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

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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

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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% dissolvation). 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 ($1.019 < d < 1.063$), HDL_2 ($1.063 < d < 1.125$), and HDL_3 ($1.125 < d < 1.225$) 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, HDL_2, and HDL_3 (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% CO_2. 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 $CuSO_4$ 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 HDL_3 showed 2-fold higher fluorescence than HDL_3 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 HDL_3 (lane 2) showed slower electrophoretic mobility than HDL_3 alone (lane 1) in SDS-PAGE. Furthermore, the apoA-I band in $PM_{2.5}$-treated HDL_3 (lane 2) showed a smeared band pattern with more upward shift than HDL_3 alone (lane 1).

In the presence of fructose, $PM_{2.5}$ extract-treated HDL (lane 4) showed up to 16-fold higher fluorescence than HDL_3 alone

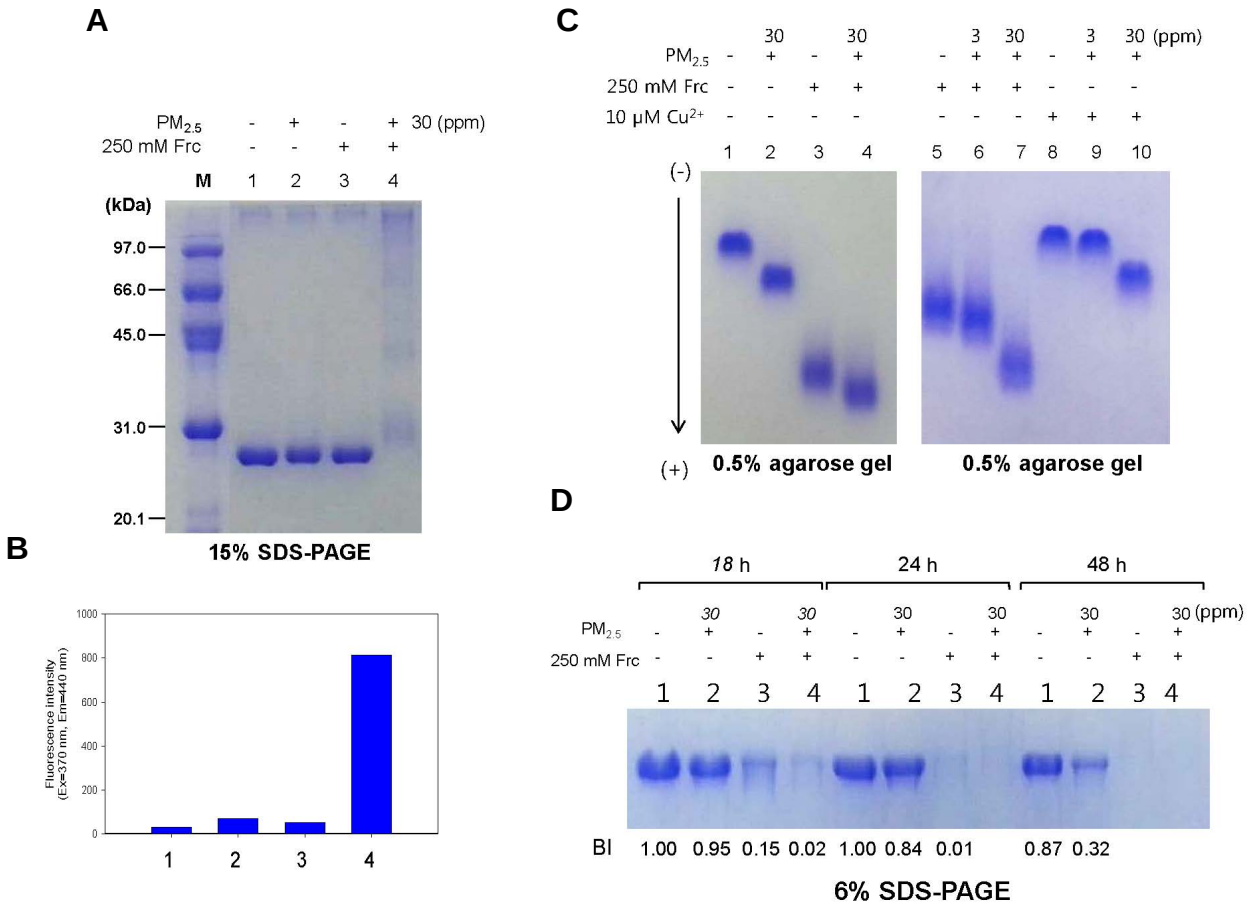

Fig. 1. Modification of HDL and LDL by $PM_{2.5}$ treatment in the presence of fructose (final 250 mM). (A) Electrophoretic patterns of HDL_3 in the presence of $PM_{2.5}$ extract (15% SDS-PAGE). (B) Measurement of glycation extent of HDL_3 treated with $PM_{2.5}$ extract based on fluorescence intensity. (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 HDL_3 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

