OXIDATIVE STRESS INDUCED BY AIR POLLUTION-DERIVED ULTRAFINE PARTICLES SUBCHRONIC EXPOSURE IN MICE: A MULTIORGAN APPROACH

Although there has been a significant reduction in air pollution since the 1990s, it remains a major public health problem, responsible for over 4.2 million premature deaths worldwide every year. At present, experts' attention is focused on ultrafine particles (*i.e.*, UFP) because of their ability to translocate into the systemic circulation and reach peripheral organs, where they are likely to have harmful effects. Nevertheless, our knowledge of the cellular and molecular mechanisms involved in the toxicity of these particles is still very patchy, and most often remains focused on their main target, the lungs. In this work, a multi-organ approach was undertaken to provide innovative insights into the toxicokinetics (*i.e.*, biodistribution) and toxicodynamics (*i.e.*, pathophysiological mechanisms) of UFP collected in urban environment in mice subchronically exposed during 3 months.

The intrinsic oxidative potential of UFP, which corresponds to their capacity to generate reactive oxygen species and/or to oxidize biological molecules, was analyzed by chloromethyl derivative of 2',7'-dichlorodihydrofluorescein diacetate, dithiothreitol, ascorbic acid, and GSH/GSSG-Glo™ (Promega) assays. Balb/cJRj mice (male, specific and opportunistic pathogen free, 10 weeks, n=6/group) were exposed for 3 months by 2 intranasal instillations/week to 0, 10 or 30 µg of UFP/40 µl of sterile saline. Mice were sacrificed 24h after their last exposure. The biodistribution of UFP was studied in 5 targets organs (*i.e.*, lungs, heart, brain, liver, and kidneys) through direct (*i.e.*, BAL cell numeration, histological observations and transmission electron microscopy analyses) and indirect (*i.e.*, metals quantification by ICP-MS, methallothioneins and cytochromes P450 gene expressions by RT-qPCR) methods. Oxidative stress was studied in all these organs by: (1) nuclear factor erythroid 2-related factor 2 (NRF2) signaling pathway activation by RT-qPCR, (2) antioxidant enzyme activities (*e.g.*, superoxide dismutase, glutathione peroxidase and glutathione reductase) by ELISA, (3) glutathione status by GSH/GSSG-Glo™ assay (Promega), and (4) oxidative damage (*e.g.*, 8-hydroxy-2'-deoxyguanosine, carbonylated protein, and 4- hydroxynonenal) by ELISA/MSD.

All the methods we used suggested a preferential pulmonary and cerebral distribution, while those in the heart, liver and kidneys were weaker, appearing to be linked only to the passage of UFP during systemic distribution or elimination. In the lungs and, to a lower extent, in the other target organs (*i.e.*, heart, brain, liver and kidneys), the intrinsic oxidative potential of UFP undeniably induced the production of pro-oxidant species, the activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated antioxidant response signaling pathway and the biosynthesis of non-enzymatic antioxidant defenses, such as reduced glutathione, in sufficient quantities to restore redox homeostasis. Taken together, these results emphasized the importance of considering not only pulmonary effects, but also cardiovascular and cerebral effects, and even hepatic and renal effects, when assessing health risks in situations involving UFP exposure.