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Long-term accumulation and low toxicity of single-walled carbon nanotubes in intravenously exposed mice

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ABSTRACT

The biomedical application of single-walled carbon nanotubes (SWCNTs), such as drug delivery and cancer treatment, requires a clear understanding of their fate and toxicological profile after intravenous administration. In this study, the long-term accumulation and toxicity of intravenously injected SWCNTs in the main organs (such as liver, lung and spleen) in mice were carefully studied. Although SWCNTs stayed in mice over 3 months, they showed low toxicity to mice. The long-term accumulation of SWCNTs in the main organs was evidenced by using Raman spectroscopy and TEM technique. Statistically significant changes in organ indices and serum biochemical parameters (LDH, ALT and AST) were observed. The histological observations demonstrate that slight inflammation and inflammatory cell infiltration occurred in lung, but the serum immunological indicators (CH 50 level and TNF-α level) remained unchanged. No apoptosis was induced in the main organs. The decreasing glutathione (GSH) level and increasing malondialdehyde (MDA) level suggest that the toxicity of SWCNTs might be due to the oxidative stress.

1. Introduction

With the development of nanotechnology, numerous nanomaterials are created for various applications. Among these nanomaterials, single-walled carbon nanotube (SWCNT) is one of the most concerned, for its unique physicochemical properties and promises in technological applications (Endo et al., 2008). SWCNTs can be used in sensor, electronic device, wastewater treatment and many other industrial applications. Importantly, broad biomedical uses of SWCNTs, such as in drug delivery systems (Lacerda et al., 2006), bone cell growth (Saito et al., 2008) and cancer treatment (Gannon et al., 2007), have been investigated. With the rapid advances in SWCNT-based new materials and technologies, there is a growing recognition that a fundamental understanding of the toxicological properties of SWCNTs is imperative (Warheit, 2006).

However, the toxicity of SWCNTs is barely known when they are introduced into the blood circulation, which is especially vital for their biomedical applications. Previous studies have reported the cytotoxicity, pulmonary and skin toxicity of SWCNTs (Smart et al., 2006). Pristine SWCNTs have been proven to be cytotoxic, inducing the cell viability loss (Jia et al., 2005), oxidative damage (Pulskamp et al., 2007), inflammation (Brown et al., 2007) and apoptosis (Cui et al., 2005). The cytotoxicity depends on the aggregation degree and pretreatment of SWCNTs samples (Wick et al., 2007). It is also suggested that the cytotoxicity of pristine SWCNTs could be reduced via chemical functionalization (Sayes et al., 2006). In the pulmonary and skin toxicity studies, pristine SWCNTs also show considerable toxicity, including animal death, inflammation and other clinical signals (Smart et al., 2006). Only a very recent pilot study shows polyethylene glycol (PEG) modified SWCNTs are non-toxic after intravenous (i.v.) injection by using very limited animals (Schipper et al., 2008).

In fact, most of i.v. exposure studies just focus on the biodistribution and pharmacokinetics of SWCNTs (Lacerda et al., 2006). We have reported that the surfactant suspended SWCNTs accumulate in liver, lung, and spleen and retain for at least one month without any sign of degradation after mice are i.v. exposed (Yang et al., 2007). Except for lung, the clearance of SWCNTs in body is rather

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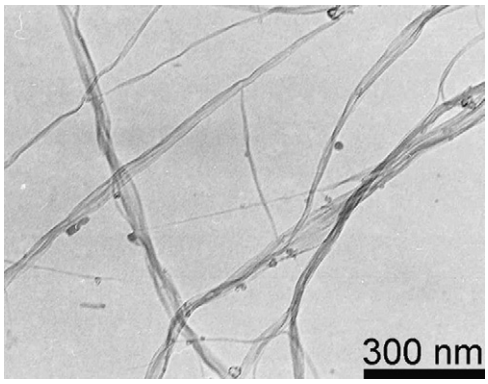

Fig. 1. A representative TEM image of the purified SWCNTs sample.

low, manifesting that the long-term fate and bio-effect of SWCNTs should be concerned. The experience of pulmonary toxicity studies, in which SWCNTs retain in lungs for 90 days and lead to the formation of granuloma (Lam et al., 2004; Warheit et al., 2004), also suggests that the study of long-term consequence is of prime importance.

Herein, we focus on the toxicity of SWCNTs to main organs, including liver, lung and spleen, to provide a general toxicological profile. Liver, lung and spleen are selected here mainly because of the high accumulation of SWCNTs in these organs (Yang et al., 2007). After i.v. administration, the long-term accumulation of SWCNTs was proved by Raman spectroscopy and transmission electron microscopy (TEM). Organ indices, serum biochemical parameters, histological observations, apoptosis, immunological indicators, and oxidative stress indicators were recorded to evaluate the toxicity.