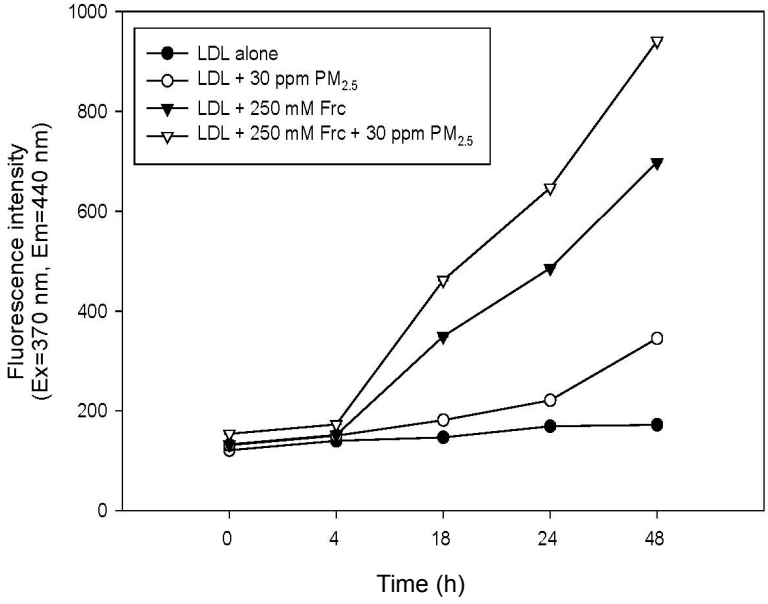

Fig. 2. Measurement of glycation extent of LDL modified by $PM_{2.5}$ in the presence of fructose based on fluorescence intensity.

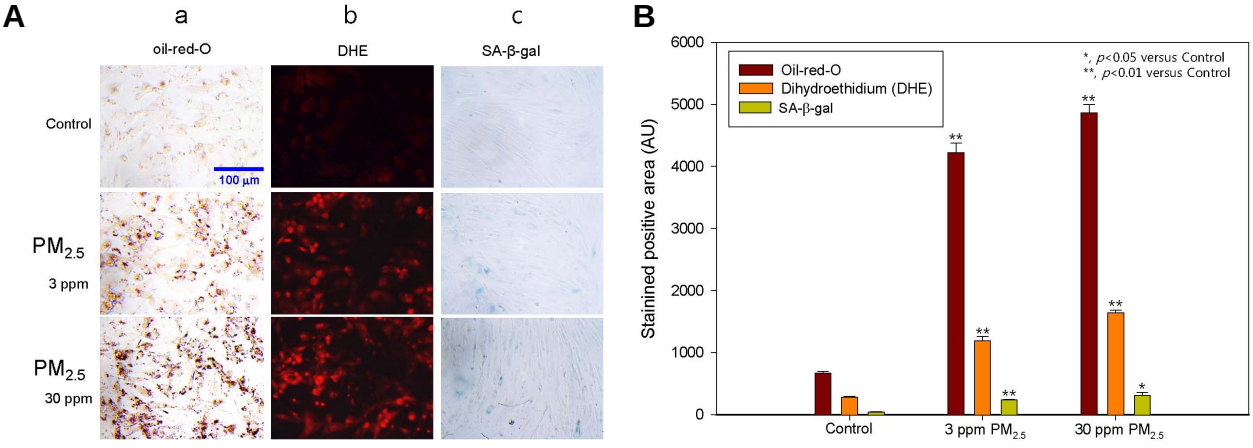

Fig. 3. (A) Treatment of human macrophages (photos a and b) and dermal fibroblasts (photo c) with $PM_{2.5}$ to visualize cellular uptake of oxLDL, ROS production, and cellular senescence in the presence of $PM_{2.5}$ by oil-red O staining, DHE staining, and SA-beta-gal staining, correspondingly. (B)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

