



Carbon Nanotubes: Toxicological Properties, Use as Drug Delivery Material and Computational Contribution to Predict their Properties Including Structures with Topological Defects

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Abstract

Carbon nanotubes (CNTs) are a material that surprises with their properties. They can transport drugs to specific targets which makes them very useful in biomedicine. The CNTs consist of hexagonal rings that can be of a single-wall (SWCNT) or concentric multi-walls (MWCNT). They are stable structures that however could be toxic. Fortunately, they can be functionalized with different molecular fragments that make them soluble and nontoxic molecules. Several toxicity studies of CNTs are known with results that still need further research. Several experimental and theoretical examples of drugs that have been adsorbed on the surface of the nanotubes or encapsulated within them are also known. There are also CNTs that have non-hexagonal rings (called defects) embedded in the network of hexagonal rings. Several types of defects (with different combinations of four, five, seven, or eight membered rings) are known and their presence modifies the properties of the perfect nanotubes. So far, some theoretical studies of CNTs with defects are reported aimed at assessing their behavior as drug carriers; but no experimental work is known to assess its toxicity, efficacy and real mechanism of action. Herein, an updated but not exhaustive review of different studies of nanotube toxicity, their influencing factors and some proposed mechanisms is made. Cases of toxicity and non-toxicity are shown, SWCNT and MWCNT are compared and the important effect of functionalization, purity and dose among other factors are discussed. Further, a brief review of some cases of drug delivery by regular CNTs and some theoretical drug delivery studies of Density Functional Theory (DFT) and molecular dynamics simulation (DM) for defective nanotubes is presented with the aim of predicting CNTs molecular parameters that favor drug-nanotube molecular interactions. Finally, a recommendation is done to be followed before any clinical trial is undertaken.

Keywords: Nanotechnology; Toxicity; Drug Delivery; Defective Carbon Nanotubes

Introduction

Carbon nanotubes (CNTs) are now one of the more used materials for a wide range of new applications in fields like engineering, biology, medicine, electronic, and mechanics. It is therefore very important to understand the scientific knowledge of their molecular behavior and the safety of these compounds for the health of diverse living species in different environments. However, there exist several factors that need to be clarified such as the toxicity they may have on the organisms, the effect of their time of exposition and the degradability they may have in the organism of different species.

This mini review will focus mainly on the toxicity and applications of carbon nanotubes (CNTs) as drug carriers in biomedicine considering new nanotube structures having non-hexagonal rings (called defects) together with the normal hexagonal rings which causes variations in the nanotube diameter and its electronic distribution. In principle, this provides a model that attempts to control the structural parameters that allow predicting the energies of drug-nanotube interaction to, in this way, increase the bioavailability of the drug, before proceeding to bioassays. In this context, computational tools are considered that can help to better understand the molecular interactions of some new structures that do not have known experimental studies so that this knowledge can help predict their properties and make a primary selection or a rational design of new structures that could reveal a more predictive understanding of their effects on living species.

Carbon Nanotubes Toxicity

Carbon nanotubes (CNTs) are organic molecules formed by rings of six carbon atoms with sp^2 hybridization that can be organized in various ways giving rise to structures with great hardness and high thermal and electrical conductivity and also with the ability to penetrate membranes and adsorb different types of drugs. These properties have increased their production, handling and use worldwide, for example in electronics, mechanics, biomedicine, which makes a large number of people, animals and the environment in general, to be exposed to their presence. CNTs are stable molecules due to their electronic composition and distribution and could accumulate in the body causing adverse effects. There are several toxicity studies of CNTs that analyze on the one hand the effect of the different structural factors of CNTs on toxicity and on the other, the type of toxicity considered, the mechanisms by which it is produced and how to avoid them.

