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Acute toxicological effects of copper nanoparticles in vivo

Zhen Chen [a,e,1], Huan Meng [a,e,1], Gengmei Xing [a], Chunying Chen [a], Yuliang Zhao [a,e,*], Guang Jia [b], Tiancheng Wang [d], Hui Yuan [a], Chang Ye [a], Feng Zhao [a], Zhifang Chai [a], Chuanfeng Zhu [c], Xiaohong Fang [c], Baocheng Ma [c], Lijun Wan [c]

[a] *Lab for Bio-Environmental Health Sciences of Nanoscale Materials, Institute of High Energy Physics, Chinese Academy of Sciences, P.O. Box 918, Beijing 100049, China*
[b] *Department of Occupational and Environmental Health Sciences, School of Public Health, Peking University, Beijing 100083, China*
[c] *Key Lab of Molecular Nanostructures and Nanotechnology, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China*
[d] *School of Public Health, Department of Clinical Laboratory, Third Hospital of Peking University, Beijing 100083, China*
[e] *The Graduate School, Chinese Academy of Sciences, China*

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Abstract

To assess the toxicity of copper nanoparticles (23.5 nm) in vivo, LD_{50}, morphological changes, pathological examinations and blood biochemical indexes of experimental mice are studied comparatively with micro-copper particles (17 μm) and cupric ions ($CuCl_2 \cdot 2H_2O$). The LD_{50} for the nano-, micro-copper particles and cupric ions exposed to mice via oral gavage are 413, >5000 and 110 mg/kg body weight, respectively. The toxicity classes of nano and ionic copper particles both are class 3 (moderately toxic), and micro-copper is class 5 (practically non-toxic) of Hodge and Sterner Scale. Kidney, liver and spleen are found to be target organs of nano-copper particles. Nanoparticles induce gravely toxicological effects and heavy injuries on kidney, liver and spleen of experimental mice, but micro-copper particles do not, on mass basis. Results indicate a gender dependent feature of nanotoxicity. Several factors such as huge specific surface area, ultrahigh reactivity, exceeding consumption of H^+, etc. that likely cause the grave nanotoxicity observed in vivo are discussed.

Keywords: Nanotoxicity; LD_{50}; Target organs; Copper nanoparticles; In vivo

1. Introduction

Health effects of nanoparticles are attracting considerable and increasing concern of the public and government worldwide. So far, most of the nanotoxicity research focused on respiratory tract exposures for assessing the health effects of nanoparticles. Other exposure routes, e.g., gastrointestinal tract also needs to be considered as potential portals of entry. There are many ways that nanoparticles can be ingested into the gastrointestinal tract. For instance, nanoparticles cleared from the respiratory tract via the mucociliary escalator can subsequently be ingested into the gastrointestinal tract; nanomaterials can be ingested directly via water, food, cosmetics, drugs, drug delivery devices, etc. (Peter et al., 2004; Oberdörster et al., 2005). Uptake of particles of different size via the gastrointestinal tract can also lead to different toxicological effects (Jani et al., 1994; Böckmann et al., 2000). But the reports about

* Corresponding author. Fax: +86 10 8823 3191.
E-mail address: zhaoyuliang@mail.ihep.ac.cn (Y. Zhao).
[1] Equal contributions.

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toxicological research of nanomaterials by the gastrointestinal tract are few.

Nanosized copper particles (herein after refer to as "nano-copper"), one of the manufactured nanoparticles, are now industrially produced and available commercially. Recently, nano-copper particles are used as the additive in lubricants, polymers/plastics, metallic coating and inks, etc. Due to excellent mending effects of nano-copper particles, they are added into lubricant oil as an additive to effectively reduce friction and wear, or to mend a worn surface (Liu et al., 2004). Nano-copper particles are homogeneously deposited on the surface of graphite to improve the charge–discharge property significantly, such as coulombic efficiency, cycle characteristics, and high rate performance as an anode material for lithium ion batteries (Guo et al., 2002). The copper-fluoropolymer nano-composite is employed as bioactive coatings that are capable of inhibiting the growth of target microorganisms such as *Saccharomyces cerevisiae*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria* (Cioffi et al., 2005). Accordingly, nano-copper particles, similar to any of other nanomaterials, are likely to enter the environment and human body via different paths such as effluent, spillage during shipping and handling, consumer products and disposal, etc.

