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

Chen Guo
School of Public Health, Peking University, Beijing 100191, China

Xiaohong Zhao, Shijie Chen, Yanli Zhou, and Shengquan Mi
Research Institute for Science and Technology of Functional Food, Beijing Union University, Beijing 100191, China
Email: xiaohong@buu.edu.cn

Ter-Yun Lin
Biology Department, North Park University, Chicago, IL 60625, USA

***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 caus3es 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.) (Sigma) 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 mg/mL

Manuscript received July 1, 2017; revised October 12, 2017.

doi: 10.18178/ijfe.4.2.161-164

streptomycin at 37 ℃ in a humidified incubator containing 5% CO_2.

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℃) with 50 mL sterilized pure water. $PM_{2.5}$suspensions were dried in a freeze vacuum and stored at -20℃.

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 withPM2.5 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.

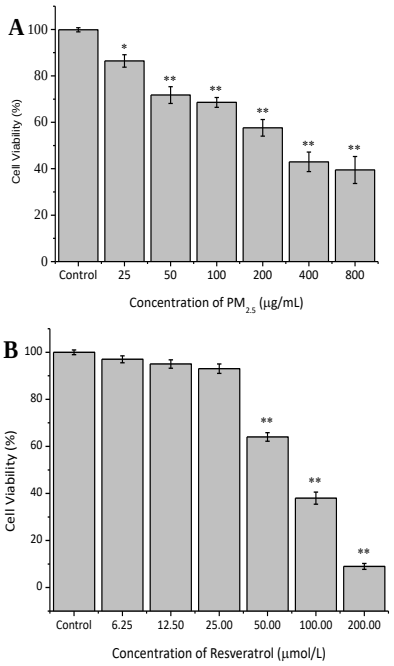

Figure 1. Effects of $PM_{2.5}$ and resveratrol on CHO cell proliferation

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

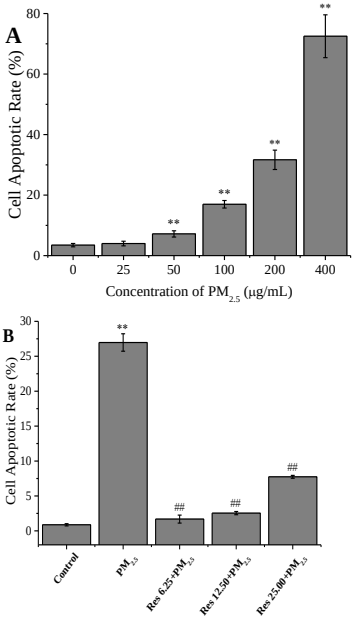

Figure 2. $PM_{2.5}$- induced CHO cell apoptosis and the anti-apoptosis effects of resveratrol

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

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 μmol to 134 μmol (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.

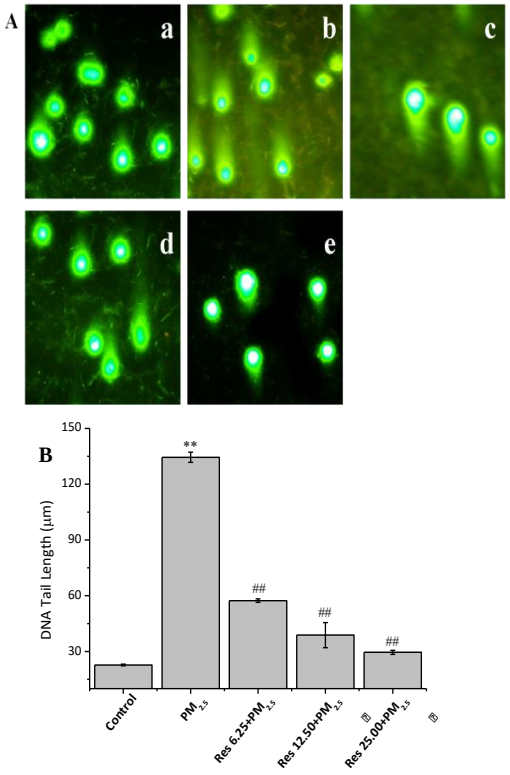

Figure 3. Effects of resveratrol on PM2.5-induced DNA damage

Note: A: 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 $PM_{2.5}$. B: * P<0.05, ** P<0.01, compared to untreated control, ##P<0.01, compared to 100 μg/mL of $PM_{2.5}$.