Wani, et al. summarizes many evidence of CNTs toxicity. One of the plausible mechanisms for cellular toxicity by CNTs involves the generation of free radicals which in turn leads

to oxidative stress. These free radicals are known to oxidize DNA, proteins and lipids in cells. They are also responsible for activating transcription factors AP-1 which causes inflammatory responses [1]. Another mechanism which has been proposed in relation to toxicity generation is the formation of reactive oxygen species (ROS) [1]. Since CNTs increase the oxidative stress inside the cell, it in turn increases the ROS level. However, when comparing samples of SWCNTs and MWCNTs having very different toxicity under the same experimental conditions, the ROS level was not different, and they did not cause any inflammatory responses [2]. Warheit, et al. reported the development of multifocal granulomas in the mouse lungs after a one-month instillation study using highly purified SWCNTs [3]. CNTs have also been reported to cause granulomas resulting in toxic effects inside the body [4]. In contrast, Huczko and Lange found no pulmonary toxicity effect after intratracheal instillation of CNTs on guinea pigs [5] and no significant toxic effects were observed after incubation of SWCNTs with cultured A549 human lung epithelial cells [6]. Pulskam, et al. & Dumortier, et al. found no toxic behavior in CNTs and functionalized CNTs, respectively [7,8].

In a number of studies, dose-dependent effects have been reported such as cytotoxicity, reduction in cell viability, inhibition of cell proliferation, apoptosis, nitric oxide release, oxidative stress, and a reduction in the level of antioxidants. For instance, Lam et al after testing a variety of SWCNT samples concluded that all SWCNT preparations induced dose-dependent lung granulomas in mice [9]. Kim, et al. investigated the lung retention kinetics of tangled MWCNTs on Male Sprague Dawley rats and found dose-dependent inhalation toxicity and an estimated retention half-life for the high concentration (4.253 mg/m^3) of about 35 days [10].

To avoid CNTs adverse effects researchers recommend for knowing the full bio distribution and the toxicological and pharmacological profiles of the CNTs in use before undertaking any clinical study [11,12]. Nayak, et al. analyzed different samples, cell lines, cell viability methods and polar chains and found that the purity of the sample was the most crucial parameter necessary to guarantee a safe application of CNTs in biology and medicine [13]. Prakash and Devasena state in addition, as a number of researchers does, that the toxicity of CNTs also depends on their properties such as length, aspect ratio, surface area, degree of aggregation, concentration, and dose and focus on cytotoxicity and pulmonary effects after intratracheal administration of CNTs [14]. Further, Mamidi analyze cytotoxicity of CNTs related to tissue engineering applications [15]. Kobayashi et al reviewed studies on pulmonary, reproductive, and developmental toxicity caused by carbon nanotubes (CNTs). The findings of reproductive and developmental toxicity studies indicate that exposure to CNTs induced inflammation, fibrosis, lung

cancer following long-term inhalation, and gene damage in the lung in animal studies. However, the carcinogenicity of CNTs may attenuate in the case of shorter fiber length [16].

Yang et al reported a review about current in vitro and in vivo research of biodegradation of CNTs by macrophages through enzymatic oxidation, particularly in the brain, the lung and the liver. They consider factors such as CNT type, length, surface functionalization, and impurities in the biodegradation of CNTs and explain the biodegradation mechanism where radicals are generated that can attack CNT defects and unsaturated carbon bonds on the sidewalls of CNTs creating holes in the graphitic structure, finally causing the degradation of CNTs to carbon dioxide. Importantly, they inform that neither CNTs undergoing biodegradation nor the byproducts of their degradation have been reported to be cytotoxic in vitro and in vivo [17]. Parwez and Budihal extend the review of respiratory toxicity of CNTs to toxicity investigations in animal cell lines and also in bacteria and yeast cells, including a review of the ecotoxicity of carbon nanotubes on different organisms with the conviction that additional studies are necessary to help the public regulatory organization establish standards of importance in the safety of aquatic and aerial animals [18].

Recently, Bergeson and Hutton in tune with the Organization for Economic Cooperation and Development (OECD), Test No. 413, reported on the availability of a Technical Report on toxicity studies of MWCNTs administered by inhalation to Sprague Dawley (Hsd: Sprague Dawley@SD®) Rats and B6C3F1/N Mice announced by the National Toxicology Program (NTP) that selected as standard the 1020 Long Multiwalled Carbon Nanotube (L-MWNT-1020) (Sun Innovations, Fremont, CA) "based on availability, high purity (97%), and the low amount of residual nickel catalyst (0.52% by weight)" and reported as 0.3 milligrams per cubic meter (mg/m³) the no-observed-adverse-effect-level (NOAEL) for L-MWNT-1020 [19,20].

