

Effects of the Particulate Matter_{2.5} (PM_{2.5}) on Lipoprotein Metabolism, Uptake and Degradation, and Embryo Toxicity

Jae-Yong Kim^{1,2,3,4}, Eun-Young Lee^{1,2,3,4}, Inho Choi^{1,2,3}, Jihoe Kim^{1,2,3}, and Kyung-Hyun Cho^{1,2,3,*}

Particulate matter_{2.5} (PM_{2.5}) is notorious for its strong toxic effects on the cardiovascular, skin, nervous, and reproduction systems. However, the molecular mechanism by which PM_{2.5} aggravates disease progression is poorly understood, especially in a water-soluble state. In the current study, we investigated the putative physiological effects of aqueous PM_{2.5} solution on lipoprotein metabolism. Collected PM_{2.5} from Seoul, Korea was dissolved in water, and the water extract (final 3 and 30 ppm) was treated to human serum lipoproteins, macrophages, and dermal cells. PM_{2.5} extract resulted in degradation and aggregation of high-density lipoprotein (HDL) as well as low-density lipoprotein (LDL); apoA-I in HDL aggregated and apo-B in LDL disappeared. PM_{2.5} treatment (final 30 ppm) also induced cellular uptake of oxidized LDL (oxLDL) into macrophages, especially in the presence of fructose (final 50 mM). Uptake of oxLDL along with production of reactive oxygen species was accelerated by PM_{2.5} solution in a dose-dependent manner. Further, PM_{2.5} solution caused cellular senescence in human dermal fibroblast cells. Microinjection of PM_{2.5} solution into zebrafish embryos induced severe mortality accompanied by impairment of skeletal development. In conclusion, water extract of PM_{2.5} induced oxidative stress as a precursor to cardiovascular toxicity, skin cell senescence, and embryonic toxicity via aggregation and proteolytic degradation of serum lipoproteins.

INTRODUCTION

Particulate matter (PM) is a well known form of air pollution that is closely associated with incidence of cardiovascular disease (Pope et al., 2004). Epidemiological studies have revealed that

fine PM < 2.5 μm in aerodynamic diameter (PM_{2.5}) is associated with increased risks of myocardial infarction (MI), stroke, arrhythmia, and heart failure within hours to days of exposure in susceptible individuals (Peters et al., 2001). Although the main pathways leading to cardiovascular toxicity upon PM_{2.5} exposure are based on oxidative stress and systemic inhalation (Nurkiewicz et al., 2011), the molecular mechanism by which PM_{2.5} aggravates disease progression is poorly understood. Thus, the health effects of PM_{2.5} in human blood should be investigated.

PM_{2.5} triggers oxidative stress in response to inhalation and release of metals such as rhodium, palladium, and platinum (Bozlaker et al., 2014). PM and fine particles are also active carriers of toxic compounds such as nitrogen oxide (NOX) and sulphur oxide (SOX) (Jalava et al., 2008), which are acidifying substances with high corrosive and oxidative potentials. Urban PM has several properties, including cytotoxic and mutagenic activities (Traversi et al., 2011) as well as genotoxicity in *S. typhimurium* TA98 (Ramos de Rainho et al., 2013).

More specifically, the American Heart Association (AHA) Scientific Statement writing group reported a consistent and causative relationship between PM_{2.5} exposure and cardiovascular morbidity and mortality (Brook et al., 2010). However, there has been no sufficient report on PM_{2.5} toxicity in the circulation system, especially with regards to lipid metabolism.

Lipoproteins are key players in serum lipid metabolism, which is closely associated with pathogenesis of cardiovascular disease and diabetes. It is well known that HDL functionality is closely and directly related with incidence of metabolic diseases, including coronary heart disease and diabetes mellitus (Groop et al., 2007). HDL is a protein-lipid complex in plasma that exerts potent antioxidant, anti-inflammatory, and anti-atherosclerotic activities (Cho, 2009; Yoo et al., 2015). Apolipoprotein (apo) A-I, the major protein of HDL, also has strong antioxidant and anti-infection activities. Many researchers, including our group, have reported that HDL quality is highly dependent on the structural and functional characteristics of apoA-I during the aging process. (Park et al., 2010; van Leuven et al., 2008). Modification of apoA-I is directly related with production of dysfunctional HDL, which has higher atherogenic and inflammatory properties that exacerbate cellular senescence (Jang et al., 2011; Park and Cho, 2011b). Taken together, reports have strongly suggested that HDL functionality is highly affected by its composition.

Regarding the antioxidant activities of apoA-I and HDL in a live animal model, we previously reported a highly sensitive and

¹School of Biotechnology, Korea, ²Research Institute of Protein Sensor, ³BK21plus Program Serum Biomedical Research and Education Team, Yeungnam University, Gyeongsan, 712-749, Korea, ⁴These authors contributed equally to this work.

*Correspondence: chok@yu.ac.kr

Received 10 July, 2015; revised 24 October, 2015; accepted 3 November, 2015; published online 26 November, 2015

Keywords: embryo, glycation, lipoprotein, oxidation, particulate matter 2.5, zebrafish

effective animal model based on zebrafish and its embryos (Park and Cho, 2011a). Zebrafish is a popular animal model to test acute toxicity (Park et al., 2014b) and hyperlipidemia (Jin and Cho, 2011). Furthermore, zebrafish embryos can be applied rapidly and economically to screen antioxidant and anti-inflammatory agents against oxLDL (Park and Cho, 2011a) and sterilizer (Kim et al., 2013). Although PM_{2.5} exposure is associated with increased cardiovascular mortality, the precise pathological mechanism is still unknown, especially at the molecular level of serum proteins. Our current study was designed based on the fact that PM_{2.5} inhaled into the lungs dissolves into the pulmonary blood, which then circulates throughout the whole blood system. Due to its amphiphilic nature, PM_{2.5} likely binds with serum lipoproteins, a unique vehicle for lipid transport via blood. In order to investigate the molecular mechanism of PM_{2.5} toxicity to serum lipoproteins, we evaluated the effects of aqueous PM_{2.5} solution on human lipoproteins, macrophages, dermal cells, and zebrafish embryos.

MATERIALS AND METHODS

Collection and extraction of PM_{2.5}

A glasswool filter containing PM_{2.5} was provided by Dr. J.Y. Ahn from the National Institute of Environmental Research (Korea), which was collected on roadside between Jan 12 and Jan 20 in 2014 at Seoul, Rep of Korea. To prepare PM_{2.5} solution in water, the glasswool filter was sliced and vortexed in deionized water (Millipore, USA) to extract PM_{2.5} solution to 300 µg/ml (300 µg of total PM_{2.5} mass was weighed and dissolved in 1 ml of water under assumption of 100% dissolution). After extraction for 24 h, the tube was allowed to stand for 2 h to allow precipitation of the glasswool fragment. The PM_{2.5} solution was collected and diluted before treatment.

Purification of human lipoproteins

LDL (1.019 < d < 1.063), HDL₂ (1.063 < d < 1.125), and HDL₃ (1.125 < d < 1.225) were isolated from sera of young human males (mean age, 22 ± 2 years) who voluntarily donated blood after fasting overnight via sequential ultracentrifugation as detailed in our previous report (Park and Cho, 2011b).

