concentrations to the body's immune system provides references for the feasibility assessment of exercises in a polluted environment and the protection of crowd movement.

Materials and methods

Sampling and suspension preparation of PM2.5

In the school sports center of the author's unit, PM2.5 was collected using particulate matter intelligent sampler TH-150 C (produced by Beijing Zhongxi Huada Technology Co., Ltd.).

When sampling, ensure that there is no accumulation around the sampling point to prevent air circulation and ensure that the collection point is a pollution-free source. To imitate the height of the human breathing belt, the instrument height is 1.5 m, which is the height of the human breathing belt. Take 5 points in a circle area as the selection objects, ensure that the distance between the two points is about 5 m, and maintain the gas flow rate at a constant value following the operation rules of the instrument gas generator, i.e. the distance from the wall is >1 m, the distance from the door and window is >3 m, and the airflow is 1.13 m^3 min^{-1}. Mass concentration of particulate matter was derived based on quality difference and sampling volume of the filter membrane before and after sampling.

After sampling, collected PM2.5 was placed on the glass fiber filter membrane, then the filter membrane was cut into small pieces and immersed in deionized water, followed by ultrasonic vibration 4 times for 30 min each time. After the particle elution, the filtrate was centrifuged at 1000 $r \cdot min^{-1}$ for 20 min (centrifuge radius 59 mm). After vacuum drying, it was weighed and stored at -20°C.

Disinfect and sterilize with 0.9% normal saline to prepare it to the required concentration, ultrasonically vibrate it for 15 min before use to make the poison suspension uniform, and sterilize it for later use.

Exposure experiment dose and method

PM2.5 was poisoned by intratracheal instillation, the indoor temperature was kept between 20~26°C, and the humidity was 44%~70%.

The temperature of the test substance was kept at about 37°C before installation, the toxic suspension was preheated to 37°C, and after ether anesthesia, the experimental animals were divided into three groups, namely the low dose group (5 $mg \cdot kg^{-1}$), the medium-dose group (10 $mg \cdot kg^{-1}$), the high dose group (15 $mg \cdot kg^{-1}$). The experimental group was intratracheally instilled with PM2.5 particle suspension, the rats were exercised for 1 hour, and the control group was given the same dose of normal saline.

Experimental animal grouping and exercise plan

36 healthy adult male Wistar rats of SPF grade at 7 weeks of age were selected, weighing 180~220 g. Feed them adaptively in a conventional breeding cage in the breeding room for 7 d. The indoor temperature was kept at 20~26°C, and the humidity was 44%~70%. The rats were given free access to food and water, and fed in an environment with 12 h light/dark alternation. The contact lasted until 1 hour before feedback of history training.

The rats were randomly divided into 4 groups, namely the control group, low dose (5 $mg \cdot kg^{-1}$), medium dose (10 $mg \cdot kg^{-1}$), and high dose (15 $mg \cdot kg^{-1}$) groups, each with 8 rats. The experimental rats needed to undergo 2 d adaptive training of 10 $m \cdot min^{-1}$, 5 min treadmill training in terms of speed and time points. Then all animals recovered 1 d before training, followed by 15 $m \cdot min^{-1}$ for a total of 15 min (equivalent to 45% of the maximum oxygen consumption), 18 $m \cdot min^{-1}$ for a total of 20 min

(equivalent to 50% of the maximum oxygen uptake), 21 $m \cdot min^{-1}$ for a total of 30 min (equivalent to 65% of maximum oxygen consumption), 24 $m \cdot min^{-1}$ for a total of 40 min (equivalent to 70% of maximum oxygen uptake), 27 $m \cdot min^{-1}$ for a total of 50 min (equivalent to 76% of the maximum oxygen uptake).

Preparation and index test of serum and alveolar lavage fluid (BALF)

After administration, the rats were injected intraperitoneal with 20% uranium urea solution. After anesthesia, the rats were sacrificed.

