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Potential cytotoxicity of water-soluble fraction of dust and particulate matters and relation to metal(loid)s based on three human cell lines

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HIGHLIGHTS

- The cytotoxicity of dust aqueous extract was dependent on metal(loid) content.
- $PM_{2.5}$ possessed much higher cytotoxicity than road dust and AC filter dust.
- KERTr cells were more sensitive than HepG2 cells.
- Other components beside metal(loid)s might enhance the toxicity of dust and PM.

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ABSTRACT

Hepatocellular liver carcinoma (HepG2), human skin derived keratinocyte (KERTr,) and lung epithelial carcinoma (A549) were employed in MTT assay to evaluate the cytotoxicity of water-soluble fraction of road dust, air-conditioning (AC) filter dust and $PM_{2.5}$ via ingestion, dermal contact and inhalation. Their effects on cell growth were dependent on exposure time and concentration. The LC_{20}s of $PM_{2.5}$ for A549 cell were approximately one order of magnitude lower than those of road dust and AC filter dust for KERTr cell and HepG2 cell. The LC_{20}s of aqueous extracts were negatively correlated to the water-soluble metal(loid)s contained in dust coarse particles (KERTr: $p = 0.004$; HepG2: $p < 0.001$). However, no significant correlation between soluble metal(loid)s and LC_{20}s of $PM_{2.5}$ was observed for A549 cell ($p > 0.05$). Other water-soluble components in dust and PM might cause the cell hazards synergistically or additively with metal(loid)s.

1. Introduction

Environmental particulate matter (PM) is composed of inert carbonaceous cores with layers of various adsorbed molecules including metals, organic pollutants, acid salts and biological elements such as endotoxins, allergens, and pollen fragments (Spurny, 1996). Suspended PM is often preferred as airborne dust in atmosphere (WHO, 1999). There have been substantial epidemiological studies indicating that the high PM level (particularly respiratory PM, <10 μm) is associated with mortality (Medina et al., 2004; Pope III et al., 2004) or cardiovascular diseases (Lanki et al., 2006; Zanobetti and Schwartz, 2006; Hoffmann et al., 2007). Toxicological study is essential to further evaluate the mechanisms of risks derived from PM and the components in it.

Exposure to dust and PM may occur through non-dietary ingestion, dermal adsorption and inhalation (USEPA, 1997). The cell lines applied to model these exposure routes usually include human hepatocellular liver carcinoma (HepG2) (Kang et al., 2010; Nobels et al., 2012), human skin derived keratinocyte (KERTr,) (Arlian et al., 2008; Kang et al., 2010) and human lung epithelial carcinoma (A549) (Calcabrini et al., 2004; Gualtieri et al., 2009). Liver is the major detoxification organ in the digestive

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system. The human hepatoma is one of the most promising choices for cell model, as it possesses a number of characteristic enzyme pathways of human hepatocytes and is regarded as a "gold standard" in toxicology (Kang et al., 2010; Nobels et al., 2012). Hence, the human hepatoma can be a suitable cell model of ingestion exposure route. In response to physical and chemical stimulation, skin derived keratinocytes can release cytokines and chemokines, which affect vascular endothelial cell function and thus induce cutaneous inflammatory and immune reactions (Arlian et al., 2008). Hence, it is a suitable *in vitro* system to study the response of dermal exposure to xenobiotics. The A549 cell line, which originates from the neoplastic transformation of type II alveolar cells, possesses the typical features of the first step of transformation induced by the pulmonary inflammatory process (Calcabrini et al., 2004). It has been widely used to examine the cytotoxic mechanisms resulted from inhalation of fine PM (such as $PM_{2.5}$) (Adamson et al., 1999; Calcabrini et al., 2004; Gualtieri et al., 2009).

In the present study, road dust sample represents the outdoor coarse particles, while air-conditioning (AC) filter dust represents household coarse particles, which can be re-suspended along with air current and settle on the air-conditioner filter. The two types of particles can enter human body through ingestion and adsorption on the skin. The coarse particles (with diameter larger than 2.5 μm) can be directly swallowed or finally reach the gastrointestinal tract after a short stay in tracheal and bronchial regions via inhalation. Therefore, HepG2 cell line and KERTr cell line were used to model the oral ingestion and dermal contact via the coarse dust particles (road dust and AC filter dust). On the other hand, A549 cell line was only employed to model the inhalation of $PM_{2.5}$, because more than 80% of particles smaller than 2.5 μm can reach the pulmonary alveoli via inhalation, where they can be deposited and stay for months to years (Huang et al., 2014). The samples of road dust, AC filter dust and $PM_{2.5}$ were collected from Guangzhou (GZ) urban area, south China, where is one of the largest mega cities in Pearl River Delta (PRD) region, with serious anthropogenic contaminations, in particular high levels of PM_{10} and $PM_{2.5}$ (Huang et al., 2007; Andreae et al., 2008), and high concentrations of metals contained in urban dust and PM (Huang et al., 2014).

