RESEARCH ARTICLE

Evaluation of Cardiopulmonary Toxicity Following Oral Administration of Multi-walled Carbon Nanotubes in Wistar Rats

Ehsan Zayerzadeh[1]*, Azadeh Fardipour[2], Meisam Shabanian[3], Mohammad Kazem Koohi[4]

[1] Department of Biology, Faculty of Food Industry and Agriculture, Standard Research Institute, Karaj, Iran
[2] Department of Quality Control, Razi Vaccine and Serum Research Institute, Karaj, Iran
[3] Faculty of Chemistry and Petrochemical Engineering, Standard Research Institute, Karaj,Iran
[4] Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

ARTICLE INFO

Article History:
Received 25 December 2015
Accepted 18 May 2016
Published 1 July 2016

Keywords:
Carbon nanotubes
Cardiorespiratory toxicity
Myocytolysis
Lactate dehydrogenase
Rats

ABSTRACT

Objective(s): Carbon nanotubes have unique mechanical, electrical, and thermal properties, with potential different applications in nanomedicine, electronics, and other industries. These new applications of carbon nanotubes in different industries lead to the increased exposure risk of nanomaterials to human. Up to now, all aspects of carbon nanotubes toxicity are not completely clear following human and animal exposures with these novel compounds. The aim of this study was to assess cardiopulmonary toxicity of multi-walled carbon nanotubes following oral administration in rats with respect to the histopathological and biochemical evaluation.

Methods: In the present investigation, we studied cardiorespiratory toxicity of multi-wall carbon nanotubes (MWCNT) with regard to histopathological changes and some biomarkers including TnT, CK-MB and LDH in experimental rats following oral administration. One dose per 24 h of MWCNT suspension was administered orally (gavage technique) to animals at the doses of 500, 1000 and 2000 mg/kg/day BW for 5 days.

Results: The results of these study showed oral administration of MWCNT induces histopathological complications such as severe alveolar edema and hemorrhage in lungs and myocytolysis in heart of all experimental groups of animals. In all of the groups, troponin T level showed no changes when compared to baseline. Lactate dehydrogenase and CK-MB activity showed significant increment in all of animal groups following oral administration of carbon nanotubes.

Conclusions: It can be concluded that oral exposure of MWCNT may be toxic for cardiovascular and respiratory systems, because MWCNT induced biochemical alterations and histopathological abnormalities in these vital systems.

How to cite this article
Zayerzadeh E, Fardipour A, Shabanian M, Kazem Koohi M. Evaluation of Cardiopulmonary Toxicity Following Oral Administration of Multi-walled Carbon Nanotubes in Wistar Rats. Nanomed Res J, 2016; 1(1): 47-51. DOI: 10.7508/nmrj.2016.01.007

INTRODUCTION

Carbon nanotubes (CNTs), including single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) are allotropes of carbon from the fullerene family. CNTs have physicochemical properties that are highly desirable for use within the commercial, environmental, and medical sectors. With the inclusion of CNTs to improve the performance of many products, as well as potentially in medicine, it is likely that occupational and public exposure to CNT-based nanomaterials will increase dramatically in the

* Corresponding Author Email: zayerzadeh@standard.ac.ir

future [1]. Hence, it is important to explore the possible toxicity and toxicity mechanisms of CNTs. Because of their morphological similarity to asbestos fibres, the question of possible potential health hazard increased to become an important concern in public health [1]. As a consequence, CNTs are extremely aerosolized, making respiratory contamination by inhalation rather likely to occur [1]. Some investigations cleared that purified MWCNTs as well as SWCNTs could induce inflammatory and fibrotic reactions [2, 3]. On the other hand, numerous other reports failed to exhibit any toxicological impact, while no ROS production was detected when macrophage cells were stimulated with purified SWCNTs [4]. The MWCNTs NPs displayed different pulmonary toxicity but induced procoagulant effects, suggesting different mechanisms of affecting hemostasis [5]. The MWCNTs accumulation and chronic inflammatory changes were observed in the lungs of experimental rats exposed to MWCNTs by intravenous injection [6]. However, toxicity of carbon nanotubes is still controversial. In the present study here, we evaluated cardiopulmonary toxicity of multi-walled carbon nanotubes with regard to histopathological and some biomarkers changes including TnT, CK-MB and LDH in experimental rats following oral administration.