Treatment of lipoproteins with PM_{2.5}

Purified LDL, HDL₂, and HDL₃ (1 mg/ml of protein) were each treated with water extract of PM_{2.5} (final 3 and 30 ppm) under absence or presence of fructose, followed by incubation at 37°C for the designated times in the presence of 5% CO₂. After incubation, lipoproteins were analyzed by electrophoresis (SDS-PAGE and agarose gel) and spectroscopy. Aliquots of lipoproteins were stored at 4°C after extensive dialysis against tris-buffered saline (TBS, pH 8.0).

LDL oxidation and uptake of LDL into macrophages

Purified human LDL was incubated with PM_{2.5} in the presence of 10 µM CuSO₄ and extent of oxidation was compared as our previous report (Yoon and Cho, 2012).

Cell culture of THP-1 cells and primary human dermal fibroblasts (HDFs) were carried out as our previous report (Cho, 2011). Differentiated and adherent macrophages were then rinsed with warm PBS and incubated with 400 µl of fresh RPMI-1640 medium containing 1% FBS, 50 µl of oxLDL (50 µg of protein in PBS), and PM_{2.5} at its designated concentration for 48 h at 37°C in a humidified incubator. After incubation, cells were stained with oil-red O solution (0.67%) to visualize the amounts of lipid species. Cell media (0.2 ml) were then analyzed by TBARS assay in order to evaluate changes in levels of oxidized

species using a malondialdehyde (MDA) standard.

Anti-senescence assay

Cellular senescence-associated (SA)-beta-gal activity was compared with extent of senescence. For induction of senescence, cells in passage 9 (at approximately 60% confluence) were exposed to PM_{2.5} solution for the designated period.

Zebrafish and embryos

Wild-type zebrafish and embryos were maintained according to standard protocols (Nüsslein-Volhard and Dahm, 2002). Zebrafish maintenance and experimental procedures were approved by the Committee of Animal Care and Use of Yeungnam University (Gyeongsan, Korea). Zebrafish and embryos were maintained in a system cage (3 L volume, acrylic tank) and 6-well plates, respectively, at 28°C during treatment under a 14:10 h light:dark cycle.

Microinjection of zebrafish embryos

Embryos at 1 day post-fertilization (dpf) were individually subjected to microinjection using a pneumatic picopump equipped with a magnetic manipulator and a pulled microcapillary pipette-using device as our previous report (Park and Cho, 2011a) To minimize bias, injections were performed at the same position on the yolk area of each embryo

Exposure of zebrafish embryo in water containing PM_{2.5}

Zebrafish embryos were treated with water extract of PM_{2.5} (final 3 and 30 ppm, wt/vol) according to our previous report (Kim et al., 2015a), based on the assumption that all components of PM_{2.5} were completely dissolved in the aqueous medium. In a preliminary study, we found that a final concentration of 3 ppm of PM_{2.5} was lethal to zebrafish after 10-fold serial dilutions from 0.0003 to 300 ppm.

After 72 h waterborne exposure of embryo to PM_{2.5} solution, the larvae were homogenized using a plastic pestle (Biomasher-II, Optima Inc., Japan) in PBS. Protein content in supernatant, which was collected by spun down, was determined by the Bradford method using human serum albumin as a standard. The same amount of the supernatant was subjected to the ferric ion reducing activity and the TBARS assay as described in our previous report (Kim et al., 2015b) to compare antioxidant activities.

Statistical analysis

All data are expressed as the mean ± SD of at least three independent experiments with duplicate samples. Data were evaluated via one-way analysis of variance (ANOVA) using SPSS (version 14.0; SPSS, Inc., USA), and the differences between the means were assessed using Duncan's multiple-range test. Statistical significance was defined as $p < 0.05$.

RESULTS

Modification of HDL by PM_{2.5}

In the absence of fructose (Fig. 1A), PM_{2.5} extract-treated HDL₃ showed 2-fold higher fluorescence than HDL₃ alone, indicating that the water soluble fraction of PM_{2.5} (final 30 ppm) modified the fluorescence properties of HDL via putative interaction during 72 hr of incubation. PM_{2.5}-treated HDL₃ (lane 2) showed slower electrophoretic mobility than HDL₃ alone (lane 1) in SDS-PAGE. Furthermore, the apoA-I band in PM_{2.5}-treated HDL₃ (lane 2) showed a smeared band pattern with more upward shift than HDL₃ alone (lane 1).

In the presence of fructose, PM_{2.5} extract-treated HDL (lane 4) showed up to 16-fold higher fluorescence than HDL₃ alone