The abdominal aortic blood was taken out, added with heparin anticoagulant, placed in a 37°C water bath for 5 min, centrifuged for 15 min at 3500 $r \cdot min^{-1}$ to separate the serum and stored in a refrigerator at 4°C for later use. The automatic biochemical analyzer was the Japanese Olympus AU 400 model. Double antibody sandwich method (ABC-ELISA) was used to detect Clara cell protein (CC16), high-sensitivity C-reactive protein (hs-CRP), rat monocyte chemoattractant protein-1 (MCP-1), rat macrophage inflammatory protein 1α (MIP-1α) and neutrophil elastase (NE).

Statistical analysis

SPSS 22.0 was used for statistical analysis of the data, the measurement data was indicated by $\bar{x} \pm s$, and the differences between the groups were compared by one-way analysis of variance. $P<0.05$ suggests a statistically significant difference.

Results

Effect of PM2.5 on immune proteins in serum of different groups

As shown in Table 1, compared with the control group, MCP-1, MIP-1α, and hs-CRP in the low, medium and high dose groups increase in a dose-dependent manner, while NE and CC16 decrease with the increasing dose.

Group	MCP-1/ ($pg \cdot \mu g^{-1}$)	MIP-1α/ ($ng \cdot mL^{-1}$)	IL-10/ (ng/L)	hs-CRP/ ($mg \cdot L^{-1}$)	IL-1β/ (ng/L)	CC16/ ($\mu g \cdot L^{-1}$)	NE ($ng \cdot mL^{-1}$)
Control group	4.14± 0.05	13.08± 0.08	1.39± 0.52	3.87± 1.04	2.94± 3.87	24.21± 1.98	25.81± 2.31
Low dose group	5.29± 0.34	12.87± 1.02	1.69± 0.68	5.32± 1.14	3.24± 1.27	19.32± 1.85	23.58± 3.52
Medium dose group	5.47± 0.69	17.02± 1.21*	2.24± 1.52	8.07± 3.71	5.01± 2.07	17.31± 1.15*	0.99± 2.68*
High dose group	5.67± 0.81	17.21± 1.42*	10.58± 3.08*	11.01± 5.47*	12.39± 3.01*	12.72± 0.68	15.21± 2.99

Table 1: The effect of PM2.5 on immune proteins in rat serum.

The effect of PM2.5 on immune proteins in BALF of different groups

As can be seen from Table 2, compared with the control group, as the PM2.5 instillation dose increases, MCP-1, MIP-1α, and hs-CRP in the low, medium, and high dose groups display an increasing trend, showing statistically significant difference ($P<0.01$), while NE and CC16 decrease with increasing PM2.5 dose.

Group	MCP-1/ ($pg \cdot \mu g^{-1}$)	MIP-1α/ ($ng \cdot mL^{-1}$)	IL-10/ (ng/L)	hs-CRP/ ($mg \cdot L^{-1}$)	IL-1β/ (ng/L)	CC16/ ($\mu g \cdot L^{-1}$)	NE ($ng \cdot mL^{-1}$)
Control group	5.21± 0.02	15.95± 0.31	253.14± 30.16	2.79± 0.54	268.42± 29.42	22.08± 1.99	19.64± 1.92
Low dose group	6.01± 0.04	19.62± 0.88	269.24± 31.68	3.21± 1.37	341.05± 31.08	19.96± 2.05	16.52± 1.57
Medium dose group	6.25± 0.53*	20.27± 3.67	287.15± 35.74	4.99± 2.04	401.55± 34.82	17.51± 2.92*	14.35± 1.05
High dose group	6.97± 0.89	20.69± 2.57*	507.34± 39.53*	7.98± 2.72*	902.14± 40.21*	13.14± 1.82	13.96± 1.31

Table 2: Effect of PM 2.5 on immune proteins in rat BALF.

IgA, IgM, IgG, C3, C4, C5 content in rat BALF by PM2.5 determination

The content of IgM and IgG in BALF is significantly higher compared to the control group. IgA content shows no statistically significant change. The contents of C3, C4 and C5 in BALF are significantly higher compared to the normal saline control group. There is no statistically significant difference between the low, medium-dose groups and the control group, Table 3.

