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Airborne quinones induce cytotoxicity and DNA damage in human lung epithelial A549 cells: The role of reactive oxygen species

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HIGHLIGHTS

- Different airborne quinones exerted different biological responses in A549 cells.
- Quinones caused cell membrane damage, cell death, DNA damge, and Ca^{2+} release.
- These biological responses could be abolished by the treatment of NAC.
- Quinone-induced *in vitro* responses is mediated by elevated production of ROS.
- Quinones participate in adverse health effects of particles through ROS generation.

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ABSTRACT

Ambient particulate matter (PM) is associated with adverse health effects. Quinones present in PM are hypothesized to contribute to these harmful effects through the generation of reactive oxygen species (ROS). However, whether the ROS induced by quinones is involved in mediating DNA damage as well as other biological responses in pulmonary cells is less well known. In this study, the toxic effects of five typical airborne quinones, including 1,2-naphthoquinone, 2-methylanthraquinone, 9,10-phenanthrenequinone, 2-methyl-1,4-naphthoquinone, and acenaphthenequinone, on cytotoxicity, DNA damage, intracellular calcium homeostasis, and ROS generation, were studied in human lung epithelial A549 cells. An antioxidant N-acetylcysteine (NAC) was used to examine the involvement of ROS in adverse biological responses induced by quinones. The quinones caused a concentration-dependent viability decrease, cellular LDH release, DNA damage, and ROS production in A549 cells. 1,2-Naphthoquinone, but not the other four quinones, increased intracellular calcium (Ca^{2+}) levels in a dose-dependent manner. These toxic effects were abolished by administration of NAC, suggesting that ROS played a key role in the observed toxic effects of quinones in A549 cells. These results emphasize the importance of quinones in PM on the adverse health effects of PMs, which has been underestimated in the past few years, and highlight the need, when evaluating the effects on health and exposure management, to always consider their qualitative chemical compositions in addition to the size and concentration of PMs.

1. Introduction

Ambient particulate matter (PM) is associated with cardiopulmonary morbidity and mortality (Brunekreef and Holgate, 2002). However, the underlying mechanisms remain poorly understood because of their complex and heterogeneous properties. Previous studies have indicated that PM interacts with biological systems through direct generation of reactive oxygen species (ROS) and further results in oxidative stress (OS) (Li et al., 2008). Quinones are typical oxygenated derivatives of polycyclic aromatic hydrocarbons (PAHs) in air (Walgraeve et al., 2010). Recently, quinones are hypothesized to contribute to these adverse health effects caused by PM because of their high redox potency in producing ROS (Li et al., 2012) and are believed to some extent to be more toxic than their parent PAHs (Sidhu et al., 2005).

Most airborne quinones are polycyclic aromatic chemicals, such as anthraquinone, naphthoquinone, and phenanthrenequinone. They are released during the incomplete combustion process of

Abbreviations: ACQ, acenaphthenequinone; DMSO, dimethyl sulfoxide; LDH, lactate dehydrogenase; LMA, low melting agarose; MAQ, 2-methylanthraquinone; MNQ, 2-methyl-1,4-naphthoquinone; MTT, 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide; NAC, N-acetylcysteine; NMA, normal melting agarose; 1,2-NQ, 1,2-naphthoquinone; OS, oxidative stress; PM, particulate matter; PQ, 9,10-phenanthrenequinone; ROS, reactive oxygen species; tBHP, tert-butyl hydroperoxide.

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fossil fuels and/or formed via atmospheric photochemical conversions from PAHs (Walgraeve et al., 2010). Quinones could also be formed *in vivo* from PAHs by enzymes (Walgraeve et al., 2010). The reported concentrations of quinones are typically from pg m^{-3} to several ng m^{-3} in air (Walgraeve et al., 2010). A study in Birmingham, in UK, measured 11 quinones during January 2010 (Alam et al., 2013), including the most abundant 9,10-phenanthrenequinone (6.1 ng m^{-3}), 1,2-naphthoquinone (3.0 ng m^{-3}), 1,4-naphthoquinone (2.1 ng m^{-3}), and 2-methylanthraquinone (2.8 ng m^{-3}).

The ability of ROS production are usually used to assess the following toxic effects resulting from PM exposure. Although most studies are focused on ROS generation through the Fenton reaction of transition metals, several studies have focused on the mechanisms caused by quinones present in PM. Using the dithiothreitol (DTT) assay, Chung et al. (2006) found that ROS generation from PM showed a strong positive correlation with quinones detected in particles. Recently, through electron spin resonance and capillary electrophoresis, the semiquinone radical and ·OH were detected directly in a physiological buffer solution, providing direct evidence for the redox cycling hypothesis of ROS generation by quinones (Li et al., 2012). Additionally, 1,4-naphthoquinone (1,4-NQ) was found to cause significant cell death in Ana-1 macrophages through the generation of ROS via redox cycling (Shang et al., 2012).

Calcium ion (Ca^{2+}) is a second messenger and plays important roles in the regulation of many physiological processes in cells. Recent studies suggest that Ca^{2+} may participate in cytotoxicity and inflammation induced by ambient particles (Happo et al., 2013). Sakamoto et al. (2007) determined that PM_{10} increased cellular Ca^{2+}, which is associated with IL-1β and IL-8 production in human bronchial epithelial cells.

