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Short communication

Synergic effects of 9,10-phenanthrenequinone and cadmium on pro-inflammatory responses in airway epithelial cells

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ABSTRACT

We investigated the synergic effects of components of particulate matter with aerodynamic diameters ≤2.5 μm (PM2.5) on airway inflammation. Co-exposure to cadmium (Cd) and 9,10-phenanthrenequinone (9,10-PQ) additively/synergistically increased pro-inflammatory responses in airway epithelial cells, whereas co-exposure to Cd and phenanthrene resulted in no acceleration. These results suggest that the combination of metal and a quinone derivative can contribute to the exacerbation of respiratory diseases by PM2.5.

1. Introduction

Particulate matter with aerodynamic diameters ≤2.5 μm (PM2.5) is composed of elemental carbons, organic carbons and metals. It has been demonstrated that single-exposures to each component contribute to respiratory diseases (Inoue et al., 2005; Hiyoshi et al., 2005; Honda et al., 2015). However, considering the constituents of PM2.5, it is important to identify the combined effects of components of PM2.5. Interestingly, it was reported that the interleukin (IL)-6 and IL-8 release from airway epithelial cells caused by organic extracts from PM2.5 were reduced by a metal chelator (Rodríguez-Cotto et al., 2015). That finding indicates that a combination of organic components and metals in PM2.5 may lead to stronger pro-inflammatory responses. However, it is not clear which of the organic and metal components of PM2.5 play critical roles in the combined effects on the exacerbation of respiratory diseases.

Among the PAHs, 9,10-phenanthrenequinone (9,10-PQ) is directly emitted from vehicles and is contained in PM2.5 that includes diesel exhaust particles (Cho et al., 2004). Koike et al. (2014) reported that exposure to 9,10-PQ but not phenanthrene (Phe) induces cytotoxic effects on airway epithelial cells. Hiyoshi et al. (2005) suggested that 9,10-PQ exacerbates the pathogenesis of asthma, with effects on airway inflammation in the presence of ovalbumin (OVA) as an antigen. The deterioration of respiratory health induced by 9,10-PQ in PM2.5 is thus a public health concern.

Among the wide variety of metals, previous research detected cadmium (Cd) in ambient air and has examined the health effects of Cd (Suvarapu and Baek, 2017). It was also reported that the lead (Pb) and Cd levels in PM2.5 and in study participants' blood were higher in an electronic waste-exposed area, and that the prevalence of respiratory symptoms such as coughing and phlegm were higher (Zeng et al., 2016). Another research group demonstrated that Cd levels in the blood are significantly associated with the pathogenesis of asthma (Park et al., 2016). It is thus possible that Cd in PM2.5 affects airway epithelial cells and can thus exacerbate respiratory diseases.

In the present study, we therefore focused on the combined effects of metal and PAHs (especially Cd and 9,10-PQ) as the components of PM2.5 on airway epithelial cells. We also compared the respiratory effects of 9,10-PQ and those of the parent PAH, i.e., Phe, in the presence and absence of Cd.

2. Materials and methods

2.1. Chemicals

The quinone derivative i.e. 9,10-PQ and its parent PAH i.e. Phe, were purchased from Sigma (St. Louis, MO, USA) and Tokyo Chemical Industry (Tokyo), respectively. Cadmium sulfate hydrate was purchased from Sigma.

