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Carbon nanotubes as delivery systems for respiratory disease: do the dangers outweigh the potential benefits?

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Abstract

Nanoparticle drug-delivery systems offer the potential for improved efficacy of treatment, and yet there are also potential risks associated with these novel therapeutic strategies. An attractive property of carbon nanotubes (CNTs) is that the tube- or fiber-like structure allows for extensive functionalization and loading of cargo. However, a large body of evidence indicates that CNTs may have adverse effects if used in drug delivery as they have been shown to cause pulmonary fibrosis and exacerbate lung disease in rodents with pre-existing lung diseases. Major factors that cause these toxic effects are the high aspect ratio, durability and residual metal content that generate reactive oxygen species. Therefore, careful consideration should be given to the possibility that lung inflammation or fibrosis could be significant side effects caused by a CNT-based drug-delivery system, thereby outweighing any potential beneficial effects of therapeutic treatment. However, functionalization of CNTs to modulate aspect ratio, biodegradability and to remove residual metals could allow for safe design of CNTs for use in drug delivery in certain circumstances.

Keywords

carbon nanotubes; disease risk; drug delivery; health benefits; nanoparticles

Nanotechnology offers significant benefits for improving drug delivery and therapy of respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis. Nanoparticles (NPs) have been of great interest for some time as they can be designed to simultaneously carry a drug payload, specifically target features of diseased tissues and carry an imaging molecule to track drug accumulation and clearance in tissues. Moreover, they can be engineered for more sustained drug delivery to improve pharmacokinetics and reduce the overall amount of drug administered during treatment of disease. A variety of NPs have been investigated in experimental animal models as tools to improve the therapeutic efficacy of drugs or genes delivered to the lung or other organ systems [1]. The nanotechnology platform for drug delivery contains a number of very different types of nanostructures with widely varying properties, including dendrimers,

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fullerenes, polymeric NPs and carbon nanotubes. While nano-based drug-delivery systems offer the potential for improved efficacy of treatment, there are also potential risks associated with these novel therapeutic strategies [2]. Engineered NPs are desirable because they easily enter cells and they can be designed to interact with specific cellular structures (e.g., receptors) to allow accumulation within specific regions of the cell. NPs can also be designed as pH-labile structures so that they degrade within the more acidic microenvironment of the cell to release drug payloads. Biocompatible NPs that are used for drug delivery, such as poly-(ethylene glycol)-block-lactide/glycolide copolymer (PEG-PLGA) NPs, generally have low toxicity but often do not persist in tissues long enough for sustained drug or gene payload delivery. Nevertheless, PEG-PLGA NPs might be useful for delivering metabolic inhibitors of inflammation for the treatment of pulmonary hypertension [3]. Carbon nanotubes (CNTs) are durable and persist in tissues after administration by aerosol inhalation. An attractive property of CNTs, particularly of single-walled CNTs (SWCNTs), is that their tube- or fiber-like structure allows for extensive functionalization and loading of cargo. The versatile physicochemical features of CNTs allow for covalent and noncovalent functionalization to simultaneously carry several agents: a drug, an imaging agent (to track the course of delivery) and a specific targeting agent (e.g., an antibody selective for diseased tissue) [4]. Despite the versatility of CNTs as delivery platforms, some of the same unique properties that make CNTs desirable for therapeutic applications also make them potentially toxic. Thus, the toxic effects of CNTs might ultimately outweigh any beneficial effects as drug-delivery systems. While no information yet exists on the adverse effects of CNTs in humans, growing evidence from *in vivo* exposure studies with rodents and *in vitro* cell culture models demonstrates that CNTs cause numerous adverse effects, including lung fibrosis, exacerbation of pre-existing lung disease, adverse immune reactions and DNA damage. Several recent reviews have overviewed the potential risks and benefits of CNTs [5–7]. This perspective is focused on the risks and potential benefits of CNTs as drug-delivery systems for respiratory disease and the hurdles that would need to be overcome given the growing evidence of pathologic effects in the lungs of rodents. If CNTs are to be ultimately developed as a viable drug-delivery platform, functionalization will be required to ameliorate toxicity, minimize the risk of side effects and target therapeutic agents to the desired organs, tissues or cells. This anticipated transformation of CNTs from a toxic insult to a therapeutic remedy is illustrated in Figure 1. A better understanding of how these nanomaterials interact with biological systems (i.e., the subcellular architecture) at the nanoscale level will be essential [8], along with adequate *in vitro* screening techniques to predict toxicity and thereby allow for safe design of CNTs in drug delivery for lung diseases [9,10].

