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RESEARCH PAPER

Toxicological effects of multi-wall carbon nanotubes in rats

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Abstract The aim of this study was to evaluate the lung toxicity of multi-wall carbon nanotubes (MWCNTs). The present work exposed MWCNTs into the rats in intratracheal instillation administration modes. We systematically studied the distribution of nanoparticles in vivo, target organs and time-effects of nanotoxicity, dose-effects of nanotoxicity, etc. These results indicate that under the conditions of this test, pulmonary exposures to MWCNTs in rats by intratracheal instillation produced a series of multiple lesions in a dose-dependent and time-dependent manner, evidence of a foreign tissue body reaction.

Keywords Multi-wall carbon nanotubes · Lung toxicity · Nanomaterials · Intratracheal instillation · MWCNT · Nanomedicine

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Introduction

With the rapid development of nanotechnology, nanomaterials have achieved the wide application in electronics, chemical industry, environmental protection, and biological medicine, etc. How these diverse nanoparticles act in vivo after entering the human body? This newly emerging issue is drawing more and more attention of worldwide governments and scientists. Carbon nanotubes (CNTs) are an example of a carbon-based nanomaterial, whose unique properties have sparked enormous popularity in nanotechnology since the first radix carbon nanotube was found in 1991(Iijima 1991). Much attention has been paid on CNTs, due to their small size and extraordinary physicochemical properties, in combination with their unique tubular morphology, not only for their huge potential industrial application, but also in biological application including biosensors, drug, and vaccine delivery and the preparation of novel biomaterials for bone tissue engineering (Smart et al. 2006; Lin et al. 2004; Aryal et al. 2006; Zhao et al. 2005; Zhao et al. 2004; Bianco et al. 2005; Khabashesku et al. 2005) . Therefore, it is imperative to thoroughly investigate the toxicity and the biocompatibility of CNTs. However, the data about the toxicity of carbon nanotubes (CNTs) in vivo is poor or contradictory. Although both the nature of carbon and positive experiences to date with various forms of carbon would suggest also a good biocompatibility of nanotubes (Fiorito et al. 2006; Cglopek et al. 2006;

Koyama et al. 2006), some researchers (Lam et al. 2004; Warheit et al. 2004; Jia et al. 2005; Muller et al. 2005) demonstrated that CNTs could pose potential health problem. They published studies on pulmonary toxicity of CNTs proved that inhaled CNTs induced the formation of epithelial granulomas and inflammation, and some nanoparticles might be toxic to human keratinocytes (Monteiro-Rivierea et al. 2005). Previous study has demonstrated that a water-soluble hydroxylated SWNTs can moving easily among the compartments and tissues of the body, behaving as small active molecules though their apparent mean molecular weight is tremendously large (Wang et al. 2004). Assessing whether this is true for CNTs is important, not only for determining the potential of CNTs-based drug delivery, but also for assessmenting the risk of potential toxicity and related environmental pollution of CNTs.

In this article, the lung toxicity of multi-wall carbon nanotubes (MWCNTs) was studied. We exposed MWCNTs into the rats in intratracheal instillation administration modes, an accepted route of exposure commonly used to value ultrafine particles for potential lung toxicity.

Materials and methods

Multi-wall carbon nanotubes (MWCNTs) in our experiments were purchased from Shenzhen Nanotech Port Co, Ltd, Limited, and its parameter is as follows: diameter: 40 ~ 60 nm; length: 0.5 ~ 500 μm; purity: 95%; agraphitic carbon: <3%; ash: ≤0.2 wt%. The analysis by the X-ray diffraction (XRD) technique showed the presence of MWCNTs (Fig. 1). Furthermore, the structure and the texture of MWCNTs were observed by Transmission Electron Microscopy (TEM) (Fig. 2). The TEM observation showed that the central canal is quite clear (from 10 to 15 nm in diameter), with the outer diameters mostly in the range form 40 to 60 nm, and the morphology of MWCNTs is linear. All the MWCNTs were freshly suspended in saline and 1% Tween 80 and sonicated for 60 min, with a short break every 10 min. A stable suspension of MWCNTs was obtained in this way and used immediately.