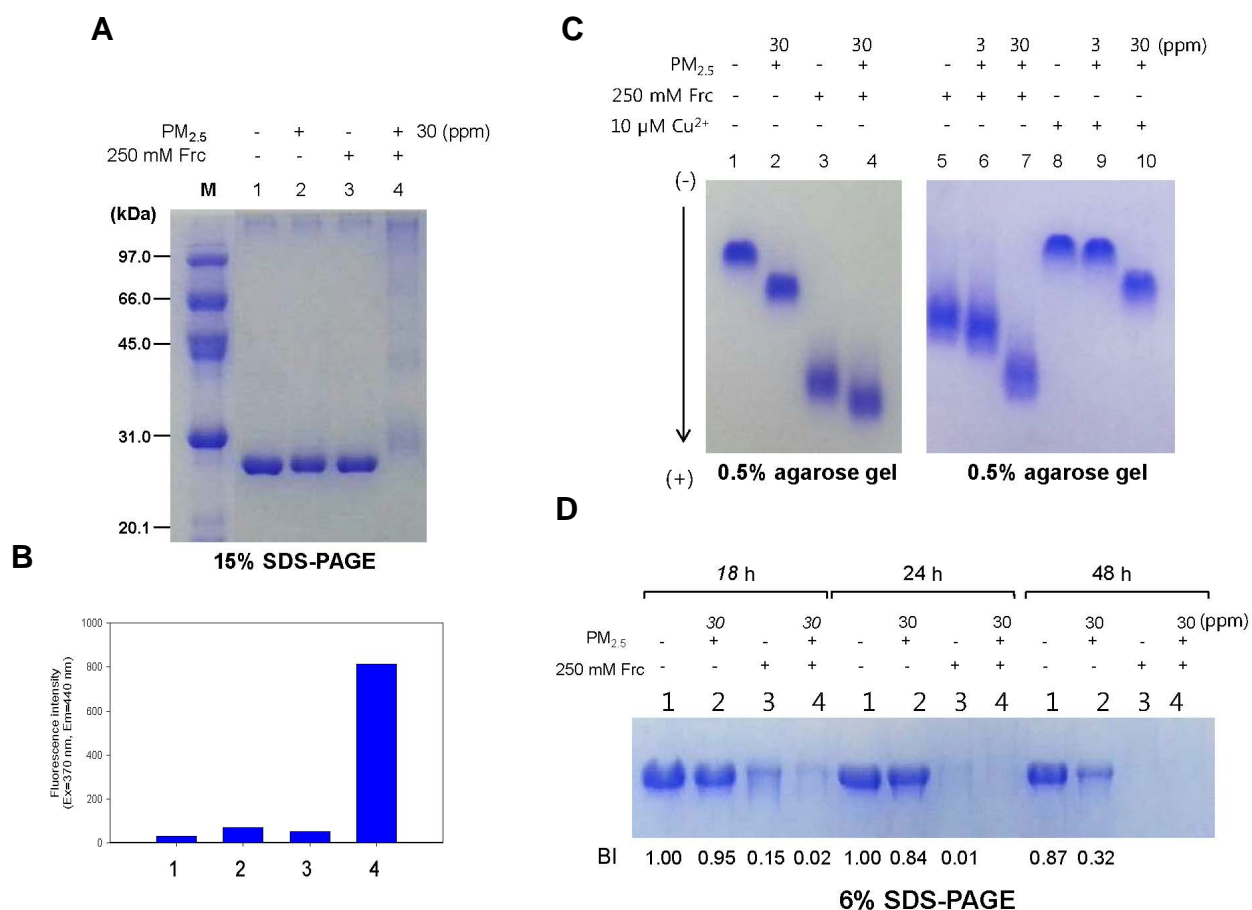


Fig. 1. Modification of HDL and LDL by PM_{2.5} treatment in the presence of fructose (final 250 mM). (A) Electrophoretic patterns of HDL₃ in the presence of PM_{2.5} extract (15% SDS-PAGE). (B) Measurement of glycation extent of HDL₃ treated with PM_{2.5} extract based on fluorescence intensity. (C) Electrophoretic patterns of LDL in the presence of PM_{2.5} solution with and without cupric ion and fructose during 48 h of incubation (0.5% agarose gel). (D) Electrophoretic patterns of LDL in the presence of PM_{2.5} solution with and without fructose during 48 h of incubation (6% SDS-PAGE). BI, Band intensity.

(lane 3) as shown in Fig. 1B. This result indicates that the fructation was more rapidly facilitated by PM_{2.5}. SDS-PAGE analysis also revealed that PM_{2.5}-treated HDL₃ showed severe disappearance of the apoA-I band with multimerization and aggregation (lane 4, Fig. 1A). The apoA-I band (28 kDa) was shifted upward with increased smear intensity, suggesting that its protein structure and electroproperties were severely modified by PM_{2.5} and fructose treatment.

PM_{2.5} accelerates oxidation of LDL

In the absence of fructose and cupric ion, PM_{2.5} alone induced slight changes in the electromobility of LDL (lane 2, Fig. 1C) compared to LDL alone (lane 1). Co-treatment with fructose and PM_{2.5} resulted in increased electromobility of LDL in a dose-dependent manner (lanes 4, 6 and 7, Fig. 1C). Cupric ion-mediated LDL oxidation was more accelerated by PM_{2.5} treatment in a dose-dependent manner (lanes 9 and 10). Interestingly, co-treatment with fructose and PM_{2.5} induced severe modification of LDL, as evidenced by increased electromobility in a time-dependent manner in agarose gel electrophoresis (Fig. 1C).

PM_{2.5} accelerates degradation of LDL

During 18 hr of incubation, as shown in Fig. 1D, PM_{2.5} (final 30

ppm)-treated LDL (lane 2) and fructose-treated LDL (lane 3) showed 5% and 95% reduced apo-B band intensities, respectively, compared to control LDL (lane 1). However, co-treatment with fructose and PM_{2.5} caused almost complete disappearance of the LDL band (lane 4, Fig. 1D), indicating a putative synergistic effect between fructosylation and PM_{2.5} on LDL proteolysis. Similar but increased protein degradation was detected in a time-dependent manner. After 48 h of incubation, PM_{2.5} treatment resulted in almost 68% disappearance of the LDL band. Further, the apo-B band completely disappeared upon co-treatment with fructose and PM_{2.5} (lane 4, Fig. 1D), indicating that proteolysis was accelerated by PM_{2.5} in a time-dependent manner.

After 48 h of incubation, fluorescence intensity increased by 5.5-fold upon co-treatment with fructose and PM_{2.5} extract (Fig. 2) as well as by 2- and 4-fold upon PM_{2.5} and fructose co-treatment, respectively. These results suggest that extent of LDL glycation was accelerated by PM_{2.5} and fructose in a synergistic manner.

PM_{2.5} causes atherogenesis and cellular senescence

THP-1 cells were next stained with oil-red O to evaluate the extent of LDL uptake in the presence of PM_{2.5} solution (final 3

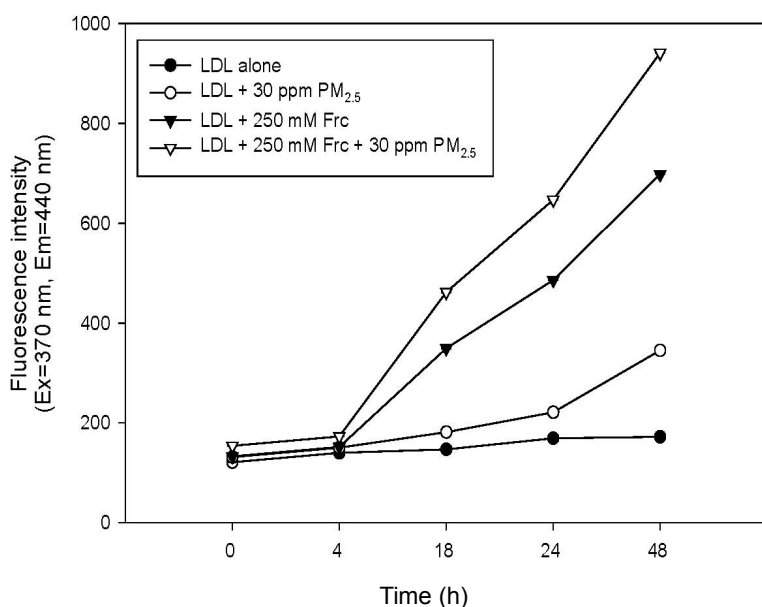


Fig. 2. Measurement of glycation extent of LDL modified by PM_{2.5} in the presence of fructose based on fluorescence intensity.

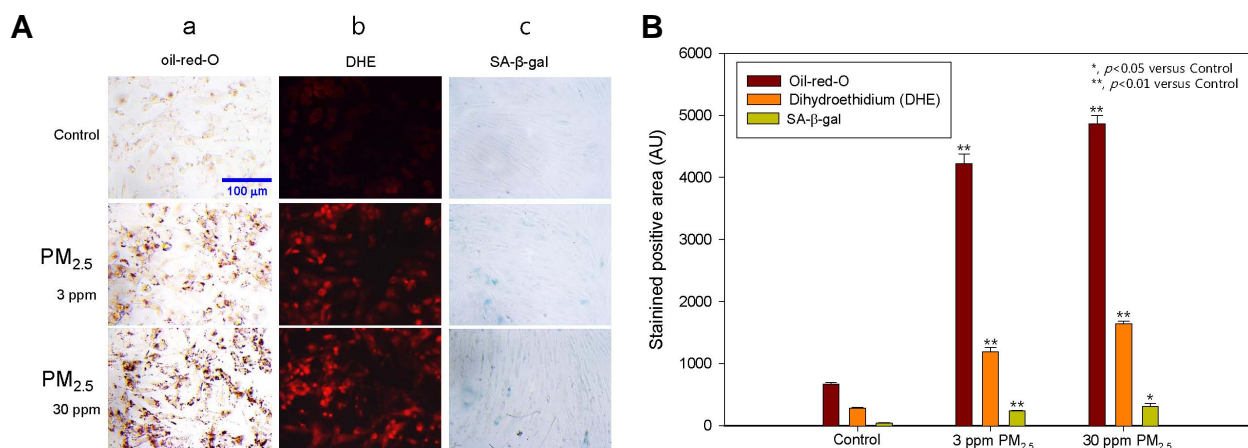


Fig. 3. (A) Treatment of human macrophages (photos a and b) and dermal fibroblasts (photo c) with PM_{2.5} to visualize cellular uptake of oxLDL, ROS production, and cellular senescence in the presence of PM_{2.5} by oil-red O staining, DHE staining, and SA-beta-gal staining, correspondingly. (B) Quantification of stained areas in photos a, b, and c by computer-assisted image analysis.