Carbon Nanotubes as Drug Carriers

The development of new and efficient drug delivery systems is of fundamental importance in the biomedical field and many different types of drug delivery systems are currently available. Within the family of nanomaterials, carbon nanotubes (CNTs) have emerged as a new alternative and efficient material for transporting and translocating therapeutic molecules. CNTs can be functionalized with drugs, bioactive peptides, proteins and nucleic acids, and used to deliver their cargos to cells and organs. Of course, since nano systems interact in different ways with biological components it is essential to test, on time, for toxicity using validated methods [21]. Since Doxil, a PEGylated liposome used for the release of doxorubicin (DOX) (an anticancer

drug) and approved by the FDA in 1995, great progress has been made in nano medicines [22]. The ability of CNTs to deliver anti-cancer drugs has been evaluated in model organisms and the results showed them to be better or equivalent to the usually employed nanoliposomes [23]. Further, polyethylenimine functionalized CNTs successfully delivered siRNA into the HeLa-S3 cells [24], and SWCNTs successfully delivered acetylcholine (Ach) into the mice brain to control Alzheimer's disease [25].

Recently, Simon et al have highlighted the use of CNTs as nanocarriers because of their exceptional cell transfection capabilities [26]. CNTs have the capacity to enter cells [27], independently of the functional groups they may have on the surface [28], allowing intracellular drugs delivery but also genes and proteins. Liu et al. have shown that drugs such as DOX can be loaded onto the surface of poly(ethylene glycol) (PEG) conjugated SWCNT thanks to DOX-CNT non-covalent interactions with an enhanced permeability and retention (EPR) effect. Next, a pH-release dependency would also permit DOX release close to the tumor tissues [23,29]. Meng, et al. discuss and evaluate the relationship of the biological safety of SWNTs with their physicochemical properties including the cellular uptake mechanism, bio distribution and metabolism of SWNTs considering pharmacokinetic, cancer targeting and therapeutic properties both in vitro and in vivo [30]. Wang and Xu investigated the adsorption and encapsulation of DOX in SWCNT using methods such as PM6-DH2 and M06-2X in the scheme of ONIUM and found that DOX encapsulation is stronger than adsorption and that more favorable DOX-CNT interactions for armchair nanotubes (n, n) were diameter dependent [31]. The optimal diameter for the encapsulation is 14 Å corresponding to (10, 10) CNT. In spite that MWCNTs as drug carriers have some limitations, studies have shown that MWCNTs are suitable carriers for poorly soluble drugs [32-34].

Carbon Nanotubes with Defects

The properties of CNTs can be strongly affected by the presence of various defects, which are usually formed during their growth process. The properties of defective CNTs have been explored in many theoretical and experimental research projects [35-39]. A five-membered group inserted into the hexagon rings in the graphene or CNTs generates apparent special reactivity. For instance, the one dimensional topological defect consisting of octagonal and pentagonal carbon rings inserted in graphene hexagons can act as a quasi-one-dimensional conducting wire [40]. Li, et al. reported in a theoretical study that together with the physical adsorption of small molecules on different defective SWCNT a chemical adsorption could also occur for instance in the case of NH₃ molecule thanks to the nitrogen lone-pair electrons [41].

Our results reveal that armchair, chiral, and zigzag perfect SWCNTs exhibit optimal drug-nanotube interactions at 14 Å diameter despite that chiral CNTs exhibit the best ability to encapsulate DOX. Pentagon/heptagon bumpy defects and PEG fragments in chiral SWCNTs enhance DOX encapsulation; CNT diameter has a more relevant effect than length; CNT chirality governs nitrogen-doping effect [42]. Interestingly, Liu et al. described a promising method with the aim of improving a spinal cord injury. For this, they used nerve ducts formed by a hydrogel containing oligo (poly (ethylene glycol) fumarate) (OPF)/CNT using the injection molding technique [43]. Armchair CNTs with one and two haeckelite defects exhibit better results compared with those with four and fifteen haeckelite defects. Each haeckelite defect consists of a pair of square and octagonal rings. For these cases DOX-CNT binding free energies are predicted to be dependent on: (i) nanotube chirality and diameter, (ii) the number of defects, (iii) nitrogen doping and (iv) the position of the encapsulated DOX inside the nanotube. Armchair (10,10) nanotubes with two haeckelite defects, doped with nitrogen, exhibit the best drug-nanotube binding free energies compared with zigzag, fully hydrogenated nanotubes and also previously reported ones with bumpy defects [44].

