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Biodistribution of functionalized multiwall carbon nanotubes in mice

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Abstract

With the application of carbon nanotubes in biomedical and pharmaceutical sciences, its basic biological properties in vivo have become an issue of strong concern. Water-soluble functionalized multiwall carbon nanotubes (MWNTs) were labeled with radioactive ^{99m}Tc atoms, and then a tracer was used to study the distribution of MWNTs modified with glucosamine in mice. It shows that MWNTs moved easily among the compartments and tissues of the body, behaving like active molecules although their apparent mean molecular weight is tremendously large. In this study, water-soluble MWNTs were labeled with ^{99m}Tc for the first time, and all results on the distribution of MWNTs in animals provide useful data for their use in the biomedical field.

Keywords: ^{99m}Tc labeled; Distribution; Multiwall carbon nanotubes; In vivo

1. Introduction

Because of their unique and fascinating one-dimensional nanostructure, carbon nanotubes are currently under careful scrutiny as novel tools for biomedical and pharmaceutical applications [1–8]. With the progress of methods for carbon nanotubes functionalization [9–12], the biocompatibility of functionalized carbon nanotubes is clearly improved [13–15]. As a consequence, functionalized carbon nanotubes were proposed as novel types of carrier system for therapeutic agents in vivo [6,7,16–18]. Thus, the biodistribution and kinetics of functionalized carbon nanotubes have raised many concerns as they are introduced into living systems.

Yet, studies about the biodistribution and kinetics of functionalized carbon nanotubes in living systems have seldom been reported because of the shortage of a suitable analytical method. In recent decades, radioisotope tracing has been an effective and straightforward method to study the in vivo quantitative distribution of xenobiotics [19]. Among the radioisotopes used in tracing studies, technetium-99 (^{99m}Tc; $T_{1/2}$=6.02 h, E_γ=141 keV) is widely used due to the stability of labeled compound and appropriate photo energies for measurement.

In this study, we modified multiwall carbon nanotubes (MWNTs) with glucosamine to get water-soluble MWNT glucosamine (MWNT-G), and then ^{99m}Tc-labeled MWNT-G (^{99m}Tc-MWNT-G) was synthesized, which was water-soluble and highly compatible with body fluid in vivo. Finally, the tissue distribution of ^{99m}Tc-MWNT-G in mice was measured. The quantity of MWNT-G accumulated in organs was determined by radioisotopes, and quantitative valuable information on MWNTs in vivo was afforded.

2. Materials and methods

2.1. Materials

MWNTs commercially prepared by chemical vaporization deposition were obtained from Shenzhen Nanotech Port Co. Ltd. China. Determined with transmission electron microscopy (TEM), MWNTs are several tens of micrometers in length, with a diameter of 20–40 nm. Purity was >95%, containing <3% amorphous carbon according to thermal gravity analysis (TGA) and ca. 0.6% Ni as determined by inductively coupled plasma mass spectrometry. The purification of MWNTs was performed according to the method of Liu et al. [20]; the purity was >96%, and the concentration of Ni decreased to <0.2%. Thionyl chloride was of analytical reagent (99% purity). Glucosamine (99% purity) was purchased from Aldrich

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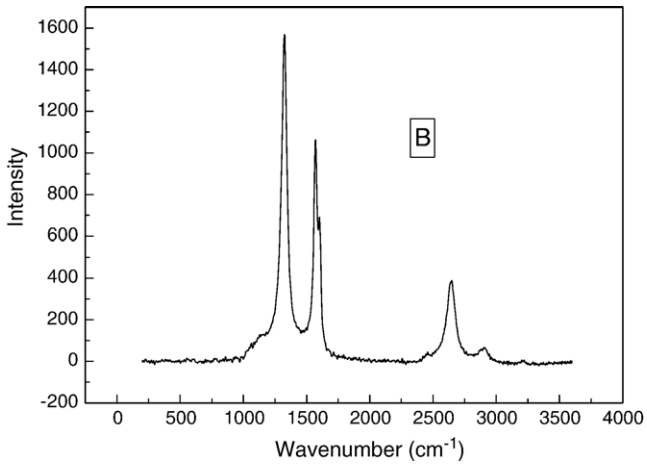

Fig. 1. Raman spectra of water-soluble MWNT-G.

(Germany). $^{99m}TcO_4^-$ (18 mCi) was purchased from the China Institute of Atomic Energy.

