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TOXICOLOGICAL IMPACT OF SECONDARY ORGANIC AEROSOLS FORMED FROM THE REACTION OF LIMONENE WITH OZONE

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Summary

Secondary organic aerosols (SOAs) formed from the reaction of limonene with O₃ were generated under weak and strong oxidation conditions in a flow reactor in order to evaluate their toxicity in relation to their physicochemical characteristics studied. SOAs were analysed with an SMPS (Scanning Mobility Particle Sizer) to measure the particle size distribution and by ESI-LC-QToF (ElectroSpray Ionization - Liquid Chromatography - Quadrupole - Time of Flight) to determine their chemical composition. Cell-free measurements of the intrinsic oxidative potential (OP) of the aerosols were performed using antioxidant depletions (DTT, AA) and a fluorescent probe (carboxyl-H2DCF-DA). *In vitro* tests, on the human bronchial epithelial BEAS-2B cells, were conducted to evaluate the oxidative and the inflammatory responses under various exposure conditions. The first results showed an intrinsic OP of the generated SOAs and the induction of antioxidant response-related parameters in exposed cells.

Introduction

Chronic exposure to PM_{2.5} at concentrations above the regulatory limit values leads to many premature deaths by causing and/or aggravating respiratory, cardiovascular or neurological pathologies and cancers (Chen et al., 2016). The regulations which set threshold concentration values of particles in µg/m³, does not yet consider the links between the chemical composition and the generated biological effects. SOAs, which can represent up to 90% of the organic carbon mass in PM_{2.5} (Gelencsér et al., 2007), are currently being studied to determine their impact on pulmonary toxicity. Indeed, their harmfulness is still poorly known, whereas their concentration in the atmosphere should raise in the future because of the concomitant increase of the emissions of volatile organic compound (VOC) precursors and the oxidizing capacity of the atmosphere in connection with the climate change. As VOCs of biogenic origin contribute to 90% of global emissions, we chose to work first on a model of SOAs resulting from the ozonolysis of limonene, one of the most emitted VOCs in the family of monoterpenes. The physicochemical characterization of the formed SOAs was carried out, as well as the measurements of their intrinsic OP and the investigation of the mechanisms involved in their lung toxicity.

Methodology and Results

The controlled generation of the SOAs produced from the limonene ozonolysis was developed in a laminar flow reactor. Two oxidation conditions were implemented, with low (1 ppmv) and high (50 ppmv) ozone concentrations. The particle size distribution of the generated SOAs showed the formation of ultrafine particles with a mean diameter of about 100 nm. The average mass concentrations obtained were 2.13 ± 0.15 mg/m³ and 3.12 ± 0.17 mg/m³ for the SOAs formed from weak and strong oxidation, respectively. The chemical composition was also studied by ESI-LC-QToF after aerosol sampling on quartz fibre filters and extraction by dichloromethane. A large range of oligomers up to 1300-1350 uma was observed. The intrinsic OP of the particles was evaluated with antioxidant depletion methods (DTT, AA) and carboxyl-H2DCF-DA fluorescent probe excitation. In addition, *in vitro* toxicity tests were conducted on BEAS-2B cells after exposure to the SOAs generated in both conditions. Two subtoxic doses of SOAs at 9 and 14 µg/cm² of cell layer (10 % and 20 % lethal concentration, respectively) were selected to evaluate the cellular effects of the particles after exposure during 6 h, 24 h or 48 h. Oxidative stress was assessed by measuring intracellular ROS, GSSG/GSH ratio, oxidative damage (4-hydroxynonenal adduct (4-HNE), 8-hydroxy-2'-deoxyguanosine (8-OHdG), carbonylated proteins), and the activation of the nuclear factor E2-related factor 2 (Nrf2) signalling pathway. The inflammatory response was also assessed by quantification of cytokine secretion and/or gene expression. Our preliminary results indicate that biogenic SOAs generated with lower ozone concentrations have a higher intrinsic OP and induce greater cellular oxidative stress than those generated with higher ozone concentrations.

Conclusions

The quantities of biogenic SOA formed in the atmosphere should strongly increase in the coming decades. Already representing a major part of the particulate mass, this study showed that SOAs produced from the ozonolysis reaction of limonene had an intrinsic OP and were able to induce a cellular oxidative stress. It is therefore necessary to further understand the toxicological impact of other SOAs generated from various VOC precursors, including anthropogenic ones.

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