or 30 ppm in cell media) after 48 h of incubation. Treatment with 3 and 30 ppm of PM_{2.5} induced 6.3- and 7.3-fold higher cellular uptake compared to control (Fig. 3). In the same manner, ROS production as visualized by DHE staining also increased by 4.2- and 5.8-fold in PM_{2.5}-treated cells in the presence of oxLDL (Fig. 3B). These results show that LDL phagocytosis by macrophages was accelerated by PM_{2.5} in a dose-dependent manner.

Cellular senescence was more highly aggravated by PM_{2.5} extract in cell culture media. HDF cells at passage 7 were treated with serially diluted PM_{2.5} solution and incubated with subculture for 9 days. After incubation, SA-beta-gal staining performed to assess cellular aging revealed that PM_{2.5} treatment induced severe cellular senescence in a dose-dependent manner. Specifically, 3 and 30 ppm of PM_{2.5} induced 5.8- and 7.6-fold higher SA-beta-gal staining, respectively, along with 28% reduction of live cell number compared to control (Fig. 3B).

This result indicates that PM_{2.5} solution was very cytotoxic to dermal cells and caused rapid cellular senescence.

Acceleration of atherogenesis by fructose and PM_{2.5}

As shown in Fig. 4, uptake of native LDL into macrophages resulted in 42% oil-red O staining. However, PM_{2.5} solution caused 2.2-fold increased LDL uptake into cells, indicating that PM_{2.5} accelerated atherogenesis. In the presence of fructose (final 50 mM in cell media), LDL uptake was also accelerated to a similar extent as PM_{2.5} treatment. Interestingly, co-presence of PM_{2.5} and fructose cause 2.4-fold higher LDL uptake with increased cell death.

Waterborne exposure of PM_{2.5} causes embryonic toxicity

Waterborne exposure to PM_{2.5} in the presence of LPS (final 20 ppm) resulted in rapid death of zebrafish embryos in a dose-dependent manner (Fig. 5A). Embryos exposed to 3 and 30 ppm

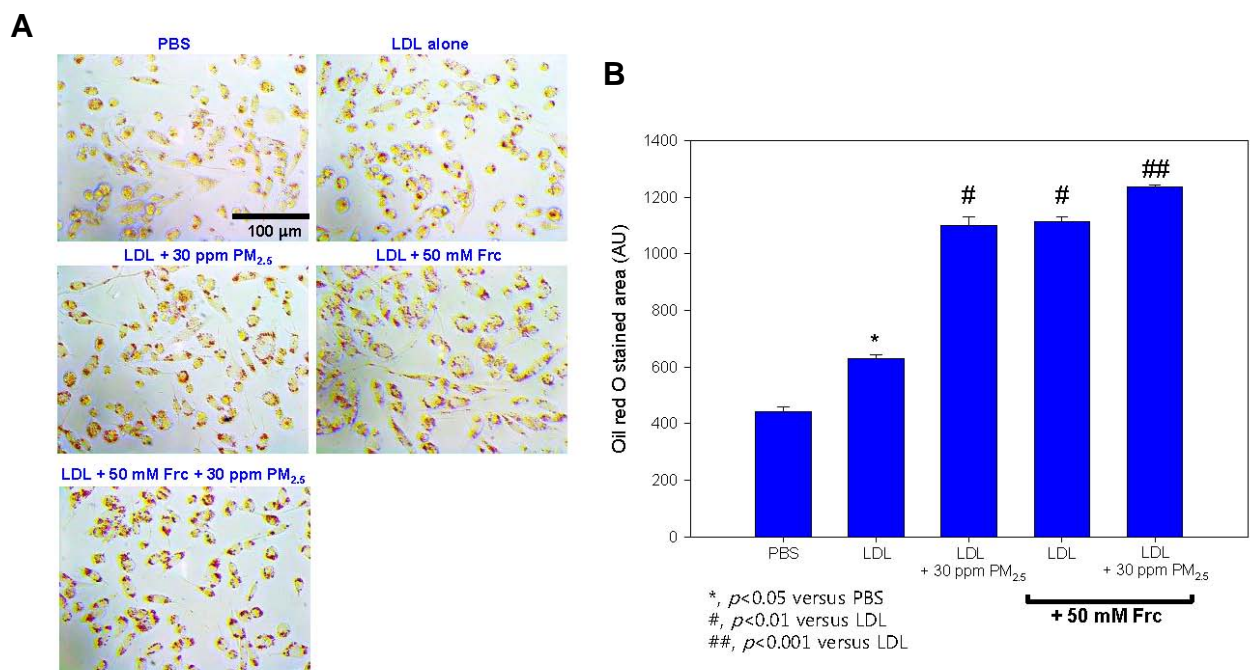


Fig. 4. Uptake of LDL into human macrophages in the presence of PM_{2.5} extract and fructose (Frc). (A) Oil red O-stained area for visualization of LDL phagocytosis. Quantification of stained area by computer-assisted image analysis.

