

Oxidative stress and inflammation induced by Diesel Exhaust Particles: pilot *in-vitro* and *in-vivo* studies

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In recent years particulate matter (PM) has been found to trigger several mechanisms responsible for neuroinflammation, an active participant in the progression of neurodegenerative diseases (Block and Calderón-Garcidueñas, 2009; Levesque et al., 2011). This project aimed to assess whether, in a model of neuronal cells, PM, specifically ultrafine particles (UFP), induces oxidative stress and inflammation.

HT22 cells, an immortalized mouse hippocampus cell line, have been treated for 3h and 24h with DEP SRM1650b (Standard Reference Material, National Institute of Standards and Technology, USA), a diesel particulate with mean aerodynamic diameter of 0,18 μm , suspended at different concentrations (10 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$) in culture medium. The following parameters have been measured: cell viability, oxidative stress related toxicity and activation of inflammation pathways.

MTT viability assay demonstrated that DEP does not induce a significant cell death neither at 3h nor at 24h of treatment, for all the doses used. After 3h of DEP treatment, protein analysis revealed the presence of oxidative stress; in fact DEP induced a significant dose-dependent increase of HO-1 levels, a stress related protein which catalyse heme degradation. The increase of iNOS protein levels suggests the activation of inflammation pathway. Moreover, we observed an increase in Cyp1B1 levels, a cytochrome of the P450 superfamily involved in polycyclic aromatic hydrocarbons metabolism, and in HSP70 levels, a protein activated in cellular stress conditions. After 24h of DEP treatment, analysis showed that HO-1 and HSP70 protein levels are still high, whereas Cyp1B1 and iNOS return almost to control levels. Indeed, we can hypothesize that exposure of neuronal cells to DEP results in oxidative stress and inflammation induction.

In in-progress experiments, BALB/c mice will be submitted to intratracheal instillation of single or repeated doses of DEP, and effects on the SNC system (in particular on hippocampus, cortex and cerebellum) will be studied.

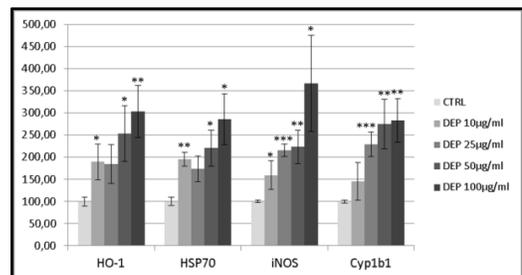


Figure 1. Protein analyses after 3h of DEP treatment. * p<0,05 vs CTRL; ** p<0,01 vs CTRL; *** p<0,001.

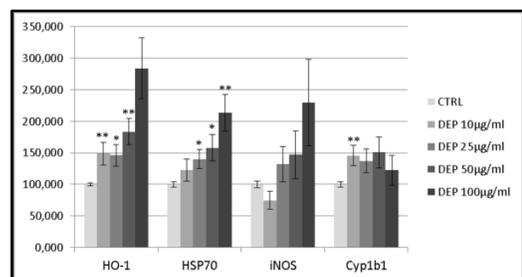


Figure 2. Protein analyses after 24h of DEP treatment. * p<0,05 vs CTRL; ** p<0,01 vs CTRL.

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