

Carbon Nanotubes: Potential Benefits and Risks of Nanotechnology in Nuclear Medicine

Targeting of tumors with radionuclides for radiotherapeutic purposes is often limited by inadequate delivery to lesions using currently available targeting vehicles (e.g., monoclonal antibodies and peptides), relatively low and heterogeneous epitope/receptor expression on cancer cells, as well as dose-limiting toxicities to normal tissues (1). Nevertheless, there have been successes, particularly for radioimmunotherapy of non-Hodgkin's B-cell lymphoma with ^{90}Y -ibritumomab tiuxetan (Zevalin; Biogen Idec) or ^{131}I -tositumomab (Bexxar; Glaxo-SmithKline) (1). Notwithstanding these

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examples, improvements in radiotherapy of malignancies are needed—these could be achieved by the introduction of versatile new delivery platforms that (i) provide opportunities for multiple target recognition on cancer cells, (ii) substantially amplify the transport of radionuclides to cancer cells with each target recognition event, and (iii) selectively route radionuclides to more radiosensitive compartments within the cells (e.g., the nucleus). The rapidly advancing field of cancer nanotechnology has generated many innovative drug delivery systems (e.g., liposomes, dendrimers, nanoshells, nanotubes, and block co-

polymer micelles) for enhanced and targeted transport of cytotoxic agents to tumors (2,3). These systems could provide the platforms needed for enhanced delivery of radionuclides to tumors. In this issue of *The Journal of Nuclear Medicine*, McDevitt et al. (4) explore the feasibility of targeted delivery of radionuclides to B-cell lymphomas using one new nanotechnology platform: carbon nanotubes (CNTs). They show that CNTs can be functionalized on their surface with 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA) chelators for complexing ^{111}In and also with the anti-CD20 antibody rituximab (Rituxan; Genentech and Biogen Idec) for targeting to malignant B-cells. The ^{111}In -labeled and rituximab-modified CNTs specifically localized in disseminated Daudi B-cell lymphoma xenografts in the bone marrow and spleen of severe combined immunodeficiency (scid) mice, after intravenous injection. These results suggest that CNTs may be useful vehicles for transporting radionuclides to malignancies; however, caution is advised because, among nanotechnology platforms being investigated, least is known about the in vivo properties and potential risks of CNTs as delivery systems.

CNTs were first described by Iijima in 1991 (5) and are essentially rolled sheets of fullerene graphene that can either be single-walled (SWCNTs) or multiwalled (MWCNTs) (Fig. 1). The diameter of SWCNTs is 1–2 nm, whereas that of MWCNTs ranges from 2 to 25 nm (6). The spacing between the layers of graphene in MWCNTs is 0.36 nm. CNTs can be synthesized and then cut by sonication in concentrated nitric acid to the desired length, ranging from $>1\ \mu\text{m}$ to a few micrometers,

or even longer (i.e., several hundreds of micrometers) (7). In the study by McDevitt et al. (4), SWCNTs with diameter of 1.4 nm and lengths from 0.2 to 1 μm were used. The high aspect ratio (i.e., length divided by width) of CNTs presents a large surface area for modification with various functionalities; moreover, cargoes can be attached to the surfaces (inner or outer) or even packaged within the core of the tubes (8). One interesting and very useful property of CNTs is their capability to penetrate cell membranes; this provides a route for delivery of cargoes into the cytoplasm and, in many cases, to the nucleus of cells (9). The mechanism by which this occurs is not well understood, but it may be mediated by endocytosis (9) or direct insertion of the nanotubes (i.e., as “microneedles”) through cell membranes (10). Therefore, CNTs have been studied for intracellular delivery of proteins and peptides (10–12), drugs (13–15), MRI or fluorescence contrast agents (16,17), and DNA (18) as well as for vaccine development (19).

A key consideration in the use of CNTs for in vivo applications is their insolubility in water; this property is responsible for their toxicity against living cells (20,21). CNTs can be functionalized to render them water-dispersable and more biocompatible by 1,3-dipolar cycloaddition of azomethidine ylides, which appends amine groups to their surface (see Fig. 1 in the article by McDevitt et al.) (22). The amine groups present sites for covalent linkage of other functional moieties (e.g., peptides or antibodies) using conventional cross-linking chemistry (22). In the study by McDevitt et al. (4), this approach was used to render the CNTs dispersible in water and to conjugate them with chelators for

