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Molecular mechanisms of dust-induced toxicity in human corneal epithelial cells: Water and organic extract of office and house dust

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ABSTRACT

Human corneal epithelial (HCE) cells are continually exposed to dust in the air, which may cause corneal epithelium damage. Both water and organic soluble contaminants in dust may contribute to cytotoxicity in HCE cells, however, the associated toxicity mechanisms are not fully elucidated. In this study, indoor dust from residential houses and commercial offices in Nanjing, China was collected and the effects of organic and water soluble fraction of dust on primary HCE cells were examined. The concentrations of heavy metals in the dust and dust extracts were determined by ICP-MS and PAHs by GC–MS, with office dust having greater concentrations of heavy metals and PAHs than house dust. Based on LC_{50}, organic extract was more toxic than water extract, and office dust was more toxic than house dust. Accordingly, the organic extracts induced more ROS, malondialdehyde, and 8-Hydroxydeoxyguanosine and higher expression of inflammatory mediators (*IL-1β*, *IL-6*, and *IL-8*), and AhR inducible genes (*CYP1A1*, and *CYP1B1*) than water extracts ($p < 0.05$). Extracts of office dust presented greater suppression of superoxide dismutase and catalase activity than those of house dust. In addition, exposure to dust extracts activated NF-κB signal pathway except water extract of house dust. The results suggested that both water and organic soluble fractions of dust caused cytotoxicity, oxidative damage, inflammatory response, and activation of AhR inducible genes, with organic extracts having higher potential to induce adverse effects on primary HCE cells. The results based on primary HCE cells demonstrated the importance of reducing contaminants in indoor dust to reduce their adverse impacts on human eyes.

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1. Introduction

The indoor environment is important for human health as people spend over 90% of their daily time indoors (Seaton et al., 1999). A large number of contaminants adsorb to particulate matter suspended in indoor air, which settles out as indoor dust (Maertens et al., 2004). Indoor dust is an important route of human exposure to contaminants (Wang et al., 2015). Numerous epidemiological data have associated the indoor dust exposures to human health issues, including cardiovascular, respiratory, and eye diseases (Gereda et al., 2000; Hansel et al., 2008). Evidence suggested that dust-induced adverse health effects may depend on contaminants in indoor dust. For instance, Kurt-Karakus (2012) showed the correlation between the carcinogenic risk for humans and Cr in indoor dust in Istanbul. Recently, concentration-dependent relationships between organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), in indoor dust and human health issues have also been established (Meeker et al., 2013; Wei et al., 2015).

Previous studies have addressed the effects of indoor dust on human respiratory system, immune system, and cardiovascular (Maertens et al., 2004; Riechelmann et al., 2007). However, few investigations focus on dust-induced eye damage. Human ocular surface epithelium (e.g., cornea and conjunctiva), covered by a tear film, acts as a protective barrier from external agents (e.g., dust, and diesel exhaust particles) (Tau et al., 2013). Ocular epithelial cells are continually exposed to environmental toxins, which may lead to adverse effect on eyes. For example, Japanese surveys showed that exposure to volcanic ash resulted in ocular symptoms including redness, discharge, foreign body sensation, and itching (Kimura et al., 2005).

Eye diseases induced by indoor air pollution (such as house and office) is also of concern, warranting more investigation on the impact of dust on corneal epithelial surface (West et al., 2013; Wolkoff et al., 2003). Mølhave et al. (2002) found that exposure to office dust, even at concentrations of normal indoor environments resulted in inflammatory cell decrease in tear fluids, corneal epithelium defects, and break-up time decrease. Also, changes of foam formation in the eyes and tear film stability were also affected by dust exposures (Franck and Skov, 1989; Pan et al., 2000). Our previous data showed that house dust

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suspension elicited oxidative stress and inflammation in human corneal epithelial (HCE) cells (Xiang et al., 2016). However, the mechanisms of dust-induced adverse effects on human cornea are still not fully understood.

To further investigate the mechanism of dust-induced toxicity, organic extracts of dust has been often exposed to human cells and cell lines (Fang et al., 2015; Suzuki et al., 2013). Kang et al. (2010) found that exposure to organic dust extract significantly decreased the viability of human hepatocellular liver carcinoma cell line (HepG2) and human skin keratinocyte cell line (KERTr). More interestingly, a negative correlation was established between total PAH concentration and LC_{50} on HepG2 and KERTr cell lines. Besides, the aromatic hydrocarbon receptor (AhR) and its downstream genes (*CYP1A1* and *CYP1B1*), which can be metabolically activated by PAHs, were also induced by organic dust extract (Mahadevan et al., 2005). The activation of the AhR signal pathway is an important toxic mode of action for organic extracts of dust. On the other hand, heavy metals in water soluble fractions of dust also contributed to the toxic effects (Mohmand et al., 2015). Recently, Huang et al. (2015) compared the effects on HepG2, KERTr, and lung epithelial carcinoma (A549) cells after exposing to water extract of dust (250 to 2000 μg/100 μL). They found a progressive decrease of cell viability with elevating concentrations of water extracts of dust, and the inhibition effect on KERTr cell appeared earlier than HepG2 and A549 cells. Additionally, oxidative stress and pro-inflammatory responses are commonly observed after exposure to water and organic extracts (Ekstrand-Hammarstrom et al., 2013; Ghio et al., 2014). It has been suggested that the pro-inflammatory responses are, at least in part, driven by oxidative stress and redox-sensitive transcription factors (e.g., NF-κB), which initiates transcription of inflammatory mediators (*IL-1β*, *IL-6*, and *IL-8*) following exposure to particulate matter ($PM_{2.5}$) or dust extracts (Wei et al., 2011). Therefore, it is important to conduct toxicity test using both organic and water extracts of dust, and to compare their adverse effects on human corneas.