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).

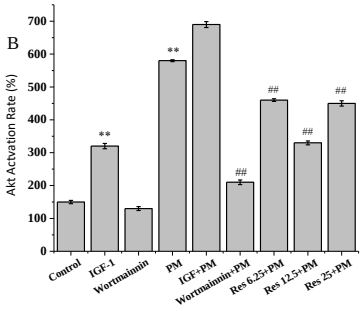

Figure 4. Effects of different concentrations of resveratrol on $PM_{2.5}$-induced akt activation

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.

ACKNOWLEDGMENT

This work was supported by Chinese Nutrition Society (CNS) Nutrition Research Foundation—DSM Research Fund and Bioactive substances and functional food Beijing key laboratory Open Foundation (Zk70201501).

REFERENCES

[1] B. B. Aggarwal, A. Bhardwaj, R. S. Aggarwal, *et al.*, "Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies," *Anticancer Res.*, vol. 5A, pp. 2783-840,2004.

[2] A. Csiszar, "Anti-inflammatory effects of resveratrol: Possible role in prevention of age-related cardiovascular disease," *Ann. NY Acad. Sci.*, vol. 1215, no. 1, pp.117-122, 2011.

[3] D. Albani, L. Polito, A. Signorini, *et al.*, "Neuroprotective properties of resveratrol in different neurodegenerative disorders," *Biofactors*, vol. 36, no. 5, pp. 370-376, 2010.

[4] A. Sgambato, R. Ardito, B. Faraglia, *et al.*, "Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage," *Mutat. Res-Gen. Tox. E.*, vol. 496, no. 1, pp. 171-180, 2001.

[5] L. Cheng, Z. Jin, R. Zhao, *et al.*, "Resveratrol attenuates inflammation and oxidative stress induced by myocardial ischemia-reperfusion injury: Role of Nrf2/ARE pathway," *Int. J. Clin. Exp. Med.*, vol. 8, no. 7, pp. 10420-8, 2015.

[6] G. Tomoaia, O. Horovitz, A. Mocanu, *et al.*, "Effects of doxorubicin mediated by gold nanoparticles and resveratrol in two human cervical tumor cell lines," *Colloids. Surf. B Biointerfaces*, vol. 135, pp. 726-734, 2015.

[7] A. K. Joe, H. Liu, M. Suzui, *et al.*, "Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker

expression in several human cancer cell lines," *Clin. Cancer Res.*, vol. 8, no. 3, pp. 893-903, 2002.
[8] C. A. Pope, R. T. Burnett, M. C. Turner, *et al.*, "Lung cancer and cardiovascular disease mortality associated with ambient air pollution and cigarette smoke: shape of the exposure-response relationships," *Environ. Health Perspect*, vol. 119, no. 11, pp. 1616-21, 2011.
[9] B. J. Malig, S. Green, R. Basu, *et al.*, "Coarse particles and respiratory emergency department visits in California," *Am. J. Epidemiol.*, vol. 178, no. 1, pp. 58-69, 2013.
[10] R. J. Delfino, J. Wu, T. Tjoa, *et al.*, "Asthma morbidity and ambient air pollution: Effect modification by residential traffic-related air pollution," *Epidemiology*, vol. 25, no. 1, pp. 48-57, 2014.
[11] P. Hystad, P. A. Demers, K. C. Johnson, *et al.*, "Long-term residential exposure to air pollution and lung cancer risk," *Epidemiology*, vol. 24, no. 5, pp. 762-72, 2013.
[12] D. Loomis, Y. Grosse, B. Lauby-Secretan, *et al.*, "The carcinogenicity of outdoor air pollution," *The Lancet Oncology*, vol. 14, no. 13, pp. 1262-1263, 2013,
[13] Z. Dagher, G. Garcon, S. Billet, *et al.*, "Role of nuclear factor-kappa B activation in the adverse effects induced by air pollution particulate matter (PM2.5) in human epithelial lung cells (L132) in culture," *J. Appl. Toxicol.*, vol. 27, no. 3, pp. 284-90, 2007.
[14] X. Deng, W. Rui, F. Zhang, *et al.*, "PM2.5 induces Nrf2-mediated defense mechanisms against oxidative stress by activating PIK3/AKT signaling pathway in human lung alveolar epithelial A549 cells," *Cell Biol, Toxicol*,, vol. 29, no. 3, pp. 143-57, 2013.
[15] S. Wang, C. Prophete, J. M. Soukup, *et al.*, "Roles of MAPK pathway activation during cytokine induction in BEAS-2B cells exposed to fine World Trade Center (WTC) dust," *J. Immunotoxicol*, vol. 7, no. 4, pp. 298-307, 2010,
[16] B. F. Cachon, S. Firmin, A.Verdin, *et al.*, "Proinflammatory effects and oxidative stress within human bronchial epithelial cells exposed to atmospheric particulate matter (PM2.5 and PM>2.5) collected from Cotonou, Benin," *Environ. Pollut.*, vol. 185, pp. 340-51, 2014.
[17] E. Corsini, S. Budello, L. Marabini, *et al.*, "Comparison of wood smoke PM2.5 obtained from the combustion of FIR and beech pellets on inflammation and DNA damage in A549 and THP-1 human cell lines," *Arch. Toxicol.*, vol. 87, no. 12, pp. 2187-99, 2013.
[18] T. L. Watterson, B. Hamilton, R. S. Martin, *et al.*, "Urban particulate matter activates Akt in human lung cells," *Arch. Toxicol.*, vol. 86, no. 1, pp. 121-35, 2012.
[19] G. Zu, W. Jiang, X. Zhao, *et al.*, "Anti-apoptosis effect of natural extracts on a549 cell apoptosis induced by PM2.5," *Asian. Journal of Ecotoxicology*, vol. 04, pp. 429-434, 2011.
[20] W. Jiang, X. Zhao, S. Mi, *et al.*, "Effect of DNA damage exposed to ambient particle matters and protecting effect of lycopene in HLF cells," *Journal of Environmental & Occupational Medicine*, vol. 06, pp. 568-571, 2008.

Chen Guo was born in 1988. He received a B.S.degree in Food science and Engineering from Tianjin Agriculture College, and M.S. in Food science and Engineering from Beijing Union University. She is doing a Ph.D Programme in the School of Public health, Peking University. She specializes in environmental health.

Xiaohong Zhao is a Researcher of Deputy Director of Research Institute for Science and Technology of Functional Food, Beijing Union University. She earned her PhD in Pollution Ecology from Lanzhou University (2000); her M.M. in Environment Medicine from Lanzhou Medical College (1989) and her Bachelor of Medicine in Public Health from Shanxi Medical Collge (1983).Her major fields of study include food and environmental toxicology. Her research interests include areas such as food nutrition, functional food, phytochemical and atmospheric particulate. Prof. Zhao is member of a council of Beijing Dietetix Association.

Dr. Ter-yun (Timothy) Lin was born in Taiwan, 1967. He received a B.S. degree in Biology from Tunghai University, and M.S. from LSU in Plant Physiology and PhD in Molecular Biology from University of Iowa. He is specialized in gene regulation and currently serves as the Program Coordinator for Molecular Biology and Biotechnology at North Park University, Chicago USA.

Shijie Chen was born in China in 1991. She graduated from He'nan University of Technology, China in 2014. She is majoring in Food science and Engineering at Beijing Union University.

Yanli Zhou was born in China 1991. She graduated from Yangtze University, China, in 2015. She is now studying at Beijing Union University and specializes in toxicological studies on bioactive substances.

Shengquan Mi was born in china 1975. He is an associate professor at Beijing Union University, China. He got his PhD from China CDC in 2011, He specializes in Food nutrition and chronic diseases.