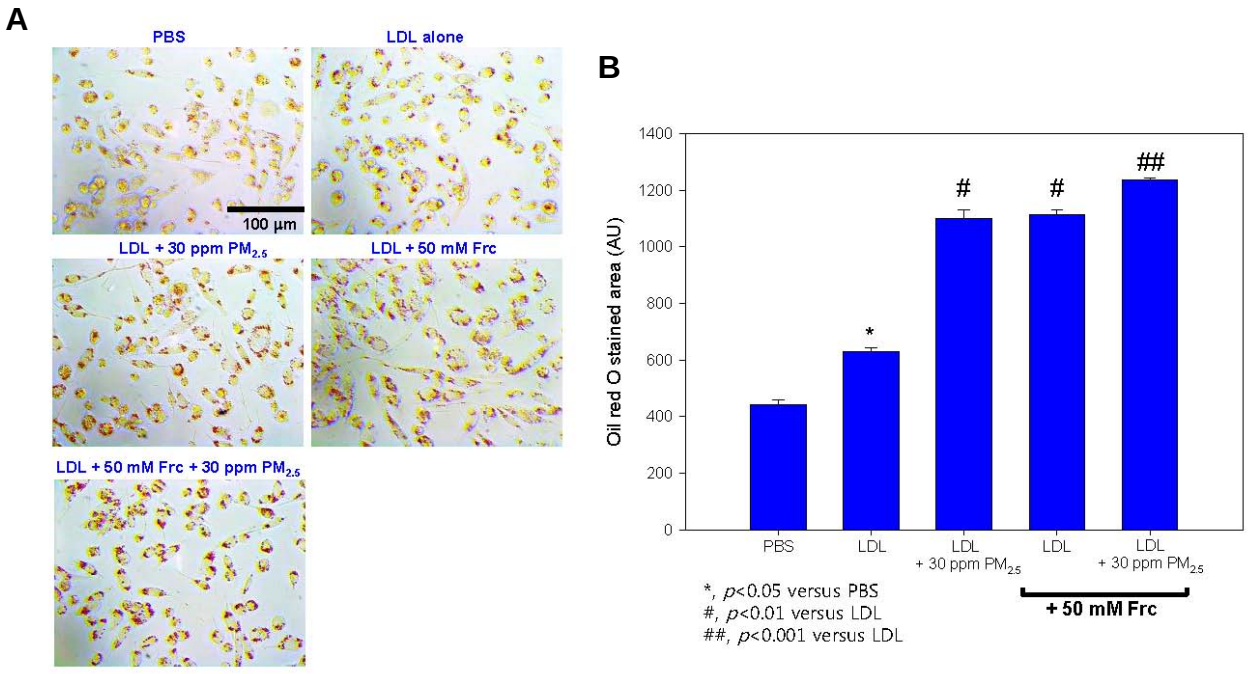

Fig. 4. Uptake of LDL into human macrophages in the presence of $PM_{2.5}$ extract and fructose (Frc). (A) Oil red O-stained area for visualization of LDL phagocytosis. Quantification of stained area by computer-assisted image analysis.