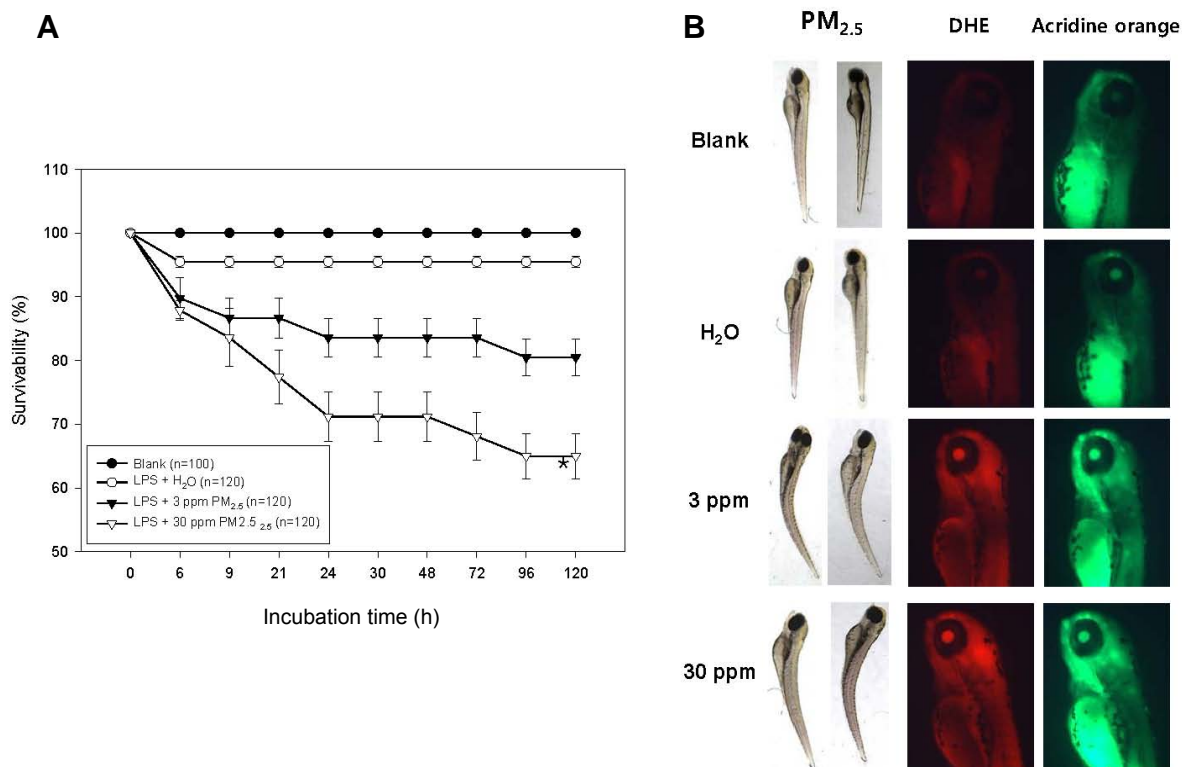


Fig. 5. Survivability of embryo in water containing PM_{2.5} water extract. (A) Survival graph of waterborne exposure to PM_{2.5} and LPS for 120 h. (B) Representative photo of embryos from stereoscopy and fluorospectroscopy. Impairment of skeletal development was observed by stereospectroscopy.

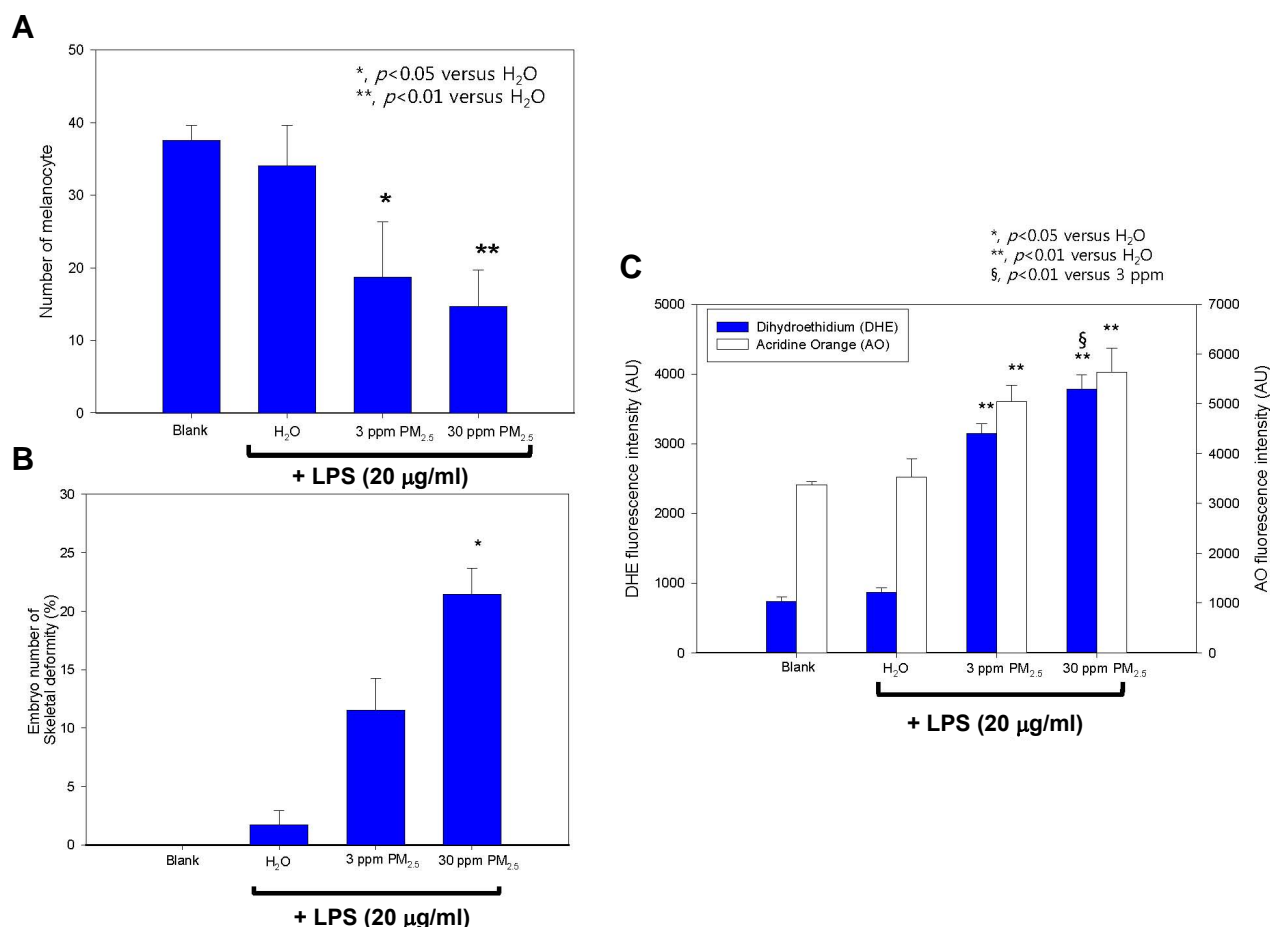


Fig. 6. Change of developmental speed and skeletal deformity induced by PM_{2.5}. (A) Development of melanocytes in embryo after 120 h post-exposure. (B) Percentage of embryonic skeletal deformity determined by spectroscopy. (C) Image analysis of stained area determined by fluorospectroscopy. Quantification of DHE-stained area and acridine orange-stained area.

of PM_{2.5} solution for 120 h showed 80% and 65% survivability, respectively, whereas embryos treated with LPS alone and blank showed 95% and 100% survivability. As shown in Fig. 6, content in the embryo abdomen, a marker of developmental speed, was significantly reduced by 48% and 60% upon exposure to 3 and 30 ppm of PM_{2.5}, respectively, compared to the control. These results suggest that embryonic survivability was more dependent on PM_{2.5} rather than LPS, a ubiquitous serum endotoxin. Developmental speed, as determined by melanin content, was attenuated upon exposure to PM_{2.5} in a dose-dependent manner.

As shown in Fig. 5B, stereoscopic observation revealed that embryos exposed to PM_{2.5} extract showed remarkable skeletal deformation in a dose-dependent manner; back skeletal bone was bent while the tail curved upward. Around 21% and 11% of embryos showed skeletal deformities at PM_{2.5} concentrations of 30 and 3 ppm, respectively, whereas H₂O control showed less than 2% incidence of skeletal deformities (Fig. 6B).

