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Responses of lung cells to realistic exposure of primary and aged carbonaceous aerosols

Lisa Künzi [a], Peter Mertes [b], Sarah Schneider [a], Natalie Jeannet [a], Carmela Menzi [a], Josef Dommen [b], Urs Baltensperger [b], André S.H. Prévôt [b], Matthias Salathe [c], Markus Kalberer [d], Marianne Geiser [a,*]

[a] Institute of Anatomy, Baltzerstrasse 2, University of Bern, 3012 Bern, Switzerland
[b] Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, OFLA, 5232 Villigen, Switzerland
[c] Division of Pulmonary, Critical Care & Sleep Medicine, Miller School of Medicine, University of Miami, Miami, FL 33136, USA
[d] Center for Atmospheric Sciences, Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, UK

HIGHLIGHTS

- Study on effects of wood and diesel combustion particles on lung cells in-vitro.
- Realistic exposure of cells to freshly emitted and atmospherically aged particles.
- Particle exposure induced subtle changes in cellular functions.
- Primary and aged particles from the same combustion induced similar effects.

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ABSTRACT

Diesel exhaust and wood burning are important sources of ambient atmospheric particles due to increasing numbers of diesel cars and the importance of wood as a source of renewable energy. Inhalation is the predominant route of entry and uptake for fine and ultrafine particles into the body. Health effects of atmospheric particles are still not completely understood. There is consistent evidence from epidemiology that particle exposure contributes to respiratory and cardiovascular diseases.

This study aimed at examining acute responses of airway epithelial cells and luminal macrophages after exposure to freshly emitted and photochemically aged carbonaceous aerosols under realistic atmospheric conditions. In addition to a bronchial epithelial cell line advanced cell cultures namely fully differentiated respiratory epithelia and primary surface macrophages were used.

Our results demonstrate that a single exposure of the cells to realistic particle doses of 0.3–3 ng diesel or 3–9 ng wood aerosol per cm^2 cell surface induces small, particle-specific responses. The release of interleukin-6 and -8 was found to be decreased in differentiated airway epithelia but not in the other cell models studied. Aerosol exposure decreased macrophage phagocytic activity by 45–90%. Cell and tissue integrity remained unaffected. Overall, primary and aged particles from the same combustion induced similar responses in the cell models tested, whereby particles from diesel exhaust affected the cells more than those from wood combustion.

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Abbreviations: ALI, air–liquid interface; BAL, bronchoalveolar lavage; BC, black carbon; DPBS, Dulbecco's phosphate-buffered saline; IL, interleukin; LDH, lactate dehydrogenase; LM, light microscopy; MCP-1, monocyte chemotactic protein-1; NO_x, nitrogen oxides; OM, organic mass; PBS, phosphate-buffered saline; PSL, polystyrene latex; RH, relative humidity; TEM, transmission electron microscopy; TNF-α, tumor necrosis factor-alpha.

* Corresponding author. Tel.: +41 31 631 84 75; fax: +41 31 631 38 07.
E-mail addresses: lisa.kuenzi@ana.unibe.ch (L. Künzi), pmertes@gmx.ch (P. Mertes), sarah.schneider@ana.unibe.ch (S. Schneider), natalie.jeannet@ana.unibe.ch (N. Jeannet), cmenzi@hotmail.com (C. Menzi), josef.dommen@psi.ch (J. Dommen), urs.baltensperger@psi.ch (U. Baltensperger), andre.prevot@psi.ch (A.S.H. Prévôt), msalathe@med.miami.edu (M. Salathe), markus.kalberer@atm.ch.cam.ac.uk (M. Kalberer), geiser@ana.unibe.ch (M. Geiser).

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1. Introduction

Despite generally improved air quality in the western world during the last decades particulate air pollution still regularly exceeds air quality standards and poses a health risk for a considerable fraction of the population (WHO, 2011). Since 1990, the number of new diesel passenger cars registered in Western Europe has increased. In 2007, 14.7 million cars were registered and 53.3% of these were diesel powered, compared to 50.8% in 2006 (ACEA, 2008). A considerable fraction of ambient airborne particles originates from combustion processes. Besides these primary emissions, additional organic particle mass is generated when gaseous components of the exhaust are oxidized in the atmosphere, so-called secondary organic or aged aerosols. Chirico et al. (2010) showed that formation of secondary organic aerosol from a diesel car without after treatment can increase the organic mass by factor 2–5. Wood burning for domestic heating is common in many parts of the world, and because of its importance as a source of renewable energy, there is an increasing interest in its usage. In some areas of Switzerland, wood burning was estimated to contribute more than 50% of the total organic aerosol mass during winter (Alfarra et al., 2007). Gaseous components of wood burning emissions are oxidized in the same way as described above for diesel exhaust, generating additional organic aerosol mass. Due to their small size, these particles deposit in the entire respiratory tract (Geiser and Kreyling, 2010).

