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Pro-inflammatory effects and oxidative stress in lung macrophages and epithelial cells induced by ambient particulate matter

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ABSTRACT

The objective of this study was to compare the toxicological effects of different source-related ambient PM10 samples in regard to their chemical composition. In this context we investigated airborne PM from different sites in Aachen, Germany. For the toxicological investigation human alveolar epithelial cells (A549) and murine macrophages (RAW264.7) were exposed from 0 to 96 h to increasing PM concentrations (0–100 μg/ml) followed by analyses of cell viability, pro-inflammatory and oxidative stress responses. The chemical analysis of these particles indicated the presence of 21 elements, water-soluble ions and PAHs. The toxicological investigations of the PM10 samples demonstrated a concentration- and time-dependent decrease in cell viability and an increase in pro-inflammatory and oxidative stress markers.

1. Introduction

As a result of a changing lifestyle with its rising demand for energy and motor vehicles the omnipresent human exposure to a multitude of different air pollutants increases (Gualtieri et al., 2010; Ristovski et al., 2012; WHO, 2006). In this context, ambient particulate matter (PM) concentration are considered as one of the most important environmental factors for adverse health effects, including cardiovascular and respiratory diseases (Brunekreef and Holgate, 2002; Pope et al., 2009; Englert, 2004; Kampa and Castanas, 2008; Simkhovich et al., 2008). For these health impacts the respiratory system constitutes the primary target of inhaled particles (Valavanidis et al., 2008; Badyda et al., 2013). The penetration depth and deposition of PM depends on particle size, shape and density (Kroll et al., 2011; Mitschik et al., 2008). Beside these physical parameters PM-induced toxicity is also affected by chemical composition and source (Li et al., 2003; Akhtar et al., 2010; Steenhof et al., 2011; Monn and Becker, 1999). Ambient particles are generally heterogeneous mixtures consisting of inorganic components (e.g., transition metals), salts, carbonaceous material, volatile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAH) and biological materials such as endotoxins, fungal spores and pollen (Anderson et al., 2011; Chirino et al., 2010; Merbitz et al., 2012). Although regulatory standards for PM10 and PM2.5 as well as specific particle constituents exist, there is still a fundamental lack of understanding of the toxicological mechanisms and their possible triggers (Li et al., 2002).

1.1. PM-induced toxicological effects

In the last years, numerous studies proposed the synergistic effects of oxidative stress and inflammation as the major biochemical pathways of PM-induced toxicity and health effects

Abbreviations: A549, Type II human alveolar basal epithelial cells; Al, Aluminum; As, Arsenic; Ca, Calcium; CAT, Catalase; Cd, Cadmium; COPD, Chronic Obstructive Pulmonary Disease; Cr, Chromium; Cu, Copper; CYP, Cytochrome; ELISA, Enzyme Linked Immunosorbent Assay; FBS, Fetal Bovine Serum; Fe, Iron; GPx, Glutathione Peroxidase; GRc, Glutathione Reductase; GSH, Glutathione; GSSG, Glutathione Disulphide; IL, Interleukin; LVS, Low Volume Sampler; Mg, Magnesium; Mn, Manganese; Na, Sodium; $NADP^+$/NADPH, Nicotinamide Adenine Dinucleotide Phosphate; $NF\text{-}_\kappa B$, Nuclear Factor-kappa B; Ni, Nickel; K, Potassium; Pb, Lead; PBS, Phosphate Buffered Saline; PAH, Polycyclic Aromatic Hydrocarbons; PM, Particulate Matter; RAW264.7, Murine macrophage cells; RNS, Reactive Nitrogen Species; ROS, Reactive Oxygen Species; SEM, Scanning electron microscopy; SOD, Superoxide-Dismutase; TC, Total Carbon; TNF-α, Tumor Necrosis Factor-α; UFP, Ultrafine Particulate Matter; V, Vanadium; VOC, Volatile Organic Compounds; Zn, Zinc.

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(Castell et al., 2005; Happo et al., 2010; Jalava et al., 2007; Scapellato and Lotti, 2007). Fig. 1 summarizes these findings and illustrates the process of PM-induced oxidative stress and inflammation with their associated triggers using the lung as an example.

1.1.1. Oxidative stress

Basically, PM-mediated oxidative stress is mainly caused by reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet -}$), and hydroxyl radicals ($OH^{\bullet}$), which are generated by free radicals present on particle surfaces as well as by the chemical reaction of specific PM-constituents from anthropogenic or natural sources (Akhtar et al., 2010; Li et al., 2003, 2008). Both, redox-active metals (e.g., Fe, Cu, Cr, Ni and Zn) and organic compounds, particularly polycyclic aromatic hydrocarbons (PAH), are directly and indirectly associated with ROS production (DiStefano et al., 2009; Ghio, 2004; Gualtieri et al., 2009). Aromatic compounds like PAHs are converted by enzymatic biotransformation to redox active peroxides and free radicals (Kelly, 2003; Steenhof et al., 2011).

The antioxidant defense system of most cells consists of different antioxidants (Risom et al., 2005; Ayres et al., 2007; Yi et al., 2012), like superoxide dismutase (SOD), catalase (CAT), and the antioxidant glutathione (GSH), scavengers of the highly reactive oxidants superoxide and hydrogen peroxide (Fig. 1) (Yang and Omaye, 2008).