Adult pathogen-free healthy male Vister rats (250–300 g) were purchased from Laboratory Animal Center of Shandong University. The animals were

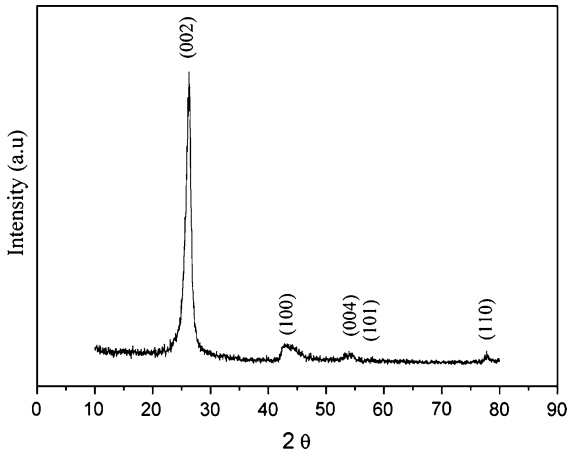

Fig. 1 The XRD pattern of MWCNTs

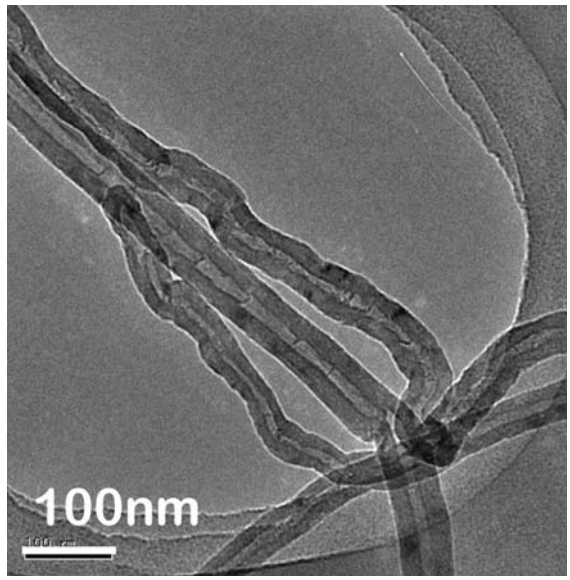

Fig. 2 The TEM micrograph of CNTs

kept in a conventional animal facility (The second hospital of Shandong University) and housed in positive-pressure air-conditioned units (25 °C, 50% relative humidity) on a 12:12-h light/dark cycle. The experimental protocol has been approved by the local ethical committee for animals in research. The rats were allowed to acclimate at this facility for one week before being used in the experiment.

We used intratracheal instillation, an accepted route of exposure commonly used to value ultrafine particles for potential lung toxicity (Lam et al. 2004). For conventional histology, tissue were taken immediately after sacrifice, fixed in 10% formaldehyde,

embedded in paraffin, cut into 5 μm section, stained with hematoxylin and eosin, and examined by light microscopy.

Results and discussion

The potential hazards related to inhalation of CNTs are unknown. Moreover, the toxicological database for most of the carbon-containing particulates is rather sparse. Preliminary studies showed that unprocessed nanotubes are very light and could become airborne and potentially reach the lungs, then damage lung tissue. Most research present cautioned that further work is essential. Concerns about the toxicity of nanoparticle must be addressed while the field is still young and exposures limited (Service 2003). Recently a rising chorus of government, industry, academic, and environmental leaders is calling for dramatic increases in funding to study possible adverse health and environmental effects of nanotechnology (Service 2005).

The objective of this lung tissue bioassay study was to evaluate the acute lung toxicity of intratracheally instilled MWCNTs in rats. We used intratracheal instillation, an accepted route of exposure commonly used to value ultrafine particles for potential lung toxicity (Lam et al. 2004). The lung of the rats was intratracheally instilled with 0, 1, 3, 5, and 7 mg/kg of MWCNTs. After exposed 1 d, 1 week, 1 month, and 3 months, the rats were killed by the method of cervical vertebra displace, and then by histopathology evaluated inflammation inflection of the lung in all CNT-exposed rats. The influence of dose and exposure time of MWCNTs to the change about the lung tissue of rats was discussed. All animals within the experiment showed no overt clinical signs except for the weights of rats increased with the increasing of age.

The light micrograph of lung tissues about rats exposed to different dose MWCNTs for 90 days were showed in Fig. 3. MWCNTs exposed at 1 mg/kg (Fig. 3a) produced mild inflammation and the structure of lung alveolus was in the course of nature with slight congestion. With the increasing of MWCNTs dose, the toxicity reflection about the lung of rats becomes severe. The lung of rats treated with high-dose CNTs showed prominent inflammation reflection. Large amount of inflammation cells (consisted of macrophages, lymphocytes, neutrophils, eosinophils, and other inflammation cells) infiltrated in lung alveolus interstitium, the alveolar septa become thicker and some lung alveolus were cracked. These phenomena can be observed in the lung tissue exposed at 3, 5, and 7 mg/kg dose (Fig. 3b–d). These results show that MWCNTs induced dose-dependent interstitial inflammation in the rats, the higher dose of MWCNTs, the higher toxicity with the lung of rats. The lung of low-dose CNTs treated (1 mg/kg) rats appeared mild inflammation reflection while the lung of high-dose CNTs treated (3–7 mg/kg) rats appeared generally severe inflammation. This suggested that there were potential health hazard in inhalation high dose CNTs