Various human cell line models, mainly cancer cells and immortalized cell lines, have been employed to elucidate the underlying molecular mechanism of dust-induced tissue specific toxicity (Kang et al., 2010; Riechelmann et al., 2007). However, published data showed that gene expression profiles between normal and neoplastic cells are strikingly diverse (Zhang et al., 1997). Proteomic study further revealed that, when compared to primary cells, immortalized cell lines are characterized with deficient mitochondria, re-arranged metabolic pathways, suppressed metabolizing enzymes, and drastically upregulated cell cycle-associated functions (Pan et al., 2009). Furthermore, after exposure to $PM_{2.5}$, primary human cells showed higher sensitivity than cell lines (De Saint Jean et al., 2004; Ekstrand-Hammarstrom et al., 2013). In this context, it is preferable to employ primary human cells to investigate dust-induced toxicity.

In this study, the contaminants in indoor dust from residential houses and commercial offices in Nanjing, China were separated into two fractions, water and organic soluble. Presumably, organic contaminants are more soluble in organic extract while heavy metals are in water soluble fractions. Our objectives were: (1) to determine the concentrations of heavy metals in water extract and prolife of PAHs in organic extract, and (2) to compare the toxicity and cellular responses of primary human corneal epithelial cells after exposure to organic or water extracts of dust.

2. Materials and methods

2.1. Chemicals and reagents

Cell culture plates and dishes were obtained from Corning Inc. (NY, USA); Cell culture medium was purchased from Life Technologies Inc. (CA, USA). Malondialdehyde (MDA) assay kit, and CCK-8 cell viability assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Human 8-Hydroxydeoxyguanosine (8-OHdG) ELISA kit was purchased from Yi Fei Xue Biotech. Co. (Nanjing, China). Reactive oxygen species (ROS) assay kit (DCFH-DA), superoxide dismutase (SOD) and catalase (CAT) assay kits, enhanced BCA protein assay kit, and cell lysis buffer were from Beyotime Institute of Biotechnology (Haimen, China). RQ1 RNase-free DNase was from Promega, USA. Chemical standards were from Aladdin Industrial Corporation (Shanghai, China) and J&K Scientific (Shanghai, China) with purity > 98%.

2.2. Indoor dust sampling, extraction and characterization

Dust samples were collected from air conditioner filter of both residential house ($n = 16$) and commercial offices ($n = 15$) in Nanjing, China using a vacuum cleaner with a paper bag (Philips Fc8222, China). All dust samples were freeze-dried, and sieved through nylon sieve (<100 μm) to remove fibrous fragments and large particles. All dust samples were then mixed into one composite sample as house and office dust. The particle size and total organic carbon (TOC) contents in dust were characterized (supporting information, SI).

Water and organic extracts were prepared from both dust samples (Fig. S1). For water extracts, 0.64 g of dust was dissolved into 40 mL Hank's balanced salt solution containing 3% antibiotic-antimycotic solution, sonicated at 4 °C for 15 min for 4 times, and filtered through a 0.22 μm disposable sterile filter (Millipore, USA). Heavy metals in the dust and water extracts were determined by inductively coupled plasma mass spectrometry (ICP-MS) (NexION300X, PerkinElmer) after digestion using USEPA Method 3050B. For organic extracts, 1.6 g of dust was extracted with 160 mL n-hexane using sonication for 30 min for 2 times, and then concentrated by a rotatory evaporator (IKA®RV10, Germany) (He et al., 2016), reconstituted in 2 mL n-hexane, and filtered by 0.22 μm Nylon filter (ANPEL, China). An aliquot of 1 mL extract was used for chemical analysis, and the detailed process for PAHs analysis can be found in SI. An aliquot of 1 mL n-hexane extract was solvent-exchanged to 0.5 mL dimethyl sulfoxide. The toxicity equivalent concentrations of individual PAHs were calculated based on the toxic equivalency factor (TEF) with respect to benzo(*a*)pyrene values (Gao et al., 2015). The water and organic extracts, house dust (WH, OH) and office dust (WO, OO) were store at −20 °C before cell assay.

Dust was acid digested using USEPA Method 3050B and analyzed for heavy metal by ICP-MS with duplicate analysis and check values (recovery of 91.7 ± 2.3%). To minimize cross contamination, only glassware was used for sample extraction, storage, and analysis of organic contaminants. Prior to use, the glassware was scrupulously washed and heated at 450 °C for 4 h. Method blanks, procedural blanks, and solvent blanks were included for quality control, and solvent blanks were analyzed every 8 samples on GC–MS. There were no PAHs being detected in blanks. The instrument limits of detection (LOD) were calculated as three times background noise level after running 7 solvent blanks. The LODs were 0.5 ± 2.1 μg/kg for PAHs. Standard reference materials for dust (SRM2858) were also measured for method recovery, which were 87 ± 5.1%−101 ± 8.7% for PAHs.

2.3. Cell culture and cell viability assay

Primary human corneal epithelial (PHCE) cells derived from corneoscleral rims of a healthy donor cornea were obtained from the Zhejiang Eye Hospital. Cells were grown in Dulbecco's modified eagle medium supplemented with fetal bovine serum (FBS) (10%), epidermal growth factor (10 ng/mL) and 1% antibiotic-antimycotic solution at 37 °C in a humidified incubator with 5% CO_2. Before exposure to dust extracts, PHCE cells were trypsinized and seeded into 6-well, 24-well, or 96-well plates overnight to allow attachment.

To determine the effects of different dust extract on cell viability, PHCE cells were replanted into a 96-well plate at density of 3×10^4 cells/100 μL/well. After overnight culture, the medium was replaced by 100 μL fresh medium containing 5 different concentrations