DNA Damage and Cytotoxicity in Fe-Containing Carbon Nanotubes and Nanofibers

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Abstract

Carbon nanotubes (CNTs) and nanofibers (CNFs) are now being synthesized and processed in large quantities, but their potential health risks have yet to be fully assessed. CNTs and CNFs are long, thin, and bio-persistent and have properties in common with asbestos, a group of crystalline, iron-containing mineral fibers that are known human carcinogens. One hypothesis for asbestos-related disease is that redox catalyzed generation of reactive oxygen species causes DNA damage and cytotoxicity. The redox-active species in asbestos is iron, which can be mobilized from the crystal lattice by natural chelators, and then redox cycles to generate free radicals, such as superoxide anion and hydroxyl, in the presence of physiologic reductants such as ascorbate. Hydroxyl radical is particularly aggressive, reacting at diffusion-limited rates with DNA and most other biological molecules. Iron is the single most common element used in CNT/CNF catalyst formulations and, despite purification procedures, most CNT/CNF samples contain significant quantities of residual catalyst.

This study examines the potential cytotoxicity of iron-containing carbon nanomaterials using a variety of molecular and cellular endpoints, including iron mobilization, induction of plasmid DNA single strand breaks, and the viability and morphology of murine macrophages. The iron in catalytically grown nanotubes varies in chemical composition (metal, oxide, carbide) and can be partially or wholly encapsulated by carbon, hindering its interaction with species in surrounding fluid media and possibly suppressing redox activity. To manage this complexity, experiments were first carried out on a model nanomaterial system in which the amount, form, and accessibility of iron were carefully controlled. The model system chosen were non-catalytic, template-fabricated carbon nanofibers in two forms: (1) high-purity as synthesized, and (2) surface doped with accessible iron nanoparticles. A second set of experiments were conducted on commercial multiwall nanotubes in four forms: (1) raw, (2) vendor purified with reduced iron content, (3) purified then partially oxidized, and (4) purified then ground. The last two samples are included to test if post-synthesis handling/processing can further expose encapsulated iron and accelerate the free radical generation process.

The doped model nanofibers showed significant Fe mobilization by ferrozine in the presence of ascorbate, a higher frequency of DNA single-strand breaks, accompanied by macrophage activation and increased cell death. The raw commercial nanotubes showed similar results to the Fe-doped model nanofibers indicating that at least some of the iron is indeed redox active and not fully passivated by carbon shells. The purified nanotubes had much lower Fe mobilization and DNA single strand break frequency. Partial oxidation of the purified sample restored some of the iron mobilization, suggesting that additional iron was made accessible by oxidative tube damage. Mechanical grinding had little effect. Overall, these results suggest practical guidelines for fabricating, purifying, and handling iron-containing carbon nanomaterials to minimize toxicity.