MATERIALS AND METHODS

Test substance

MWCNTs produced by the chemical vapor deposition (CVD) method, with an average diameter of 10 nm and lengths between 5 and 10 μm were purchased from (Nanocyl S.A., Sambreville, Belgium) and used in the present study without further purification or sieving (Fig. 1).

Animal maintenance

Healthy adult male Wister rats (with average body weight (BW) of 200±50g were used in this study. They were obtained from Razi vaccine and serum research institute and allowed to acclimate for 10 days before treatment. They were maintained in a controlled atmosphere with a 12h:12h dark/light cycle, a temperature of 22 ± 3°C and 55±5 % relative humidity with free access to standard pellet diet (commercially available from Razi vaccine and serum research institute) and fresh tap water. All animals were kept in according to the recommendation of the animal care committee of the Tehran University based on the 'Guide for Care and Use of Laboratory Animals' (NIH US publication 86-23, revised 1985)

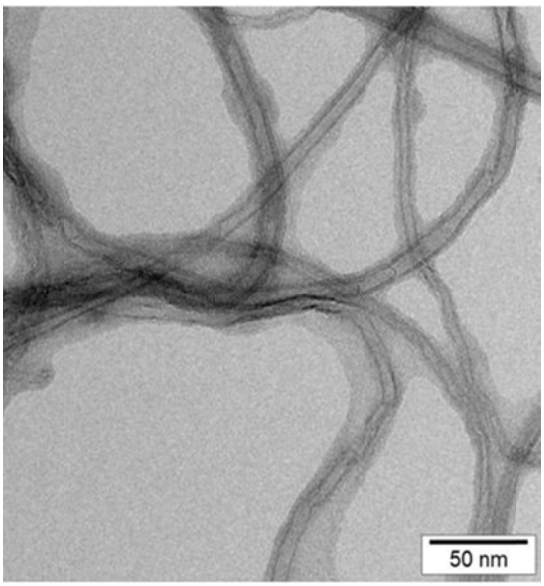

Fig. 1. TEM image of MWCNTs

Treatment

MWCNTs were suspended in a sterile saline solution containing 1% Tween-80 and were dispersed using an ultrasonic liquid processor at 4° C and 30% amplitude to read pulses (1 sec on and 1 sec off) for 30 min. Twenty four rats were randomly divided into four groups, six for each group. One group was chosen as the tween-saline control groups, and the last three were used as experimental groups. One dose per 24 h of MWCNT suspension was administered orally (gavage technique) to animals at the doses of 500, 1000 and 2000 mg/kg/day BW for 5 days.

Histopathology evaluation of tissue

The heart and lungs extracted from animals were immersed in 10% buffered formalin for 48 h at room temperature and sectioned transversely in 3–4 mm slices. Specimens were dehydrated in a graded series of alcohol and xylene and embedded in paraffin. Multiple slices were made and stained by hematoxylin and eosin stains. Sections were viewed and photographed using a Nikon E200 light microscope (Nikon E200 Japan).

Evaluation of biomarkers

Troponin T (TnT) was measured using an electrochemiluminescence immunoassay (Elecsys

2010 analyzer and Troponin T STAT kit, Roche, Germany) according to the manufacturer's instructions. Lactate dehydrogenase (LDH) and creatininekinase-musclebrain(CK-MB)wereassayed in the sera using the electrochemiluminescence immunoassay (Elecsys 2010 analyzer and CK-MB STAT kit, Roche, Germany) according to the manufacturer's instructions. The enzyme values were expressed in international units (U/L).

Statistical analysis

All results were expressed as mean ± SD. The statistical significance of differences among groups was analyzed by the Student's t-test. Data were considered statistically significant if p-values were < 0.05.