Epidemiology supported by experimental studies in humans and laboratory animals has consistently provided evidence for an association of acute and chronic health effects, i.e. respiratory and cardiovascular disease exacerbations, with fine and ultrafine particulate air pollution (Anderson et al., 2012; Brook et al., 2010; Rückerl et al., 2011). Particles from wood combustion and traffic, including diesel exhaust, seem to contribute substantially to these observed health effects (Barregard et al., 2006; McCreanor et al., 2007).

The cause-effect relationship of epidemiologically observed effects of inhaled particles on morbidity and mortality is only partially known. Experiments with well characterized in-vitro models that closely mimic the in-vivo situation in regard to aerosol exposure and cell systems may significantly expand current knowledge and also provide a valid alternative to animal testing.

The aim of this study was to investigate acute responses of lung cells after exposure to primary and aged aerosols from wood burning and diesel exhaust. To achieve realistic exposure conditions, we used our aerosol deposition chamber which allows efficient deposition of fine and ultrafine particles directly out of a conditioned air flow on several cell cultures simultaneously (Mertes et al., in press). The advanced cell models consisted of primary differentiated cells of the inner surface of conducting airways with functional innate defense like mucus production and ciliary beating by the respiratory epithelium and phagocytosis by macrophages.

2. Experimental section

Cell cultures representing the immediate targets of inhaled particles, i.e. the lung epithelium and surface macrophages were used to study cellular responses to primary and aged particles from diesel and wood burning. Particles were processed and photochemically aged in a smog chamber from where they were drawn for cell exposures. Cell cultures were exposed to the aerosol for 1.5–2 h in the particle deposition chamber at simulated physiological conditions. Cellular responses, i.e. cytotoxicity, ultrastructural cell and tissue integrity, inflammatory mediator release as well as phagocytic activity of macrophages were assessed within 24 h after exposure to the aerosols.

2.1. Lung cell cultures

The following cell culture models representing the inner lung surface were used and responses after aerosol exposure were comparatively analyzed: (i) micro-dissected epithelia from porcine tracheae, (ii) re-differentiated airway epithelia from healthy human donors, (iii) the human bronchial epithelial cell line BEAS-2B, and (iv) macrophages obtained by bronchoalveolar lavage (BAL) of pig lungs. All cell cultures were prepared according to standard protocols of our laboratory (see Supplementary material) and cultured on microporous inserts (polyester membrane, 0.4 μm pore size; 24 mm Transwell® inserts, Corning, Vitaris, Baar, Switzerland or 24-mm Falcon® inserts, Becton Dickinson AG, Milian, Geneva, Switzerland) in multiwell plates. Porcine cells were harvested from lungs obtained from the slaughterhouse. Primary human epithelial cells were obtained from organ donors whose lungs were deemed not suitable for transplant through the organ procurement team of the University of Miami, Miller School of Medicine with appropriate consent procedures approved by the local institutional review board. A more detailed description of cell origin and preparation is given in the Supplementary material. Micro-dissected and re-differentiated airway epithelia were cultured at an established air–liquid interface (ALI; permanent ALI with cells producing their own surface lining layer). The basal medium of these cultures was changed daily and mucus was removed by washing cell surfaces with Dulbecco's phosphate-buffered saline (DPBS, pH 7.4) every other day. Mucus production was regularly checked visually and ciliary beating by light microscopy (LM) to confirm cellular function of primary epithelia. The epithelial cell line and macrophages were submersed cultures. For aerosol exposure apical medium (with reduced [1%] fetal calf serum) was reduced to a minimum (<1 mm in height) to mimic ALI conditions.

2.2. Aerosol generation, particle deposition and cell exposure

Production and aging of particles from diesel car exhaust and a wood burning stove have been described previously (Chirico et al., 2010; Heringa et al., 2011). Briefly, exhaust of a passenger diesel car (EURO 2 Volkswagen Transporter TDI Syncro, December 2000) operated under idle conditions was introduced through a heated tube into the PSI smog chamber leading to a dilution of a factor of 50–100 (Chirico et al., 2010). Beech logs were burnt in a wood stove (Attika Avant, 2009, combustion chamber ~ 0.037 m^3). Emissions were sampled from the chimney during the flaming phase and injected into the smog chamber through a heated inlet tube under similar conditions as used for the diesel exhaust (Heringa et al., 2011). We have chosen to investigate the wood burning emissions from the flaming phase as this is the prevailing mode of operation of heating appliances. However, aerosol chemical composition and secondary organic aerosol production vary in different burning phases (Heringa et al., 2011) and differences in the activity of reactive oxygen species in primary wood exhaust particles from different burning phases have been observed (Miljevic et al., 2010). Particle concentrations of about 2×10^4–1×10^5 particles/cm^3 and 12–60 μg m^{-3} were obtained immediately after filling the chamber (see Fig. 1 for typical particle concentration time trends). The particle concentrations in the smog chamber were similar to ambient conditions and thus representative for ambient gas-particle partitioning. Particles from diesel car emissions showed two modes around 35 and 135 nm for primary as well as 60 and 145 nm for aged particles. In wood burning experiments particle size modes were around 170 nm for primary and 200 nm for aged particles.

The PSI smog chamber is a 27-m^3 Teflon bag suspended in a temperature-controlled housing. The chamber was operated at

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