Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

The effects of autophagy on vascular endothelial cells induced by airborne PM2.5

Zhixiang Zhou[1,*], *Tong Shao*[1], *Mengnan Qin*[1], *Xiaoyan Miao*[1], *Yu Chang*[1], *Wang Sheng*[1], *Fengshang Wu*[2], *Yunjiang Yu*[3,*]

1. *College of Life Science and Bioengineering, Beijing University of Technology, Beijing 100124, China*
2. *State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China*
3. *South China Institute of Environmental Science, Ministry of Environmental Protection, Guangzhou 510655, China*

ARTICLE INFO

Article history:
Received 24 January 2017
Revised 10 May 2017
Accepted 12 May 2017
Available online 19 May 2017

Keywords:
PM2.5
HUVEC
Cytotoxicity
Autophagy

ABSTRACT

The purpose of this study was to examine the direct toxicity of PM2.5 collected from Beijing on human umbilical vein endothelial cells (HUVEC). A Cell Counting Kit 8 (CCK8) assay demonstrated that PM2.5 exposure decreased the proliferation of HUVECs in a dose-dependent manner. We also found that PM2.5 exposure induced autophagy in HUVECs, as evidenced by: (1) an increased number of double-membrane vesicles; (2) enhanced conversion and punctuation of the microtubule-associated protein light chain 3 (LC3); and (3) decreased levels of the selective autophagy substrate p62 in a time-dependent manner. Furthermore, promoting autophagy in PM2.5-exposed HUVECs with rapamycin increased the cell survival rate, whereas inhibiting autophagy *via* 3-methyladenine significantly decreased cell survival. These results demonstrate that PM2.5 exposure can induce cytotoxicity and autophagy in HUVECs and that autophagy play a protective role against PM2.5-induced cytotoxicity. The findings of the present study imply a direct toxic effect of PM2.5 on HUVECs and provide novel insight into the mechanism of cardiovascular diseases caused by PM2.5 exposure.

Introduction

Due to the rapid industrialization and motorization of the world, air pollution has become one of the greatest environmental concerns. Particulate matter (PM) is one of the major constituents of air pollution, and there is increasing evidence demonstrating that PM is one of the most harmful pollutants (Gao et al., 2017). In addition, epidemiologic investigations have consistently demonstrated a link between PM and the increased incidence of human cardiovascular morbidity and mortality (Kaufman et al., 2016; Fang et al., 2016).

Vascular endothelial cells line the inner surface of blood vessels and provide an essential structural and biological barrier for the maintenance of vascular function and homeostasis. Moreover, endothelial dysfunction is now hypothesized to be a dominant mechanism in the development of cardiovascular diseases, including atherosclerosis and ischemic heart disease (Thornburg, 2015). The toxic effects of PMs are primarily attributed to small inhalable particulates with an aerodynamic diameter smaller than 2.5 μm (PM2.5) that can penetrate deep into the airways of the respiratory tract, reach the alveoli, and even enter into the systemic circulation

* *Corresponding authors*. E-mail: zhouzhixiang@bjut.edu.cn (Zhixiang Zhou), yuyunjiang@scies.org (Yunjiang Yu).

http://dx.doi.org/10.1016/j.jes.2017.05.019
1001-0742/

(Martinelli et al., 2013). It is possible that inhalation of PM2.5 induces inflammation in the lungs, promoting the release of inflammatory cytokines from the pulmonary tissue into the systemic circulation, leading to endothelial dysfunction and indirectly amplify vascular damage (Shimada et al., 2006). PM2.5 may also directly affect the cardiovascular system by entering into the systemic circulation and causing cardiovascular dysfunction (Nelin et al., 2012). Recently, studies have demonstrated that PM2.5 exposure is strongly associated with a perturbation in endothelial function in both human and animal models (Bai and Sun, 2016). The underlying mechanism of action by which the air pollutant, PM2.5, induces adverse cardiovascular effects is, therefore, of intense scientific interest.