RESULTS AND DISCUSSION

Carbon nanotubes are known to have superior mechanical, electrical, and magnetic properties and have applications in diverse biotechnology fields [1]. Moreover, the toxicological database and the potential for toxic effects in humans and the environment have not yet been established for most carbon nanotubes. Nanotoxicology investigations cleared toxicity of nanot ubes and nanoparticles for stable and safe development of nanotechnology [1]. In the present investigation, cardiopulmonary toxicity of MWCNTs was studied using histopathological evaluation and some biochemical markers including TnT, CK-MB and LDH in experimental rats. The histological evaluation of the heart and lungs in

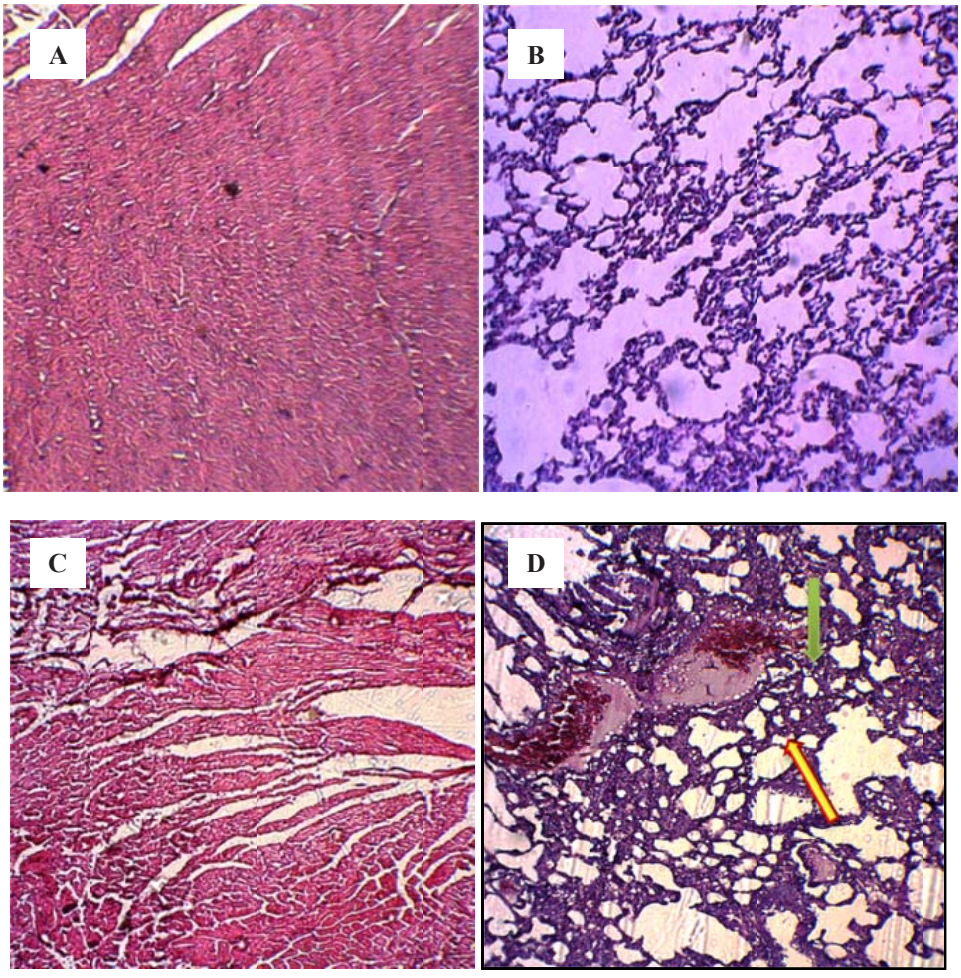

Fig. 2. Photomicrographs of heart and lung sections obtained from rats exposed to carbon nanotubes. Panels A and B: controls. Panel C: myocardium tissue of rats exposed to carbon nanotubes. Panel D: lung tissue from treated rats with carbon nanotubes. (A) Normal heart, (B) normal lung, (C) myocytolysis, (D) green arrow shows hemorrhage and blue arrow shows alveolar edema. (Staining with hematoxylin and eosin). Magnification: 40x for panels.