Evidence for respiratory toxicity of carbon nanotubes

Immune cell reactions

Macrophages are the first line of the innate immune defense system that remove inhaled particles from the lungs [11]. Some evidence indicates that SWCNTs delivered to the lungs of mice escape immune surveillance by macrophages in that they are not readily recognized and taken up by phagocytosis unless they are treated with agents such as phosphatidylserine [12]. However, other evidence indicates that SWCNTs are readily recognized and avidly engulfed by macrophages [13]. The latter study revealed that SWCNTs engulfed by rat alveolar macrophages *in vivo* form bridge structures that linked macrophages. The nature of carbon bridge formation remains to be elucidated but it is possible that the nanoscale width of CNTs might allow for interaction with subcellular structures such as cytoskeletal filaments. Interestingly, actin filaments that comprise the cytoskeleton are approximately the same diameter as SWCNTs and therefore carbon bridge structures might represent a molecular interaction between SWCNTs and actin. Whether or not CNTs are recognized by macrophages probably depends on aggregation status. Functionalization (e.g.,

carboxylation) of CNTs to reduce aggregation may result in evasion of macrophage recognition and uptake (Figure 1). However, because aggregated CNTs are more readily recognized by macrophages, the aggregation status is probably important, determining clearance and removal of CNTs from the lung. CNTs that are engulfed by macrophages are removed from the lung through two principal avenues; the mucociliary escalator and the pulmonary lymphatic system. The mucociliary escalator transports macrophages and their engulfed payload up the airways of the lungs where they are ultimately swallowed or expelled through coughing. Clearance of CNTs through the pleural lymphatic system could lead to adverse effects, such as pleural inflammation and subpleural fibrosis [14]. The pleura is also the site of mesothelioma formation in response to asbestos fibers. Whether or not CNTs behave like asbestos fibers and cause mesothelioma remains unknown. However, several reviews have speculated on the fiber-like similarities of CNTs to asbestos with regard to human disease [15–17]. Macrophage-mediated clearance via pleural lymphatics or via the airway mucociliary escalator can be impeded by particle overload. Recent work has shown that the pathologic effects of inhaled CNTs (Baytubes®) are due to volumetric overload of alveolar macrophages [18]. Therefore, reducing dose as well as reducing other toxic aspects of CNTs (high aspect [length-to-width] ratio, aggregation status and reactivity due to residual metal catalysts), are important factors for consideration of CNTs as a drug-delivery platform. Lymphocyte activation and survival is also important in evaluating the immune response to CNTs. In this regard, relatively low doses of multiwalled CNTs (MWCNTs) have been introduced into cell cultures of Jurkat T lymphocytes with few overt toxic effects, suggesting that low doses of CNTs might be tolerated by the immune system [19]. It is not possible to fully understand the effects of CNTs on the immune system based on *in vitro* studies alone given the complexity of immune reactions. *In vivo* studies are essential in this regard and it has been reported that inhalation exposure of mice to MWCNTs, using relatively low doses of CNTs, produce no significant lung pathology and yet still show a significant effect on splenic immune suppression [20]. In addition, pharyngeal aspiration of SWCNT decreased splenic T-cell proliferation and preincubation of naive T cells *in vitro* with SWCNT-treated dendritic cells also suppressed T-cell proliferation [21]. As with other engineered nanomaterials, the immune response, as well as mechanisms of cellular and biodistribution, are determined by physicochemical properties; in particular, surface characteristics [22]. Therefore, a thorough assessment of immune cell interaction with CNTs before and after functionalization will be essential towards developing a safe design for the purposes of drug delivery.