Autophagy as a biological pathway has been found to be involved in the occurrence of certain diseases. It is a process of intracellular bulk degradation, in which cytoplasmic components (*e.g.*, organelles) are sequestered within double-membrane vesicles that deliver the contents to the lysosome or vacuole for degradation (Lockshin, 2016). The LC3 protein is a homologous body of ATG8 during the formation of autophagy bodies and becomes modified into an LC3II type (Jiang and Mizushima, 2015). Moreover, when autophagy occurs, the P62 protein binds directly to LC3, serving as a mechanism for delivering selective autophagic cargo for degradation by autophagy. Therefore, the expressions of LC3II/LC3I and P62 are widely used as autophagy markers. Autophagy has conventionally been considered to be a pathway contributing to cellular homeostasis and adaptation to stress (Shintani and Klionsky, 2004). In addition, it has been demonstrated that autophagy also functions as a cytoprotective response against various types of cellular stress (Rubinstein and Kimchi, 2012). Recently, it was reported that autophagy was involved in the PM2.5-mediated cytotoxicity in human lung epithelial cells (Xu et al., 2016; Deng et al., 2014, 2017; Liu et al., 2015; Y. Wang et al., 2015; C. Wang et al., 2015; Li et al., 2016).

To elucidate the adverse cardiovascular effects induced by air pollution PM, we will further explore the autophagic effect in PM2.5-exposed human umbilical vein endothelial cell (HUVECs), and investigate its contribution to HUVEC cytotoxicity mediated by PM2.5 exposure.

1. Materials and methods

1.1. Materials

Rabbit polyclonal antibodies against GAPDH(AP0063) and P62 (BS7360), were purchased from BioWorld Biotech Co. Ltd. (Nanjing, China). Rabbit monoclonal antibodies against LC3 I/II (MP28F8) were purchased from NOVUS (MO, USA). The RDye800® conjugated goat anti-rabbit IgG (072-07-15-06) was purchased from KPL (MN, USA). Polyvinylidene difluoride (PVDF) membranes (IPVH00010) and the ultrapure water system were obtained from Millipore (Millipore Ltd. China, Beijing, China). The 3-methyladenine (3-MA) (HY-19312) and rapamycin (Rap) (HY-10219) were obtained from Selleck Chemicals (USA). All other reagents, including penicillin and streptomycin (15050-065) were obtained from Gibco (Grand Island, NY, USA), BCA protein assay kit (PC0020), SDS-PAGE gel preparation kit (B1027), and Annexin-V-FITC/PI apoptosis kits (AD10) were purchased from Zoman Biological Technology Co. Ltd. (Beijing, China).

1.2. PM2.5 sample preparation

Sampling was performed at a typical urban sampling site located on the roof of the School of Environment at Tsinghua University, Beijing, China, from January 1st to January 31st, 2015. Fig. 1 illustrates the air quality index (AQI) data in Beijing during the January of 2015. The good, lightly polluted, moderately polluted, and heavily polluted days based on the AQI of PM2.5 accounted for 9%, 13%, 52%, and 26%, respectively, of the month. The monitoring data were from the Chinese Ministry of Environmental Protection (http://www.zhb.gov.cn). PM2.5 was sampled using high-volume samplers at a flow rate of 1 m^3/min, and collected on quartz filters (47 mm, 2 μm, Whatman). The samples were stored at −80°C until further extraction. The quartz fibers containing the PM2.5 sample were moistened with a small amount of 75% ethyl alcohol. The filters were then immersed in 5 mL sterile water and subjected to an ice-bath sonication of four cycles of 5 min each to fully elute the PM. The eluate was collected and filtered through six layers of sterile gauze. We weighed the filtered eluate after freezing and vacuum drying, and added sterile water to make a PM2.5 sample stock solution of 4 mg/mL. The stock solution was then stored in the dark at −80°C.

Thirty nine elements were investigated and the concentration of each was determined in the PM2.5 sample: total carbon (TC), organic carbon (OC), elemental carbon (EC), Cl, NO_3, SO_4, Indeno(1,2,3-cd)pyrene (IP), DBahA, Coronene (COR), BbFA, Fluoranthene (FA), BaA, Chrysene (CHR), BaP, BeP, BkFA, Sb, Cr, Cu, Pb, Mg, Ca, Zn, Fe, Al, S, Sc, Ti, V, Mn, Co, Ni, As, Se, Sr, Mo, Cd, Ce, and Eu. Data remained to be published.