Group	IgA	IgM	IgG	C_3	C_4	C_5
Control group	142.08± 27.14	240.34± 42.58	189.12± 43.52	183.14± 37.05	150.57± 29.31	121.52± 24.05
Low dose group	149.21± 30.51	242.85± 37.24	243.58± 38.54	182.31± 36.92	155.02± 31.82	164.82± 27.25
Medium dose group	139.51± 29.82	254.92± 43.41	256.35± 41.98	194.62± 40.15	160.37± 36.18	174.51± 34.05
High dose group	152.57± 31.05	339.48± 47.55*	335.91± 49.48*	271.38± 45.18*	239.48± 52.97*	249.58± 46.57

Table 3: Comparison of IgA, IgM, IgG, C_3, C_4, and C_5 contents in the rat BALF by PM2.5 (mg/L).

Discussion

At present, among the 500 plus cities in China, merely less than 1% can meet the air quality guidelines of the World Health Organization, so China is facing serious air pollution. Seven of these cities have been rated as the top ten most polluted cities in the world. In particular, harm of PM2.5 closely related to human respiratory

diseases has attracted increasing attention. Studies have shown that PM2.5 of different sources may induce inflammation. After PM2.5 enters the body through the respiratory tract, it will interfere with a variety of physiological activities, inducing cellular inflammation, stimulating oxidative stress, and causing calcium homeostasis imbalance, thereby destroying subcellular structure and function, and leading to the correlation between short-term particle concentration change in disease and death rate[7]. Studies have shown that PM2.5 stimulations can reduce the viability of macrophages and induce the secretion of pro-inflammatory factors (such as IL-10, IL-8, and tumor necrosis factor (TNF-α)). The signal transduction pathway of nuclear factor NF-κB participates in the process of this immune response. Despite numerous studies abroad, there is very little research in China. In order to simulate PM2.5 of different concentrations in atmospheric particulate matter, different doses were set and injected into the bronchus and pulmonary alveoli of experimental rats through the trachea, so that the atmospheric particulate matter PM2.5 was naturally deposited in the rat trachea, bronchus and pulmonary alveoli with different dose levels[8].

Wister rats have a similar reaction to humans after exposure to PM2.5. Therefore, we selected rats as the subjects of our study. Before the experiment, the animals' 24 h condition and various experimental indexes like serum and alveolar slot lotion were observed[9] to guarantee reliability in experimental method. Based on successful model replication, 36 rats were used for experimental research. Studies have shown that with the increase in particle concentration, the phagocytic rate and phagocytic index of macrophages are significantly reduced. According to reports, PM2.5 significantly reduces the phagocytosis of alveolar macrophages in vitro and in vivo, with severe lung immune response as the main pathological feature[10, 11]. Once atmospheric particulate matter PM2.5 binds to the receptors on the surface of macrophages, it can trigger phagocytosis. Exposure of particulate matter from different sources to PM2.5 will result in enhanced AM phagocytosis. The active product attracts more inflammatory cells to PM2.5. The inflammation caused by the accumulation and processing of antigens in this site leads to antigen presentation. This study showed that compared with the control group, the levels of IL-10, IL-1β, MCP-1, MIP-1α, and hs-CRP in serum increased in a dose-dependent manner in the low, medium and high dose groups, while NE and CC16 decreased with increasing dose. Complement components (C3, C4, C5) can also help AM promote the phagocytosis of atmospheric particulate matter PM2.5. While the respiratory system enhances its defenses against atmospheric particulate matter PM2.5, AM also produces a variety of secretory products. Under normal circumstances, IL-10 is at a low level. Under hypoxia, ischemia, infection, poisoning and stress, IL-10 shows significant up-regulation and continuously increased secretion, and tissue damage is closely related to the occurrence and development of diseases[12, 13].