Following waterborne exposure to PM_{2.5}, ROS production and apoptotic signaling in embryos remarkably increased (Fig. 6C). Embryos treated with 30 ppm of PM_{2.5} and LPS showed 4.4- and 1.6-fold higher DHE and acridine orange staining, respectively, compared to an LPS control. This result suggests that PM_{2.5} toxicity was associated with oxidative stress and apoptosis along with developmental impairment.

Loss of antioxidant functions in the embryo

As shown in Fig. 7A, embryo homogenate in the PM_{2.5} group showed lower FRA. While embryo treated with LPS alone showed 70% increase of FRA, the increases in FRA in the presence of 3 and 30 ppm of the PM_{2.5} were 62% and 55%, respectively.

Production of oxidized species (malondialdehyde, MDA) in the embryo homogenate were increased in dose dependent manner of PM_{2.5} (Fig. 7B). LPS alone group showed 40% more increase of MDA than control, however, 3 and 30 ppm of PM_{2.5} treated group showed 2-fold and 2.6-fold, respectively, more increase of MDA than LPS alone group

Microinjection of PM_{2.5} with LDL and fructose

After 48 h, as shown in Fig. 8A, native LDL-injected zebrafish embryos showed around 80% survivability, whereas PM_{2.5} (final 30 ppm)-LDL-injected embryos showed 65% survival, indicating that PM_{2.5} induced putative embryotoxic changes in LDL. Fructose-LDL-injected embryos showed a similar death rate (around 62%). Co-treatment with PM_{2.5} and fructose increased embryonic death (51% survival), indicating that PM_{2.5} and fructose synergistically increased embryotoxic changes in LDL.

Stereoscopic observation revealed that PM_{2.5}-fructose-LDL-injected embryos showed the slowest developmental speed as well as the highest ROS production (Fig. 8B). PM_{2.5}-LDL injected

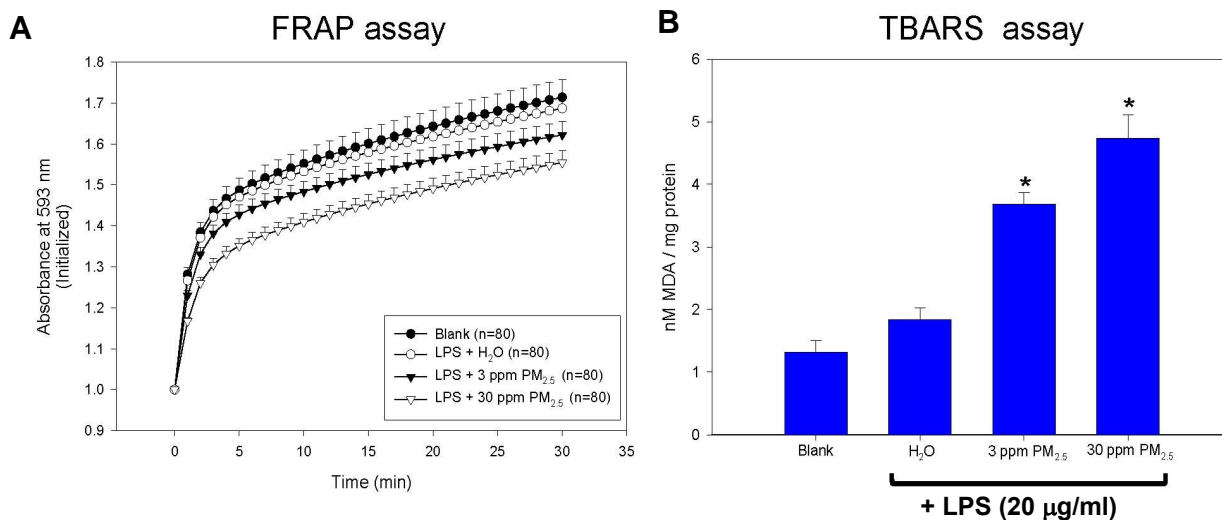


Fig. 7. Assessment of antioxidant ability and production of oxidized species in embryo by waterborne exposure to PM_{2.5} and LPS. (A) Ferric ion reduction ability of the embryo homogenate. (B) Quantification of oxidized species in the embryo homogenate.

tion and fructose-LDL injection caused 1.5-fold and 2.2-fold higher ROS production than LDL alone, whereas PM_{2.5}-fructose-LDL-injected embryos showed 2.9-fold higher ROS production than native LDL injection (Fig. 8C).

DISCUSSION

Many epidemiological studies have suggested that PM is associated with cardiovascular morbidity, respiratory disease, lung cancer, and low birth weight in infants. The Louis group estimated that 10 µg/m³ of PM₁₀ is associated with a 0.15% increase in mortality (Peng et al., 2006). Although there have been many controversies about the origin of PM, its direct relationship with incidence of many metabolic diseases has been well established.

Our current results show that PM_{2.5} modified HDL and LDL to produce aggregated HDL and oxidized LDL along with degradation of apo-B. Our results also provide clues to explain the link between PM_{2.5} as well as atherosclerosis and diabetes. Indeed, diabetic patients show increased glycation of serum apolipoproteins, and glycated and oxidized LDL is a major culprit of cardiovascular disease. The glycation pattern of HDL (Fig. 1) was very similar with our previous report showing that apoA-I undergoes multimerization and proteolysis by fructosylation (Jang et al., 2011; Park and Cho, 2011b). In our study, PM_{2.5} and fructose induced severe apoA-I multimerization and degradation along with smear band morphology, suggesting that PM_{2.5} can exacerbate glycation in serum. LDL was also proteolyzed by PM_{2.5} via acceleration of glycation and oxidation. Therefore, patients with diabetes, atherosclerosis, and Alzheimer's should avoid exposure to PM_{2.5} to avoid exacerbation of pre-existing disease symptoms via aggregation and degradation of serum apolipoproteins. Many reports have shown that long- or short-term exposure to PM_{2.5} is associated with incidence of chronic and acute metabolic diseases such as insulin resistance and visceral adiposity (Sun et al., 2009). These results clearly show that PM_{2.5} extract modified lipoproteins to aggravate atherogenesis and embryonic toxicity.

Furthermore, mental diseases in children such as autism have been shown to be associated with PM exposure. Two studies in California demonstrated strong associations between PM_{2.5} exposure during pregnancy and autism (Becerra et al., 2013; Volk et al., 2013). In the current study, waterborne exposure to PM_{2.5} or modified LDL by PM_{2.5} impaired embryonic development via oxidative stress, resulting in mortality. Moreover, PM_{2.5} caused embryonic toxicity in the presence of LPS. To the best of our knowledge, this is the first report showing that PM_{2.5} has embryonic toxicity in vertebrates via waterborne exposure.

The detailed molecular mechanisms driving toxicities in the human body are still unknown. Furthermore, there has been no report on the effects of PM_{2.5} on lipoprotein metabolism. In addition to cardiovascular disease, patients with diabetes show altered lipoprotein states, such as oxidized LDL and dysfunctional HDL. These results suggest that lipoprotein quality is a critical factor in the progression of cardiovascular disease and mortality.