1.3. Cell culture and PM2.5 exposure

In this study, human umbilical vein endothelial cells (HUVECs) were obtained from Allcells, China. The cells were routinely maintained in an HUVEC complete culture medium (Allcells, China) supplemented with 10% fetal bovine serum (FBS), 2% growth factors, 100 U/mL penicillin, and 100 U/mL streptomycin. The cells were grown at 37°C, with 5% CO_2. For exposure, the cells were seeded in triplicate 24 hr prior to exposure. A small aliquot of the PM2.5 suspension was mixed with the culture medium by sonication for 10 min and then applied evenly to the cultured cells with blank samples (unexposed filters) used as controls running in parallel. For following CCK8 cell viability assay, the final concentrations of PM2.5 suspension were 0, 5, 25, 50, 100 and 200 μg/mL, respectively. Rap (100 nmol/L, Sigma-Aldrich) or 3-MA (5 mmol/L, Sigma-Aldrich) was added for 1 hr before the addition of the PM2.5 suspension.

1.4. CCK8 cell viability assay

A WST-8 assay was applied to evaluate HUVEC cell viability using Cell Counting Kit 8 (CCK-8) (Dojindo Molecular Technologies, Inc., MD, USA). After exposing the cells to PM2.5 for 24 hr, 10 μL CCK8 reagent was added to each well. After incubating for 4 hr at 37°C, the absorbance was detected using

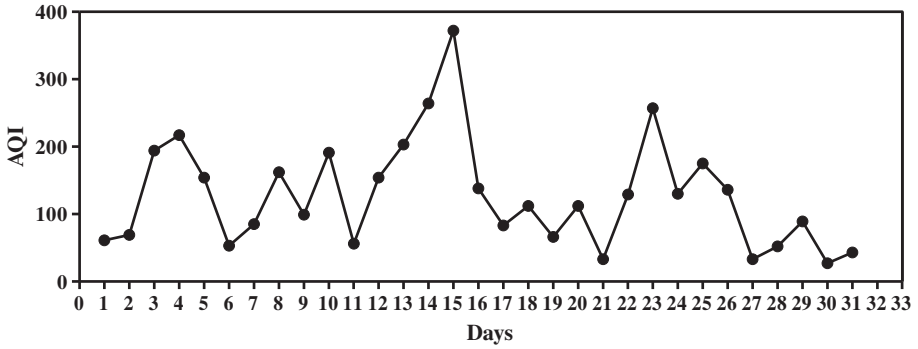

Fig. 1 – Trend chart of AQI changes from January 1st to January 31st, 2015 in Beijing. AQI: air quality index.

an EnSpire® Multimode Plate Reader (PerkinElmer Inc., MA, USA) at 450 nm. To exclude interference by sample PM and other sources of background absorbance, the absorbance was measured in comparison with the control wells containing PM2.5 without cells. The survival rate of the untreated cells was set as 100%. The WST-8 experiment was repeated three times and the final figures were presented as the mean percent (±standard deviation [SD]).

1.5. *Transmission electron microscopy (TEM)*

TEM analysis was carried out after the cells were exposed to the PM2.5 suspension for 24 hr. Treated and untreated cells were harvested and pelleted by centrifugation at 800 r/min for 10 min and fixed in ice-cold 2.5% glutaraldehyde for 2 hr. Afterward, samples were post-fixed in 1% OsO_4 for 1 hr, dehydrated through an ethanol series, and embedded in epoxy resin, then ultra-thin sections (60 nm) were double stained with uranyl acetate and lead citrate (Y. Wang et al., 2015; C. Wang et al., 2015). Representative areas were examined with an electron microscope (FEI Tecnai Spirit; USA) at 120 kV.

