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The Pulmonary Toxicity of Multi-Wall Carbon Nanotubes in Mice 30 and 60 Days After Inhalation Exposure

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The pulmonary toxicity, induced by multi-wall carbon nanotubes in mice, was studied after 30-day and 60-day inhalation exposure. The mice were exposed to multi-wall carbon nanotubes aerosol with weighted mean concentration of 32.61 mg/m^3, once in very two days, 6 hours in an exposure day. After 30-day and 60-day inhalation exposure, the pulmonary toxicity of multi-wall carbon nanotubes was assessed using biochemical indices in bronchoalveolar lavage fluid and pathological examination. It was found that the aerosolized multi-wall carbon nanotubes did not induce obvious pulmonary toxicity in 30-day exposure group, but induced severe pulmonary toxicity in 60-day exposure group.

Keywords: Multi-Wall Carbon Nanotubes, Inhalation Exposure, Pulmonary Toxicity.

1. INTRODUCTION

During carbon nanotubes (CNTs) production and application, they can be extremely small and buoyant under some conditions,[1] which might produce potential respiratory toxicity to occupational workers. In recent years, increasing effort has been made to identify the respiratory toxicity of CNTs. After intratracheal instillation, the single-wall nanotubes (SWCNTs) induced dose-dependent epithelioid granulomas in mice,[2] and a series of non-dose-dependent multifocal granulomas in rats.[3] By pharyngeal aspiration, it was found that SWCNTs-induced gramulomas in rats mainly associated with hypertrophied epithelial cells surrounding SWCNTs aggregations and diffusive interstitial fibrosis and alveolar wall thickening likely associated with dispersed SWCNTs.[4] As regards the pulmonary toxicity of multi-wall carbon nanotubes (MWCNTs), Muller et al. concluded that the MWCNTs could induce inflammatory and fibrotic reactions in rats after intratracheal administration.[5] However, there was little known about the pulmonary response to aerosolized CNTs that being chronically inhaled. The inhalation exposure was adopted in this work to assess the pulmonary toxicity of aerosolized MWCNTs in mice through 30 days and 60 days postexposure.

2. EXPERIMENTAL DETAILS

2.1. MWCNTs and Animals

The MWCNTs were obtained from Shenzhen Nanotech Port Co., Ltd, Shenzhen, China, with average external diameter of 50 nm, mean length of 10 μm, purity >95%, ash (La, Ni) <0.2 wt%, average special surface area of 280 m^2/g, and amorphous carbon <3%. The pristine MWCNTs were used directly in this experiment without any physicochemical processes before hand.

The experimental animals, female Kunming mice with weight of 30 g, were purchased from Shanghai Slack Experimental Animal Center, Shanghai, China. All mice were housed in clean cages in a ventilated animal room. Room temperature was maintained at 18–20 °C, relative humidity at 55 ± 10%, and illumination on a 12 hr light-dark cycle. The mice were supplied with sterilized food and pure water, and they were allowed to acclimate for one week prior to the onset of inhalation exposure. The study was carried out in compliance with the national regulations related to the conduct of experimentation.

2.2. Inhalation Equipment and Exposure

The inhalation equipment was mainly made up of powder generator, first depositor, second depositor, inhalation chamber, digital dust monitor, sensor of temperature and

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humidity, exhaust location I, exhaust location II, and vacuum pump.[6] Eighteen mice were exposed to MWCNTs aerosol in inhalation chamber, once in very two days, 6 hours in an exposure day. According to exposure period, the mice were divided into 2 groups, nine in each group, and denominated 30-day and 60-day group, respectively. Their actual exposure time was 15 days in 30-day group and 30 days in 60-day group. The inhalation experiment was conducted in ventilated hoods at room temperature (18–20 °C). In addition, nine mice housed in cages of the same animal room were denominated as control group.

2.3. Biochemical Indices in Bronchoalveolar Lavage Fluid (BALF)

After inhalation exposure, the mice in control and experimental groups were anaesthetized by intraperitoneal injection of 0.3 ml 0.5% pentobarbital sodium solution and exsanguinated via the abdominalis aorta. Six mice were randomly chosen from each group, then whose thoraxes were opened and bronchoalveolar lavage was performed by cannulating trachea and infusing lung two times with physiological saline (37 °C), 1 ml per time. About 1.5 ml BALF was pooled, and stored at 4 °C. The BALF were centrifuged (400 g, 10 min, 4 °C). Total protein, alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH) in supernatant were analyzed with commercial reagent kits that were obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

2.4. Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA). For analysis, each of the experimental values was compared with that in control group. Data were expressed as means ± SD. Significance was judged at $P < 0.05$ and $P < 0.01$ probability level.