Inhalation of PM_{2.5} is more dangerous compared to PM₁₀ or larger particles, as PM_{2.5} can reach deeper into the lungs, alveoli, and lung sac. As greater than 90% of particles larger than 10 µm are removed in the nostrils or nasopharynx, particles between 5-10 microns in size impact the carina. During O₂ and CO₂ exchange in the capillaries, ingredients of PM_{2.5} can be dissolved and transported directly into the bloodstream. Inhaled PM_{2.5} can be transported from lung alveoli to pneumocytes and capillaries, after which it is dissolved in the bloodstream. After inhalation, major hydrophilic components can be dissolved and circulated into blood and serum via the pulmonary artery. During blood circulation, components of PM_{2.5} may affect the function and structure of lipoproteins. Lipoproteins can also be affected by blood exchange in the placenta between mothers and the fetus.

Reduction of total suspended particulate (TSP) pollution has been shown to prevent mortality in adults and the elderly, implying a link between TSP and mortality rates (Chay et al., 2003). Specifically, an increase in TSP of 1 µg/m³ has been shown to elevate the premature death rate. In the same context, we re-

***p*<0.01 versus PBS, ##*p*<0.01 versus LDL

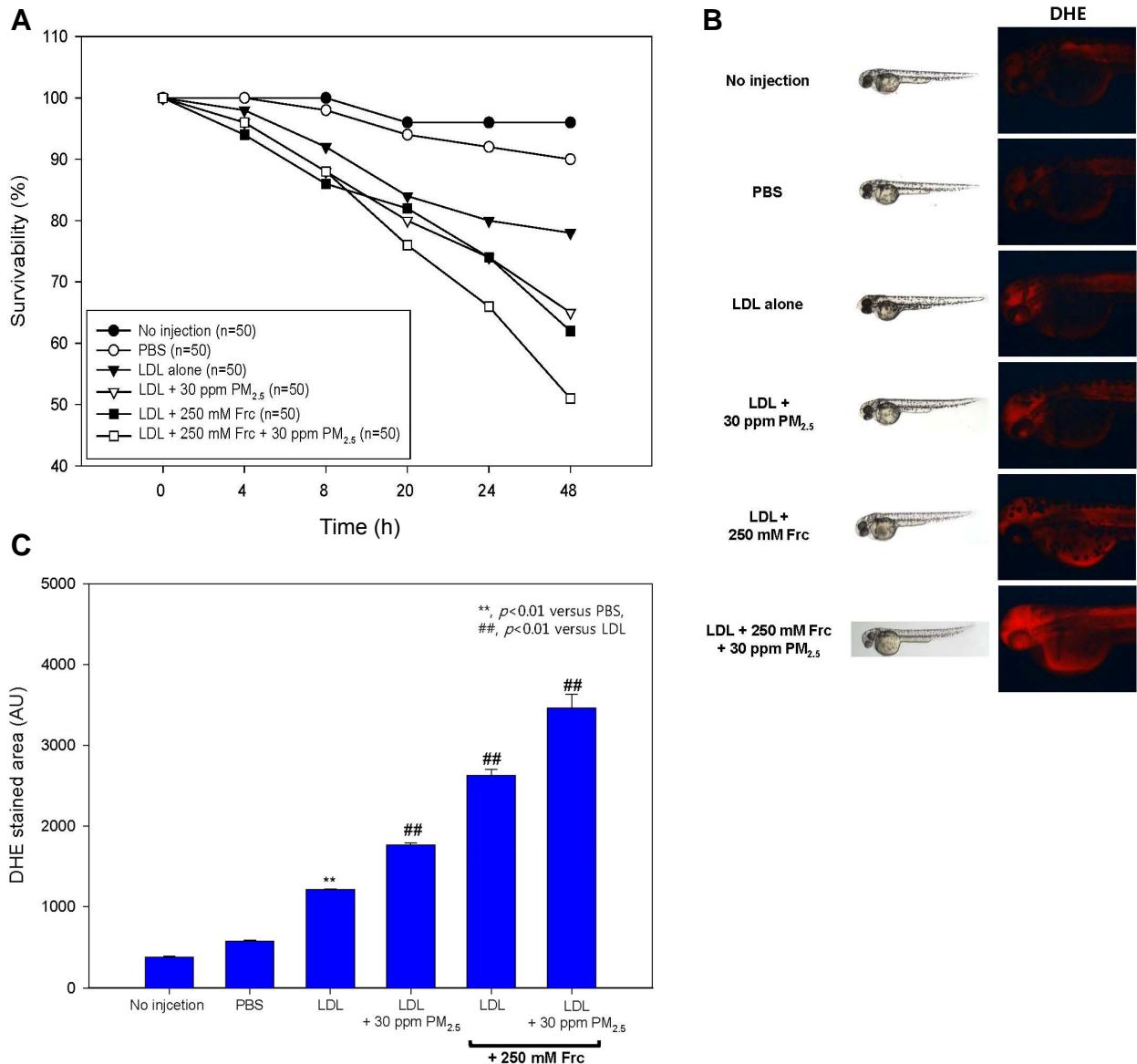


Fig. 8. Developmental stage of embryos and ROS production after co-injection of LDL and PM_{2.5} in the presence of fructose. (A) Survivability of embryos at 48 h post-injection. (B) Representative photo of embryos from stereoscopy at 48 h post-injection. Production of ROS was determined by DHE staining based on fluorescence (Ex = 588 nm, Em = 615 nm). (C) Quantification of DHE-stained area from image analysis.

cently showed that modified HDL by tobacco smoking causes dermal cell senescence via apoA-I truncation and multimerization (Park et al., 2014b). This report raises the possibility that inhalation of dangerous ingredients can exert toxic effects on blood cells and proteins. We also reported that injection of modified HDL by trans fatty acids (TFA) induces embryonic toxicity along with rapid death and ROS production (Park et al., 2014a), whereas native apoA-I and rHDL exerts anti-inflammatory effects.

The current report is also the first to show that serum lipoproteins can be modified by PM_{2.5} with synergistic effects in the

presence of fructose. These results suggest that pregnant women and patients with diabetes and/or hyperglycemia should avoid PM_{2.5} exposure, which can impair fetal development and aggravation of disease progression. Future studies should address the pathological mechanism by which PM_{2.5} aggravates disease via amyloidosis and protein aggregation.

ACKNOWLEDGMENTS

This work was supported by the Mid-career Researcher Program (2014-11049455) and the Medical Research Center Program (2015R1A5A2009124) through the National Research

Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning.