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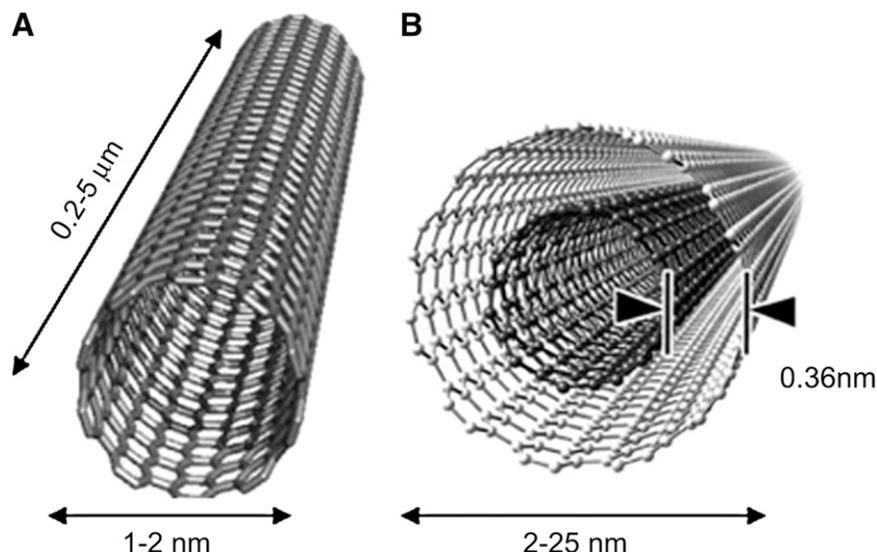


FIGURE 1. Conceptual diagram of single-walled carbon nanotube (SWCNT) (A) and multiwalled carbon nanotube (MWCNT) (B) delivery systems showing typical dimensions of length, width, and separation distance between graphene layers in MWCNTs. (Adapted from (41,42).)

^{111}In using the isothiocyanatobenzyl ester of DOTA. Residual amine groups on the CNTs after DOTA conjugation were modified with the cross-linker succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC) for subsequent covalent linkage of thiolated rituximab. Analogous approaches have been used to conjugate CNTs with peptides (22), fluorophores (13), as well as drugs such as methotrexate or amphotericin B (13,14). The benefits of CNTs as platforms for the delivery of radionuclides to tumors, compared with directly radiolabeled antibodies or peptides, are that a very large number of metal chelators could be attached to their surface, thus substantially amplifying the delivery of radionuclides to cancer cells per target recognition event. In contrast, substitution of more than a few chelators onto antibody molecules has been associated with a significant decrease in their immunoreactivity (23). In addition, CNTs could be modified on their surface with multiple antibody molecules to enhance targeting to tumors; moreover, these antibodies could recognize different epitopes, thereby addressing the issue of target heterogeneity. In the study by McDevitt et al. (4), 0.30 mmol of DOTA and 0.02 mmol of rituximab

were conjugated per gram of 0.2- μm -length CNTs—this corresponded to an incredible 114 chelators for ^{111}In and 6 antibody molecules per CNT! The immunoreactivity of the rituximab-conjugated CNTs with Daudi cells was not substantially lower than that of ^{111}In -labeled rituximab. Of course, such modifications increase the molecular size of CNTs, which may diminish extravasation and penetration, particularly into solid tumors (24). However, this effect may be counteracted by the enhanced permeability and retention (EPR) phenomenon, which describes the selective accumulation of nanosized particles (e.g., liposomes and block copolymer micelles) into tumors as a consequence of their “leaky” vasculature and poor lymphatic drainage (25). It is been suggested that the size of nanoparticles should not exceed 300 nm for optimal exploitation of the EPR effect, as larger particles are more susceptible to recognition and phagocytosis by macrophages (26).

Despite these potential benefits, the toxicity of CNTs is a major concern that needs to be more clearly understood and addressed. Pristine, water-insoluble CNTs have been found to be highly toxic in vitro to many different types of cells, including human keratino-

cytes (27), rat brain neuronal cells (28), human embryonic kidney cells (29), and human lung cancer cells (30). In addition, unmodified CNTs administered intratracheally to mice have been reported to induce the formation of lung granulomas (31). CNTs have also been shown to promote the aggregation of human platelets in vitro, and analogous carbon particulate matter found in the environment enhanced experimentally induced vascular thrombosis in rats (32). Nevertheless, in contrast to the harmful effects of pristine CNTs, recent studies have suggested that modified, water-dispersible CNTs are not toxic to cells, at least in vitro, and their toxicity appears to be ameliorated depending on the extent of surface functionalization (20,21). Sayes et al. (21) found that modification of the surface of SWCNTs with carboxylic acid or sulfonate moieties diminished their toxicity in vitro toward human dermal fibroblasts >1,000-fold compared with pristine SWCNTs dispersed in 1% Pluronic F108 (BASF Corp.) (some toxicity of the SWCNTs was attributable to the Pluronic surfactant). Dumortier et al. (20) observed that SWCNTs modified with fluorescein through the 1,3-dipolar cycloaddition of azomethidine ylides were nontoxic to cultures of mouse B- and T-lymphocytes and macrophages and preserved the function of these immune cells. Nevertheless, no formal acute and, importantly, chronic toxicology studies of functionalized water-dispersible CNTs have been performed to examine their effects on normal tissues in vivo (33)—these toxicities will be dependent on the organ distribution, metabolism, and elimination characteristics of the CNTs. It is imperative that these types of studies be performed sooner rather than later to assess the potential translatability of CNTs as platforms for the delivery of drugs and radionuclides to tumors in humans. In one report of SWCNTs modified with diethylenetriaminepentaacetic acid (DTPA) for labeling with ^{111}In , administered intravenously to mice, Singh et al. (34) briefly mentioned that “none of the animals

