In vitro studies on pulmonary- and immunotoxic effects of industrially relevant multiwalled carbon nanotubes

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Carbon nanotubes (CNTs) are among the most produced nanomaterials and their exceptional properties are exploited in many fields of technology and medicine with possible applications ranging from nanocomposites to the imaging and treatment of diseases. As a consequence, the industrial production of these novel nanomaterials will increase dramatically in the near future, raising concerns about their possible hazardous effects on the environment and living organisms. Indeed, several studies on mice and rats indicate that CNT might have a toxic potential to human health. Moreover, there is public concern that carbon nanotubes might elicit harmful responses similar to those of other toxic fibrous particles such as asbestos.

A major entry site for nanomaterials to the body is represented by the lung, thus underlining the relevance of toxicity studies on pulmonary cells. Once deposited, nanoparticles may rapidly be spread and distributed throughout the body via the circulation and lymphatic system. Our intricate immune system with numerous cell types constantly patrolling the whole body is central to the detection and elimination of foreign particles and pathogens. However, there is little knowledge on how cells of the immune system interact and cope with these novel nanomaterials.

Here, we investigated possible adverse effects of three industrially relevant multiwalled CNTs (MWNTs) on human lung cells (epithelial alveolar A549) and immune cells (Jurkat T lymphocytes). Cell viability, oxidative stress, cytoskeletal organization and cytokine production were analyzed by colorimetric or fluorimetric assays, flow cytometry, immunocytochemistry and enzyme-linked immunosorbance assay (ELISA). In addition, we compared the effects of MWNTs to those of crocidolite, a particularly potent form of asbestos.

We found that after treatment with MWNTs, both cell types showed signs of oxidative stress such as the induction of reactive oxygen species (ROS) or a decrease in the mitochondrial membrane potential (MMP). However, these MWNTs did not cause major cell death of A549 lung epithelial cells or Jurkat T lymphocytes. Measuring the production of IL-2 protein in T cells, we found that MWNTs did not activate resting T cells nor did they have major immune modulatory effects on PMA/PHA-activated cells. Asbestos slightly decreased cell viability of Jurkat cells and more strongly of A549 cells but did not induce ROS or a shift in the MMP suggesting that at least some mechanisms underlying MWNT-toxicity might be different from those of asbestos.

Our study indicates that pristine, currently large-scale produced MWNTs have low immunogenic and immunomodulatory effects on human T lymphocytes in vitro, however, they affect the basic functionality of cultured A549 lung epithelial cells. Further studies will be required to determine the basis of these cell type specific reactions.

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Introduction

Due to their exceptional properties and their wide range of applications, carbon nanotubes (CNTs) are nowadays produced in large quantities and thus the potential for a widespread exposure to these novel materials is likely to increase. Such exogenous materials may be inhaled and distributed throughout the body by the circulation and lymphatic system. Here we investigated in vitro toxicity of three large-scale produced multiwalled CNTs (MWNTs) and cococidal asbestos on human lung cells (epithelial alveolar A549) and immune cells (Jurkat T lymphocytes).

Materials

Materials MWNT were commercially available from Bayer Technologies Services (MWNT A), Cheap Tubes Inc. (MWNT B) and Nanocyl SA (MWNT C). They were dispersed in ultrapure water containing 160 ppm Pluronic F127 to a final stock solution of 300 μg/ml. Test materials MWNT effects were compared to those of toxic, fibrous cococidal asbestos. Cell culture Jurkat A3, Human Lung Cancer A549 (ATCC, CRL-1586) and A549 lung epithelial cells (ECV-304), were exposed to different concentrations of MWNT and test materials for the indicated times.

Results A549

1. MWNTs do not induce major A549 cell death

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- A549 cells were analyzed for annexin V (A′) binding and propidium iodide (PI) incorporation to distinguish between apoptotic (A′/PI−) and late apoptotic/necrotic (A′/PI+) and A′/PI− cells by flow cytometry.

2. Effects of MWNTs on the basic functionality of A549 cells might be secondary due to the loss of cells

- A549 cells were exposed to MWNTs or asbestos for 5 days, and cell viability was assayed by the MTT assay.

3. MWNTs induce the formation of ROS in A549 cells

- Formation of reactive oxygen species (ROS) was determined using the DCF assay, measuring the conversion of H2DCF to fluorescent 2′, 7′-dichlorofluorescein (DCF) by ROS. A549 cells were treated with different concentrations of particles for 2 hours. In one, a morpholin compound, was used as a positive control.

4. MWNTs do not affect the morphology of A549 cells

- A549 cells were treated with MWNTs for 6 days, and the actin cytoskeleton (green) and nuclei (blue) were visualized by phalloidin and DAPI, respectively.

Results Jurkat

1. MWNTs have no major effect on Jurkat cell viability

- The trypan blue exclusion assay was used to determine the number of viable Jurkat cells.

2. MWNTs induce the formation of ROS in Jurkat cells

- Formation of reactive oxygen species (ROS) was determined using the DCF assay, measuring the conversion of H2DCF to fluorescent 2′, 7′-dichlorofluorescein (DCF) by ROS. Jurkat cells were treated with different concentrations of particles for 2 hours. In one, a morpholin compound, was used as a positive control.

3. MWNTs do not affect the cytoskeletal organization of Jurkat cells

- Jurkat cells were treated with particles for 6 days and the actin cytoskeleton and microtubules were visualized by phalloidin and α-tubulin antibody, respectively.

4. MWNTs do not have major immunomodulatory effects

- IL-2 receptor levels were determined from supernatants of Jurkat cell cultures by specific sandwich ELISA after 24 h of exposure to MWNTs.

Conclusions

In this study we show that despite the induction of reactive oxygen species the large-scale produced MWNTs are not acutely toxic to Jurkat cells and only slightly increase cell death in A549 cells at high concentrations. MWNTs do not affect the immune competence of Jurkat cells. However, the basic functionality of A549 cells appears to be affected by MWNTs. This might be a secondary effect resulting from a reduced adhesion of A549 cells in the presence of MWNTs. However, this hypothesis remains to be tested in further experiments.