Resveratrol Ameliorates PM$_{2.5}$-Induced Detrimental Effects in CHO Cell

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Abstract—PM$_{2.5}$ was proved to cause adverse health effects and this was related to oxidative stress and inflammatory responses. Resveratrol has many beneficial activities that may ameliorate PM$_{2.5}$ caused injury. An in vitro experiment was conducted where CHO cells were exposed to PM$_{2.5}$ with or without resveratrol pretreatment. Results showed PM$_{2.5}$ caused a dose-dependent decrease in cell viability and cell apoptotic rate at concentrations from 25 to 800 μg/mL. Pretreatment with resveratrol reduced PM$_{2.5}$-induced apoptosis in cells and the optimal effect occurred at 6.25 μmol/L. Resveratrol pretreatment also significantly shortened DNA tail lengths of comet cells with optimal dosage at 25.00 μmol/L. PM$_{2.5}$ caused a significant increase in Akt activation level, resveratrol pretreatment reduced the activation rate. These results suggested that resveratrol protected CHO cells from PM$_{2.5}$ induced cell injury and DNA damage. This protective effect was shown to be related to Akt pathway.

Index Terms—resveratrol, PM$_{2.5}$, cytotoxicity, DNA damage, Akt activation

I. INTRODUCTION

Resveratrol (3,4',5-trihydroxystilbene) is a plant-derived polyphenolic compounds that highly enriched in grapes, peanuts, red wine and other food sources [1]. Researches on the physiological effects of resveratrol have shown that it has many beneficial properties including anti-oxidant, anti-inflammatory, anti-leukemic, and neuroprotective function[2-4], so resveratrol has cardiovascular protection, anti-cancer activity and others [5, 6] and its protective mechanisms were related to preventing DNA damage, regulating intracellular pathways, apoptosis, cell cycle arrest and differentiation of different cancer cell lines and tumor models[7].

Epidemiological studies have shown a correlation between adverse health effects and PM$_{2.5}$ (particulate matters with air-dynamic diameter less than 2.5 micrometers) and its exposure [8-11]). The International Agency for Research on Cancer (IARC) Working Group has classified outdoor air pollution and particulate matter as carcinogenic to humans (IARC Group 1) [12]. PM$_{2.5}$ can induce oxidative stress, subsequently activate many signaling pathways such as PI3K/Akt, NF-κB, MAPKs, inflammatory cytokines expression that will then cause3es cell cycle alteration, DNA lesions, cell apoptosis and other cell damages [13-18].

There is limited information about the mechanism by which the natural phytochemicals prevent the detrimental effects of PM$_{2.5}$. Our previous studies have shown the natural extracts from lycopene, bamboo leaf and lingonberry attenuated the cytotoxicity of PM by increasing the cell viability, reducing the apoptotic rate and preventing DNA damage[19, 20]. In this study, Chinese Hamster Ovary (CHO) cells were pretreated with the resveratrol before being exposed to PM$_{2.5}$. Cell proliferation, apoptosis, DNA damage and Akt expression were determined after treatments.

II. MATERIALS AND METHODS

A. Chemicals

F-12 medium and Fetal Bovine Serum (FBS) were purchased from GIBCO Co. (USA). Quartz filters (203×254mm) were purchased from Whatman Co. (USA). Hoechst 33342 dye and Thiazolyl Blue Tetrazolium Bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis. MO., USA). Annexin V-FITC Apoptosis Detection Kit was obtained from Beijing Biosea Biotechnology Co. (Beijing, China). Resveratrol (3, 4, 5-trihydroxy-stilbene, Res.) stock solution (100 mM) was prepared by dissolving in DMSO (Sigma).

B. Cell Culture

The CHO-Akt-EGFP cells were purchased from GE Healthcare (USA). They were routinely cultivated in Ham’s F-12 medium (GIBCO) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 1% L-glutamine, 100 IU/mL penicillin and 100 μg/mL.
streptomycin at 37 °C in a humidified incubator containing 5% CO₂.

C.  **PM$_{2.5}$ Sample Collection and Preparation**

PM$_{2.5}$ samples were collected with Quartz filters using the TH-1000 TSP high volume sampler (Wuhan Tianhong Instrument Co., Ltd, Wuhan) for 22 h with 2 h interval in 2010 winter and were extracted by sonicating for 1 min (below 25°C) with 50 mL sterilized pure water. PM$_{2.5}$ suspensions were dried in a freeze vacuum and stored at -20°C.

D.  **Cell Viability**

CHO cells were seeded in 96-well plate with a density of 1×10$^4$ cells/mL and incubated overnight. Then treated with PM$_{2.5}$ for 24 h or pre-treated with resveratrol for 6 h, followed by addition of PM$_{2.5}$ and continue incubated for 20 h. After the treatment, decanted the drugs and cell viability was assessed by the MTT assay.

E.  **Apoptosis Assay**

Cell apoptosis was detected by annexin-V/PI assay by using flow cytometer and the results were assessed by apoptotic cells rate.

F.  **Single Cell Gel Electrophoresis (Comet Assay)**

DNA strand breakages were detected by Comet Assay. The comet cells were observed using a fluorescent microscope (Leica Microsystems, Wetzlarator). For each treatment, at least 100 CHO cells were evaluated by the IMI 1.0 software automatic image analysis system (Shenzhen CDC, China). The comet cell images were digitized and tail length was determined to evaluate the degree of DNA damage.

