Environment International 89–90 (2016) 30–37

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Mechanisms of housedust-induced toxicity in primary human corneal epithelial cells: Oxidative stress, proinflammatory response and mitochondrial dysfunction

Ping Xiang [a], Rui-Wen He [a], Yong-He Han [a], Hong-Jie Sun [a], Xin-Yi Cui [a], Lena Q. Ma [a,b,*]

[a] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210046, People's Republic of China
[b] Soil and Water Science Department, University of Florida, Gainesville, FL 32611, United States

ARTICLE INFO

Article history:
Received 18 October 2015
Received in revised form 10 January 2016
Accepted 11 January 2016
Available online xxxx

Keywords:
Housedust
Primary human corneal epithelial cells
Oxidative stress
Inflammation
Mitochondrial dysfunction

ABSTRACT

Human cornea is highly susceptible to damage by dust. Continued daily exposure to housedust has been associated with increasing risks of corneal injury, however, the underlying mechanism has not been elucidated. In this study, a composite housedust sample was tested for its cytotoxicity on primary human corneal epithelial (PHCE) cells, which were exposed to dust at 5–320 μg/100 μL for 24 h. PHCE cell viability showed a concentration-dependent toxic effect, attributing to elevated intracellular ROS. Moreover, when exposed at >20–80 μg/100 μL, dust-induced oxidative damage was evidenced by increased malondialdehyde and 8-hydroxy-2-deoxyguanosine (1.3–2.3-fold) and decreased antioxidative capacity (1.6–3.5-fold). Alteration of mRNA expression of antioxidant enzymes (*SOD1*, *CAT*, *HO-1*, *TRXR1*, *GSTM1*, *GSTP1*, and *GPX1*) and pro-inflammatory mediators (*IL-1β*, *IL-6*, *IL-8*, *TNF-α*, and *MCP-1*) were also observed. Furthermore, the mitochondrial transmembrane potential was dissipated from 9.2 to 82%. Our results suggested that dust-induced oxidative stress probably played a vital role in the cytotoxicity in PHCE cells, which may have contributed to dust-induced impairment of human cornea.

Published by Elsevier Ltd

1. Introduction

Epidemiological studies have linked human health issues, including respiratory, allergenic, and ocular surface diseases with exposure to housedust (Maertens et al., 2004; Torricelli et al., 2014). Housedust is a complex heterogeneous mixture of particles of different sources, which contains both harmful microbes and various contaminants (Fang and Stapleton, 2014; Li et al., 2014; Pitkäranta et al., 2008). The contaminants in housedust are probably responsible for the adverse health effects. Accumulating evidence demonstrated that organic pollutants such as phosphorus flame retardants (PFRs) and phthalic acid esters (PAEs) in indoor dust were positively associated with human diseases (Betts, 2015; Kolarik et al., 2008; Meeker and Stapleton, 2010; He et al., 2015). Heavy metals in dust have also attracted attention due to their persistence in the environment and potential adverse health effect on human health (Yu et al., 2014).

People spend long time indoors, especially young children. It has been estimated that ~51 mg of housedust can be absorbed by adults and 28 mg for kids (Hawley, 1985). Thus, it is important to address the potential human health risk associated with exposure to dust. Most studies addressed effects of indoor dust on human respiratory and cardiovascular system, but few investigations focus on dust-induced damage on eye surface. Human eyes are a sensitive organ, which are exposed to ambient air every day and are susceptible to damage by housedust. Epidemiological data showed that air pollution and airborne particulate matters in household significantly impacts the eyes, especially ocular surface such as conjunctiva and cornea (Saxena et al., 2003). Strong association ($r = 0.62$) between the number of outpatient visits for eye surface diseases and airborne particulate matter exposure levels has been observed (Mimura et al., 2014). Gupta et al. (2007) also indicated that people exposed to high air pollutants are linked to high incidence of subclinical ocular surface disorders. Changes in foam formation in the eyes (Franck and Skov, 1989) and tear film stability were affected by dust exposure (Pan et al., 2000).