Figure 4 is the light micrograph of lung tissues from rats exposed to MWCNTs of 3 mg/kg by intratracheal instillation at different exposure time. After CNT-exposed 1 day, the alveolar septa became thicker and little inflammation cells infiltrated in lung alveolus interstitium. The alveolar septa became thicker and a large amount of inflammation cells infiltrated in lung alveolus interstitium after 1 week,

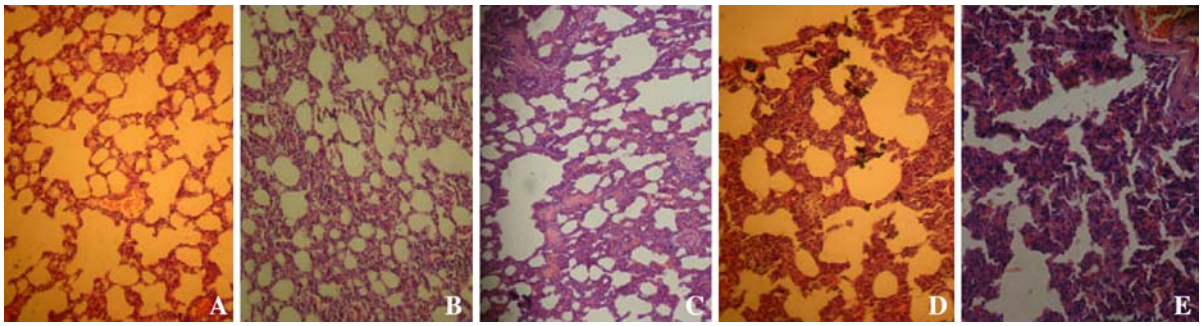

Fig. 3 Light micrograph of lung tissues from rats exposed to different dose MWCNTs by intratracheal instillation at 90 days exposure. (**a**) Control: 0 mg/kg; (**b**) 1 mg/kg; (**c**) 3 mg/kg; (**d**) 5 mg/kg; (**e**) 7 mg/kg. (Magnification = ×100)

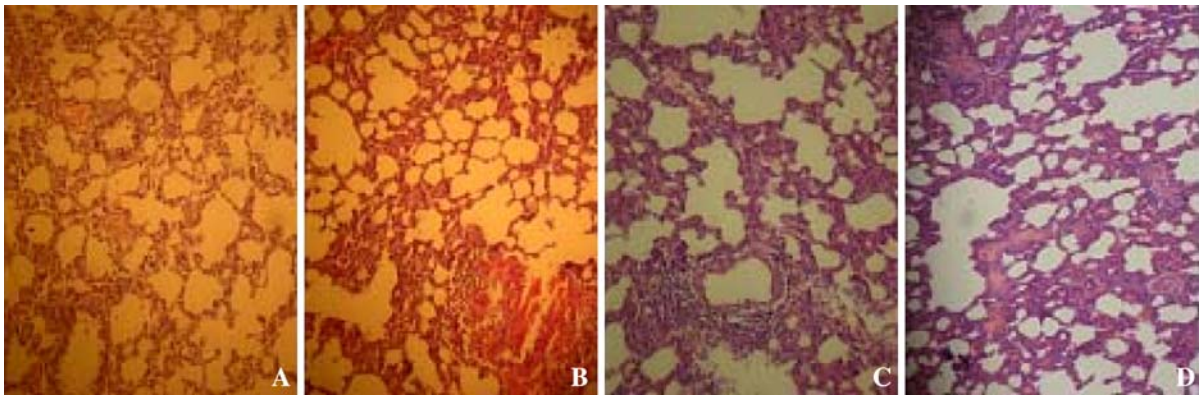

Fig. 4 Light micrograph of lung tissues from rats exposed to MWCNTs of 3 mg/kg by intratracheal instillation at different time exposure. (**a**) 1 day; (**b**) 1 week; (**c**) 1 month; (**d**) 3 months. (Magnification = ×100)

and the inflammation inflection reduced slightly. After 3 months, the damage was serious, which was expressed by an erratic and peculiar injury. This was due to the immunity of individual or the impact of experiment error. We can conclude that exposure time was critical for induction of lung pathology, which is in according with Huczko's report (Huczko et al. 2005), but the immunity of individual is not ignored.