1.6. *Western blot analysis*

Following exposure to PM2.5, the cells were collected and dissociated with RIPA lysis buffer and placed on ice for 30 min. After centrifuging for 10 min at 12,000*g* at 4°C, the proteins were separated on a 10% SDS-PAGE gel and electrotransferred to a nitrocellulose membrane. The blots were blocked for 2 hr at room temperature with 5% nonfat milk in 0.01 mol/L PBS, then incubated overnight with different dilutions of rabbit polyclonal antibodies to GADPH, LC3II/LC3I, and P62. The membranes were washed three times with TBST for 10 min each at room temperature, and incubated for 45 min with a 1:5000 dilution of RDye800® conjugated goat anti-rabbit IgG (KPL, MN, USA). The membranes were visualized using an Odyssey infrared imaging system (LI-COR Biosciences, CA, USA) following three rinses in TBST.

1.7. *Data analysis*

All results were expressed as the mean ± SD from three independent experiments. All of the study data were analyzed by one-way Analysis of Variance (ANOVA). The Least Significant Difference (LSD) test was used to calculate statistical significance between the control group and each treatment group. The statistical significance was defined as $p < 0.05$ for all tests.

2. Results

2.1. *The effects of PM2.5 on the survival rate of HUVECs*

To investigate the potential cardiovascular toxicity of PM2.5 extracted from Beijing air samples, we incubated HUVECs with various concentrations of PM2.5 and measured cell viability with a CCK8 assay after 24 hr of exposure. A progressive decrease in the cell viability was observed with increasing concentrations of PM2.5 (Fig. 2). When the concentration of PM2.5 was 25 μg/mL, the cell survival rate was significantly lower than that of the control group. Moreover, a progressive decrease in cell viability was observed following PM2.5 exposure in a concentration-dependent manner. The IC50 and IC60 values required for PM2.5 to reduce the HUVEC

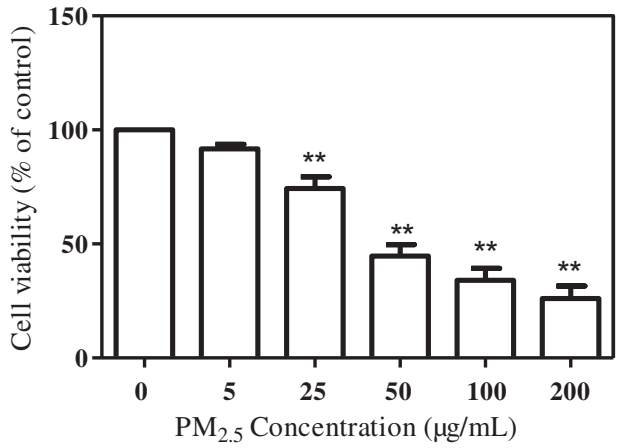

Fig. 2 – Toxicity of PM2.5 to the HUVECs. The HUVECs were exposed to 0, 5, 25, 50, 100 and 200 μg/mL for 24 hr. Cell viability was expressed as a percentage of the unexposed control (mean ± SD). (**) indicates significant difference ($p < 0.05$) compared to the control. PM2.5: particulate with an aerodynamic diameter smaller than 2.5 μm; HUVECs: human umbilical vein endothelial cells; SD: standard deviation.

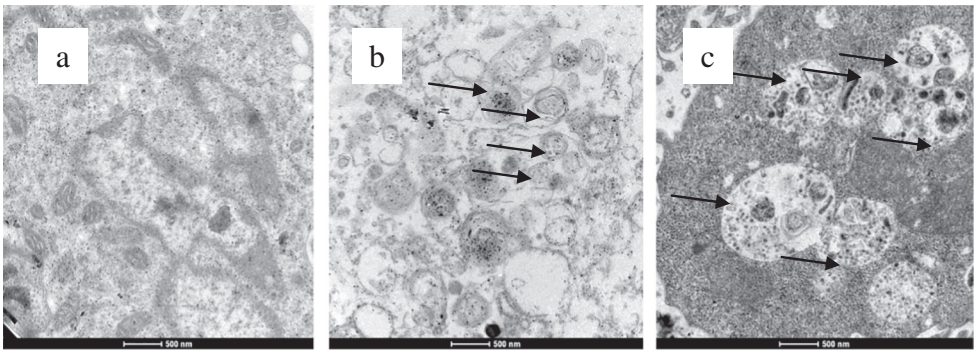

Fig. 3 – PM2.5 induced autophagosome in HUVECs. (a) the unexposed control; (b) after being treated with 100 μg/mL of PM2.5 for 6 hr; (c) after being treated with 100 μg/mL of PM2.5 for 24 hr. Cells were harvested and observed by TEM (10,000×). PM2.5: particulate with an aerodynamic diameter smaller than 2.5 μm; HUVECs: human umbilical vein endothelial cells; TEM: transmission electron microscopy.

