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Gastrointestinal actions of orally-administered single-walled carbon nanohorns

Maki Nakamura [a,b,*], Yoshio Tahara [a], Tatsuya Murakami [c], Sumio Iijima [a,d], Masako Yudasaka [a,*]

[a] Nanotube Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba 305-8565, Japan

[b] Nanosystem Research Institute, AIST, 1-1-1 Higashi, Tsukuba 305-8562, Japan

[c] Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto 606-8501, Japan

[d] Meijo University, 1-501 Shiogamaguchi, Nagoya 468-8502, Japan

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ABSTRACT

Carbon nanomaterials, such as single-walled carbon nanohorns (SWCNHs) and carbon nanotubes, demonstrate great potential as drug delivery systems. However, no reports to date have detailed the use of SWCNHs as oral drug carriers. This study shows for the first time the actions of orally-administered SWCNHs in normal mice and mice with dextran sulfate sodium (DSS)-induced colitis. SWCNHs labeled with gadolinium oxide for quantification purposes were detected in the gastrointestinal tract and the feces of mice, but not in the blood or other bodily organs. These results indicate that the nanohorns were not absorbed into the body from the gastrointestinal tract. SWCNH absorption was not influenced by the functionalization or size control of SWCNH. Neither death nor behavioral aberrations were observed in normal mice following SWCNH administration. However, histological observation of mice with DSS-induced colitis at 24 h after oral administration of SWCNHs revealed the presence of black particles, presumed to be SWCNHs, in the inflamed areas of the colon and the cecum. Thus, SWCNHs might serve as efficacious drug delivery carriers for the treatment of ulcerative colitis.

1. Introduction

The development of nanometer-sized carbon materials with a tubular shape, such as carbon nanotubes (CNTs) [1,2] and single-walled carbon nanohorns (SWCNHs) [3,4], has rapidly progressed over the past decade. These materials act as nanocontainers with a large internal volume available for loading various molecules and metallic compounds, including drugs [5–9], contrast agents [10,11], and sensitizers [12]. Moreover, the multiplicity of attainable covalent and non-covalent modifications is advantageous for the development of highly functional CNTs and SWCNHs for biomedical purposes. Indeed, experimental investigations using animals and cells have uncovered the potential
application of these materials for drug delivery, diagnostic imaging, and photodynamic therapy. Their prospective toxicity has also been discussed for the safety use of these materials as medicinal products [13–16].

Numerous administration routes of CNTs and SWCNHs have been explored in animal experiments, including intravenous [11,17–19] and intraperitoneal [13,20] injection, inhalation [14–16], and local pathways [8,9,12]. However, there

* *Corresponding authors at:* Nanotube Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba 305-8565, Japan.
E-mail addresses: ma-ki-nakamura@aist.go.jp (M. Nakamura), m-yudasaka@aist.go.jp (M. Yudasaka).
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are not many reports that address oral administration, although this is the most common and desirable route of drug administration in humans. Several toxicological and/or distribution studies have been performed regarding orally-administered single-walled carbon nanotubes (SWCNTs) [20–23], multi-walled carbon nanotubes (MWCNTs) [23–27], and SWCNHs [15]. Only a few studies focused on the inherent capacity of CNTs as oral drug delivery carriers [28–30].

Despite the paucity of data, CNTs and SWCNHs appear to be useful oral drug carriers because they are chemically stable, graphene-based materials that can tolerate the acidic and alkaline environments in the gastrointestinal tract created by stomach acids and digestive enzymes. Therapeutic compounds loaded into CNTs and SWCNHs could then purportedly be protected from degradation under harsh conditions and delivered intact to areas affected by gastrointestinal disorders (e.g., ulcerative colitis (UC) and colon cancer).

SWCNHs are single graphene tubules with horn-shaped tips and diameters of 2–5 nm and lengths of 40–50 nm [3]. About 2000 SWCNHs assemble to form a spherical aggregate with a diameter of 80–100 nm (Fig. 1a). High-purity SWCNHs can be produced in large quantities by a simple procedure, without metal catalysts [31]. Therefore, these nanotubules do not contain any contaminating, toxicity-inducing metals, which is advantageous for their use in drug delivery applications [8,9,12].

