Toxicity of single walled carbon nanotubes to human epithelial cells as affected by Norfloxacin

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Abstract. SWCNTs are widely used in the fields of biomedicine, electronics and energy storage. With the increasing of SWCNTs production, SWCNTs are likely to present in the environment. In this work, a series of concentrations of SWCNTs were used to test the toxicity to human epithelial cells (A549). Results showed that SWCNTs can inhibit cells growth and medium lethal concentration (IC_{50}) is 65 mg/L at 24 h. Norfloxacin (NOR) can increase the viability of cells exposed to SWCNTs and relieve the oxidative stress of SWCNTs induced.

Introduction

Single walled carbon nanotube (SWCNT) is one-dimensional cylinder nanostructure formed by rolling one sheet of graphene (Dai, 2002). CNTs with a lot of extraordinary properties are considered as excellent candidates in a wide range of fields (Tasis et al., 2006), such as energy storage (Che et al., 1998), electronics (Li et al., 1999), biomedicine (Liu et al., 2009). With increasing CNTs production, they are likely toreleaseinto the environment and affect human health (Petersen et al., 2011). It is reported that CNTs exhibited harmful effects on human lung epithelial cells (Cavallo et al., 2012). The toxic mechanism of CNTs to human cells remainsundetermined, however, some research demonstrate that CNTs could damage cell membrane and induce oxidative stress. Mu et al. (2009) reported that SWCNT-COOH can inhibit the growth of human bone cells by disturb genes and protein expression and Zhu et al. (2014) observed CNTs could change the cell membrane structure and composition. Norfloxacin (NOR) as the first member of fluoroquinolones, is increasingly used and widely present in environments (Gustot, 2014). CNTs that can adsorb other contaminantshas been reported (Yang et al., 2006), so the combined toxicity of CNTs and these adsorbed contaminants should be paid attention.

The main objective of this study was to investigate whether environment pollutant(NOR) could affect the toxicity of SWCNTs to human epithelial cells. The related toxic mechanism of CNTs to A549 cells will also be addressed.

Materials and methods

Cell culture and growth inhibitiontest

SWCNTs were purchased from Shenzhen nanotube supermarket (China). The human lung epithelial (A549) cell line was obtained from Shanghai Biological Sciences Institute, Chinese Academy of Science. A549 cells were cultured in an improved F-12K medium supplemented with 10% (v/v) fetal bovine serum (PAA Laboratories GmbH, Austria) at 37°cin a 95% (v/v) humidified atmosphere and 5% CO2. The cell viability was evaluated using cell counting kit (CCK-8, Beyotime Institute of Biotechnology). A549 cells were cultured in the 96-well plates (5×103 cells/well). SWCNTs were introduced to A549 cells with final concentration of 0, 10, 20, 40, 100, 150, 175 and 200 mg/L, respectively. After incubated for 6, 12, 24 and 48 h, the cells were incubated with 11uL cholecystokinin octapeptide for 4 h, the optical density of each well was recorded at 450 nm on Multiskan Spectrum (Thermo, Varioskan Flash, USA).

Detection of intracellular ROS and MDA

The generation of intracellular reactive oxygen species (ROS) in A549 cells was measured by the DCF method. In brief, cells were cultured in 96-well plates and exposed to 65 mg/L SWCNTs.

After exposed for 1, 2, 4, 8, 16 and 24 h, all the cells were then incubated with 50 um DCFH-DA for 40 min and then washed twice in pH 7.2 phosphate buffered saline (PBS), the fluorescent intensity of DCF was detected using flow cytometry (Becton Dickinson, Mountain View, USA). The concentration of lipid peroxides was quantized in terms of the Methane Dicarboxylic Aldehyde (MDA) concentration, the MDA were measured by the thiobarbituric acid reactive substance (TBARS) assay (Vavilin et al., 1998).

Statistical analysis

Statistical analysis was analyzed using a one-way analysis of variance (ANOVA) and compared with LSD test. All treatments included three replicates and standard error was reported ($p<0.05$).

