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In vitro responses of rat alveolar macrophages to particle suspensions and water-soluble components of dust storm $PM_{2.5}$ ☆

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Abstract

A study was conducted to investigate the in vitro toxicities of dust storm particulate matter with an aerodynamic diameter $\leqslant 2.5$ μm ($PM_{2.5}$) on rat alveolar macrophages (AM). This was based on the ambient $PM_{2.5}$ collected in March 2004 from Baotou city, Inner Mongolia Autonomous Region, China. The particles were classified as normal (from sunshiny and clean days) and dust storm samples according to the dust storm classification, and the local weather and air quality monitoring data. The cell viability, levels of cellular thiobarbituric acid-reactive species (TBARS), glutathione (GSH), and cytosolic free calcium ions (Ca^{2+}), and the plasma membrane ATPase activities and membrane lipid fluidity were determined 4 h following the in vitro treatment of AM with differing dosages of the collected samples. Results revealed that dust storm $PM_{2.5}$ and their water-soluble fractions at high dosages generated oxidant stress on AM, induced leakage of lactate dehydrogenase (LDH), significantly decreased activities of plasma membrane Na^+K^+-ATPase, increased intracellular Ca^{2+} levels, and led to significant alterations in membrane lipid fluidity, finally resulting in cytotoxicity. A two-way ANOVA showed that there was no significant difference in the above indices measured between the normal and dust storm $PM_{2.5}$, suggesting the deleterious effects of ambient $PM_{2.5}$ from Baotou city were based on the dose used rather than the type of particles. But due to a higher concentration of airborne $PM_{2.5}$ mass concentration in dust storm days than that in normal days, it concluded that during dust storm episodes, a much more serious effect on AM was imminent.

Keywords: Dust storm; Fine particulate matter; Rat alveolar macrophages; Oxidant stress; Cell viability; Calcium ion

1. Introduction

The dust storm is a natural event that is common in the arid and semi-arid regions of the earth's land surface. It is characterized by great wind velocity, lots of dust and sand, and deteriorated visibility (<1 km). Besides destroying construction, causing traffic jam and doing harm to crops, the dust storm also makes adverse effects on human health

Abbreviations: AM, alveolar macrophages; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; GSH, glutathione; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; ANS, 8-anilino-1-naphthalene-sulfonic acid; DPH, 1,6-diphenyl-1,3,5-hexatriene; FBS, fetal bovine serum; ATPase, adenosinetriphosphatase; ROS, reactive oxygen species; RNS, reactive nitrogen species; LPO, lipid peroxidation; TBARS, thiobarbituric acid-reactive species; EGTA, ethylene glycol-bis(β-amino-ethylether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid; HEPES, *N*-2-hydroxy-ethylpiperazine-*N*′-2-ethanesulfonic acid; BSA, bovine serum albumin; Fura-2/AM, fura-2 penta-acetoxymethyl ester.

☆ We assure that the experimental animals in the study were conducted in accordance with Chinese and local institutional guidelines for the protection of animal welfare.

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(Park et al., 2003). Epidemiological studies showed that respiratory diseases and mortality, cardiovascular diseases in the elderly, Al Eskan disease (desert storm pneumonitis) and even stroke were associated with dust storms (Chen et al., 2004; Hefflin et al., 1991; Kwon et al., 2002; Korenyi-Both et al., 1992; Yang et al., 2005).

It is thought that an abrupt increase of airborne fine particulate matter ($PM_{2.5}$; particles $\leqslant 2.5$ μm in aerodynamic diameter) or coarse particles (PM_{10}, between 2.5 and 10 μm in diameter) concentration during dust storm events is responsible for these diseases or deaths because the ambient fine particles can pass easily into the deepest regions of the lungs (the alveoli or alveolar sacs) and no clearance mechanisms effectively remove them (Englert, 2004; Chen and Yang, 2005). Animal experiments in vivo and in vitro have revealed that ambient $PM_{2.5}$ or PM_{10} could result in pulmonary inflammation, airway hyperreactivity, alveolar macrophage impairment and epithelial cell damage (Okeson et al., 2003; Becker et al., 2005; Kwon et al., 2002). In addition, Lei et al. (2004) proposed that the composition of dust storm $PM_{2.5}$ or the microorganisms such as fungi and bacteria adsorbed onto the surface of the particles might contribute to pulmonary inflammation. Becker et al. (2005) proposed that components of Gram positive and Gram negative bacteria were related to the ambient particles, including the ubiquitously present lipopolysaccharide (LPS). By analyzing the chemical composition of ambient $PM_{2.5}$ collected in Beijing city during dust storm occurrence period in April 2000, it was found that the crustal species (ions such as K^+, Ca^{2+}, Mg^{2+}, Cl^- and elements such as Fe, Al, Ca, Ti, Zn) were the primary chemical components in fine particle matter, which accounted for 66.4% of $PM_{2.5}$ concentration, and followed organics while the contribution ratio of SO_4^{2-} and NO_3^- was much less than that in non-dust storm days (Xie et al., 2005). All the researches suggested that compositions of dust storm $PM_{2.5}$ played important roles in their toxicities on health.