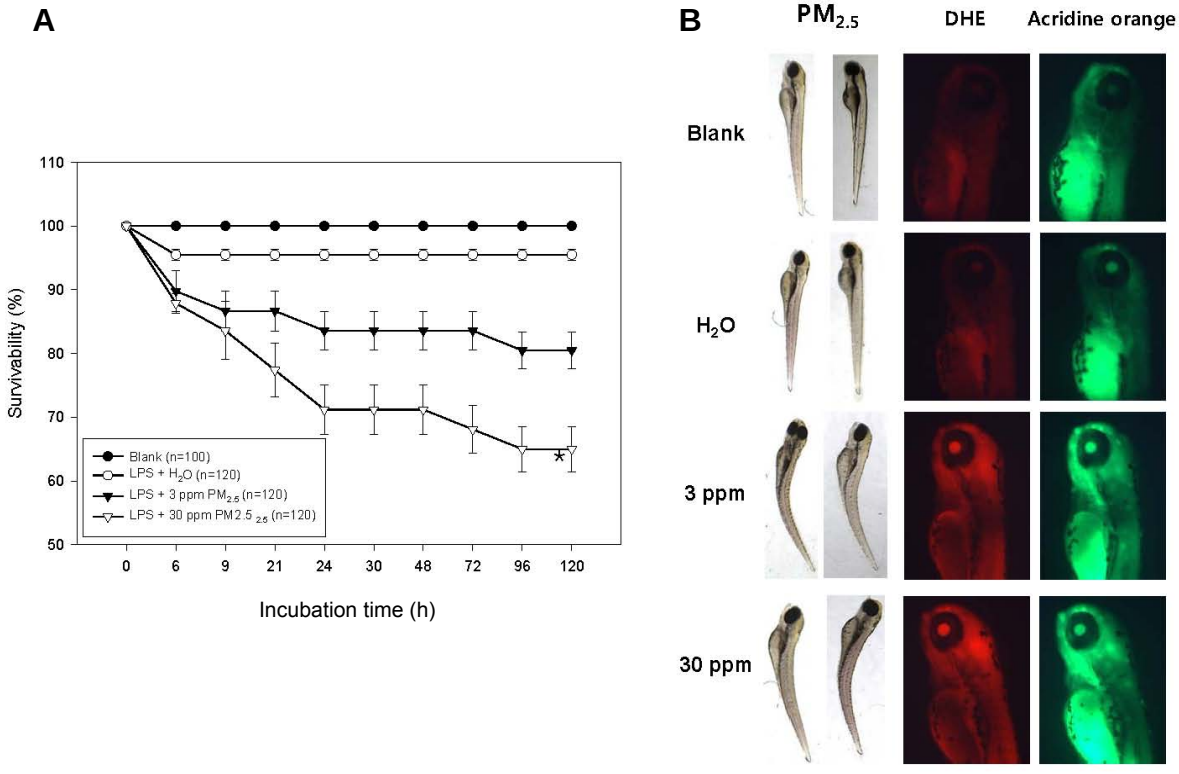

Fig. 5. Survivability of embryo in water containing $PM_{2.5}$ water extract. (A) Survival graph of waterborne exposure to $PM_{2.5}$ and LPS for 120 h. (B) Representative photo of embryos from stereoscopy and fluorospectroscopy. Impairment of skeletal development was observed by stereospectroscopy.