Results and Discussion

Characterization of SWCNTs

Characterization of SWCNTs was analyzed by transmission electron microscopy (TEM JEM-2100, Japan) and Nanosizer (Malvern Instruments Ltd.). From the TEM image can be clearly seen that the length is 5-15 um and the diameter is less 2 nm (Fig. 1). The hydrodynamic diameter is 495.6 nm and 6743 nm of SWCNTs in water and NOR solution, respectively. The zeta potential of SWCNTs in water and NOR solution is -10.9 mV and -36.1 mV, respectively.

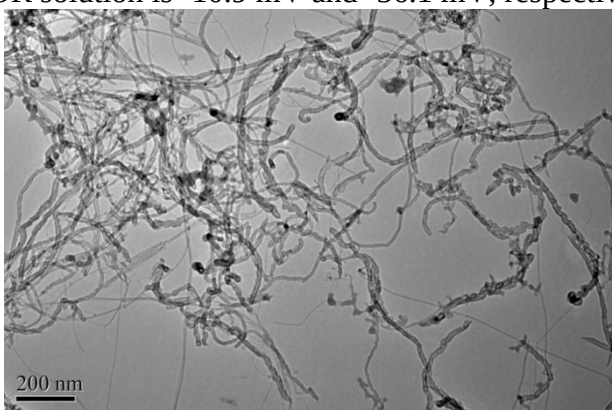

Fig. 1 TEM images of SWCNTs.

Toxicity of SWCNTs to A549 cells

Fig. 2 shows the toxicity of SWCNTs to A549 cells at different exposure concentrations and times. A549 cells viability decreased with increasing SWCNTs concentrations at 24 h (Fig. 2A). The viability of A549 cells exposed to SWCNTs showed both dose-effect relationship and time-effect relationships (Fig. 2B). The 24 h median lethal concentration (IC50) of SWCNTs was 65 mg/L. Therefore, A549 cells exposed to 65 mg/L SWCNTs for 24 h was chosen for the following experiments.

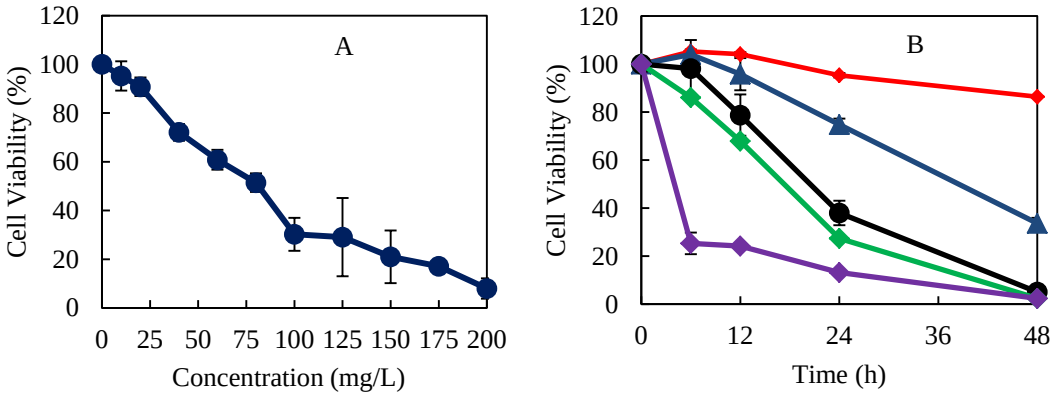

Fig. 2 (A) A549 cells viability after 24 h exposed to SWCNTs at different concentrations. (B) A549 cells viability after exposed to 0~200 mg/L SWCNTs for different time.Red, blue, black, green, purple is 10, 40, 100, 150, 200 mg/L.

Toxicity of SWCNTs in the presence of NOR

NOR significant increased the viability of cells exposed to SWCNTs; the viability of cells is

increased 17.6% and 22.7% after additional 10 and 40 mg/L NOR in SWCNTs suspension (Fig. 3).