Table1. Serum TnT, CK-MB and LDH in rats following carbon nanotubes treatment

	TnT	CK-MB	LDH
Control	0.7±0.1	386.2±51.9	462.8±41.3
First group	0.6±0.2	434.5±47.5*	580.8±55.2*
Second group	0.7±0.1	522.8±62.2*	640.6±59.2*
Third group	0.8±0.1	777.1±81.6*	899±75.6*

the control group following treatment revealed no observable changes. In all of experimental groups of animals, oral administration of MWCNTs induced histopathological complications such as severe alveolar edema and hemorrhage in lungs. MWCNTs oral administration also induced myocytolysis in heart. However, histopathplogial abnormalities in third group were more than another two groups (as in Figure 2). Myocytolysis is a specific histological marker of congestive heart failure without relation to coronary blood flow, myocardial hypoxia and myocardial fibrosis [7]. Pulmonary edema is a condition caused by excess fluid in the lungs. This fluid collects in the numerous air sacs in the lungs, making it difficult to breathe. Two factors can induce pulmonary edema: a cardiogenic factor due to dysfunction of the left ventricle and a non-cardiogenic factor related to the inflammatory response [8]. The MWCNTs NPs displayed different pulmonary toxicity but induced procoagulant effects, suggesting different mechanisms of affecting hemostasis [5]. The MWCNTs accumulation and chronic inflammatory changes were observed in the lungs of experimental rats exposed to MWCNTs by intravenous injection [6]. Tong et al demonstrated oropharyngeal aspiration exposure of acid-functionalized single-walled carbon nanotubes (AF-SWCNTs) induces signs of focal cardiac myofiber degeneration in mice. These substances also induce patches of cellular infiltration and edema in both the small airways and in the interstitium. In addition, AF-SWCNT increases in the percentage of BAL neutrophils in mice. AF-SWCNT exposure also induced slightly edematous in areas where cellular accumulation was evident [9]. The cellular findings reported that purified carboxylate-functionalized SWCNT has the potential to induce hepatotoxicity in Swiss-Webster mice through activation of the mechanisms of oxidative stress [10]. Chen et al recently reported histopathological complications in abdominal arteries of rats 30 days after exposure of MWCNTs [11]. In another study, exposures to SWCNT produced transient inflammatory and cytotoxicity in lungs of treated rats [12]. The histological observations demonstrate that slight inflammation and inflammatory cell infiltration occurred in lung of intravenously exposed mice with MWCNTs [13]. It has been reported that carbon nanotubes damaged the endothelial cells with a resultant increase in permeability *in vitro* [14]. Hence, nanoparticles may translocate from the systemic circulation to the organs by crossing the vasculature. However, in our investigation, we did not detect any MWCNTs in heart and lung tissue. Table 1 summarizes the effects of carbon nanotubes on markers (TnT, CK-MB and LDH). In all of the animal groups, troponin T levels showed no changes when compared to the baseline. Lactate dehydrogenase activity showed significant increment in all of the animal groups. However, in the third group of rats, lactate dehydrogenase activity increased significantly more than another groups. In all of the groups also, CK-MB showed significant increase. In the third group of rats, CK-MB level increased significantly more than first and second groups. There was a significant increment in plasma creatine kinase in mice exposed to 40 μg of SWCNT. Aspartate aminotransferase (AST) was increased in mice exposed to 40 μg of SWCNT. Patlolla et al demonstrated that exposure to carboxylate-functionalized SWCNT induced ROS, enhanced the activities of serum amino-transferases (ALT/AST), alkaline phosphatases (ALP) and concentration of lipid hydroperoxide [10]. Statistically significant changes in organ indices and serum biochemical parameters (LDH, ALT and AST) were observed [9] Changing of enzymes in the blood is usually used as a marker for the diagnosis of tissue damages. Troponins (TnT and TnI) are the gold biomarkers for myocardial infarction. In this study, we observed no significant changes in troponin T concentration because there was no observable necrosis in the heart tissue. The amounts of LDH and CK-MB levels are indices for identifying the cell injury and membrane integrity. When toxicants destruct cell membrane, these enzymes are leaked out of cells [15, 16].