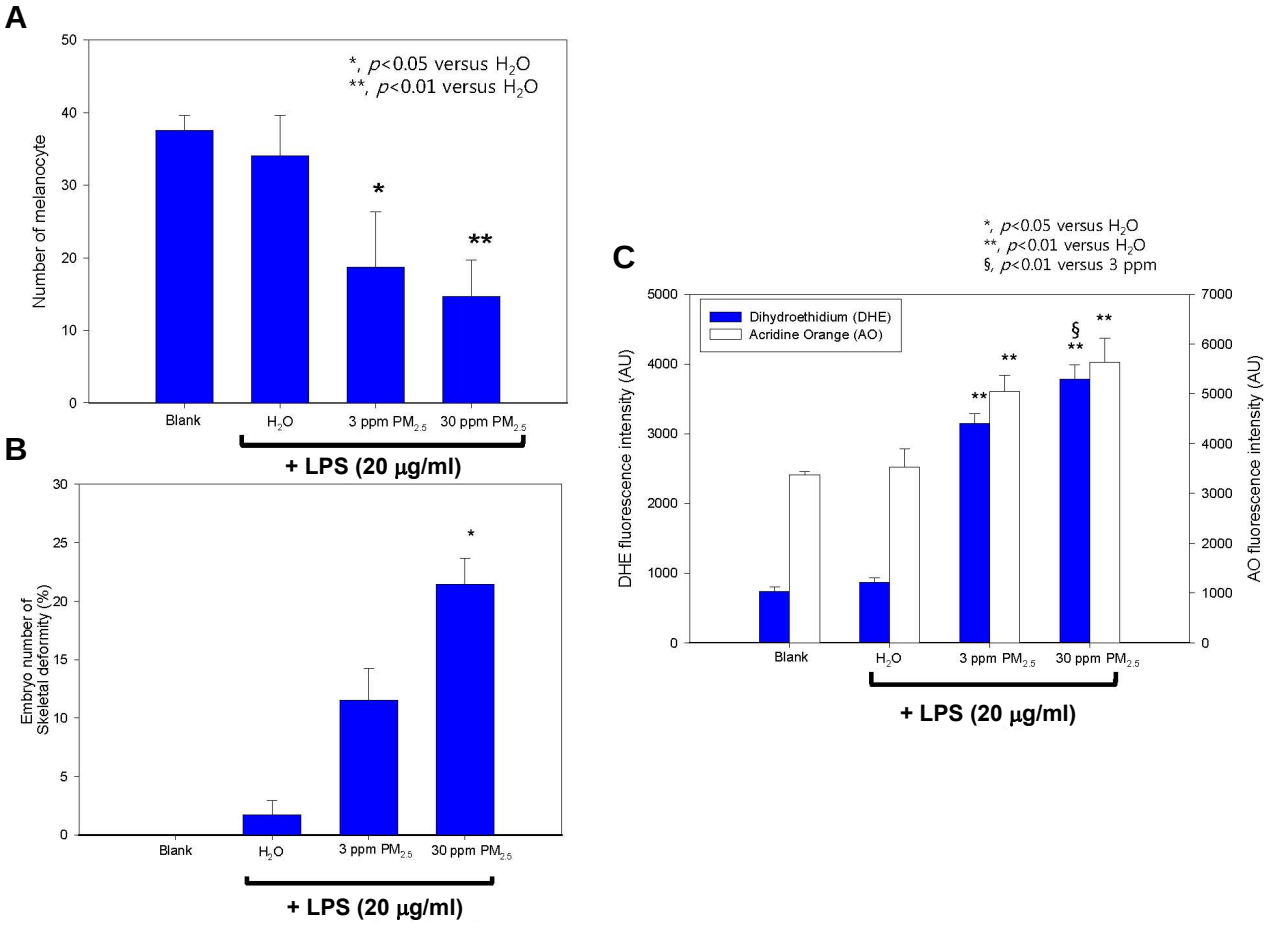

Fig. 6. Change of developmental speed and skeletal deformity induced by $PM_{2.5}$. (A) Development of melanocytes in embryo after 120 h post-exposure. (B) Percentage of embryonic skeletal deformity determined by spectroscopy. (C) Image analysis of stained area determined by fluorospectroscopy. Quantification 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 H_2O 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 injec-

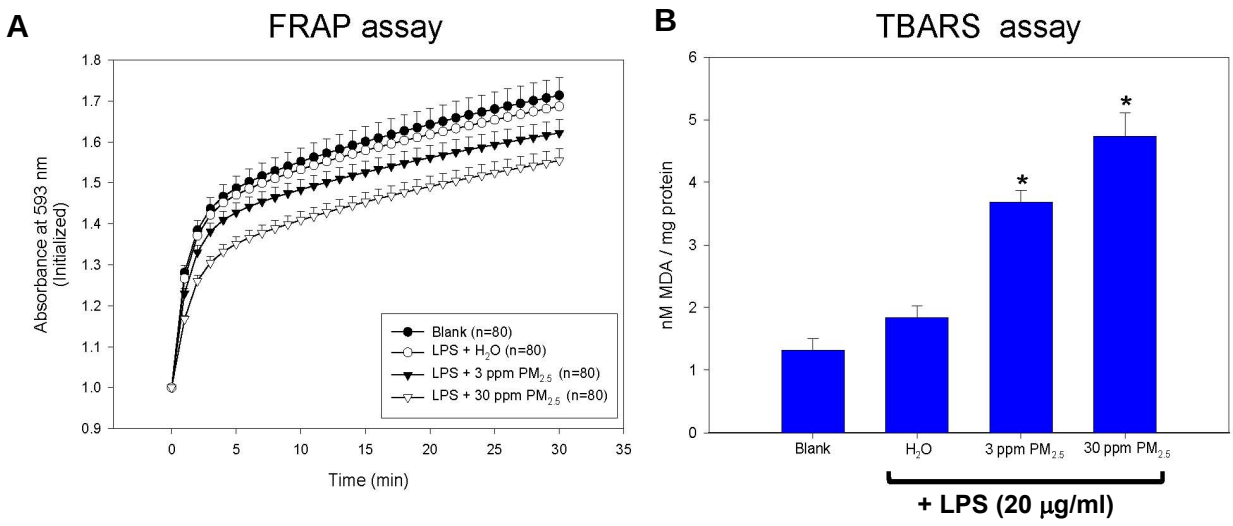

Fig. 7. Assessment of antioxidant ability and production of oxidized species in embryo by waterborne exposure to $PM_{2.5}$ and LPS. (A) Ferric ion reduction ability of the embryo homogenate. (B) Quantification of oxidized species in the embryo homogenate.