Fibrogenic reactions

An increasing number of reports indicate that CNTs cause lung fibrosis in rodents. In most of these studies, CNTs were delivered to the lung by intratracheal instillation or pharyngeal aspiration [13,23–26]. These studies showed that micron-sized CNT aggregates stimulated the formation of lung inflammatory and fibrotic foci known as granulomas. Pulmonary fibrosis and/or granuloma was reported in mice or rats within days after intratracheal instillation of SWCNTs [25,26] or MWCNTs [24]. CNTs also stimulated the production of profibrotic mediators such as PDGF and TGF- β 1 [13,25]. Granulomas were associated with micron-sized CNT aggregates easily visible by light microscopy, while interstitial pulmonary fibrosis was associated with dispersed CNTs that could only be visualized by electron microscopy. Aggregated CNTs are not suitable for drug-delivery purposes and a variety of modifications have been introduced to increase the dispersion of CNTs in biological media. However, increased dispersion reduces granuloma formation but apparently does not prevent interstitial fibrotic reactions. CNTs that are more dispersed have an altered deposition pattern and cause more interstitial pulmonary fibrosis and alveolar thickening as opposed to nondispersed CNTs, which cause more focal granulomatous lesions [27]. Inhalation studies in rodents have also been extremely valuable in predicting

and modeling 'real world' exposure scenarios to CNTs. Inhaled CNTs show a more diffuse pattern of deposition in the lungs of mice with less severe focal pathological lesions than those caused by intratracheal instillation and are more likely to produce diffuse fibrotic effects within the lungs [28]. Inhalation studies also produce subpleural fibrosis, while this pathologic effect is not readily observed in instillation or aspiration studies in mice [14]. Therefore, increasing the dispersability of CNTs for drug delivery without further functionalization for biodegradability might not necessarily reduce toxicity, but might simply alter the site of toxicity, and indeed it has been proposed that increasing dispersibility of CNTs could increase the risk of fibrosis [29]. If CNTs are to be used in drug delivery, two major obstacles that are likely to determine toxicity must be overcome. First, the length of CNTs must not exceed several micrometers as fibers longer than 10–15 μm (the approximate width of an alveolar macrophage) are difficult to clear from lung tissues via macrophage-mediated mechanisms. Second, the composition of CNTs must be carefully considered. Metals such as nickel, cobalt and iron are commonly used as catalysts in the manufacture of CNTs and can be present as impurities in pristine (i.e., unmodified) CNTs. These metals are well known to cause pulmonary diseases in humans including pulmonary fibrosis, asthma or cancer. Thus, if CNTs are to be used for drug delivery, an intelligent design must include a thorough consideration of multiple factors such as removal of residual metal, increasing biodegradability, controlling dispersibility and modifying aspect ratio.

Asthma exacerbation

Individuals with pre-existing respiratory diseases such as asthma, bronchitis and COPD represent susceptible populations to CNT exposure (Figure 2). However, these are also some of the same individuals that might benefit from improved drug-delivery strategies using nanotechnology. Several issues should be considered when delivering NPs to individuals with pre-existing airway disease. For example, altered airway flow in patients with obstructive lung disease may increase airway deposition and relative concentration of inhaled NPs in these subjects compared with healthy individuals [30]. In addition, the physicochemical characteristics of CNTs may be affected by the presence of mucus and cellular debris in diseased airways that could change the aggregation status and surface charge or surface area. Also, individuals with asthma or COPD have impaired mucociliary clearance and therefore might not be expected to remove CNTs as effectively as healthy individuals. Finally, the presence of activated inflammatory cells and structural airway cells may alter the response to NPs. As a case in point, airway fibrosis in mice is increased by the combination of ovalbumin allergen and MWCNT, whereas ovalbumin or MWCNT alone do not significantly increase airway fibrosis [31]. This study suggested that CNTs pose a hazard to individuals with allergic lung inflammatory diseases such as asthma. Another report indicated that intratracheally administered MWCNT significantly increased ovalbumin-induced T-lymphocyte proliferation and amplified lung Th2 cytokines and chemokines compared with ovalbumin exposure alone [32]. More recent work by these investigators suggested that SWCNT also can exacerbate murine allergic airway inflammation via enhanced activation of Th2 immunity and increased oxidative stress, and that this exacerbation may be partly through the inappropriate activation of antigen-presenting cells, including dendritic cells [33]. MWCNTs delivered to the lungs of mice may also induce allergic responses through B-cell activation and production of IgE in the absence of any allergen pre-exposure [34]. SWCNT also promote ovalbumin-induced allergic immune responses in the lungs of mice [35]. Finally, a recent study demonstrated that MWCNTs delivered to the lungs of mice impair airway function [36]. Therefore, studies from several different laboratories indicate that CNTs exacerbate allergic airway inflammation in rodents, suggesting that CNTs are not likely to be a viable option for drug delivery in asthma unless extensive functionalization can overcome the effects on allergic airway diseases described in these reports. It is unknown if CNTs will cause or exacerbate asthma in humans. However,