CONCLUSION

Enzyme assays and histopathological analysis play crucial role in toxicological evaluation. This study provides a detailed overview about the biochemical alterations and histopathological complications resulting from oral acute exposure of multi-walled carbon nanotubes in cardiopulmonary organs of rats. From our findings, it can be concluded that MWCNTs may be toxic for cardiopulmonary organs. However, more nanotoxicological investigations need to clear essential mechanisms of pathological and biochemical changes following exposure to MWCNTs.

ACKNOWLEDGEMENTS

This work was financed by Standard Research Institute.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1.Lam C-W, James JT, McCluskey R, Arepalli S, Hunter RL. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. Crit Rev Toxicol. 2006;36(3):189-217.

2.Lam C-W, James JT, McCluskey R, Hunter RL. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol Sci. 2004;77(1):126-34.

3.Muller J, Huaux F, Moreau N, Misson P, Heilier J-F, Delos M, et al. Respiratory toxicity of multi-wall carbon nanotubes. Toxicol Appl Pharmacol. 2005;207(3):221-31.

4.Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, et al. Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. Am J Physiol Lung Cell Mol Physiol. 2005;289(5):L698-708.

5.Luyts K, Smulders S, Napierska D, Poels K, Scheers H, Hemmeryckx B, et al. Pulmonary and hemostatic toxicity of multi-walled carbon nanotubes and zinc oxide nanoparticles after pulmonary exposure in Bmal1 knockout mice. Part Fibre Toxicol. 2014;11(1):1.

6.Gu XM, Hu P, Shen T, Xu YY, Zhang J, Zheng YF, et al. [Pulmonary toxicity in high-fat diet SD rats induced by intravenous injection of multi-walled carbon nanotubes]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2012;30(6):413-7.

7.Turillazzi E, Baroldi G, Silver MD, Parolini M, Pomara C, Fineschi V. A systematic study of a myocardial lesion: colliquative myocytolysis. Int J Cardiol. 2005;104(2):152-7.

8.Ware LB, Matthay MA. Acute pulmonary edema. N Engl J Med. 2005;353(26):2788-96.

9.Tong H, McGee JK, Saxena RK, Kodavanti UP, Devlin RB, Gilmour MI. Influence of acid functionalization on the cardiopulmonary toxicity of carbon nanotubes and carbon black particles in mice. Toxicol Appl Pharmacol. 2009;239(3):224-32.

10.Patlolla A, McGinnis B, Tchounwou P. Biochemical and histopathological evaluation of functionalized single□walled carbon nanotubes in Swiss–Webster mice. J Appl Toxicol. 2011;31(1):75-83.

11.Chen R, Zhang L, Ge C, Tseng MT, Bai R, Qu Y, et al. Subchronic toxicity and cardiovascular responses in spontaneously hypertensive rats after exposure to multiwalled carbon nanotubes by intratracheal instillation. Chem Res Toxicol. 2015;28(3):440-50.

12.Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GAM, Webb TR. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. Toxicol Sci. 2004;77(1):117-25.

13.Yang S-T, Wang X, Jia G, Gu Y, Wang T, Nie H, et al. Long-term accumulation and low toxicity of single-walled carbon nanotubes in intravenously exposed mice. Toxicol Lett. 2008;181(3):182-9.

14.Walker VG, Li Z, Hulderman T, Schwegler-Berry D, Kashon ML, Simeonova PP. Potential in vitro effects of carbon nanotubes on human aortic endothelial cells. Toxicol Appl Pharmacol. 2009;236(3):319-28.

15.Downey JM, Cohen MV. Free radicals in the heart: friend or foe?. Expert Rev Cardiovasc Ther. 2008;6(5):589-91.

16.Rossoni G, Manfredi B, Civelli M, Berti F, Razzetti R. Combined simvastatin–manidipine protect against ischemia–reperfusion injury in isolated hearts from normocholesterolemic rats. Eur J Pharmacol. 2008;587(1):224-30.