cell viability by 50% and 60% were 63 μg/mL and 100 μg/mL, respectively.

2.2. PM2.5 induced autophagy in HUVECs

To detect whether the PM2.5 could induce autophagy in the HUVECs, the cells were exposed to a PM2.5 concentration of 100 μg/mL (IC60) for 6 and 24 hr, respectively. TEM was used to observe the morphological changes in the cells. As shown in Fig. 3, a typical autophagosome with a bilayer membrane structure was exhibited by the cells from both exposures of 6 and 24 hr (Fig. 3b, c). To further confirm this observation, the autophagy-associated proteins LC3I, LC3II and P62 were detected by performing a Western blot. The conversion of LC3I to LC3II and changes in P62 expression were reflective of autophagy. After the HUVECs had been exposed to 100 μg/mL of PM2.5 for 0, 6, 12, and 24 hr, the ratio of LC3II/LC3I expression increased (Fig. 4a, b); whereas P62 expression was reduced (Fig. 4a, c), confirming that PM2.5 exposure had induced autophagy in HUVECs.

2.3. Effects of the autophagy on PM2.5-induced HUVEC toxicity

We further investigated the role of autophagy in the process of PM2.5-mediated HUVEC toxicity. 5 mmol/L of 3-MA was added as an inhibitor of autophagy, while 100 nmol/L of Rap was used to induce autophagy. When autophagy was inhibited by 3-MA, the cell survival rate significantly decreased after PM2.5 exposure (Fig. 5a). However, pretreating the cells with Rap significantly improved the cell survival rate (Fig. 5b). These results suggest that autophagy reduced the cytotoxicity in PM2.5-exposed HUVECs.

3. Discussion

The exposure to PM is currently one of the most pressing public health issues, particularly in relation to the effects on the cardiovascular system. Human exposure to PM, particularly PM2.5, has been linked to a number of cardiovascular conditions (Kaufman et al., 2016; Fang et al., 2016; Ghauri et al., 2012). The pooled effect estimate expressed as the excess risk per 10 μg/m^3 increase in PM2.5 exposure was 6% (95% Confidence Interval (CI): 4%, 8%) for all-cause and 11% (95% CI: 5%, 16%) for cardiovascular mortality (Hoek et al., 2013). Although it has been reported that PM2.5-mediated oxidative stress and inflammation are potential causes of various cardiovascular diseases (Deng et al., 2017), the mechanisms underlying PM2.5-induced health effects remain unknown.

It was reported that the toxic effect of PM on the cardiovascular system could be subdivided into two general pathways: 1) direct; and 2) indirect (Nelin et al., 2012). The majority of these studies have focused on the indirect effects of PM on the cardiovascular system and are thought to be the only mechanism whereby particulate exposure to the respiratory system induced a release of an inflammatory/cytokine milieu that circulated through the bloodstream and affected the heart (Bai and Sun, 2016; Sun et al., 2008; Y. Wang et al., 2015; C. Wang et al., 2015). Recently, it was demonstrated that small, inhalable PM2.5 could penetrate deeper into the airways of the respiratory tract and reach the alveoli. Furthermore, such particles can even enter into the systemic circulation, and cause a direct toxic effect on the heart and blood vessels (Shimada et al., 2006).

