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Ambient fine particulate matters induce cell death and inflammatory response by influencing mitochondria function in human corneal epithelial cells

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ABSTRACT

Ambient fine particulate matter (AFP) is a main risk factor for the cornea as ultraviolet light. However, the mechanism of corneal damage following exposure to AFP has been poorly understood. In this study, we first confirmed that AFP can penetrate the cornea of mice, considering that two-dimensional cell culture systems are limited in reflecting the situation in vivo. Then, we investigated the toxic mechanism using human corneal epithelial (HCET) cells. At 24 h after exposure, AFP located within the autophagosome-like vacuoles, and cell proliferation was clearly inhibited in all the tested concentration. Production of ROS and NO and secretion of pro-inflammatory cytokines were elevated in a dose-dependent manner. Additionally, conversion of LC3B from I-type to II-type and activation of caspase cascade which show autophagic- and apoptotic cell death, respectively, were observed in cells exposed to AFP. Furthermore, AFP decreased mitochondrial volume, inhibited ATP production, and altered the expression of metabolism-related genes. Taken together, we suggest that AFP induces cell death and inflammatory response by influencing mitochondrial function in HCET cells. In addition, we recommend that stringent air quality regulations are needed for eye health.

1. Introduction

The cornea, which is the eye's outermost layer, is responsible for 65–75% of total our vision by helping focus the light that comes into the eye (DelMonte and Kim, 2011; Castro-Muñozledo, 2013; Eghrari et al., 2015), it also serves as a filter that blocks damage of the lens and the retina by ultraviolet light from the sun. The cornea is arranged in five layers including epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium, and the epithelium, which is the outermost layer of the cornea, primarily functions as a barrier against foreign materials, such as dust, water, particles, and bacteria that can harm the eye. The epithelium is also filled with thousands of tiny nerve endings, which is why our eye may hurt when it is rubbed or scratched. Additionally, the cornea receives its nourishment from tears and the aqueous humor (a fluid in the front part of the eye that lies behind the cornea), but not blood vessels, and the epithelium provides a smooth surface for absorbing oxygen and nutrients from tears. Therefore, the corneal epithelium should be well protected for our eye health.

Oxidative stress is caused by an imbalance between the generated reactive oxygen species (ROS) level and the biological systems' antioxidant ability (Amico et al., 2015; Wakamatsu et al., 2008). Accumulating evidences show that oxidative stress may initiate or develop ocular injury resulting in decreased visual acuity or even vision loss (Amico et al., 2015; Li et al., 2008; Pastori et al., 2015; Porcu et al., 2007; Serbecic and Beutelspacher, 2005; Uchino et al., 2012). For example, composite house dusts induced the toxic effects via oxidative stress in primary human corneal epithelial cells and altered antioxidant enzymes- and pro-inflammatory mediators-related mRNA expression (Xiang et al., 2016a). Additionally, supplementation of vitamins, such as vitamin A, C and E, inhibited apoptotic cell death by preventing the free radical generation and lipid peroxidation in corneal endothelial cells (Serbecic and Beutelspacher, 2005).

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Ambient fine particulate matters (AFP), which are particulate matters less than 2.5 μm in aerodynamic diameter (Auchincloss et al., 2008), can directly contact with our eyes as ultraviolet light, thus it can play as a risk factor for our eyes. As well, previous studies propose that the corneal epithelial damage may depend on the constituents of ambient particulate matters. For example, oxidative stress-related genes and pancreatic and eye-lens gene markers appeared de-regulated in embryos exposed to urban extracts, whereas exposure to rural extracts affected genes related to basic cellular functions (Mesquita et al., 2015). Additionally, water and organic soluble fractions of dusts caused cytotoxicity, oxidative damage, inflammatory response, and activation of ligand-activated transcription factors on primary HCET cells, and the organic extracts showed higher potential to induce adverse effects compared to water extracts (Xiang et al., 2016b). Furthermore, epidemiological studies suggested that the number of patient with dry eye disease, which is caused by a chronic lack of sufficient lubrication and moisture on the surface of the eye, is rapidly increasing (Mantelli et al., 2013; Versura et al., 2013; Labbé et al., 2007) and air pollution was raised over the course of the past 20 years, coinciding with increased incidence of dry eye (Cejka and Cejkova, 2015). However, the underlying mechanism of corneal damage following exposure to AFP has not been fully elucidated. Considering that the cornea consists of multilayer which functions as a barrier against foreign materials and that conventional 2D cell cultures may be too simple to predict biological response in living tissues (Cao et al., 2015), we first confirmed that AFP can penetrate the cornea of mice (Fig. S1). Then, we investigated the toxic mechanism using human corneal epithelial (HCET) cells.

2. Material and methods

2.1. AFP preparation

As previously reported (Park et al., 2011), AFP was weekly collected using PM2.5 high volume samplers (Sibata HV 1000F, Tokyo, Japan) with PTFE filters (Zefluor™, 2.0 μm, PALL life Sciences, NU, USA) on the top of a university building (from January to May, 2008, approximately 12 m above the ground). A sampling site is close to big apartment complexes, where about 2200 households live and is approximately 300 m from the busy traffic road (Park et al., 2008, 2011). All the filters were dispersed in autoclaved deionized water (DW) for 1 h using a sonicator (37 °C). After removing filters, the extract was lyophilized and stored in a deep freezer (below −80 °C) until used in the experiment. For this study, AFP (1 mg/mL) was resuspended in autoclaved DW and sterilized by autoclaving. Endotoxin level in the suspended AFP was below the detection limit (0.1 EU/mL) as was assessed by a Limulus amebocyte lysate (LAL) chromogenic endpoint assay kit (Pierce LAL Chromogenic Endotoxin Quantitation Kit, Thermo Scientific, Waltham, MA, USA).