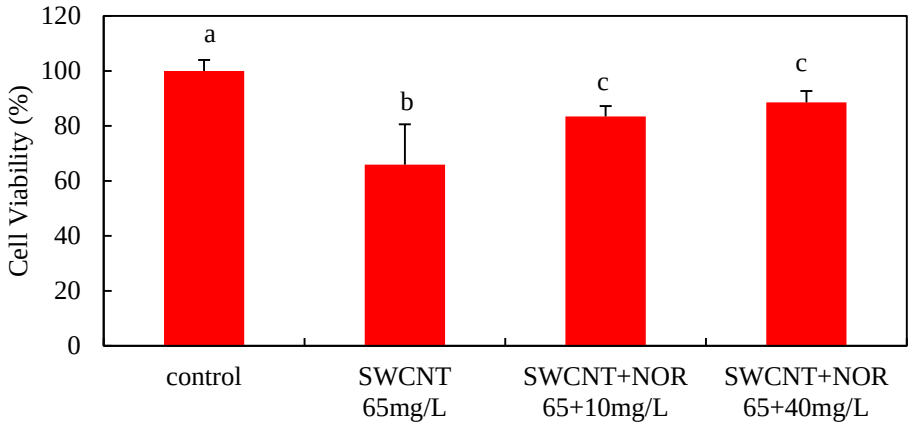

Fig. 3 A549 cells viability after exposed to 65 mg/L SWCNTs alone and SWCNTs in presence of NOR (10, 40 mg/L).

Effect of NOR on SWCNTs-induced oxidative stress

Level of intracellular ROS in A549 cells was raised by 9 times after exposure to SWCNTs for 4 h. The activity of antioxidant enzymes such as superoxide dismutase and thioredoxin peroxidase increased by feedback regulation and they can scavenging excessive free radical and enhanced the antioxidant capacity of the cell. However, as time increased, ROS could be accumulated again (Fig. 4A). With the accumulating of ROS, lipid peroxidation of cells was caused. NOR can significantly relieve ROS accumulation and lipid peroxidation (Fig. 4B). This is attribute to NOR enhanced agglomeration of SWCNTs and lead to decreased interaction of SWCNTs with the cells.

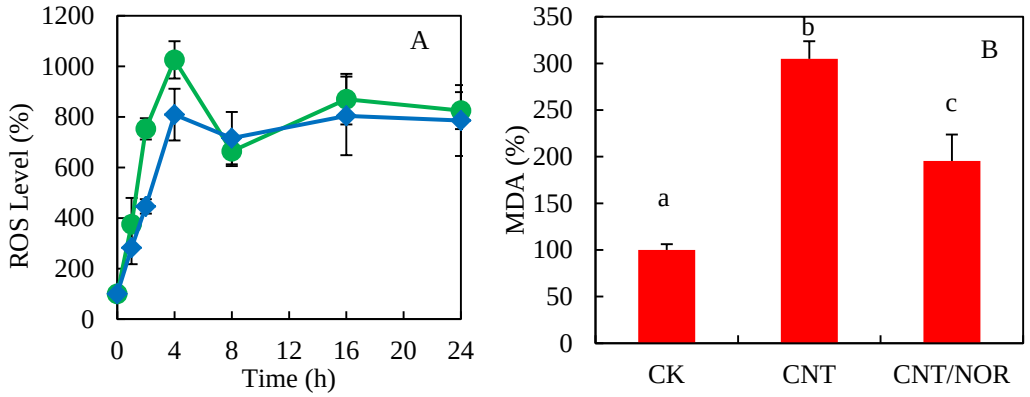

Fig. 4 Level of intracellular ROS (A) and MDA (B) in A549 cells after exposure to SWCNTs (65 mg/L) alone and SWCNTs (65 mg/L) in presence of NOR (40 mg/L).

Conclusions

The results of this study demonstrated that toxicity of SWCNTs to A549 cells is time-effect relationship and dose-effect relationship. NOR can increase the viability of cells exposed to SWCNTs, NOR can enhanced agglomeration of SWCNTs, which may lead to decreased interaction of SWCNTs with the cells. Oxidative stress is responsible for the toxicity of SWCNTs, NOR can decrease the level of oxidative stress to cells.

Acknowledgements

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