exhibited any signs of renal or other severe acute toxicity responses". However, the observation period was very short—only 24 h—and no objective measurements of toxicity (i.e., hematologic and biochemical testing) were performed. McDevitt et al. (4) similarly did not evaluate the toxicity of the ^{111}In -labeled rituximab-conjugated CNTs in scid mice—only their tumor targeting and biodistribution properties were examined, although these properties were studied for up to 15 d after injection. It is also important to consider the potential immunogenicity of CNTs. Although CNTs do not appear to be immunogenic themselves, in one study (19), they elicited an immune response in mice against a peptide epitope of the foot-and-mouth-disease virus conjugated to the CNTs, suggesting that they could promote similar immune responses in humans to conjugated antibodies or peptides used for tumor targeting, especially if these were not humanized.

The elimination characteristics of CNTs are fascinating. McDevitt et al. (4) showed that SWCNTs conjugated to ^{111}In through DOTA (but not modified with rituximab) were rapidly cleared with only about 1% of the injected dose (%ID) remaining in the blood at 20 h after injection (assuming a blood volume of 3 mL in a mouse). This rapid elimination was associated with relatively high uptake in the kidneys, modest accumulation in the liver, and excretion of ^{111}In into the urine. ^{111}In -Labeled CNTs were detected in the urine of the mice by instant thin-layer chromatography. Moreover, radioactivity in the kidneys and liver diminished over time. Singh et al. (34) found an even more rapid elimination of DTPA-conjugated nontargeted SWCNTs labeled with ^{111}In , with <0.1 %ID circulating in the blood of mice at 24 h after intravenous administration. In their study, kidney uptake diminished dramatically (20- to 30-fold), and liver radioactivity decreased 2- to 3-fold over 24 h. Especially intriguing was that they identified intact CNTs in the urine of the mice by transmission electron microscopy. These pharmaco-

kinetic properties of CNTs are starkly different from those of other drug delivery nanosystems (e.g., liposomes or block copolymer micelles), which exhibit high liver uptake unless they are surface-modified with polyethylene glycol (PEG) and have relatively slow blood clearance (3). In some instances, CNTs have been modified similarly with PEG to decrease non-specific interactions with cells and improve their biocompatibility (20,35). The rapid elimination of functionalized CNTs from the blood and most normal tissues is expected to diminish their toxicity; however, it should be kept in mind that modification with antibodies or peptides may alter these properties. For example, in the study by McDevitt et al. (4), conjugation of the CNTs to rituximab decreased kidney accumulation 4-fold but increased liver uptake more than 2-fold. The ^{111}In -labeled rituximab-modified CNTs were slightly less effective at targeting lymphoma-infiltrated bone marrow in mice than ^{111}In -labeled rituximab, but splenic sequestration was similar. Because DOTA can also complex ^{90}Y , these results suggest that these CNTs could be used to target B-cell lymphomas for radiotherapeutic applications.

One application of CNTs not explored by McDevitt et al. (4) is their potential for inserting nanometer- to micrometer-range Auger electron-emitting radionuclides, such as ^{111}In into cancer cells for targeted radiotherapy. The unique capability of CNTs to penetrate cell membranes and localize around the nuclear membrane of cancer cells (10,13,22,36) would amplify the DNA damaging effects of the Auger electrons. Our group has been studying targeted Auger electron radiotherapy of epidermal growth factor receptor (EGFR)-positive breast cancer using ^{111}In -labeled EGF (^{111}In -DTPA-hEGF) (37,38) and acute myelogenous leukemia (AML) using ^{111}In -labeled anti-CD33 antibodies (39). One could imagine that CNTs could be functionalized on their surface with multiple DTPA chelators to transport large amounts of ^{111}In into cancer cells and with EGF or anti-

CD33 antibodies to specifically direct them either to breast cancer or AML cells, respectively. Routing these ^{111}In -labeled CNTs to other malignancies would only require substitution of an alternative targeting moiety. Nevertheless, issues of toxicity need to be more clearly understood to move forward with this and other applications of CNTs. In 2000, the National Aeronautical and Space Administration proposed CNTs as the ideal strong and ultralight material to construct a 35,000-km-long cable forming the "space elevator" linking Earth with outer space (40). As fantastic as this idea seems, the application of CNTs for improving the treatment of malignancies is no less daunting. Will this nanotechnology be the breakthrough we have been awaiting? Only time will tell.

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