G.  **Akt Activation**

The Akt activation was measured with IN Cell Analyzer 1000 system (GE Healthcare). IGF-1 (agonist of AKT1) and Wortmannin (antagonist of AKT1) were applied as positive control and negative control, respectively. The plasma membrane spot analysis module was used to determine the level of active Akt signal and the fluorescence intensity. The Akt activity was represented as the percentage of the cells containing green fluorescence among all counted cells.

H.  **Statistical Analysis**

All data were analyzed using SPSS.15.0 and presented as mean ± S.D. The statistical differences were determined by ANOVA. Least Significant Difference (LSD) was used to compare the effects between the exposed group and the control group (significance P <0.05 or P <0.01).

III.  **RESULTS**

A.  **Effects of PM$_{2.5}$ and Resveratrol on Cell Viability**

25µg/mL-800µg/mL PM$_{2.5}$ caused a concentration dependent decrease in cell viability (r=-0.840, p<0.05). The cell viability was approximately 70% at 100 µg/mL PM$_{2.5}$ which was set as the PM$_{2.5}$ exposure concentration (Fig. 1A). Resveratrol also caused a dose-dependent decrease in cell viability (r=-0.925, P<0.01) (Fig. 1B) and 6.25µg/mL - 25.00 µmol/L resveratrol had no significant effects on CHO cell viability and were used in the further experiments. But 50.00 µmol/L or higher concentrations of resveratrol significantly decreased cell viability (P<0.01). IC$_{50}$ value of resveratrol was 64.90µmol/L.

![Figure 1](image1.png)

**Figure 1.** Effects of PM$_{2.5}$ and resveratrol on CHO cell proliferation

Note: * P<0.05, ** P<0.01, compared to untreated control.

B.  **PM$_{2.5}$ - Induced CHO Cell Apoptosis and the Anti-apoptosis Effect of Resveratrol**

![Figure 2](image2.png)

**Figure 2.** PM$_{2.5}$ induced CHO cell apoptosis and the anti-apoptosis effects of resveratrol

Note: **P<0.01, compared to untreated control, ##P<0.01, compared to 100 µg/mL PM$_{2.5}$. 

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As shown in Fig. 2A, PM$_{2.5}$ induced a dose-dependent CHO cell apoptosis (r=0.964, P<0.01). When the concentration raised to 50 μg/mL or higher, cell apoptotic rate was increased significantly (P<0.01). Apoptotic rate was increased to 26.97 % at 100 μg/mL PM$_{2.5}$. The anti-apoptotic effects of resveratrol on PM$_{2.5}$-induced apoptosis were shown in Fig. 2B. The results indicated that the apoptotic rates were significantly decreased to 1.69 %, 2.54 % and 7.74 % compared to PM$_{2.5}$ caused apoptosis (P<0.01), when pretreated with 6.25 μmol/L, 12.50 μmol/L and 25.00 μmol/L resveratrol, respectively. The result suggested that resveratrol protects CHO cells against PM$_{2.5}$-induced apoptosis.

C. Effects of Resveratrol on PM$_{2.5}$-Induced DNA Damage

Results of Comet assay (Fig. 3) showed that the comet tail length of PM$_{2.5}$ treated cells was significantly increased from 22 μm to 134 μm (P<0.01). When CHO cells pre-treated with resveratrol, the tail length was decreased significantly, which indicating the protective effect of resveratrol on PM$_{2.5}$-induced DNA damage. The most effective concentration of resveratrol was 25.00 μmol/L.

![Figure 3. Effects of resveratrol on PM$_{2.5}$-induced DNA damage](image)

**Note:** A: untreated control; b: 100 μg/mL PM$_{2.5}$ alone; c: 6.25 μmol/L Res $+$ 100 μg/mL PM$_{2.5}$; d: 12.50 μmol/L Res $+$ 100 μg/mL PM$_{2.5}$; e: 25.00 μmol/L Res $+$ 100 μg/mL PM$_{2.5}$; * P<0.05, ** P<0.01, compared to untreated control, ###P<0.01, compared to 100 μg/mL PM$_{2.5}$ treatment.

D. Effects of Resveratrol on PM$_{2.5}$-Induced Akt Activation

By detecting the fluorescence expression using IN Cell Analyzer 1000 system in CHO-Akt-EGFP cells, the Akt activation status can be quantified. As expected, IGF-1 promoted Akt activation and Wortmannin inhibited Akt activation significantly. PM$_{2.5}$ significantly increased Akt activation rate (P<0.01) and resveratrol inhibited the Akt activation rate increased by PM$_{2.5}$ with the highest inhibitory effects at 12.50 μmol/L resveratrol pre-treatment cells (Fig. 4).

![Figure 4. Effects of different concentrations of resveratrol on PM$_{2.5}$-induced akt activation](image)

**Note:** * P<0.05, ** P<0.01, compared to untreated control ###P<0.01, compared to 100 μg/mL PM$_{2.5}$ treatment.

In conclusion, PM$_{2.5}$ significantly caused a decrease in CHO cells viability, and an increase in apoptosis and DNA damage (P<0.01). Resveratrol was shown to reduce the cell damage caused by PM$_{2.5}$. Results from this study indicated Akt pathway is involved in PM$_{2.5}$ mediated cytotoxicity. Resveratrol plays a protective role by alleviated the over activation caused by PM$_{2.5}$. This finding provided evidence that bioactive substance such as resveratrol may help to prevent health problems caused by air pollution.

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REFERENCES


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