Original Article

The Cellular Toxicity of $PM_{2.5}$ Emitted from Coal Combustion in Human Umbilical Vein Endothelial Cells*

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Abstract

Objective To explore the relationship between different components of fine particulate matter ($PM_{2.5}$) emitted from coal combustion and their cytotoxic effect in the vascular endothelial cells.

Methods Coal-fired $PM_{2.5}$ was sampled using a fixed-source dilution channel and flow sampler. The sample components were analyzed by ion chromatography and inductively coupled plasma atomic emission spectroscopy (ICP-AES). The $PM_{2.5}$ suspension was extracted using an ultrasonic water-bath method and then human umbilical vein endothelial cells (*EA.hy926*) were treated with various concentrations of the $PM_{2.5}$ suspension. Cell proliferation, oxidative DNA damage, and global DNA methylation levels were used to measure the cellular toxicity of $PM_{2.5}$ emitted from coal combustion.

Results Compared to other types of coal-fired $PM_{2.5}$ preparations, the $PM_{2.5}$ suspension from Yinchuan coal had the highest cytotoxicity. $PM_{2.5}$ suspension from Datong coal had the highest toxic effect while that from Yinchuan coal had the lowest. Exposure to coal-fired $PM_{2.5}$ from Jingxi coal resulted in lower 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels. At the same dose, $PM_{2.5}$ emitted from coal combustion could produce more severe DNA impairment compared to that produced by carbon black. Cell survival rate was negatively correlated with chloride and potassium ions content. The 5-methylcytosine (5-mC) level was positively correlated with Mn and negatively correlated with Zn levels. The 8-OHdG% level was positively correlated with both Mn and Fe.

Conclusion $PM_{2.5}$ emitted from coal combustion can decrease cell viability, increase global DNA methylation, and cause oxidative DNA damage in *EA.hy926* cells. Metal components may be important factors that influence cellular toxicity.

Key words: $PM_{2.5}$; Coal combustion; Vascular endothelial cell; Cytotoxicity; DNA methylation

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INTRODUCTION

In 2004, the American Heart Association (AHA) published its first scientific statement concluding that exposure to particulate matter (PM) air pollution contributes to cardiovascular morbidity and mortality[1]. Increasing evidence has shown that the overall absolute risk for mortality attributable to PM air pollution is much higher for cardiovascular-caused than for

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respiratory-caused[2-3]. Heart rate, blood pressure, myocardial infarction, and atherosclerosis are the main health outcome posed by PM exposure[4]. Research has shown that the main pathway by which PM contributes to increased cardiac risk is by promoting atherosclerotic progression via vascular endothelial cell injury[5-10]. Studies have shown that emission from coal burning is mainly responsible for fine particle pollution in China[11-14]. Thus, this study was aimed at exploring the relationship of different components of $PM_{2.5}$ emitted from coal combustion and the cytotoxic effect in the vascular endothelial cells by evaluating cell proliferation, oxidative DNA damage, and global DNA methylation levels.

MATERIALS AND METHODS

Coal Samples and Technical Analysis

Raw coal from four typical coal fields (Yinchuan, Datong, Jingxi, and Zhijin) in China was purchased from state-owned coal mines[15]. Volatile matter, one of the main indexes in the technical analysis of coal, was determined on a dry basis[16].

Sample Collection of Coal-fired $PM_{2.5}$

$PM_{2.5}$ emitted from coal combustion was sampled by the dilution tunnel system, which was designed and utilized to measure and analyze the emission status and characteristics of particulate matters emitted from stationary sources[16-17]. The dilution tunnel system had a dynamic dilutor to introduce flue gas into the smog chambers to avoid the loss of particles. Dilution and sampling continued until the combustion finished and the coal samples were broken into pieces, after which they were ignited in the stove.

Emission Characteristics of Coal-fired $PM_{2.5}$

Particle size distribution and concentration were measured using Model 3090 EEPS™ (TSI, USA). Water-soluble inorganic ions and metal elements were analyzed by ion chromatograph (761 Compact IC, Metrohm, Switzerland) and mass spectrometer (DRC-e, PerkinElmer, USA).

Sample Extraction

The $PM_{2.5}$ filters were extracted with ultra-pure water in an ultrasonic bath. After ultrasonic elution and freeze-drying, coal-fired $PM_{2.5}$ suspension was prepared and stored at -20 ˚C until it was prepared for cell exposure. The effective particle weight after the extraction divided by the total weight of the particles on the filter was determined to calculate the extraction efficiency.

Cell Line and Cell Culture

Human umbilical vein endothelial cell line (*EA.hy926*) was purchased from ATCC (CRL-2922, Manassas, Virginia, USA). When 80% confluency was achieved, the cells were washed with phosphate-buffered saline (PBS), treated with 0.25% parenzyme (Sigma, USA), and split in a 1:3 ratio of culture to Dulbecco's Modified Eagle's Medium (DMEM) plus 10% fetal bovine serum (FBS). The cells were maintained at 37 °C and 5% CO_2[18].

Experimental Groups

The experimental design had six separate treatments consisting of the following: a solvent control group (PBS); $PM_{2.5}$ emitted from Yinchuan coal (YC); $PM_{2.5}$ emitted from Datong coal (DT); $PM_{2.5}$ emitted from Jingxi coal (JX); $PM_{2.5}$ emitted from Zhijin coal (ZJ); and $PM_{2.5}$ carbon black control group (CB, Degussa, Germany). *EA.hy926* cells were treated at concentrations of 0 (PBS), 10, 25, and 50 μg/mL for 24 h.

Cell Proliferation Assay

Cell viability was determined by MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay kit, Promega, USA) according to the manufacturer's instruction[19]. The MTS reaction was measured using a microplate reader to measure the absorbance at 490 nm.

DNA Extraction

DNA was purified using Cell/Tissue DNA Extraction Kit (A&D Technology Corporation, Beijing, China) according to the manufacturer's instruction. DNA concentration and purity was determined by comparing the ratio of optical density measurements at 260 and 280 nm.

Determination of Global DNA Methylation

Global DNA methylation was determined using the Methyl Flash Methylated DNA Quantification Kit (Epigentek, New York, USA) according to the manufacturer's instruction[20]. The kit measures the methyl-cytosine content as a percentage of total cytosine content.