OXIDATIVE STRESS INDUCTION IN CACO-2 CELLS BY SINGLE WALL CARBON NANOTUBES

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Carbon nanotubes (CNTs) are among the nanoparticles with higher potential for biomedical uses. They consist exclusively of carbon atoms arranged in a series of condensed benzene rings rolled-up into a tubular structure, and belongs to the third allotropic form of carbon along with graphite and diamond. CNTs can be classified in two general categories: single-walled nanotubes (SWNT) which have diameters from 0.4 to 2.0 nm and lengths in the range of 20–1000 nm, and multi-walled nanotubes (MWNT) that are bigger objects with diameters in the range of 1.4–100 nm and lengths from 1 to several μm.

CNTs have some interesting physicochemical properties such as: ordered structure with high aspect ratio, ultralight weight, high mechanical strength, high electrical conductivity, high thermal conductivity, metallic or semi-metallic behaviour and high surface area, which make CNTs a unique material with the potential for diverse applications, including biomedical [1].

Another point is the status of CNTs dispersion in solution because CNTs are highly hydrophobic [2]. Some studies show that well-dispersed SWNT is associated with lesser cytotoxicity compared with the same SWNT present in an agglomerated form [3]. This high hydrophobicity of pristine CNTs has induced the need to modify the surface chemistry of CNTs to improve their aqueous solubility, which is a very important point in terms of their subsequent potential toxicity. Thus, CNTs are functionalized to obtain more biocompatible products.

CNTs toxicity has been previously studied mainly in pulmonary and dermal cells. Some i*n vitro* studies highlight that CNTs can be toxic for macrophages [4], lymphocytes [5], keratinocytes [6], type II alveolar epithelial cells [4], mesothelial cells [7], aortic smooth muscle cells [8], skin fibroblasts [9] and embryo kidney cells [10], but the gastrointestinal tract is also one of the prime targets for direct interactions with nanomaterials and studies on it are still scarce. In terms of the intracellular mechanism, oxidative stress is frequently proposed as a key mechanism of CNT-induced toxicity, usually linked to the metallic impurities of CNTs [11].

Single wall CNTs functionalized with carboxylic acid (COOH-SWCNT) have shown to induce cytotoxicity on the human intestinal cell line Caco-2 [12] with a reduction of the cell viability and cell membrane injure. The present study explores the oxidative stress as toxic mechanism involved in COOH-SWCNT observed damage in Caco cells. Cells were exposed to concentrations between 0 and 1000 μg/mL COOH-SWCNT for 24h of exposure and the following oxidative stress biomarkers were studied: lipid peroxidation (LPO); antioxidant enzymatic activities such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST); and cellular glutathione content (GSH).

Results showed that LPO significantly increased with COOH-SWCNT concentrations from 50 μg/mL (Fig.1). Exposure to COOH-SWCNT induced CAT activity in a concentration-dependent manner up to 500 μg/mL, but with 1000 μg/mL depletion was observed (Fig.2). SOD, GPx and GR showed a similar trend; reaching their maximum level at 100 μg/mL (Fig.3); although the increase was not significant for GR. At higher concentrations, however, they experienced a reduction. No significant changes were observed in the activity of GST with the different concentrations of COOH-SWCNT assayed. Finally, GSH content of the cells decreased at all exposure levels but only with 1000 μg/mL this reduction was significantly different from control values (Fig.4).

COOH-SWCNT induced oxidative stress in Caco-2 cells from 50 μg/mL. The increase in the antioxidant enzymatic activities could be due to an adaptative response of the cells to the toxic injury, whereas the depletion at the highest concentration mainly observed in CAT activity could reflect not a functional damage but a structural one, indicating a higher sensitivity of this enzyme to this compound. Further investigations are necessary to elucidate whether the oxidative stress observed is induced by the COOH-SWCNT per se or by potential metallic traces contained in the product and derived from the synthesis process.

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Figures:

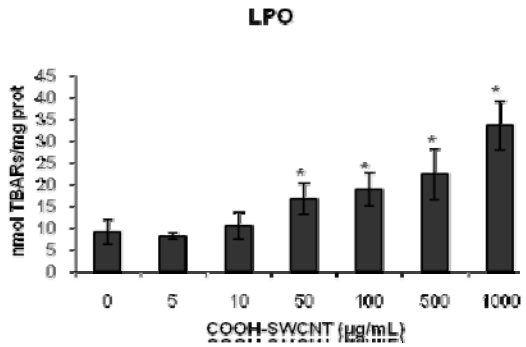

Figure 1. Lipid peroxidation (LPO) on Caco-2 cells exposed to COOH-SWCNT for 24h. The values are expressed as mean ± S.E. LPO value is expressed as nmol TBARS/mg protein.

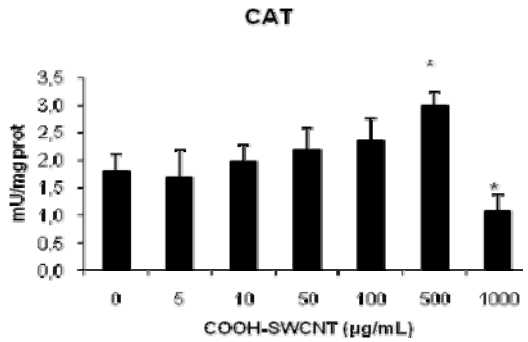

Figure 2. Catalase activity (CAT) on Caco-2 cells exposed to COOH-SWCNT for 24h. The values are expressed as mean ± S.E. CAT activity is expressed as mU/mg protein.

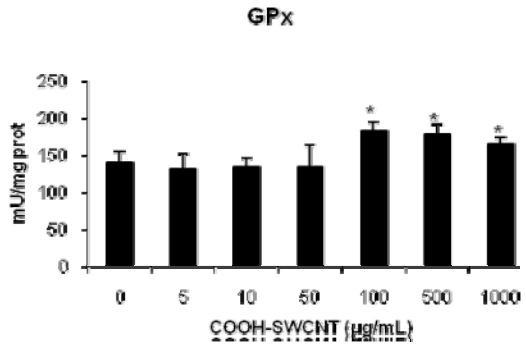

Figure 3. Glutathione peroxidase activity (GPx) on Caco-2 cells exposed to COOH-SWCNT for 24h. The values are expressed as mean ± S.E. GPx activity is expressed as mU/mg protein.

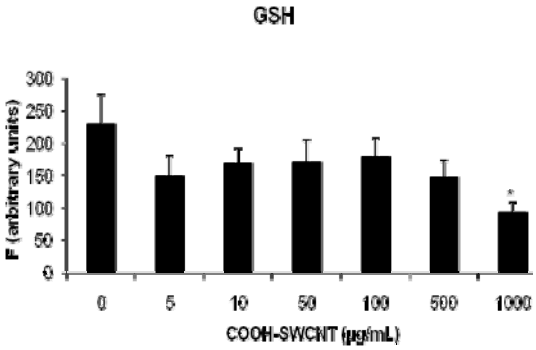

Figure 4. Glutathione (GSH) on Caco-2 cells exposed to COOH-SWCNT for 24h. The values are expressed as mean ± S.E. GSH is expressed as arbitrary units.

*Significantly different from control ($p \leq 0.05$)