In order to assess the biological effect of MWCNTs on lung tissue, we observed the ultrastructural features of the lung tissue by TEM. Figure 5 showed the TEM images of the ultrastructural features of the lung tissue exposed to CNTs. We can observe that the CNTs still remain in the lungs after 3 months, some in capillary vessel and some in cytoplasmic vacuoles of lung tissue. The distribution of carbon was nonuniform and the hollow space inside CNTs can be clearly observed. These results confirmed that CNTs could traverse the wall of capillary vessel and enter to the cytoplasmic vacuoles of lung tissue. There are a lot of inflammation cell appear in the wall of lung vacuole, such as multinuclear giant cell and acidophilic. The ultrastructural features of most cell appeared pathological changes. The experiment demonstrated that MWCNTs exposed to rats by intratracheal instillation could be reserved in lung tissue after 3 months. The residuum could induce the change of the ultrastructural features

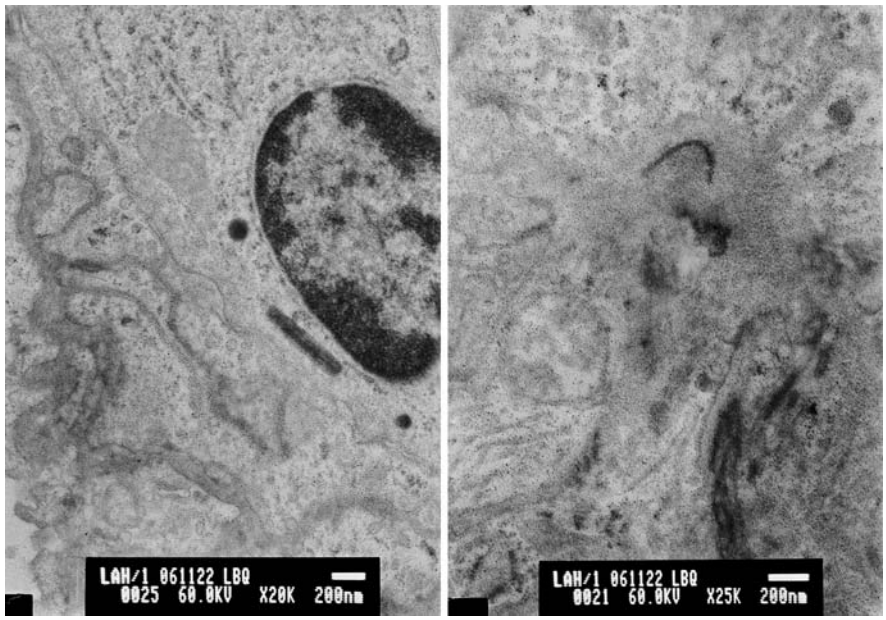

Fig. 5 TEM micrograph of the ultrastructural features of the lung tissue exposed to CNTs

of the lung tissue cell, as a result of inflammation reflection of lung in histopathological studies.

Results from the lung histopathology component of the study indicated that pulmonary exposures to MWCNTs produced dose-dependent inflammatory responses, and this phenomenon persisted within all the experiment time. Lung (high-dose) histology reported multiple lesions in CNTs-exposed animals at 3 months, concomitant with lung tissue thickening at the sites of normal particle deposition and a plentiful of inflammation cells infiltrated in lung alveolus interstitium. Instillation of MWCNT at 1 mg/kg (low-dose) did produce mild inflammation reflection to CNTs-exposed rats. These results were slightly contradictory with Warheit's results (Warheit et al. 2004) but in accordance with Lam's (Lam et al. 2004) results. In Warheit's study, pulmonary exposures to SWCNT in rats produced a non-dose-dependent series of multifocal granulomas, which were proof of a foreign tissue body reaction and were nonuniform in distribution and not progressive beyond 1 month postexposure. Lam et al. investigated the pulmonary toxicity of three batches of SWCNTs and found that all three SWCNTs products induced dose-dependent lung lesions, characterized by interstitial granulomas.

Conclusions

In conclusion, the results demonstrated that under the conditions of this test, pulmonary exposures to MWCNTs in rats by intratracheal instillation produced a series of multiple lesions in a dose-dependent and time-dependent manner, evidence of a foreign tissue body reaction. Although there are enormous differences between the lungs of rodents and human beings, the results may draw attention to the workers at occupational potential risk for exposure to MWCNTs.

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