Historically, autophagy was exclusively considered to be a cell death process. However, accumulating evidence suggests that autophagy can be involved in both cell survival and cell death (Rubinstein and Kimchi, 2012). For example, autophagy is transiently induced as a survival response to various stress stimuli, including nutrient starvation and oxidative stress (Galluzzi et al., 2016). Moreover, autophagy is now acknowledged to perform a largely cytoprotective function in physiologically relevant conditions by negatively regulating apoptosis. Recently, autophagy was reported to play a critical role in the pulmonary toxicity induced by environmental toxicants, such as increased air pollution (Xu et al., 2016; Deng et al., 2014, 2017; Liu et al., 2015; Y. Wang et al., 2015; C. Wang et al., 2015; Li et al., 2016). Additionally, it was reported that autophagy might be a survival mechanism for PM2.5-induced cytotoxicity in human bronchial epithelial cells (A549) (Xu et al., 2016; Liu et al., 2015). However, it remains unclear whether PM2.5 can induce autophagy in vascular endothelial cells.

In the present study, we revealed that *in vitro* short-term exposure to airborne PM2.5 collected from Beijing, China,

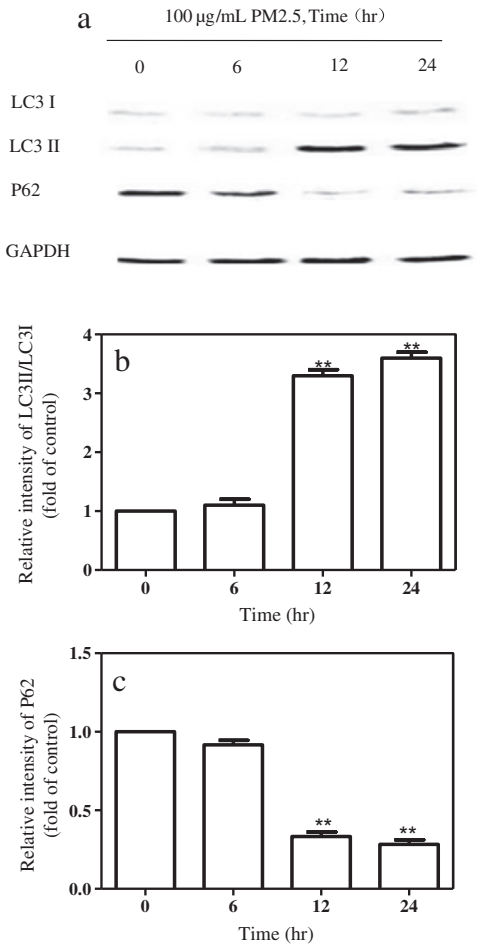

Fig. 4 – Western blot of the LC3 II/LC3 I and P62 following exposure to 100 μg/mL of PM2.5 for 0, 6, 12, and 24 hr. (a) Western blot representatives of the LC3I, LC3II and P62 expression in HUVECs; (b and c) Densitometric analysis was used for calculating the ratio between LC3 II to LC3 I and the ratio of P62 to GAPDH from three independent experiments. The quantitative data are presented as the mean ± SD (n = 3), where $^{**}p < 0.05$ *versus* the unexposed control group (0 hr).