The corneal epithelium, the outermost cell layer of the eyes, serves as the mechanical barrier to environmental agents including dust to minimize interior damage (Black et al., 2011; Leong and Tong, 2015). Exposure to indoor dust, even at concentrations of normal indoor environments led to corneal epithelium defects and breakup time decrease (Mølhave et al., 2002). Housedust exposure can also irritate human corneal epithelium (HCE) and damage its histological structure (Cao et al., 2015), which may lead to impaired vision and eventual blindness (Cullen, 2002; Lu et al., 2001). Therefore, dust-induced corneal diseases are of concern and warrant more investigation (West et al., 2013; Wolkoff et al., 2003).

* Corresponding author at: State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210046, People's Republic of China.
E-mail address: lqma@ufl.edu (L.Q. Ma).

http://dx.doi.org/10.1016/j.envint.2016.01.008
0160-4120/Published by Elsevier Ltd

To elucidate the underlying molecular mechanism of dust-induced toxicity in human eyes, various human cell line models have been employed including cancer cells and immortalized cell lines (Kang et al., 2010a; Riechelmann et al., 2007). However, recent study showed different pattern and magnitude of cytokine expression from immortalized and primary HCE (PHCE) cells responding to particulate matter (Ekstrand-Hammarstrom et al., 2013), with PHCE showing higher sensitivity than immortalized cell lines (De Saint Jean et al., 2004). Others also demonstrated that immortalized human epithelial cell lines have lost or changed some features after long-term in vitro culture under changed physiological conditions (Proulx et al., 2004). In short, the differences observed between primary cells and immortalized cell lines suggest that it is important to use primary cultures to assess contaminant toxicity (Cree and Andreotti, 1997).

Oxidative stress is a vital molecular mechanism of environment-mediated corneal epithelium injury, attributing to the localized production of ROS by HCE cells (Lee et al., 2014). Several studies have demonstrated that increase of ROS directly suppressed several antioxidant enzymes including catalase (CAT), and superoxide dismutase (SOD) in HCE cells (Black et al., 2011; Čejková et al., 2004). Moreover, ROS can also damage DNA and oxidize lipids and key proteins, resulting in excessive production of inflammatory mediators, malondialdehyde and 8-hydroxy-2-deoxyguanosine, causing toxic effects (Cejkova et al., 2000; Ye et al., 2012). Excessive ROS induces the production of proinflammatory mediators, contributing to many corneal inflammatory diseases such as corneal inflammation, dry eye disease, and bullous keratopathy (Shoham et al., 2008). Nevertheless, the potential role of oxidative damage induced by housedust in PHCE cells has not been investigated.

In this study, we hypothesized that oxidative stress was responsible for housedust-induced cytotoxicity in PHCE cells. We tested the impact of housedust on PHCE cells after exposure to various dust concentrations for 24 h. The specific objectives of this study were: (1) to investigate the toxicity of PHCE cells after exposure to housedust, and (2) to evaluate dust-induced toxicity mechanisms by evaluating the oxidative toxicity and inflammatory mediator expression, and mitochondrial transmembrane potential depolarization of PHCE cells after exposure to housedust. This is the first study using PHCE cells to test dust-induced toxicity and its associated mechanisms.

2. Materials and methods

2.1. Chemicals and reagents

Cell culture reagents and plates were obtained from Life Technologies Inc. (CA, USA) and Corning Inc. (NY, USA). CCK-8 cell viability assay kit was perchased from Yi Fei Xue Biotech. Co., Ltd. (Nanjing, China). Malondialdehyde (MDA) assay kit and total antioxidant capacity assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). JC-1 mitochondrial membrane potential assay kit, reactive oxygen species assay kit (DCFH-DA), RNase-Free DNase I and cell lysis buffer were purchased from Beyotime Institute of Biotechnology (Haimen, China). Chemical standards were purchased from Aladdin Industrial Corporation (Shanghai, China) and J&K Scientific (Shanghai, China) with purity >98% including five phthalic acid esters (PAEs) [dimethyl phthalate-(DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP)] and five PFRs [tris(2-chloroethyl) phosphate (TCEP), tris(chloroisopropyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), triphenyl phosphate (TPP), and 2-ethylhexyl diphenyl phosphate (EHDPP)]. Other chemicals were obtained from Sigma-Aldrich, Inc. (MO, USA).