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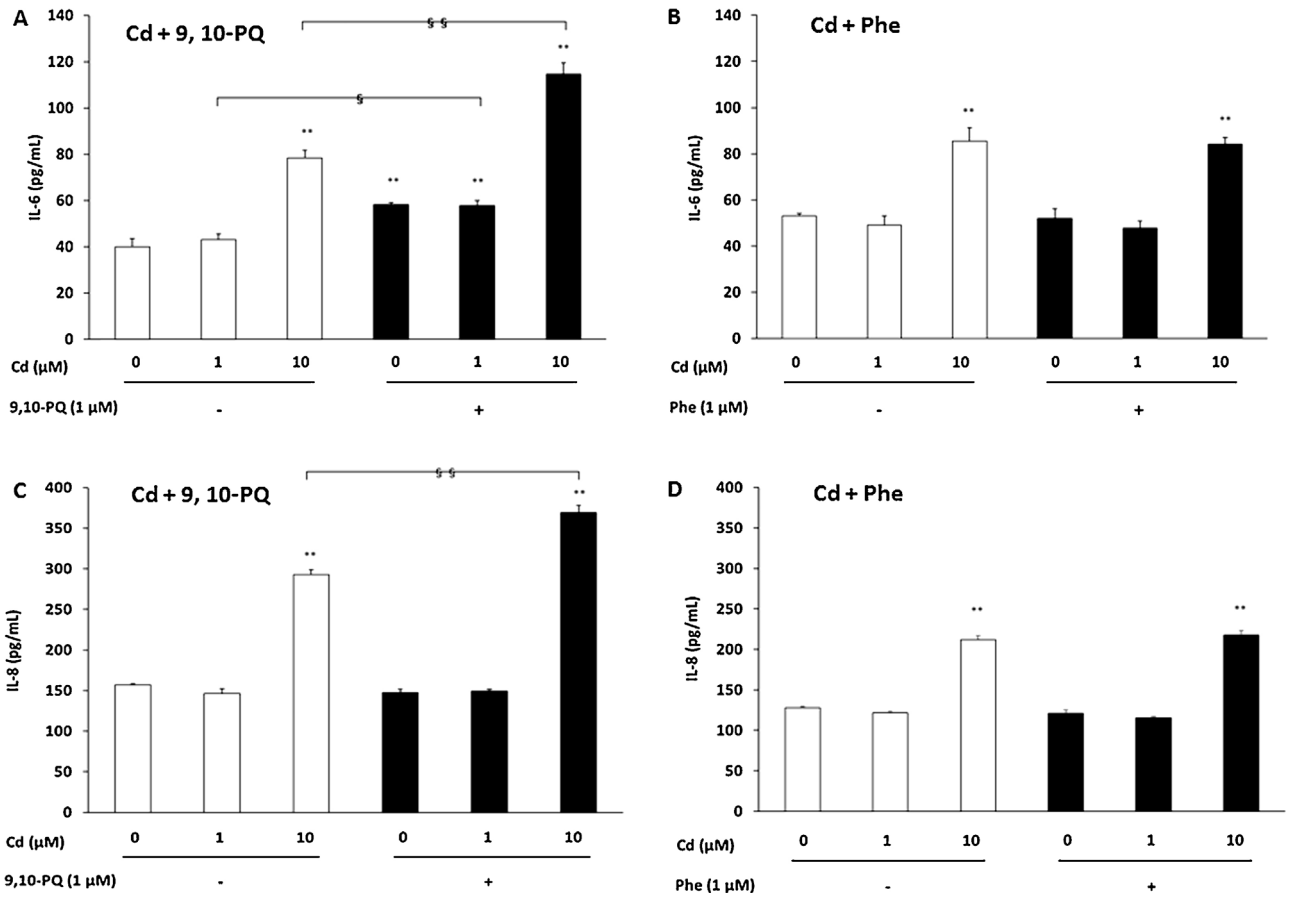

Fig. 1. Combined effects of Cd plus 9,10-PQ (A, C) or Phe (B, D) on the IL-6/IL-8 production from airway epithelial cells. $^{**}p < 0.01$ vs. 0 μM Cd in the absence of PAHs, $^{§}p < 0.05$, $^{§§}p < 0.01$ vs. each other.

2.2. Experimental protocol

After the BEAS–2 B cell line as airway epithelial cells grew to semi-confluence in LHC-9 medium in collagen I-coated plates, they were exposed to Cd (0, 1, 10 μM), and 9, 10-PQ (1 μM) or Phe (1 μM) for 3 h or 24 h. We then evaluated the releases of IL-6 and IL-8 in the culture supernatants, the generation of reactive oxygen species (ROS), and the metallothionein 2A (MT-2A) mRNA expression by performing enzyme-linked immunosorbent assay (ELISA), a CM-H_2DCFDA fluorescent probe, and a real-time polymerase chain reaction (RT-PCR), respectively. Our previous study showed that extracts of PM2.5 collected from two areas in Japan induced IL-6 release from airway epithelial cells under nontoxic conditions (Honda et al. in press), and we therefore used lower doses of Cd, 9,10-PQ and Phe without cytotoxicity in the present experiment (Suppl. Fig. S1A,B in the online version at DOI: http://dx.doi.org/10.1016/j.etap.2017.04.019).

2.3. Quantitation of inflammatory proteins in the culture supernatants

After exposure for 24 h, the levels of IL-6 and IL-8 in the culture medium were measured by ELISA (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. The detection limits of IL-6 and IL-8 were < 1 pg/mL and < 2 pg/mL, respectively.

2.4. ROS generation

We used a fluorescent probe, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA), to measure the intracellular ROS generation. The fluorescence intensity during 0–3 h (excitation 485 nm, emission 530 nm) was measured.

2.5. Extraction of RNA and quantitative RT-PCR analysis

After exposure for 3 h, total RNA was extracted with an RNeasy Mini Kit (Qiagen, Hilden, Germany) and was reverse-transcribed to cDNA using a High Capacity RNA-to-cDNA kit (Life Technologies) according to the manufacturer's instructions. The quantitation of mRNA expression was carried out using the ABI Prism 7000 Sequence Detection System (Life Technologies). The relative intensity was normalized to β-ACTIN as an endogenous control gene. TaqMan probes and pair primers for MT-2A and β-ACTIN were designed by Life Technologies, which does not disclose these sequences.

2.6. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) for each experimental group (n = 4). Differences among groups were analyzed using the Tukey multiple comparison test (Excel Statistics 2010, Social Survey Research Information, Tokyo). A p-value < 0.05 was accepted as significant.

3. Results

3.1. The combined effects of Cd and 9,10-PQ or Phe on the cytokine production

Both, Cd at a dose of 10 μM and 9,10-PQ alone increased the protein release of IL-6 compared to the controls (Fig. 1A). Cd in the presence of 9,10-PQ also induced the release of IL-6 compared to the controls. Cd at the doses of 1 or 10 μM in the presence of 9,10-PQ elevated the protein release of IL-6 compared to Cd alone at the same doses. IL-6 release was