In human body, copper is maintained in homeostasis (Jesse and Mary, 2004). If the intake of copper exceeds the range of the human tolerance, it would cause toxic effects such as hemolysis, jaundice and even death. Most recently, the study indicates that the overload of common copper in vivo can induce a set of toxicological activities such as hepatocirrhosis (Björn et al., 2003), changes in lipid profile, oxidative stress, renal dysfunction (Galhardi et al., 2004) and stimulation of mucous membrane of alimentary canal, etc. However, recent toxicological investigations of manufactured nanoparticles revealed such a nature that compared with the larger particles of the same chemical composition (on the identical mass basis), nanoparticles tends to exhibit quite different toxicological effects in vivo (for example, Oberdörster, 1994; Donaldson et al., 1998; Warheit et al., 2004). Specifically, for nano-copper particles, compared with the micro-copper, their primary alteration in biochemical property is the higher reactivity originated from a larger specific surface area. How this property can alter the toxicological effects in vivo? In this paper, the mice are exposed to nanoscale and micro-sized particles of copper via gastrointestinal tract, the nanotoxicity in vivo as well as differences in toxicological effects of nano- and micro-copper particles are investigated comparatively on mass basis.

2. Materials and methods

2.1. Tested chemicals

The nano-copper particles (25 nm) were purchased from Shenzhen Junye Nano Material Co., Ltd. Before the use, the nanoparticles were weighed and divided into several ampoules (each weigh 1 g) under an air-free condition of a specially designed glove box that was filling with dry argon gas, where the nano-copper-loaded ampoules were stored until the animal experiments. The size distribution and specific surface area of nano-copper were analyzed by atomic force microscopy (AFM, Nano III a SPM, Digital Instruments Inc. USA 3A). Micro-copper particles (200-mesh) were purchased from Beijing HaoYun Co. Ltd. Before the experiment, the size was measured using transmission electron microscopy (TEM, Hitichi H-700 electron microscope) techniques. The impurities (e.g. aluminum, barium, calcium, cadmium, chromium, iron, magnesium, manganese, molybdenum, sodium, potassium, nickel, lead, strontium, zinc, etc.) in both nano- and micro-copper particles were analyzed using X-ray fluorescence spectroscopy (XRFS). The results indicate that the purity of nano- and micro-copper both are better than 99.9%. $CuCl_2 \cdot 2H_2O$, provided from Shanghai Chemicals Co. Ltd. (purity >99.9%), was used as ion-copper in the experiment.

Hydroxypropylmethylcellulose K4M (HPMC, analytical grade), the suspending agent for copper particles, is obtained from Shanghai Colorcon Coating Technology Limited. To insure the non-reaction to oxygen, the nano-copper particles were dispersed into the 1% w/v HPMC solution inside the glove box which was filled with dry argon gas. The suspending solutions containing copper nanoparticles were treated by ultrasound for 10 min and vibrated for 2 min. Then, these solutions in different doses were subsequently exposed to mice via oral gavage. To ensure non-ionization and non-aggregation of nano-copper before administration, the time interval from preparation to oral gavage was strictly limited in less than 20 min. In addition, 20 min after the preparation, the particle size and surface property of nano-copper were analyzed by AFM, and the cupric ion in the solution was measured by the chemical titration method which was used to monitor if copper is transformed into ions in the suspending agent before oral administration.

2.2. Animals

ICR mice of either sex (provided by Weitong-Lihua Experimental Animal Center), aged 8 weeks and weighing 20–22 g, were used in the experiments. Every five mice with same sex were housed in stainless steel cages containing sterile paddy husk as bedding in ventilated animal rooms. They were acclimated in the controlled environment (temperature: $22 \pm 1\,^{\circ}C$; humidity: $60 \pm 10\%$ and light: 12 h light/dark cycle) with free access to water and a commercial laboratory complete food. All animal experiments were performed in compliance with the local ethics committee.