2.2. Sampling and characterization of indoor dust

Sixteen indoor dust samples were collected from Nanjing, China. In each house, dust was obtained from air conditioner filter by 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, it was then mixed into one composite sample and stored in clean aluminum foil at −20 °C until analysis. For preparation of dust suspension, 200 mg of dust was suspended into 40 mL Hank's balanced salt solution containing 3% antibiotic–antimycotic solution and sonicated at 4 °C for 15 min 4 times and vortexed before exposure to the cells.

Concentrations of heavy metals including As, Pb, Cr, Cd, Cu, Zn, Ni, Sb, Ti, and Mn were determined by inductively coupled plasma mass spectrometry (ICP-MS) (NexION300X, PerkinElmer) after digestion using USEPA Method 3050B. Total concentrations of PAEs and PFRs were quantified (Guo and Kannan, 2011). Sample of 200 mg was extracted with 20 mL n-hexane in ultrasonic bath (SCOENTZ, SB-800 DTD, China) for 30 min three consecutive times. The extracts were collected after centrifugation at 3000 rpm for 5 min, and filtrated through anhydrous sodium sulfate for dehydration into 150 mL flask bottle. The combined extract was concentrated to near dryness by rotatory evaporimeter (IKA®RV10, Germany), and then reconstituted in 2 mL n-hexane, which was transferred to 2 mL amber vials through 0.45 mm Nylon filter (ANPEL, China) and stored at −20 °C until analysis. Ten organic compounds including five PAEs and five PFRs were detected using gas chromatography coupled with mass spectrometry (Agilent Technologies, 7890A) (GC–MS) in selective ion-monitoring mode. After carbonate carbon was removed using 0.5 M HCl, total organic carbon (TOC) contents in dust samples were also measured using element analyzer (vario TOC select, Elementar, Germany).

The concentration of endotoxin (lipopolysaccharide, LPS) in dust sample was tested using Kinetic Turbidimetric LAL Assay Kit (Chines Horseshoe Crab Reagent Manufactory, CO., Ltd., Xiamen, China). Endotoxin concentrations were expressed in EU per milligram of dust.

2.3. Cell culture and exposure to housedust

PHCE cells originally isolated from corneas of a healthy donor were obtained from the Zhejiang Eye Hospital. They were cultured in DMEM medium containing high glucose (4.5 g/L), fetal bovine serum (10%), epidermal growth factor (10 ng/mL) and 1% antibiotic–antimycotic solution in an incubator with 5% CO_2 at 37 °C. For the following studies, PHCE cells were seeded into 6/24/96-well plates and cultured for 24 h to reach ~80% confluence. Subsequently, the culture medium was changed and HCE cells were incubated with fresh medium containing 5 to 320 μg/100 μL of housedust suspension for 24 h.

2.4. Analysis of cell viability and intracellular ROS

To test the effect of dust on cell viability, PHCE cells were seeded in a 96-well plate at density of 1×10^4 cells/100 μL/well. After 24 h exposure, the cell morphology was observed via an inverted microscopy (TS-100, Nikon, Japan). Cell viability was measured by CCK-8 cell viability assay kit.

Changes in intracellular ROS level after dust exposure were detected using a well-characterized probe 2′,7′-dichlorofluorescein diacetate (DCFH-DA). DCFH-DA is permeated into cytoplasm and hydrolyzed into non-fluorescent DCFH. Under ROS, DCFH is converted into fluorescent dichlorofluorescein. Briefly, PHCE cells were cultured with control medium and different concentrations of dust in a 24-well plate for 24 h. Then, cell culture medium was aspirated and washed by HBSS. DCFH-DA was added into cells for 30 min in the dark. Subsequently, the fluorescence image was visualized by inversed fluorescent microscopy (Eclipse Ti-U, Nikon, Japan).

2.5. Malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8-OHdG) and total antioxidant capacity (T-AOC)

The level of MDA, a marker of toxic byproduct of lipid peroxidation was detected using MDA assay kit (TBA method). Briefly, PHCE cells