2. Materials and methods

2.1. Synthesis, purification and characterization of SWCNTs

SWCNTs were synthesized by arc-discharge method and purified to a carbonaceous purity of 95% following the reported method with minor modifications (Wang et al., 2004). Briefly, graphite powder was mixed with FeS and Ni_2Y at a mass ratio of 1:5:8.6. The obtained raw SWCNTs were refluxed in 15% H_2O_2 for 2 h, then in 6 mol/L HCl for 12 h and finally in 2.6 mol/L HNO_3 for another 12 h. The resulting residues were heated under air at 400 °C for 1 h and annealed under N_2 at 1000 °C for 2 h. The purified SWCNTs were characterized by TEM (JEM-200CX, Japan), Brunauer–Emmett–Teller (BET) technique (ASAP2010, Micromeritics, USA), thermogravimetric analysis (TGA) (SDT 2900, Thermal, USA), Raman spectroscopy (Renishaw micro-Raman instrument, England), inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Elemental X7, Thermo Electron Co., USA) and infrared spectroscopy (IR) (Magna-IR 750, Nicolet, USA). The purified SWCNTs were suspended in 1.0 wt% Tween® 80 aqueous solution by sonication for 30 min for the following animal exposure.

2.2. Animal administration and sampling

All animal experiments were performed in compliance with the institutional ethics committee regulations and guidelines on animal welfare (Animal Care and Use Program Guidelines of Peking University).

Male CD-ICR mice (~25 g) were obtained from Peking University Animal Centre, Beijing, China. They were housed in plastic cages (5 mice/cage) and kept on a 12 h light/dark cycle. Food and water were provided *ad libitum*. Following acclimation, mice were randomly divided into four groups (5 mice/group).

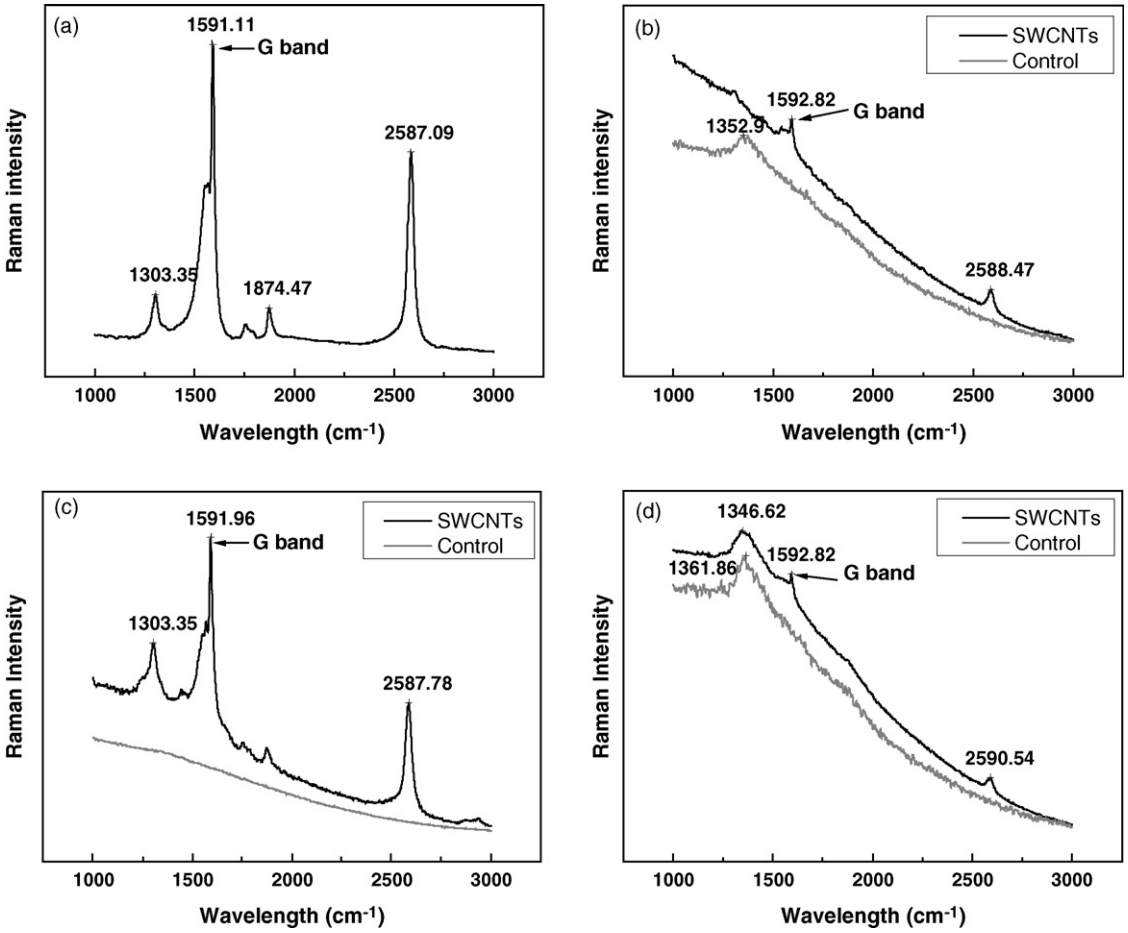

Fig. 2. Representative Raman spectra of SWCNTs suspended in Tween® 80 aqueous solution (a) and Raman spectra of tissue homogenates of liver (b), lung (c) and spleen (d) of both SWCNTs exposed mice at 90 days post-exposure and control mice.