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 $\mu g/m^3$ of PM_{10} 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 PM_{10} 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 nasopharinx, particles between 5-10 microns in size impact the carina. During O_2 and CO_2 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 $\mu g/m^3$ has been shown to elevate the premature death rate. In the same context, we re-

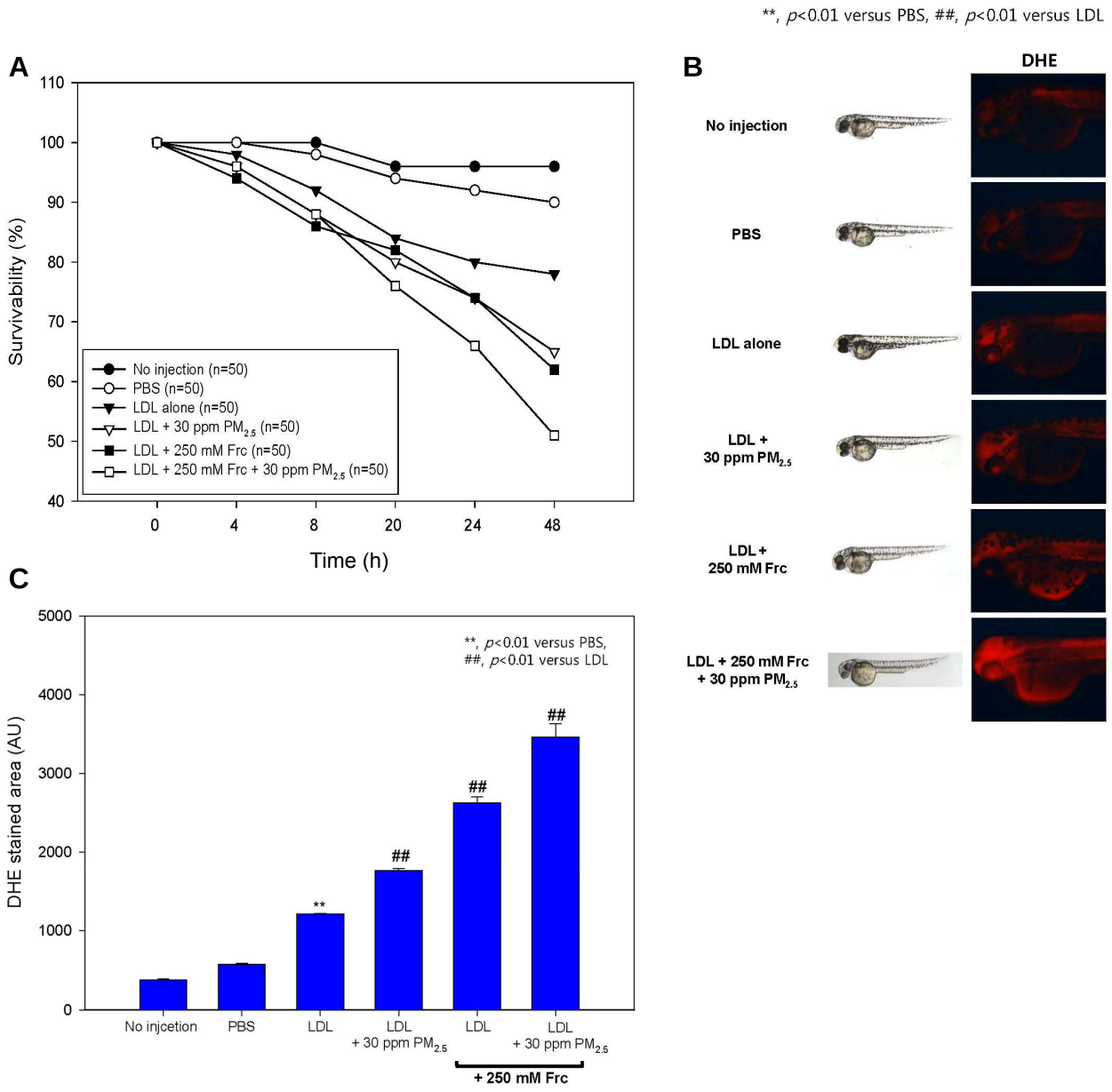

Fig. 8. Developmental stage of embryos and ROS production after co-injection of LDL and $PM_{2.5}$ in the presence of fructose. (A) Survivability of embryos at 48 h post-injection. (B) Representative photo of embryos from stereoscopy at 48 h post-injection. Production of ROS was determined by DHE staining 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.

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