Respiratory diseases will cause mortality. The cell deformation caused by the adhesion, migration, and toxicity of PM 2.5 in tissue cells will drive lipid reorganization of the cell membrane and change its ability to pass through the cell membrane gap. BALF and serum are the representatives of lung tissue and blood circulatory system, whose mechanical barrier will be attacked by PM 2.5. Immune protein is a marker protein of immune stress response. This study found that the effects of different doses of PM 2.5 exposure on the immune indexes of rats can effectively indicate the health risks of exercise rats exposed to environmental pollutants. The changes of MCP-1, MIP-1α, hs-CRP, NE and CC16 further explain that the mechanism of PM 2.5's inflammatory effect on organisms is related to the expression level of immune proteins. Previous studies have shown that free radicals, metals, and organic components of PM2.5 can induce the production of free radicals to oxidize lung cells, which can be the primary cause of body damage. The surface of environmental particles itself will generate free radicals. In addition, PM2.5 surface is rich in iron, copper, zinc, manganese, and other transition elements, as well as polycyclic aromatic hydrocarbons and lipopolysaccharides.

These ingredients can increase the production of free radicals in the lungs, consume antioxidants and cause oxidative stress. Studies have confirmed that reactive oxygen species (ROS) generated by particles (especially water-soluble particles) produce hydroxyl radicals (•OH) by activating metals. Hydroxyl free radicals are the main cause of DNA oxidative damage. When damaged DNA cannot be repaired effectively in time, it can cause teratogenicity, carcinogenicity, mutagenesis and other irreversible damage[14]. Studies have found that particles can not only damage DNA and inhibit DNA repair but also promote the replication of damaged DNA fragments, thereby promoting canceration. For imbalance of intracellular calcium

homeostasis, calcium acts as one of the important second messengers, which mediates and regulates cell function physiologically and pathologically. Abnormally high calcium concentration will activate a series of inflammatory reactions, leading to inflammation and cell damage. PM2.5 can induce excessive production of free radicals or ROS, and reduce the antioxidant capacity of cells, which in turn leads to lipid peroxidation on cell membranes and increased intracellular Ca^{2+} concentration. In addition, increased intracellular Ca^{2+} concentration can further increase the production of free radicals or ROS. Studies have shown that ROS-mediated regulation of intracellular Ca^{2+} concentration may be one of the mechanisms of PM2.5-induced cell damage, which indicates that apoptosis and necrosis are related to the overexpression of Ca^{2+} sensitive receptors[15, 16].

Because PM2.5 is closely related to inflammatory cytokines, it can stimulate the overexpression of many transcription factor genes that cause inflammatory damage and cytokine genes related to inflammation. Studies have found that PM2.5-induced inflammation leads to an increased number of neutrophilic granulocyte. It is reported that exposure to pine dust leads to an increased number of eosinophils, T cells, and mast cells in bronchoalveolar lavage fluid; PM2.5 and its microenvironment affect the phenotype and function of two types of alveolar macrophages. The first type is known as M1 polarized alveolar macrophages, which are mainly induced by Th1 type cytokines (IL-12, IFN-γ) and internal pathogens to promote inflammation. The second type, M2 polarized alveolar macrophages, is closely related to Th2-type cytokines and immunomodulatory cytokines (IL-10) that inhibit inflammation. According to reports, human alveolar macrophages treated with PM2.5 express high levels of M1-related cytokines and low levels of M2-related cytokines (IL-10 and IL-13). These results suggest that cytokines can not only induce migration of neutrophils, T cells, and eosinophils to the lung and other tissues but can also migrate to the lungs by themselves, exhibiting higher cell activity and releasing more inflammatory cytokines and chemokines.

The interaction between inflammatory cells and cytokines can synergistically destroy lung cells. Therefore, the mechanism by which PM2.5 harms human health is still one of the main focuses in many current studies. Epidemiological studies have found that inhalable particulate matter is closely related to the incidence of human diseases and mortality. The "Six Cities Study of Harvard University" published in 1996 indicates that PM2.5 is one cause of abnormal human deaths. This study provides support for the linear correlation between non-accidental deaths and PM2.5, and respiratory diseases account for a large proportion of non-accidental deaths caused by air pollution.