there is a correlation between particulate air pollution (especially the ultrafine or nanoscale fraction) and the incidence and severity of asthma attacks [37]. Moreover, pulmonary exposure to nanometals exacerbates airway hyper-responsiveness in mice [38]. Therefore, unless CNTs can be functionalized to overcome these effects, they will probably not be viable delivery platforms for asthma therapy. However, other NPs, particularly fullerenes, have been shown to inhibit allergic airway inflammation in mice [39] and should be further explored as a drug-delivery system for the treatment of asthma.

Respiratory bacterial infection

Bacterial infections are a common cause of acute lung injury and sepsis, and individuals with pre-existing microbial infection also represent a possible target population for CNT exposure (Figure 2). Therefore, if CNTs are to be used for delivery platforms for these types of lung diseases, then it is important to know how CNTs will interact with bacteria or bacterial products. Lipopolysaccharide (LPS), a component of Gram-negative bacteria cell walls, is ubiquitous in the environment and has been implicated in a number of occupational and environmental lung diseases in humans, including bronchitis, COPD and asthma. SWCNTs or MWCNTs have been shown to exacerbate lung inflammation, pulmonary vascular permeability and lung expression of proinflammatory cytokines induced by LPS [40]. Pre-exposure to LPS also exacerbates the fibrogenic potential of MWCNT delivered to the lungs of rats [41]. In this study, LPS alone did not cause fibrosis when delivered by intranasal aspiration but significantly increased MWCNT-induced fibrosis and levels of PDGF, a mitogen and chemo-attractant for mesenchymal cells that promotes the development of pulmonary fibrosis. The precise mechanism through which LPS enhances CNT-induced lung fibrosis remains to be elucidated. The interaction between CNTs and microbial infection has also been investigated. Mice given SWCNT by aspiration and then infected with *Listeria monocytogenes* had increased severity of pulmonary inflammation compared with mice that received either SWCNTs or bacteria alone [42]. This study provided evidence that microbial infection is an important susceptibility factor when considering environmental, occupational or medicinal exposures to nanomaterials. Future studies should also focus on viral infection as a possible susceptibility factor in exposure to nanomaterials since pulmonary responses to airborne particulate matter are exacerbated by viruses such as respiratory syncytial virus or influenza [43]. On the other side of the coin, it may be important to consider functionalized CNTs as antiviral agents or for the treatment of infectious diseases such as TB.

Extrapulmonary effects

CNTs could have adverse affects on distant organ systems other than the lung. For example, SWCNTs or MWCNTs deposited into the lung have been shown to induce or exacerbate acute cardiovascular dysfunction and disease [44]. SWCNTs delivered to the lungs of mice by intrapharyngeal aspiration induced heme-oxygenase-1, a biomarker of oxidative stress, and caused aortic mitochondrial DNA damage [45]. Moreover, this same study showed that *ApoE*-deficient mice exhibited accelerated atherosclerosis when fed an atherogenic diet. Delivery of CNTs to the lung also affects immune reactions in the spleen. As mentioned previously in the discussion of immune cell reactions, inhaled MWCNTs have been reported to cause systemic immunosuppression and splenic oxidative stress [20]. The mechanism involves the release of TGF- β 1 from the lungs of MWCNT-exposed mice, which enters the bloodstream to signal cyclooxygenase-2-mediated increases in prostaglandin-E₂ and IL-10 in the spleen, both of which play a role in suppressing T-cell proliferation [46]. This same study used a relatively low dose of CNTs delivered by inhalation and a low level of lung inflammation was observed. However, even a relatively low concentration of inhaled MWCNTs had a significant effect on the systemic immune response.