2.5. Pathological Examination

The remained 3 mice in each group were intraperitoneally anesthetized with 0.3 ml 0.5% pentobarbital sodium solution, and their lungs were excised and fixed by 4% formalin. The formalin-fixed lungs were embedded in paraffin, thin-sectioned, and mounted on glass microscope slides. Sections were stained with hematoxylin and eosin, and then examined by light microscopy.

3. RESULTS AND DISCUSSION

3.1. Characterization of Aerosolized MWCNTs and Its Concentration Descending

In this work pieces of silicon slice were placed horizontally on the bottom of the inhalation chamber filled with MWCNTs aerosol for 90 minutes, then the aspect of MWCNTs was observed with Scanning Electron Microscopy (SEM, LEO 1530VP). As showed in Figure 1, the aerosolized MWCNTs were predominately in respirable sizes.

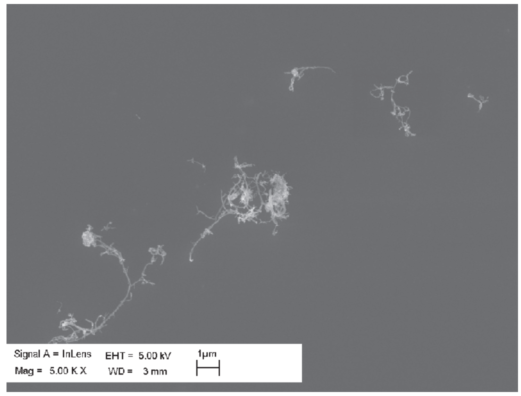

Fig. 1. Aerosolized MWCNTs well dispersed on the silicon slice after 90-minute deposition.

During mice inhalation exposure, the concentration of MWCNTs aerosol in the inhalation chamber decreased regularly from about 80 mg/m^3 to 13 mg/m^3 per 90 minutes. Similar exposure was continually repeated for 4 times in an exposure day, namely, the cumulative exposure time was 6 hours in the exposure day. The intra-lung deposition dose of aerosolized MWCNTs can be calculated following formula:

$$D_{\text{intra}} = F \cdot V \cdot \bar{\rho} \cdot T$$

Where D_{intra} was intra-lung deposition dose, F was a fractional deposition ratio of aerosolized MWCNTs, V was volume of aerosol inhaled by the mouse in unit time, $\bar{\rho}$ was weighted mean concentration of MWCNTs, and T was exposure time. In present work, the weighted mean concentration in 90-minute exposure was calculated from the following formula:

$$\bar{\rho} = \sum \Delta Ti\bar{\rho}i / \sum \Delta Ti$$

Where ΔTi was time interval (around 5 minutes) and $\bar{\rho}i$ was arithmetic mean concentration in that time interval.

Table I. Changes of biochemical indices in BALF in control, 30-day and 60-day groups.

	Total protein (g/L)	ALP (U/L)	ACP (U/L)	LDH (U/L)
Control group	0.14 ± 0.04	1.36 ± 0.44	2.13 ± 0.43	582.04 ± 82.49
30-day group	0.16 ± 0.04	1.56 ± 0.19	2.36 ± 0.84	667.09 ± 40.59
60-day group	0.24 ± 0.06*	2.91 ± 0.92*	4.96 ± 0.36**	1248.74 ± 28.05**

*means significant difference at $P < 0.05$ level; **means significant difference at $P < 0.01$ level.

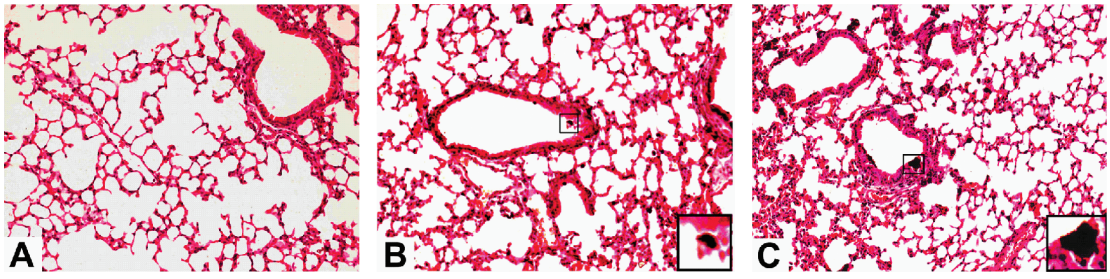

Fig. 2. Micrographs of lung in control, 30-day and 60-day groups. A: lung tissue in control group ×200, B: lung tissue in 30-day group ×200 (inset ×600), C: lung tissue in 60-day group ×200 (inset ×600).

Based on the above formulas, the weighted mean concentration ($\bar{\rho}$) of MWCNTs, in a typical test, was calculated to be 32.61 mg/m^3. Following the recent article,[7] the fractional deposition ratio (F) of aerosolized CNTs into lung was about 4%. It was also assumed that a 30-g mouse breathes in 30 ml air per minute.[8] Thus intra-lung deposition dose of mice in the 30-day and 60-day group were roughly 0.21 mg and 0.42 mg, respectively.