Until now, it is at least partially clear that airborne quinones contribute to the oxidative potential of PM. However, whether quinone-induced adverse biological responses are correlated with irregular ROS generation is still not well documented. In this study, we investigated the cytotoxicity and DNA damage in human lung epithelial A549 cells exposed to five typical airborne quinones, including 1,2-naphthoquinone (1,2-NQ), 2-methyl-1,4-naphthoquinone (MNQ), 2-methylanthraquinone (MAQ), acenaphthenequinone (ACQ), and 9,10-phenanthrenequinone (PQ). To explore the mechanisms of quinone-induced cellular biological responses, their ability to elevate Ca^{2+} and generate ROS was also examined. The protective role of NAC towards ROS generation induced by quinones was also studied. These results should contribute to better understanding of the potential cellular mechanisms of potential toxic effects of human exposure to airborne quinones.

2. Materials and methods

2.1. Materials

A549 cells (type II pulmonary epithelial cell line) were from American Tissue Type Culture Collection (USA). RPMI 1640 medium and fetal bovine serum (FBS) were from Gibco (UK). Lactate Dehydrogenase (LDH) Assay Kit was from Nanjing Jiancheng Bio-engineering Research Institute (Nanjing, China). Dimethyl sulfoxide (DMSO), trypsin, 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide (MTT), and 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) were from Sigma (USA). 1,2-NQ, MAQ, PQ, MNQ, and ACQ were from Sigma–Aldrich (USA). All other reagents were analytical grade chemicals from Sinopharm Chemical Reagent Company (Shanghai, China).

2.2. Cell culture and treatment

A549 cells were cultured in RPMI 1640 medium supplemented with 10% FBS and penicillin/streptomycin (100 units mL^{-1}) in an atmosphere of 5% CO_2 and 100% relative humidity at 37 °C.

Quinones were dissolved in DMSO and working solutions were freshly diluted by RPMI 1640 with 2% FBS. The final concentration of DMSO was 0.1% (v/v). Treatment dosages were 5, 10, 15, 20 μM for 1,2-NQ and MNQ; 10, 20, 40, 60 μM for MAQ and ACQ; 1, 2, 3, 4 μM for PQ. The cells were exposed for 24 h except for Ca^{2+} and ROS measurements, which were 1 h. DMSO-treated cells (0.1%, v/v) served as control. Tert-butyl hydroperoxide (tBHP) was employed as positive control in Comet assay and ROS measurement.

NAC is a cell-permeable thiol antioxidant involved in the induction of glutathione synthesis and scavenging of ROS, which inhibits the effects of endogenous ROS. To determine the involvement of ROS in the biological responses of quinones, A549 cells were treated with quinones with or without NAC. A 10 mM concentration was chosen based on the viability of A549 cells caused by NAC. A549 cells treated with 10 mM NAC for 24 h showed no significant decrease in viability. NAC was dissolved in PBS, and the filter-sterilized NAC was freshly diluted in culture medium before use.

2.3. MTT assay

Cells were plated in 96-well plates (5×10^3 cells/well) and cultured for 24-h to allow cell adhesion. Subsequently, the culture medium was replaced with fresh medium containing various concentrations of quinones with or without 10 mM NAC. After the 24-h treatment, cells were incubated with 100 μL of freshly prepared MTT (1 mg mL^{-1}) in medium for 4 h in the dark at 37 °C. The formazan crystals formed in the cells were solubilized by 100 μL DMSO. Optical density (OD) was read using a Multi scan Mk3 plate reader (Thermo Electron Corporation, USA) at 570 nm. Based on the concentration–response curves, LC_{50} values (lethal concentration where cell viability was reduced by 50%) were determined.

2.4. LDH release

LDH release was assessed to indicate plasma membrane damage. A549 cells were plated in 24-well plates (1×10^4 cells/well) and cultured for 24 h to allow cell adhesion. Subsequently, the culture medium was replaced with fresh medium containing quinones with or without 10 mM NAC. After 24 h, LDH in culture medium was measured according to the manufacturer's procedure as previously described (Shang et al., 2013). LDH catalyzes the conversion of lactic acid into pyroracemic acid, which reacts with 2,4-dinitrophenylhydrazine to form brownish red dinitrophenylhydrazone. Subsequently, LDH was assayed by colorimetry at 400 nm using a Quant microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA). LDH activity was calculated according to the following formula: LDH activity (U/L) = $[(OD_{treated} - OD_{control})/(OD_{standard} - OD_{blank})] \times$ standard concentration × dilution factor × 1000. The control was culture medium without cells. The standard was sodium pyruvate, and the blank was distilled water.

2.5. Comet assay

DNA damage was evaluated by alkaline single cell gel electrophoresis (Comet assay) according to the recommended procedure (Tice et al., 2000). Briefly, cells were plated in 35-mm culture dishes and treated with quinones with or without NAC. Following treatment, cells were resuspended in low melting agarose (LMA) and placed on slides coated with 1% normal melting agarose (NMA), and LMA was then added as the top layer. Cells were lysed in cold (4 °C) lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% Triton X, and 10% DMSO, pH 10.0) for 1 h. After lysis, the slides were subjected to horizontal gel electrophoresis in cold (4 °C) alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na_2-EDTA, pH 12.5) at 25 V and 300 mA for 20 min. The slides were