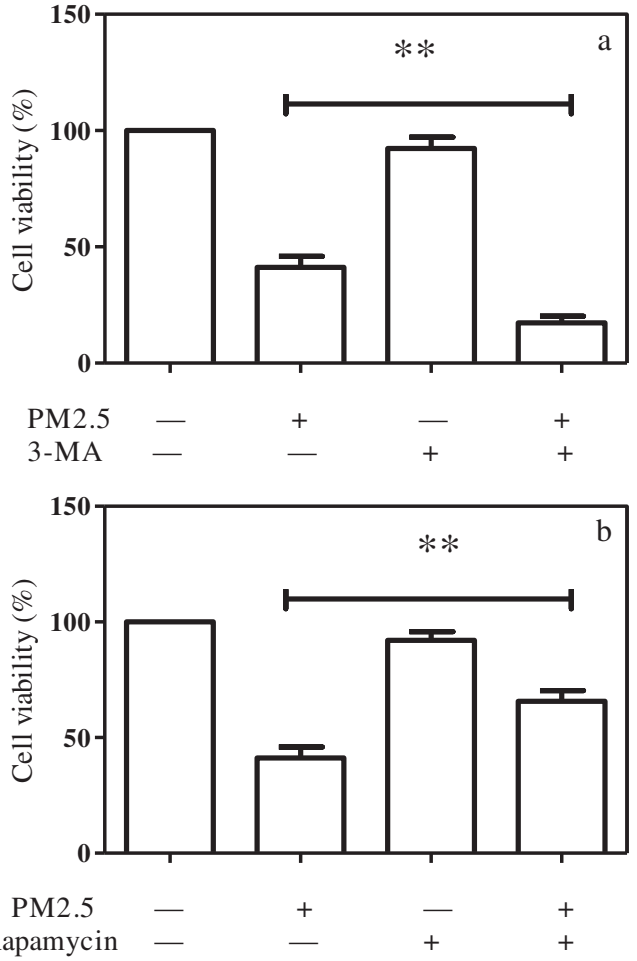

Fig. 5 – Effects of the autophagy on cell death of HUVECs induced by PM2.5. The HUVECs were exposed with 100 μg/mL of PM2.5 for 24 hr following exposures to 5 mM 3-MA (a) and 100 nM Rap (b) for 1 hr. Cell death was measured by CCK8 assay. The quantitative data are presented as the mean ± SD from three independent experiments, where $^{**}p < 0.05$ *versus* PM2.5 exposure only group. HUVECs: human umbilical vein endothelial cells; PM2.5: particulate with an aerodynamic diameter smaller than 2.5 μm; 3-MA: 3-methyladenine; Rap: rapamycin; SD: standard deviation.

caused significant cytotoxicity to HUVECs. We also found that the exposure to PM2.5 induced an increased conversion of LC3 I to LC3 II, decreased expression of p62, and the appearance of multivesicular and membrane-rich autophagosomes in HUVECs. Furthermore, the inhibition of autophagy by 3-MA significantly decreased the cell survival rate, whereas the induction of autophagy by Rap enhanced the cell survival. Our findings clearly demonstrate that PM2.5 is effective at inducing cytotoxicity and an autophagic effect in HUVECs. Moreover, autophagy appears to be protective against PM2.5-induced cytotoxicity.

4. Conclusions

The results of the present study provide the first evidence indicating that PM2.5 induces autophagy in human endothelial cells. Autophagy may act as a protective mechanism to reduce the damage mediated by PM2.5 on vascular endothelial cells. This work provides novel insight into the mechanisms underlying PM2.5-induced cardiovascular disease. As the direct pathways are poorly characterized but important in triggering severe cardiovascular events, our ongoing research is concentrated on elucidating the signaling pathway associated with the induction of autophagy in PM2.5-exposed HUVEC cells, and exploring its role in PM2.5-induced cardiovascular disease.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 21677006, 21177119), the State Key

Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences (No. SKLECRA2016OFP), and the State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (No. KF2015-19).

REFERENCES

Bai, Y., Sun, Q., 2016. Fine particulate matter air pollution and atherosclerosis: mechanistic insights. Biochim. Biophys. Acta 1860 (12), 2863–2868.

Deng, X., Zhang, F., Wang, L., Rui, W., Long, F., Zhao, Y., et al., 2014. Airborne fine particulate matter induces multiple cell death pathways in human lung epithelial cells. Apoptosis 19 (7), 1099–1112.

Deng, X., Feng, N., Zheng, M., Ye, X., Lin, H., Yu, X., et al., 2017. PM2.5 exposure-induced autophagy is mediated by lncRNA loc146880 which also promotes the migration and invasion of lung cancer cells. Biochim. Biophys. Acta 1861 (2), 112–125.

Fang, D., Wang, Q., Li, H., Yu, Y., Lu, Y., Qian, X., 2016. Mortality effects assessment of ambient PM2.5 pollution in the 74 leading cities of China. Sci. Total Environ. 570, 1545–1552.

Galluzzi, L., Bravo-San Pedro, J.M., Kepp, O., Kroemer, G., 2016. Regulated cell death and adaptive stress responses. Cell. Mol. Life Sci. 73 (11–12), 2405–2410.