3.2. Biochemical Indices in BALF

After inhalation exposure to the aerosolized MWCNTs, four biochemical indices (total protein, ALP, ACP and LDH) in supernatant of BALF were measured. These biochemical indices in 30-day group increased slightly comparing with that in control group. However, in 60-day group the total protein and ALP concentration elevated significantly at $P < 0.05$ level, ACP and LDH concentration elevated significantly at $P < 0.01$ level (Table I).

3.3. Pathological Examination

After the same inhalation exposure, in 30-day group the aggregations of MWCNTs adhered to bronchial wall and entered into alveolar wall, respectively, and they could result in alveolar wall slightly thickening (Fig. 2(B)). In 60-day group, the aggregations that deposited in the bronchi became more obvious as their size was larger than that in 30-day group (Figs. 2(B and C)). On the other hand, in 60-day group the aggregations in alveolar wall were fur smaller than that adhered to bronchial wall, and they resulted in alveolar wall obviously thickening (Fig. 2(C)).

3.4. Pulmonary Toxicity Induced by MWCNTs

Based on the biochemical indices and pathological examination, the aerosolized MWCNTs could induce severe pulmonary toxicity in 60-day group but not in 30-day group. The different toxic effect in the two groups might be due to the following reasons. Firstly, MWCNTs, as a kind of insoluble and non-biodegraded nanomaterails, once deposited in lung are difficult to be cleared from it.[5] During inhalation exposure, the intra-lung deposition dose of mice was roughly 0.21 mg in 30-day group and 0.42 mg in 60-day group. Perhaps the 0.42 mg deposition dose exceeded the limitation that lung could clear. Secondly, the high surface area of MWCNTs makes them easily adhere to bronchial and alveolar wall and easily accumulate therein such as the larger size aggregations of MWCNTs in the lining wall of bronchi (Fig. 2(C)). These aggregations were too large to be phagocytized by alveolar macrophages, which might persistently stimulate lung tissue, thereby led to more significant pulmonary toxicity with longer-term inhalation exposure (Fig. 2 and Table I).

4. CONCLUSIONS

When the mice were exposed to MWCNTs aerosol with weighted mean concentration of 32.61 mg/m^3, once in very two days, 6 hours in an exposure day, the intra-lung deposition dose of mice in 30-day and 60-day groups was roughly 0.21 mg and 0.42 mg. The changes of total protein, ALP, ACP and LDH in supernatant of BALF and pathological lesions in lung tissue showed that the aerosolized MWCNTs induced severe pulmonary toxicity in 60-day exposure group but not in 30-day exposure group. Perhaps the different toxic effect was attributed to the physicochemical characteristics of MWCNTs, such as non-solution, non-biodegradation, and high surface area.

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References and Notes

1. A. D. Maynard, P. A. Baron, M. Foley, A. A. Shvedova, E. R. Kisin, and V. Castranova, *J. Toxicol. Environ. Health A* 67, 87 (**2004**).
2. C. W. Lam, J. T. James, R. McCluskey, and R. L. Hunter, *Toxicol. Sci.* 77, 126 (**2004**).
3. B. D. Warheit, B. R. Laurence, K. L. Reed, D. H. Roach, G. A. Reynolds, and T. R. Webb, *Toxicol. Sci.* 77, 117 (**2004**).
4. A. A. Shveodova, E. R. Kisin, R. Mercer, A. R. Murray, V. J. Johnson, A. I. Potapovich, Y. Y. Tyurina, O. Gorelik, S. Arepalli,

D. Schwegler-Berry, A. F. Hubbs, J. Antonini, D. E. Evans, B. K. Ku, D. Ramsey, A. Maynard, V. E. Kagan, V. Castranova, and P. Baron, *Am. J. Physiol. Lung. Cell. Mol. Physiol.* 289, L689 (**2005**).

5. J. Muller, F. Huaux, N. Moreau, P. Misson, J. F. Heilier, M. Delos, M. Arras, A. Fonseca, J. B. Nagy, and D. Lison, *Toxicol. Appl. Pharmacol.* 207, 221 (**2005**).

6. J. G. Li, W. X. Li, J. Y. Xu, X. Q. Cai, R. L. Liu, Y. J. Li, Q. F. Zhao, and Q. N. Li, *Environ. Toxicol.* 22, 415 (**2007**).

7. C. W. Lam, J. T. James, R. McCluskey, S. Arepalli, and R. L. Hunter, *Crit. Rev. Toxicol.* 36, 189 (**2006**).

8. Y. L. Lai, Comparative Biology of the Normal Lung, edited by R. A. Parent, CRC Press, Boca Raton FL (**1992**), p. 217

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