1.1.2. Inflammatory response

Moreover, oxidative stress itself or other extracellular stimuli (endotoxins or pollen) can activate further redox-sensitive signaling cascades, which result in inflammatory response (Gerlofs-Nijland et al., 2009; Garçon et al., 2006; Li et al., 2008). Inflammation is generally characterized by local recruitment of pro-inflammatory cells such as neutrophils and macrophages, which are involved in the up regulation of various signaling molecules, such as cytokines (TNF-α, IL-6), chemokines (IL-8) and adhesion molecules (Gualtieri et al., 2010; Schins et al., 2002; Smith et al., 2000). These mediators in connection with an increasing ROS formation play a key role in the development of several inflammatory diseases like acute lung injury, COPD, chronic bronchitis and asthma (Gualtieri et al., 2010; Valavanidis et al., 2008). Based on these mechanisms, increased production and release of antioxidant enzymes and inflammatory mediators by cells are relevant and often used parameters suggesting PM-induced toxicity.

The objective of this study was to compare the toxicological effects of different source-related particles with regard to their chemical composition. In this context we investigated airborne PM from urban traffic and rural sites in the region of Aachen. For the toxicological characterization alveolar basal epithelial cells (A549) and lung macrophages (RAW264.7), were exposed to increasing PM10 concentrations followed by analysis of cell viability, oxidative stress and pro-inflammatory response.

2. Materials and methods

2.1. Reagents

Cell culture medium Dulbecco's modified Eagle's medium (with/without phenol red), penicillin–streptomycin, trypsin, L-Glutamine and phosphate buffered saline (PBS) were purchased from GIBCO® (Darmstadt, Germany). Fetal bovine serum (FBS) was obtained from Invitrogen (Darmstadt, Germany). Trypan blue (0.4%) for the viability staining test as well as the assays for glutathione and superoxide-dismutase detection were purchased from Sigma–Aldrich (Steinheim, Germany). The assay kit

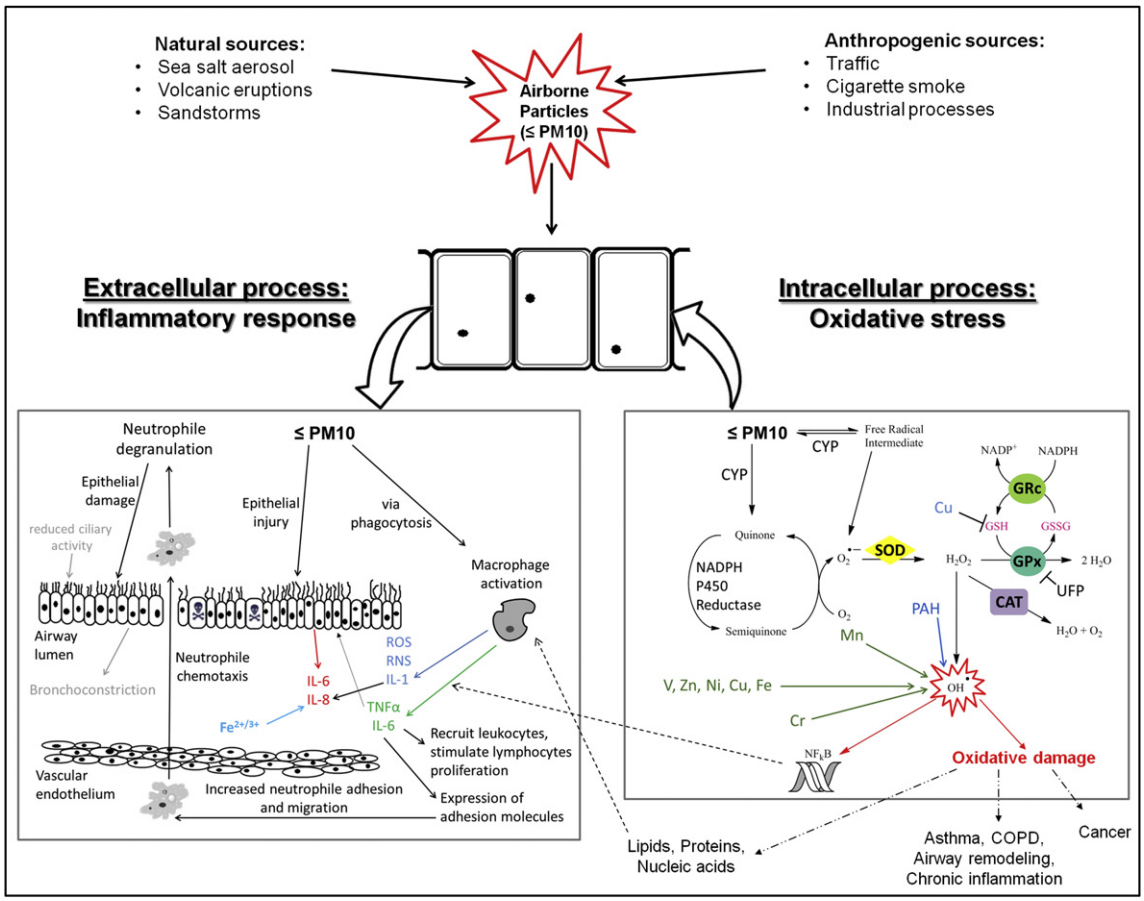

Fig. 1. Illustration of PM-induced intracellular and extracellular toxicological mechanisms in the lung.

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