Gao, J., Woodward, A., Vardoulakis, S., Kovats, S., Wilkinson, P., Li, L., et al., 2017. Haze, public health and mitigation measures in China: a review of the current evidence for further policy response. Sci. Total Environ. 578, 148–157.

Ghauri, B., Mansha, M., Khalil, C., 2012. Characterization of cytotoxicity of airborne particulates from urban areas of Lahore. J. Environ. Sci. 24 (11), 2028–2034.

Hoek, G., Krishnan, R.M., Beelen, R., Peters, A., Ostro, B., Brunekreef, B., et al., 2013. Long-term air pollution exposure and cardio-respiratory mortality: a review. Environ. Health 12 (1), 43.

Jiang, P., Mizushima, N., 2015. LC3- and p62-based biochemical methods for the analysis of autophagy progression in mammalian cells. Methods 75, 13–18.

Kaufman, J.D., Adar, S.D., Barr, R.G., Budoff, M., Burke, G.L., Curl, C. L., et al., 2016. Association between air pollution and coronary artery calcification within six metropolitan areas in the USA (the Multi-Ethnic Study of Atherosclerosis and Air Pollution): a longitudinal cohort study. Lancet 388 (10045), 696–704.

Li, X., Lv, Y., Hao, J., Sun, H., Gao, N., Zhang, C., et al., 2016. Role of microRNA-4516 involved autophagy associated with exposure to fine particulate matter. Oncotarget 7 (29), 45385–45397.

Liu, T., Wu, B., Wang, Y., He, H., Lin, Z., Tan, J., et al., 2015. Particulate matter 2.5 induces autophagy via inhibition of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin kinase signaling pathway in human bronchial epithelial cells. Mol. Med. Rep. 12 (2), 1914–1922.

Lockshin, R.A., 2016. Programmed cell death 50 (and beyond). Cell Death Differ. 23 (1), 10–17.

Martinelli, N., Olivieri, O., Girelli, D., 2013. Air particulate matter and cardiovascular disease: a narrative review. Eur. J. Intern. Med. 24 (4), 295–302.

Nelin, T.D., Joseph, A.M., Gorr, M.W., Wold, L.E., 2012. Direct and indirect effects of particulate matter on the cardiovascular system. Toxicol. Lett. 208 (3), 293–299.

Rubinstein, A.D., Kimchi, A., 2012. Life in the balance — a mechanistic view of the crosstalk between autophagy and apoptosis. J. Cell Sci. 125 (22), 5259–5268.

Shimada, A., Kawamura, N., Okajima, M., Kaewamatawong, T., Inoue, H., Morita, T., 2006. Translocation pathway of the intratracheally instilled ultrafine particles from the lung into the blood circulation in the mouse. Toxicol. Pathol. 34, 949–957.

Shintani, T., Klionsky, D.J., 2004. Autophagy in health and disease: a double-edged sword. Science 306 (5698), 990–995.

Sun, Q., Yue, P., Kirk, R.I., Wang, A., Moatti, D., Jin, X., et al., Schecter, A.D., Lippmann, M., Gordon, T., Chen, L.C., Rajagopalan, S., 2008. Ambient air particulate matter exposure and tissue factor expression in atherosclerosis. Inhal. Toxicol. 20, 127–137.

Thornburg, K.L., 2015. The programming of cardiovascular disease. J. Dev. Orig. Health Dis. 6 (5), 366–376.

Wang, Y., Lin, Z., Huang, H., He, H., Yang, L., Chen, T., et al., 2015a. AMPK is required for PM2.5-induced autophagy in human lung epithelial A549 cells. Int. J. Clin. Exp. Med. 8 (1), 58–72.

Wang, C., Tu, Y., Yu, Z., Lu, R., 2015b. PM2.5 and cardiovascular diseases in the elderly: an overview. Int. J. Environ. Res. Public Health 12 (7), 8187–8197.

Xu, X., Wang, H., Liu, S., Xing, C., Liu, Y., Zhou, W., et al., 2016. TP53-dependent autophagy links the ATR-CHEK1 axis activation to proinflammatory VEGFA production in human bronchial epithelial cells exposed to fine particulate matter (PM2.5). Autophagy 12 (10), 1832–1848.