2.2. Preparation of MWNT-G

MWNTs were functionalized with glucosamine according to the method of Pompeo and Resasco [21]. MWNTs were placed in a mixture of concentrated sulfuric and nitric acids (3:1; 98% and 70%) and heated at 40°C with ultrasonic for 24 h to synthesize MWNTs with carboxyl. In order to synthesize MWNTs functionalized with acyl chloride, 100 mg of MWNTs was suspended in 30 ml of thionyl chloride ($SOCl_2$). This suspension was stirred at 70°C for 24 h. After centrifugation, the brown-colored supernatant was decanted, and the remaining solid was washed with anhydrous tetrahydrofuran (THF). After centrifugation, the pale yellow-colored supernatant was decanted. The remaining solid was dried at 60°C under vacuum. Finally, a mixture of resulting MWNTs and 10 g of glucosamine was dissolved in anhydrous THF, which was kept in the presence of Na wires to eliminate any traces of water. The mixture was then refluxed for 48 h at room temperature. Glucosamine excess was removed by washing the product with anhydrous THF four times. The remaining solid was dried at 60°C under vacuum, and the final MWNT-G was obtained. Fourier transform infrared (FTIR) spectroscopy, Raman spectra, element analysis, TGA and TEM were applied to determine the characteristics of MWNT-G.

2.3. ^{m}Tc labeling of MWNT-G

MWNTs-G were labeled with ^{99m}Tc according to the method of Li et al. [22]. In brief, MWNTs were dissolved in deionized water with ultrasonic device for 5 min, and then ascorbic acid, stannous chloride and $^{99m}TcO_4^-$ were added to the suspension. This mixture was stirred at 90°C for 20 min. After centrifugation, the supernatant was decanted, and the remaining solid was ^{99m}Tc-MWNT-G.

2.4. Biodistribution study in mice

All animal experiments were performed in compliance with the local ethics committee. Kunming mice (female, 18–22 g, 5–6 weeks old) were obtained from the Shanghai SLAC Laboratory Animal Co. Ltd., Chinese Academy of Sciences (Shanghai, China).

In this study, each mouse in all five groups (five mice per group) was intraperitoneally injected with 300 μl of ^{99m}Tc-MWNT-G suspension (0.5 mCi). They were sacrificed at 1, 3, 6, 10 and 24 h postinjection, respectively. Their tissues, including the heart, lung, liver, spleen, kidney, stomach (emptied), intestine (emptied), skin, muscle (leg) and enterogastric area, were immediately dissected, and blood, feces and urine were collected. Each tissue was wrapped in foil, weighted and counted for ^{99m}Tc activity. Data were corrected for physical decay of radioactivity. Distribution in tissues was presented in percent injected dose per gram of wet tissue (% ID/g), which could be calculated by the percent injected dose (tissue activity/total activity dosed) per gram of wet tissue. The excretion of MWNT-G from mice was investigated by counting ^{99m}Tc in the urine and feces of mice at different time intervals from 0 to 24 h postdosing. Results were expressed as percent injected dose per gram of wet tissue.

2.5. In vivo stability

Normal female mice were intraperitoneally injected with 300 μl of the ^{99m}Tc-MWNT-G suspension containing 0.5 mCi of radioactivity. The mice were then sacrificed at 6, 10 and 24 h postinjection, and urine was collected. Urine radioactivity was measured with NaI(Tl) scintillor. Urine was centrifuged at 10,000 rpm for 4 min, the supernatant was decanted and the remaining solid was washed with

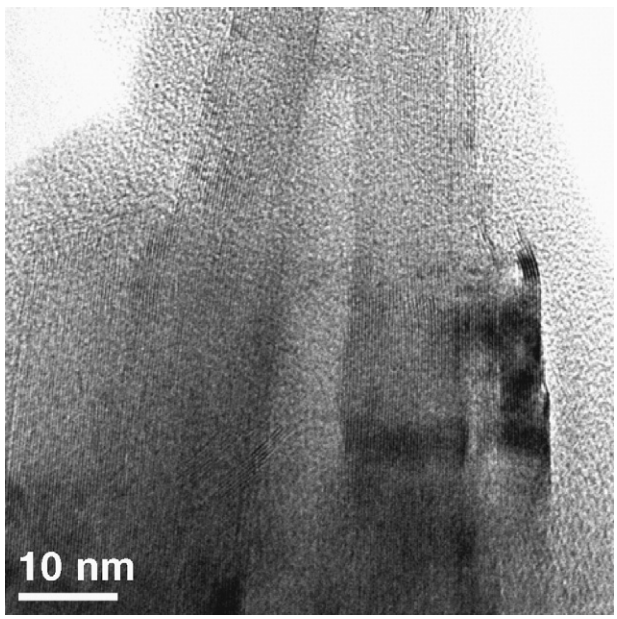

Fig. 2. TEM image of MWNT-G.