Stress-relieving effect of negatively charged aerosols on in-vitro cell cultures

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Abstract

The aim of this study was to investigate the stress-relieving effect on exposed lung-epithelial cells when exposed to an aerosol mix containing an aqueous and a combination aerosol from an ultrafine Diesel-engine. The study clearly demonstrates the toxic effects of nano-sized Diesel particles (WHO, 2012), yet at the same time documents the stress-relieving properties of this exhaust aerosol mix when negatively charged water aerosol. Negatively charged water aerosols are toxic at large concentrations, yet at low levels they can have beneficial effects. In the case of this study, a negatively charged water aerosol mixed with Diesel exhaust was found to be beneficial for cell viability and cell proliferation. The study also demonstrates the importance of the electrical properties of the aerosol, as well as the importance of the duration and intensity of the exposure.

Methods

A cell culture consisting of human alveolar-butted epithelial cells (A549 cell line) is cultivated under CO2-enriched conditions (5% CO2, 3% O2, 92% N2) using a culture-medium mix consisting of RPMI-1640 and 10% fetal calf serum (FCS), 1% glutamine and 1% antimicrobial mix (penicillin and streptomycin). After a few days of incubation the cells are mature enough (Fig. 1, step-A) that a sample (taken with a pipette) can be transferred to the upper compartment of the 24-well plates (step-B). Following a 3-day cultivation interval, during which the cells adhere and form a tight monolayer, the cells are set up for exposure. In order to render the cells more vulnerable to the toxic effects of the Diesel-exhaust mix, the neutralizer is turned on and the culture broth is replaced by the above medium mix containing an additional buffer (40 mM HEPES). Three of the six wells are filled to a level where the cells are submerged under the medium, whereas the remaining three wells are filled to a medium with a minimum of a 30 μm cell layer. Finally, Diesel-exhaust particles are mixed with and without a negatively charged water aerosol onto an epithelial-lung-cell culture to determine their effects. The additional feed of a H2O-aerosol into the setup stimulates the production of reactive oxygen species (ROS) that aids in the cell’s metabolic activity to better counteract the harmful influence during Diesel-exposure. The beneficial effects of reactive oxygen species (ROS) have only recently been recognized as playing a fundamental role in normal physiological processes. Indeed, they are indispensable in the operation of all bio-regulatory processes (Voeikov & DelGiudice, 2009).

Results

Although this investigation is still ongoing, the following preliminary observations show that upon exposure to a Diesel-aerosol, cell viability is drastically reduced (Fig. 4 – left panel). At the same time cytotoxicity increases dramatically (Fig. 5 – left pane). In both cases the submerged cell cultures are less affected than the control cultures that are directly exposed to the Diesel exhaust. Adverse effects are reduced upon repeating the setup with a H2O-Diesel aerosol mix – less in terms of cell viability, but more pronounced in terms of cytotoxicity (Fig. 4 & 5 – right pane). The water-enriched aerosol not only softens the adverse effects of Diesel (Fig. 4 – left pane) but has also a stimulating effect on proliferation of the submerged cells due to the additional humidification of the chamber air (Fig. 4 – right pane).

As shown in Fig. 5, cytotoxicity on the other hand is drastically reduced in both submerged and directly exposed samples. Although the water enriched aerosol drastically reduced the toxic effects of Diesel-exposure, it softens the toxicity potential of the air-exposed cultures from a factor of 2.2 and 1.5 to about 1.3 and 1.1 respectively. Hereby, the aqueous fraction of the mixed aerosol drastically buffer the toxic cocktail of Diesel exhaust. The drastic reduction in cytotoxicity is most likely related to the properties of nano-droplets—a concept briefly outlined in the conclusion.

Conclusions

According to the theory on the formation of Coherence Domains (CD, Voeikov & DelGiudice, 2009), aerosolized water produces a reservoir of almost free electrons, which in turn drive recombination-reactions. The energy potential of released electrons is tunneled into the surrounding medium, creating the electronegative effect of water. Due to electromagnetic coherence, the energy stored in a CD can not be released normally. Here CD becomes the privileged receptor of these tunnelling electrons. The coherent system (H2O + guest molecules) becomes discharges via the interaction of an ion pair consisting of H2O+ and O2−.

References


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Fig. 1: Normalized cytotoxicity of Diesel-exposed cells. The left shoulder represents the controls, whereas the right shoulder is dominated by the Diesel aerosol. The functional elimination of the toxic effects of the Diesel-exposed cells is determined by the presence of the negatively charged water aerosol.

Fig. 6: Normalized cytotoxicity of Diesel-exposed cells. The left shoulder represents the controls, whereas the right shoulder is dominated by the Diesel aerosol. The functional elimination of the toxic effects of the Diesel-exposed cells is determined by the presence of the negatively charged water aerosol.