Carcinogenicity

Some studies have shown that CNTs have carcinogenic potential. SWCNTs have been shown to cause fragmented centrosomes, multiple mitotic spindle poles, anaphase bridges and aneuploid chromosome number in cultured primary or immortalized human airway epithelial cell types [47]. This work was the first to show disruption of the mitotic spindle by SWCNTs and the authors noted that the similar size and geometry of SWCNT bundles might account for physical interaction of SWCNTs with the microtubules that form the mitotic spindle. MWCNTs can accumulate and induce apoptosis in mouse embryonic stem cells and activate the tumor suppressor protein p53, a marker of DNA damage [48]. Therefore, both SWCNTs and MWCNTs can exert genotoxic effects in certain *in vitro* systems. More recent studies demonstrated that CNTs can cause genotoxic effects *in vivo*. Long and thick MWCNTs caused greater DNA damage and inflammation than short and thin MWCNTs, or SWCNTs, in mice [49]. Repeated intraperitoneal injections of nonfunctionalized and COOH-functionalized MWCNTs to mice caused dose-dependent genotoxic effects of structural chromosome aberrations, DNA damage and micronuclei formulation [50]. In this study, the incidence of genotoxic effects was higher for COOH-functionalized MWCNTs. However, other evidence indicates that polyethylene glycol-coated SWCNTs reduce toxicity in neuronal PC12 cells [51]. Therefore, increased or decreased toxicity due to modification of CNTs depends on the specific type of functionalization. Since genotoxicity plays a role in the development of cancer, high-throughput screening to identify markers of DNA damage should be implemented in order to better predict the carcinogenicity of a wide variety of functionalized CNTs.

Pleural disease

A possible side effect of using CNTs in drug delivery in the lung may be localization and retention at the pleura, which is the site of mesothelioma formation. The durable nature of CNTs along with their fiber-like shape could pose a problem of long-term lung persistence, which might result in asbestos-like behavior and carcinogenicity (i.e., mesothelioma). Injection of long MWCNTs into the peritoneal cavity of mice (a surrogate for the pleura) was shown to cause inflammation, suggesting that MWCNTs have asbestos-like pathogenicity [52]. The authors demonstrated that long MWCNTs, but not short MWCNTs, caused granuloma formation at the pleura similar to that caused by long asbestos fibers. It was further demonstrated that MWCNTs inhaled by mice reached the pleura (the anatomic site of mesothelioma) and caused significant increases in pleural fibrosis [14,53]. MWCNTs have since been found to penetrate the pleural lining and remain in the pleural region for weeks after exposure [54]. The aspect ratio of CNTs also plays a role in determining toxicity, as the pathogenicity of long CNTs is due to length-dependent retention at the pleura [55]. However, no indication of mesothelioma was found in any of these studies with MWCNT. Longer-term, low-dose studies with CNTs are required to evaluate pleural carcinogenicity. Whether CNTs cause mesothelioma is a major issue that must be addressed if CNTs are to be used in drug delivery.

Discussion & conclusion

Engineered nanomaterials, including CNTs, represent novel drug-delivery platforms. CNTs are a 'double-edged sword' in that they can be highly functionalized for drug delivery and yet their fiber-like shape or high aspect ratio makes them similar in some ways to asbestos fibers, which are a known cause of fibrosis and mesothelioma in humans. Therefore, the potential risks of CNTs should be carefully considered when designing nanoscale drug-delivery platforms. The inflammatory and fibrogenic responses due to oxidative stress induced by CNTs in the lung suggest that their medical applications may be limited [56]. Moreover, as described above, there are numerous physicochemical factors (e.g., aspect