2.2. Characterization of water-soluble components bound to AFP

The chemical and physical characterization of all samples was investigated by using transmission electron microscopy with energy dispersive X-ray spectroscopy (TEM with EDS, Tecnai G2 F30 S-Twin, FEI, Hillsboro, OR, USA). The morphology of samples was analyzed with scanning electron microscopy (SEM, SNE-3000M, SEC, Korea). The hydrodynamic diameter (HDD) and zeta potential (i.e., surface charge) was measured by dynamic light scatting (DLS, ELS-Z, Photal, Japan) and electrophoretic light scattering (ELS, ELS-Z, Photal, Japan), respectively. Additionally, we measured the concentration of water soluble components bound to AFP. Briefly, autoclaved AFP-contained solution was filtered by using a syringe filter (pore size, 0.45 μm, 16555 K, Minisart, Sigma-Aldrich, St. Louis, MO, USA), and then water soluble fraction was digested in a microwave digestion system (Milestone, Sorisole, Italy) according to EPA method 3052. The concentration of elements contained in the soluble component was measured using inductively coupled plasma mass spectrometry (ICP-MS, 7700, Agilent Technologies, Santa Clara, CA, USA) at the Korean Basic Science Institute (Supplementary Table 1, Park et al., 2011). To ensure the reliability of the analysis results, standard reference materials (NIST 1646a and USGS BCR-2) were digested and measured under the same condition (Supplementary Table 2).

2.3. Cell culture

HCET cells were kindly provided by Kaoru Araki-Sasaki (Osaka University, School of Medicine, Osaka, Japan) and maintained in a DMEM/F12 (3:1) media containing 5% heat-inactivated fetal bovine serum (FBS), 500 ng/mL hydrocortisone, 5 μg/mL insulin, 30 ng/mL choleratoxin, 10 ng/mL epithelial growth factor (Sigma-Aldrich), and 1% penicillin streptomycin (Welgene, Gyeongsan-si, Gyeongsangbuk-do, Korea) at 37 °C in a humidified 5% CO_2 incubator.

2.4. Uptake of AFP

HCET cells (70–80% of confluency in 100 mm dishes) were exposed at a dose of 40 μg/mL for 24 h, and total cells in 3 dishes were pooled to make TEM images. After washing with phosphate-buffered saline (PBS), HCET cells were immediately fixed in a mixture (1:1, pH 7.2) of glutaraldehyde (2%) and sodium cacodylate buffer (0.1 M) for 2 h. The next, the cells were stained for 30 min in aqueous uranyl acetate (0.5%), dehydrated in graded ethanol solutions, and embedded in Spurr's resin (Cat. 14300, Low Viscosity Embedding Kit, Hatfield, PA, USA). Then, thin sections were cut using an ultramicrotome (MT-X, RMC, Tucson, AZ, USA), stained with aqueous uranyl acetate (2%) and Reynolds's lead citrate, and finally imaged with a LIBRA 120 TEM (Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 80 kV.

2.5. Real-time proliferation assay

As previously reported (Park et al., 2015), cells (5000 cells/200 μL/well) were placed into an E-Plate 16 and stabilized overnight. After removing the medium, fresh medium was added to each well with or without AFP (5, 10, 20 and 40 μg/mL, 4 wells/concentration), and then the proliferation was monitored using an xCELLigence RTCA DP system (Roche Applied Science, Indianapolis, IN, USA) for 24 h.

2.6. Cell cycle and LDH assay

At 24 h after exposure to AFP (60–70% of confluency/60 mm dish), harvested total cells were treated with RNase A (200 μg/mL, Sigma-Aldrich) for 10 min and stained with propidium iodide (20 μg/mL, Sigma-Aldrich) (Park et al., 2011, 2016). The cell cycle was analyzed by measuring the DNA content using the FACSCalibur system and CellQuest software (BD Biosciences, Franklin Lakes, NJ, USA). Additionally, cells (1×10^6 cells/mL) were incubated in a 96 well-plate for 24 h with or without AFP (5, 10, 20, and 40 μg/mL, 4 wells/concentration). A part of the supernatants (10 μL/well) was transferred to a new 96-well plate (Park et al., 2015), lactate dehydrogenase (LDH) reaction solution (100 μL, Biovision, Milpitas, CA, USA) was added to each well, and the plate was incubated for 30 min at room temperature (RT). The absorbance values were measured using a microplate spectrophotometer (450 nm, Molecular Devices, Sunnyvale, CA, USA), and finally, the LDH level of the treated groups was calculated with the absorbance value of the control group (100%).

2.7. Generation of ROS and NO

HCET cells (60–70% of confluency/60 mm dish) were exposed to AFP (0, 5, 10, 20 and 40 μg/mL) for 24 h. FBS-free media containing 5 μM carboxy-2'7'-dichlorodihydrofluorescein diacetate (DCFH-DA, Invitrogen, Waltham, MA, USA) was added to each well and further