In summary, there is a direct relationship between the body's immune response and PM 2.5 environmental behavior, metabolic toxicology. After the atmospheric particulates in rats are treated with different concentrations of PM 2.5, there are obvious inflammatory and immune changes. Although this inflammatory immune response itself is a defensive and protective response of the body, different PM 2.5 concentrations in sports venues can damage the body's immune system. Regular exercise can increase immune function and reduce PM 2.5 exposure-induced immunosuppression in a way subject to many factors.

References

1) Pam K., Zhao J.Z., Effect of exercise on PM_ (2.5)-induced changes in cellular immune function of mice, Shanghai Journal of Preventive Medicine, 2020, 32(04), 294-298.
2) Li F., You W.H., Liu Y.L., Impact of environmental behavior and acute toxicological effects of PM_ (2.5) in stadiums on immune indexes of exercise rats, Asian Journal of Ecotoxicology, 2019, 14(06), 248-253.
3) Gu N., Zhang G.X., Shi P.C., Study on NLRP3 inflammasome activation of alveolar macrophages in rat model of lung injury induced by PM2.5, Tianjin Medical Journal, 2018, 46(11), 1171-1175 +1257.
4) Zhang X.J., Study on health effects of atmosphere PM 2.5 on outdoor sports fitness crowd, Chinese Journal of Public Health Management, 2017, 33(6), 781-784.
5) Bao Z.J., Hu X., Shen X.Q., The stimulation effect of PM2.5 oxidative stress on the expression of innate immune regulatory factors in the airway epithelium of allergic asthma, Zhejiang Medical Journal, 2018, 40(02), 142-144.
6) Wu J., Yang X.J., Wang J., Liu J., Lu J., Cheng L.F., Immunoregulatory effect of TGF-β_1/Smads signaling pathway on PM2.5-induced lung tissue injury in rats, Northwest Pharmaceutical Journal, 2017, 32(03), 316-321.

7) Li F., Antagonistic effect of zinc-metallothionein on PM_ (2.5)-induced oxidative-immune damage in exercise rats, Journal of Environmental and Occupational Medicine, 2016, 33(12), 1148-1151.
8) Pozzi R., Berardis B.D., Paoletti L., Inflammatory mediators induced by coarse (PM2.5-10) and fine (PM2.5) urban air particles in RAW 264.7 cells, Toxicology, 2003, 183(1-3), 243-254.
9) Ogino K., Nagaoka K., Ito T., Involvement of PM2.5-bound protein and metals in PM2.5-induced allergic airway inflammation in mice, Inhal Toxicol, 2018, 30(13-14), 498-508.
10) Pan M.M., Lv T., Zhang J.C., IL-1β promotes the secretion of angiogenic and inflammatory factors in human dermal fibroblast, Geriatrics & Health Care, 2017, 23(4), 286-289.
11) Song Y., Li F., Liu L., Study on the mechanism by which PM2.5 induces mouse spleen cell NF-ϰB activation through the TLR4-MyD88 pathway, Hebei Medical Journal, 2019, 41(10), 1445-1449.
12) Xian M., Ma S.Y., Wang Y., Wang C.S., Zhang L., PM2.5 undermines the barrier function of cultured normal human nasal epithelium, Chinese Archives of Otolaryngology-Head and Neck Surgery, 2019, 26(7), 386-390.
13) Yan S.Y., Yang B., Sun Z.Y., Chen D.M., Niu J.Y., Xiao C.L., Effect of PM2.5 exposures on the immune function of tumor-bearing lung cancer mice, Journal of Liaoning University of Traditional Chinese Medicine, 2017, 19(02), 126-129.
14) Wang Y.X., Design of risk assessment model for outdoor sports under severe excessive PM_ (2.5), Environmental Science and Management, 2018, 43(02), 186-189.
15) Xu L., Effects of exercise and PM2.5 exposures on liver fat function in rats, Systems Medical, 2018, 3(04), 19-21.
16) Li R.J., Zhao L.F., Wang Z.T., Effect of PM_ (2.5) on lung oxidative damage and inflammation related indexes in exercise rats, Journal of Environment and Health, 2016, 33(02), 100-102.

Corresponding Author:
Xun Xu
Email: zjhzxx01@163.com
(China)