This study investigated the behavior of orally-administered SWCNHs in normal mice and mice with experimentally-evoked colitis. The possible use of SWCNHs as oral drug carriers targeted to specific parts of the body is also discussed. To the best of our knowledge, this is the first report to show the results of biodistribution of SWCNHs administered by gavage.

2. Experimental

2.1. *Preparation and characterization of carbon nanohorn materials*

SWCNHs were prepared by CO_2 laser ablation of graphite in the absence of metal catalysts in an argon atmosphere (760 Torr) at room temperature. The resultant materials (as-CNHs) were used without further purification [3,31]. Gadolinium (Gd) oxide-embedded SWCNHs (Gd-CNHs) were prepared according to previously reported methods [17], with some modifications (Supplementary data I-1). Oxidized SWCNHs (ox-CNHs) were prepared by a slow combustion method [32] (Supplementary data I-2). SWCNHs functionalized with abundant oxygenated groups, such as carboxylic acid groups (COOH-CNHs), were prepared by a light-assisted oxidation method [33] (Supplementary data I-3). Ox-CNHs functionalized with polyethylene glycol (PEG-ox-CNHs) were prepared by coating ox-CNHs with 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt) (Avanti) (Supplementary data I-4). Small-sized SWCNHs (small-CNHs) were obtained by oxidative exfoliation of as-CNHs [34] (Supplementary data I-5).

Structures of carbon nanohorn materials were observed using a high resolution transmission electron microscope (TEM) (EM-002B model; Topcon) operated at 120 kV. The size and dispersibility of the carbon nanohorn materials were estimated using a dynamic light scattering (DLS) method (FPAR-1000; Otsuka Electronics). As "Supplementary data II" shows, DLS revealed that the particle sizes of the Gd-CNHs, COOH-CNHs, and PEG-ox-CNHs were approximately 160, 110, and 160 nm, respectively, suggesting that these materials (~100 nm) were well dispersed in water. On the other hand, the as-CNHs and ox-CNHs formed large agglomerates (~1000 nm), indicating the poor dispersibility. The size of the small-CNHs was approximately 70 nm, which was larger than the size of individual small-CNHs (20–50 nm) reported in a previous study [34]. In our study, the small-CNHs were highly concentrated (10 mg/mL); therefore, they probably underwent a certain amount of agglomeration.

2.2. *Animals*

Female BALB/cA mice (6-weeks-old) were purchased from CLEA Japan. Mice were housed under conditions of controlled temperature, humidity, and day–night cycles, with free access to standard laboratory feed and drinking water. Feed and water were freely available during the experiment even after the administration of carbon nanohorn materials. The mice were acclimated to this environment for 7–18 days before initiating the study on Day 0. On Day 0, the animals weighed 18–24 g. All animal experiments were approved by the Animal Care and Use Committee at the National Institute of Advanced Industrial Science and Technology.

Colitis was induced by providing the mice with drinking water supplemented with 5% dextran sulfate sodium (DSS,

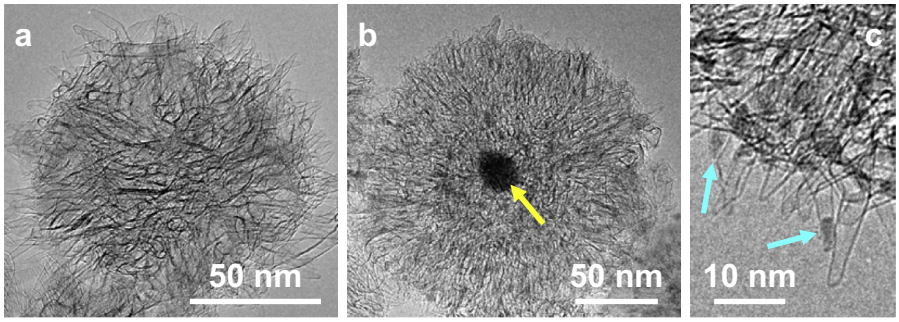

Fig. 1 – TEM images of as-CNHs (a) and Gd-CNHs (b and c). Gd oxide nanoparticles are embedded near the center of the CNH aggregate (b, yellow arrow) and at the tip of the individual horns (c, light blue arrows). (A color version of this figure can be viewed online.)