In one of our previous studies, the risks of metal(loid)s derived from exposure to dust and PM mentioned above were characterized and assessed according to their bioaccessibilities and exposure levels (Huang et al., 2014). Furthermore, the present study attempts to identify their toxicity on human epithelial cells via the three kinds of exposure ways. The cytotoxicity will be measured with MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. MTT assay is the most commonly used in detecting the alterations in cellular metabolism, proliferation and activation. It evaluates the mitochondrial dehydrogenase (MDH) activity, which depends on the degree of cell activation (Kang et al., 2010).

The studies evaluating the cytotoxicity of dust and PM, usually focused on the organic extracts (Kang et al., 2010; Wang et al., 2013) or the particle itself (Gualtieri et al., 2009). Recently, the water-soluble fraction and the metals in it deserve increasing attention (Taylor et al., 2013). Even though the mass of metal elements constitutes a small portion in PM (Calcabrini et al., 2004; Gualtieri et al., 2009) compared with other components (such as NO_3^-, SO_4^{2-}, NH_4^+, organic carbon and elemental carbon), they can pose serious human health risks (Rashed, 2008). On the cellular level, the transition metals, such as nickel (Ni), zinc (Zn), copper (Cu) and manganese (Mn), contained in PM contribute to the induction of reactive oxygen species (ROS), which damage membrane lipids, proteins and DNA, even lead to cell death (De Kok et al., 2006; Gualtieri et al., 2009). Continuous exposure to PM-induced ROS can result in inflammation (De Kok et al., 2006). Adamson et al. (1999) speculated that the toxicity following exposure to urban dust is related to soluble fraction rather than the insoluble particles, and that the soluble metal ions have the potential to cause pulmonary epithelial cell (A549) injury as well as inflammatory response. Moreover, Gualtieri et al. (2009) suspected that the water-soluble fraction of PM is responsible for the ROS stimulation in pulmonary epithelial cell (A549), especially in the early exposure stage. Hence, it is hypothesized in the present study that in addition to A549, the cytotoxicities on other human cells (HepG2 and KERTr) might also be derived from the aqueous extracts of dust and PM, and that these cytotoxicities might be associated with the contents of water-soluble metal(loid)s, which might contribute an important proportion of cytotoxicity potency of the aqueous extracts.

Fig. S1 shows our research design. The objectives of the present study are to (1) identify the effects on cell proliferation of the HepG2 cell line, KERTr cell line and A549 cell line after exposure to aqueous extracts of road dust, AC filter dust and $PM_{2.5}$; (2) evaluate the induced cytotoxicities (LC_{50} and LC_{20}) on the three human cell lines during early exposure period (24 h); (3) attempt to link the cytotoxicities derived from the aqueous extracts of dust and PM with soluble metal(loid)s contents; and (4) compare the cytotoxicity originated from composite aqueous extract and those from standard spike mixture to further evaluate the extent of contribution to cytotoxicity potency caused by water-soluble metal(loid)s contained in dust and PM.

2. Materials and methodology

2.1. Sampling and sample preparation

The samples of road dust, household AC filter dust and $PM_{2.5}$ were collected from Guangzhou (GZ) urban area, which had been described in detail in our previous studies (Huang et al., 2012, 2014). Briefly, the road dust samples were collected from scenic parks, educational sites, residential sites, heavy traffic sites, commercial sites and peri-urban district in the central area of GZ. The AC filter dust and $PM_{2.5}$ were collected from different houses with different construction years, human activities (cooking, religious burning and use of mosquito coil) and types of decoration (tile floor or teak floor). The road dust samples were sieved to 100 μm before analysis and treatments.

2.2. Determination of concentrations of water-soluble metal(loid)s in dust and PM

About 0.5 g of road dust samples, a strip of $1'' \times 8''$ from the $8'' \times 10''$ AC filter sample, and quarters of $PM_{2.5}$ membranes were extracted in Milli-Q water performed with Bransonic® ultrasound bath. The supernatants were filtered with 5C Whatman filter paper and 0.45 μm syringe filter, and added with an aliquot of concentrated HNO_3. The acidified water extracts were then determined using atomic absorption spectrometry (FIMS 100 Perkin Elmer) for Hg and ICP-MS (Perkin Elmer Elan 9000) for other metal(loid)s. Method blanks were proceeded in parallel with the samples. Matrix spike/matrix spike duplicates (MS/MSD) were also used for quality assurance and quality control (QA/QC). The average recovery rates of spike metal(loid)s in MS varied from 89.8% for As to 99.3% for Zn. The relative percent difference (RPD) between MS and MSD varied from 8.2% to 12.1%.

2.3. Cell culture

The HepG2 cell line, KERTr cell line, and A549 cell line were obtained from the American Type Culture Collection (ATCC, USA).