Abstract # 444

Effects of carbon black nanoparticles on human pulmonary cell lines and precision cut lung slices

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INTRODUCTION

Carbon Black nanoparticles (CBNPs) are among the most abudantly used nanomaterials and have been reported to cause adverse health effects after inhalative exposure. Pulmonary *in vitro* or *ex vivo* models are thus urgently needed to gain insights into potential mechanisms of toxicity. To this end, a joint research project is funded by the German Federal Ministry of Education and Research. In the course of this project, the effects of Printex® 90 and acetylene soot particles were comparted in human pulmonary cell lines (16HBE14o-, Calu-3 , A549) and precision cut lung slices (PCLS) of mice, rats and humans over a wide concentration range. Acetylene soot particles carry polycyclic aromatic hydrocarbons bound to the surface.

MATERIAL AND METHODS

Particle size distribution in the cell culture medium was determined by dynamic light scattering. Viability assays were LIVE/DEAD® dtaining and WST-1 assay for PCLS and WST-8 and neutral red assay in the case of cell lines. CBNP-induced formation of reactive oxygen species (ROS) was assessed in A549 and 16HBE14o- cells by flow cytometry using the DCFH-DA assay. Furthermore, the effect of CBNP exposure on the transepithelial electrical resistance (TEER) was investigated in Calu-3 cells. With PCLS, the inflammatory response was assessed by measuring pro-inflammator cytokines (i.e. IL-1α, TNF- α, IL-8).

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RESULTS

Both CBNPs tested were nearly nontoxic in physiologically relevant concentrations. Statistically significant effects were observed in the WST-8 assay for both CBNPs l after 48h, whereas no effects were found in the neutral red assay. Increased ROS formation was observed with both CBNPs after 24 and 48 h. TEER values were measured after 24, 48 and 120h treatment with 10 and 50 µg/ml. Interestingly, acetylene soot particles caused significant TEER reduction at both dose levels and all time points tested whereas Printex® 90 reduced the TEER only after 120h and in the high dose. Neither Printex® 90 nor acetylene soot particles induced the secretion of proinflammators cytokines in mouse and rat PCLS.

CONCLUSION

Cytotoxic effects of CBNPs depend on their surface properties. Furthermore, this study demonstrates, that the combination of *in vitro* and *ex vivo* models provides a valuable tool to assess the acute effects of CBNPs on lung tissue.

ACKNOWLEDGEMENT

CARBON BLACK
A project of the nanoCare funding programme

nanoCare

Federal Ministry of Education and Research

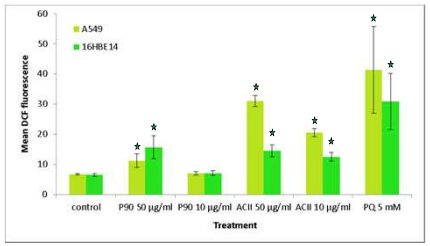

Figure 1: Effect of Printex 90 (P90) and Acetylene soot (ACII) on ROS levels in human pulmonary cell cultures. Paraquat (PQ) was used as positive control.

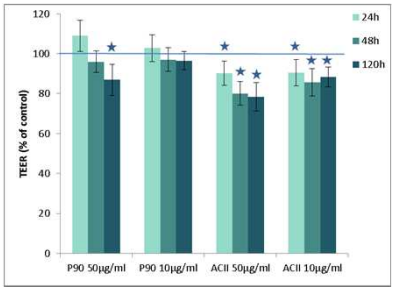

Figure 2: Effect of Printex 90 (P90) and Acetylene soot (ACII) on the barrier formation of Calu-3 cells cultured on permeable inserts.

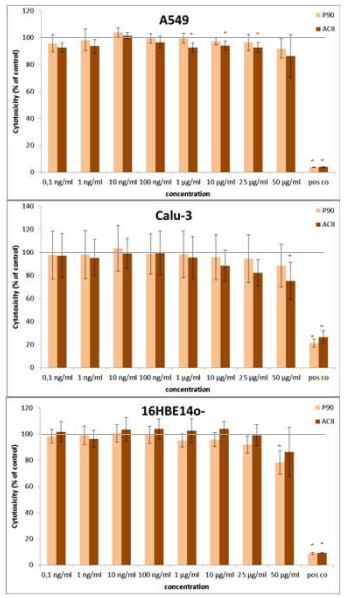

Figure 3: Cytotoxic effect of Printex 90 (P90) and Acetylene soot (ACII) on human pulmonary cell lines quantified using the WST-assay.

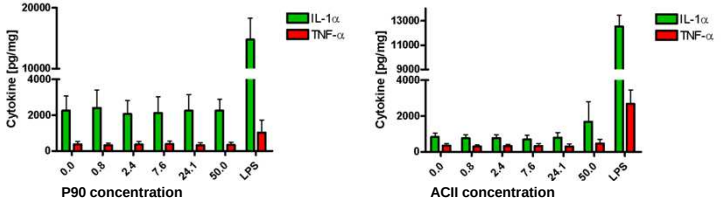

Figure 4: Production of cytokines [IL-1 and TNF-α] in mouse PCLS after 24h Printex90 (P90) and acetylene soot (ACII) exposure.

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