The aim of this study was to compare the effects of urban ambient $PM_{2.5}$ from normal and dust storm days on rat alveolar macrophages (AM) and to elucidate the mechanism through which dust storm $PM_{2.5}$ impaired AM. The samples (daily ambient $PM_{2.5}$) were collected in March 2004 from Baotou city since in China, the southern Xinjiang basin, Hexi Corridor and Helan Mountains are some of the regions easily disturbed by strong dust storms, which occur mainly between March and June (Feng et al., 2002). And the effects of normal and dust storm $PM_{2.5}$ (including the total particle suspensions and the concentrated water-soluble components) on AM were investigated. After the cell viability, plasma membrane permeability, membrane lipid fluidity, intracellular calcium ion concentration ($[Ca^{2+}]_i$) and cellular lipid peroxidation (LPO) of rat AM were determined 4 h following the in vitro treatment of AM with differing concentrations of the samples, it was believed that oxidant stress induced by dust storm $PM_{2.5}$ played an important role in destroying cell membranes and making damages on the AM.

2. Materials and methods

2.1. Chemicals

Glutathione (GSH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), dimethyl sulfoxide (DMSO), bovine serum albumin (BSA), 8-anilino-1-naphthalene-sulfonic acid (ANS), 1,6-diphenyl-1,3,5-hexatriene (DPH), trypan blue, ethylene glycol-bis(β-amino-ethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), *N*-2-hydroxy-ethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) and fura-2 penta-acetoxymethyl ester (Fura-2/AM) were purchased from Sigma Chemicals Company. RPMI 1640 medium with L-glutamine, penicillin–streptomycin and fetal bovine serum (FBS) were obtained from Gibco BRL. Adenosine-triphosphatase (ATPase) and lactate dehydrogenase (LDH) detection kits were bought from Nanjing Jiancheng Bioengineering Institute, Nanjing city, China. All other reagents and solvents used were of the analytical grade. The solutions were prepared with deionized water purified from a Milli-Q purification system (Millipore, American).

2.2. Sampling

The sampling site was located in Baotou city (109°50′–111°25′E longitude, 41°20′–42°40′N latitude), Inner Mongolia Autonomous Region, China (Fig. 1). $PM_{2.5}$ high volume air sampler (Thermon Anderson) was placed on the rooftop of a building about 10-metre tall and there were no large obstacles and no large pollution sources near it.

Samples (airborne $PM_{2.5}$) were collected on quartz filters (Whatman, American) daily from March 1 to March 31, 2004. The flow rate of the sampler was 1.13 m^3/min (±10%) and the sampling time was nominally 24 h with sampling starting at 5:00 p.m. The filters loading particles were wrapped in aluminium foil and sealed and stored in a refrigerator at −20 °C. According to data from the local

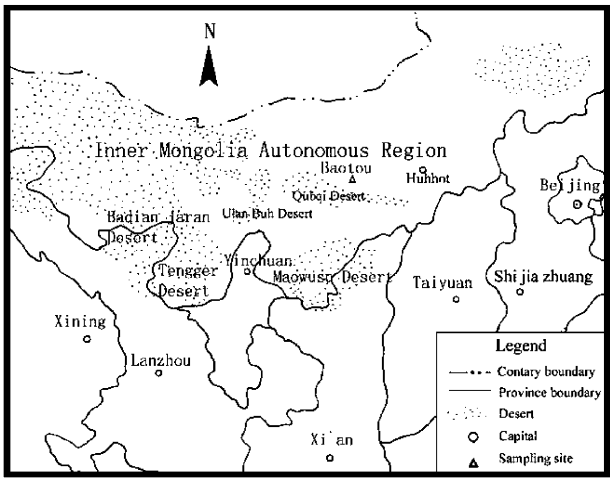

Fig. 1. The sketch map of airborne $PM_{2.5}$ sampling site (a part of China map).