REFERENCES

- Becerra, T.A., Wilhelm, M., Olsen, J., Cockburn, M., and Ritz, B. (2013). Ambient air pollution and autism in Los Angeles county, California. *Environ. Health Perspect* 121, 380-386.
- Bozlaker, A., Spada, N.J., Fraser, M.P., and Chellam, S. (2014). Elemental characterization of PM_{2.5} and PM₁₀ emitted from light duty vehicles in the Washburn Tunnel of Houston, Texas: release of rhodium, palladium, and platinum. *Environ. Sci. Technol.* 48, 54-62.
- Brook, R.D., Rajagopalan, S., Pope, C.A. 3rd., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., Holguin, F., Hong, Y., Luepker, R.V., Mittleman, M.A., et al. (2010). Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation* 121, 2331-2378.
- Chay, K., Dopkin, C., and Greenstone, M. (2003). The clean air act of 1970 and adult mortality. *J. Risk Uncertainty* 27, 279-300.
- Cho, K.H. (2009). Biomedical implications of high-density lipoprotein: its composition, structure, functions, and clinical applications. *BMB Rep.* 42, 393-400.
- Cho, K.H. (2011). Enhanced delivery of rapamycin by V156K apoA-I high-density lipoprotein inhibits cellular pro-atherogenic effects and senescence and promotes tissue regeneration. *J. Gerontol. A. Biol. Sci. Med. Sci.* 66, 1274-1285.
- Groop, P.H., Thomas, M.C., Rosengård-Bärlund, M., Mills, V., Rönnback, M., Thomas, S., Forsblom, C., Taskinen, M.R., and Viberti, G. (2007). HDL composition predicts new-onset cardiovascular disease in patients with type 1 diabetes. *Diabetes Care* 30, 2706-2707.
- Jalava, P.I., Salonen, R.O., Pennanen, A.S., Happonen, M.S., Penttinen, P., Hälinen, A.I., Sillanpää, M., Hillamo, R., and Hirvonen, M.R. (2008). Effects of solubility of urban air fine and coarse particles on cytotoxic and inflammatory responses in RAW 264.7 macrophage cell line. *Toxicol. Appl. Pharmacol.* 229, 146-160.
- Jang, W., Jeoung, N.H., and Cho, K.H. (2011). Modified apolipoprotein (apo) A-I by artificial sweetener causes severe premature cellular senescence and atherosclerosis with impairment of functional and structural properties of apoA-I in lipid-free and lipid-bound state. *Mol. Cells* 31, 461-470.
- Jin, S., and Cho, K.H. (2011). Water extracts of cinnamon and clove exhibits potent inhibition of protein glycation and anti-atherosclerotic activity *in vitro* and *in vivo* hypolipidemic activity in zebrafish. *Food Chem. Toxicol.* 49, 1521-1529.
- Kim, J.Y., Kim, H.H., and Cho, K.H. (2013). Acute cardiovascular toxicity of sterilizers, PHMG, and PGH: severe inflammation in human cells and heart failure in zebrafish. *Cardiovasc. Toxicol.* 13, 148-160.
- Kim, J.Y., Park, K.H., Kim, J., Choi, I., and Cho, K.H. (2015a). Modified high-density lipoproteins by artificial sweetener, aspartame, and saccharin, showed loss of anti-atherosclerotic activity and toxicity in zebrafish. *Cardiovasc. Toxicol.* 15, 79-89.
- Kim, S.M., Lim, S.M., Yoo, J.A., Woo, M.J., and Cho, K.H. (2015b). Consumption of high-dose vitamin C (1,250 mg/day) enhances functional and structural properties of serum lipoprotein to improve anti-oxidant, anti-atherosclerotic, and anti-aging effects via regulation of anti-inflammatory microRNA. *Food Funct.* 6, 3604-3612.
- Nurkiewicz, T.R., Porter, D.W., Hubbs, A.F., Stone, S., Moseley, A.M., Cumpston, J.L., Goodwill, A.G., Frisbee, S.J., Perrotta, P.L., Brock, R.W., et al. (2011). Pulmonary particulate matter and systemic microvascular dysfunction. *Res. Rep. Health Eff. Inst.* 164, 3-48.
- Nüsslein-Volhard, C., and Dahm, R. (2002). Zebrafish: a practical approach. (New York: Oxford University Press).
- Park, K.H., Shin, D.G., Kim, J.R., and Cho, K.H. (2010). Senescence-related truncation and multimerization of apolipoprotein A-I in high-density lipoprotein with an elevated level of advanced glycated end products and cholesteryl ester transfer activity. *J. Gerontol. A. Biol. Sci. Med. Sci.* 65, 600-610.
- Park, K.H., and Cho, K.H. (2011a). A zebrafish model for the rapid evaluation of pro-oxidative and inflammatory death by lipopolysaccharide, oxidized low-density lipoproteins, and glycated high-density lipoproteins. *Fish Shellfish Immunol.* 31, 904-910.
- Park, K.H., and Cho, K.H. (2011b). High-density lipoprotein (HDL) from elderly and reconstituted HDL containing glycated apolipoproteins A-I share proatherosclerotic and pro-senescent properties with increased cholesterol influx. *J. Gerontol. A. Biol. Sci. Med. Sci.* 66, 511-520.
- Park, K.H., Kim, J.M., and Cho, K.H. (2014a). Elaidic acid (EA) generates dysfunctional high-density lipoproteins and consumption of EA exacerbates hyperlipidemia and fatty liver change in zebrafish. *Mol. Nutr. Food Res.* 58, 1537-1545.
- Park, K.H., Shin, D.G., and Cho, K.H. (2014b). Dysfunctional lipoproteins from young smokers exacerbate cellular senescence and atherogenesis with smaller particle size and severe oxidation and glycation. *Toxicol. Sci.* 140, 16-25.
- Peng, R.D., Dominici, F., and Louis, T.A. (2006). Model choice in time series studies of air pollution and mortality. *J. R. Statist. Soc. A.* 169, 179-203.
- Peters, A., Dockery, D.W., Muller, J.E., and Mittleman, M.A. (2001). Increased particulate air pollution and the triggering of myocardial infarction. *Circulation* 103, 2810-2815.
- Pope, C.A.3rd., Burnett, R.T., Thurston, G.D., Thun, M.J., Calle, E.E., Krewski, D., and Godleski, J.J. (2004). Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. *Circulation* 109, 71-77.
- Ramos de Rainho, C., Machado Corrêa, S., Luiz Mazzei, J., Alessandra Fortes Aiub, C., and Felzenszwalb, I. (2013). Genotoxicity of polycyclic aromatic hydrocarbons and nitro-derived in respirable airborne particulate matter collected from urban areas of Rio de Janeiro (Brazil). *Biomed. Res. Int.* 2013, 765352.
- Sun, Q., Yue, P., DeJulius, J.A., Lumeng, C.N., Kampfrath, T., Mikolaj, M.B., Cai, Y., Ostrowski, M.C., Lu, B., Parthasarathy, S., et al. (2009). Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. *Circulation* 119, 538-546.
- Traversi, D., Schilirò, T., Degan, R., Pignata, C., Alessandria, L., and Gilli, G. (2011). Involvement of nitro-compounds in the mutagenicity of urban Pm_{2.5} and Pm₁₀ in Turin. *Mutat. Res.* 726, 54-59.
- van Leuven, S.I., Stroes, E.S., and Kastelein, J.J. (2008). High-density lipoprotein: A fall from grace? *Ann. Med.* 40, 584-593.
- Volk, H.E., Lurmann, F., Penfold, B., Hertz-Picciotto, I., and McConnell, R. (2013). Traffic-related air pollution, particulate matter, and autism. *JAMA Psychiatry* 70, 71-77.
- Yoon, J.H., and Cho, K.H. (2012). A Point Mutant of Apolipoprotein A-I (V156K) Showed Enhancement of Cellular Insulin Secretion and Potent Activity of Facultative Regeneration in Zebrafish. *Rejuvenation Res.* 15, 313-321.
- Yoo, J.A., Lee, E.Y., Park, J.Y., Lee, S.T., Ham, S., and Cho, K.H. (2015). Different functional and structural characteristics between apoA-I and apoA-4 in lipid-free and reconstituted HDL state: apoA-4 showed less anti-atherogenic activity. *Mol. Cells* 38, 573-579.