Among the factors to consider in order improving the design of drug delivery systems (DDS) based on CNTs, it is very valuable and useful to know the molecular non-covalent drug-nanotube attraction forces that occur in the adsorption of the drug. Through the knowledge of the way in which the molecular structure of the nanotube favors that interaction, it is possible to predict, as a first approximation, scales of relative reactivity of the nanotubes as drug receptors and provide necessary information to design DDS systems using CNTs with molecular structures that adsorb/encapsulate drugs more efficiently.

Computational Methods for Property Predictions

Within applications in biomedicine, for a systematic approach with minimal risk to patients, a detailed molecular level understanding is required to reduce the potential for adverse effects. From this perspective, computer simulation is a good initial step prior to chemical synthesis and clinical research. Several computational alternatives have been implemented considering CNTs and DOX [31,45-48]. Density Functional Theory (DFT) and molecular dynamics (MD) simulation methods are the main approaches used. Some of them calculate the formation energies or the reactivity descriptors that account for the stability and reactivity of the isolated systems under study and others determine the drug-receptor interaction energies for the conjugates or complexes.

Comparative MD studies of drug-nanotube non-covalent interactions through the MM/PBSA and MM/GBSA methods implemented in the AMBER programs [49], considering the ligand-receptor conjugate solvated by explicit solvent (i.e. water in an octahedral box) proved to be an adequate tool to rank structures indifferent if they have not been evaluated experimentally yet [50]. Note that the mentioned methods have been validated for biological systems, in the prediction of an activity ranking experimentally corroborated [51].

Nanoethics

From a scientific perspective, nanotechnology in biomedicine has several physical, chemical and biological components that must be considered to understand their real advantages and risks. Scientists and the general public believe that applications of nano compounds must consider both the benefits and risks of nanotechnology to achieve an ethical application of such technology.

In this way it has been established that in the nanotechnology development there are at least four categories of products in which the ethical concerns of its use have been concentrated [52]. The first category includes surface coatings such as paints and ceramics; the second includes chemical and biological sensors, and some drugs. The third category includes biological circuit networks, and the fourth includes certain molecular devices for health. For instance, magnetic nanoparticles, bound to a suitable antibody are used to label specific molecules, structures or microorganisms. Specifically, silica nanoparticles are inert from the photophysical point of view and might accumulate a large number of dye(s) within the nanoparticle shell [52]. Also, gold nanoparticles tagged with short segments of DNA can be used for detection of genetic sequence in a sample.

A global regulatory framework is necessary to ensure that new technological materials having an appropriate benefit could be prepared, manipulated and used avoiding unnecessary risks [53]. Scientists and their national and international associations of research working in nanobiotechnology are among the most suitable people for developing such rules. Fortunately, some countries with greater knowledge in this regard (USA, UK, Germany, Switzerland, and China for example) have promoted local classifications, safety standards, and evaluation of associated risk and international institutions like OECD, UNESCO, ISO, and FOE, also contribute to a more comprehensive ethical approach. When countries do not have well-established knowledge about new nanobiotechnologies, the possibility of a good regulation of the eventual negative effects of new innovations diminishes and the risks in their use will be present [54].

Conclusion

Because functionalized CNT display low toxicity and are not immunogenic, they hold great potential in the field of nanobiotechnology and nanomedicine, but it is imperative to fully know the biodistribution and the toxicological and pharmacological profiles of the CNTs in use before undertaking any clinical study. This mini review shows that carbon nanotubes constitute a convenient molecular option for obtaining a good drug delivery system before performing bioassays on animals, indicating that if the chemical or physical parameters study model predicted is appropriate, the system could have a better chance of giving good results and be approved by the corresponding authority that follows the ethical guidelines. In this way, medications for different pathologies could have a better chance of obtaining a good benefit, according to well-established norms.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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