ratio, surface charge and biodegradability) that need to be taken into account in the design of a functional and safe CNT for drug delivery [57]. Owing to the infancy of the field of nanotechnology, there are no epidemiologic data to indicate health hazards for the majority of nanomaterials, including those that could be used for drug delivery. CNTs contain nanosized metals (e.g., nickel and cobalt) that are present as residual catalysts and many of these metals in their native form are known to have fibrogenic, allergic or carcinogenic effects in humans. Moreover, some CNTs have shown inflammatory, immune and/or fibrogenic effects in the lungs of mice and rats, indicating that caution should be taken in the handling of these materials. The durability of CNTs could prolong drug delivery, but also presents a problem in that it promotes chronic lung disease. Therefore, functionalization of CNTs to render them more biodegradable will probably be necessary for drug delivery and reducing toxicity. Recent studies have shown that enzymes such as human neutrophil myeloperoxidase or horse-radish peroxidase are capable of degrading CNTs and reducing toxic effects [58,59]. The development of nanotechnology-based therapeutics, including those based on CNTs, will be an ongoing challenge owing to the anticipated evolution of nano-specific regulatory guidelines and safety testing protocols. However, this challenge is expected to be overcome as experience is gained and quality physicochemical and toxicity data become more widely available, thus leading to the development of effective nanotechnology-based therapies for a range of respiratory diseases.

Expert commentary

If CNTs are to be used for drug delivery, careful consideration should be given to the possibility that respiratory diseases or systemic immune responses could be caused by the CNT-delivery system itself, thereby outweighing any potential beneficial effects of therapeutic treatment of an existing disease condition. The majority of research related to CNTs and drug delivery are focused on SWCNTs rather than MWCNTs. The latter have received considerable attention owing to their similarities to asbestos fibers, whereas SWCNTs are more flexible and therefore are more likely to fold and tend not to form rigid structures that would persist in tissues for months. However, it remains unknown how long either SWCNTs or MWCNTs remain in human tissues after inhalation exposures. Studies with mice show that MWCNTs remain in lung tissues for several months [14]. It would be desirable to have CNTs designed through functionalization to have pharmacokinetic properties that would allow for payload delivery and then biodegradation or efficient clearance from tissues. Simply dispersing CNTs in surfactant does not appear to prevent lung fibrosis but seems to result in more diffuse alveolar thickening rather than focal lesions caused by aggregated CNTs [60]. For effective therapy, it is desirable to have an NP-delivery system that remains in the target tissues long enough to achieve sustained and targeted release of the drug, but NP persistence in tissues for too long will increase the possibility of toxic side effects and pathogenesis. Biodegradable NPs are currently considered to be the best choice for targeted drug delivery but biopersistent NPs might also be considered valuable if they are cleared from tissues quickly enough to minimize exposure and retention [61]. The latter would exclude high-aspect-ratio NPs, such as CNTs. It would be ideal to design functionalized CNTs for drug delivery that also have a limited half-life in tissues – that is, are less biopersistent and therefore pose less of a threat for chronic side effects such as tissue fibrosis. However, it is also possible that if functionalized CNTs are degraded too quickly then they might not be effective as drug-delivery platforms. Durability and degradation half-life must therefore be carefully considered in the intelligent design of functionalized CNTs in order to maximize beneficial pharmacologic effects while minimizing pathogenic side effects. Controlling the aspect ratio of CNTs would also likely determine persistence, as has been shown for other fiber-like materials. Furthermore, increasing solubility and decreasing aggregation status through functionalization will probably be important for developing CNTs as delivery platforms. Some of these issues are

highlighted in Figure 1. The big question is: can functionalization of CNTs overcome respiratory toxicity? While this remains to be determined, the most likely benefit of CNTs in disease therapy in the near future will be derived from their use in treating cancer. Tumor-targeted SWCNT constructs have been synthesized from water-soluble CNTs by covalent attachment of tumor-specific monoclonal antibodies [62]. Moreover, this strategy may be used along with thermal ablation of cancer cells by selectively heating CNTs taken up by cancer cells with infrared radiation [63]. While fibrotic reactions may still be a risk of these functionalized CNT platforms in some patients, overall, the benefits could outweigh the risks. Other issues will also ultimately determine the potential success of treatment strategies that might utilize CNTs, such as the cost of CNTs versus current biopolymers or other types of NPs. In the words of Paracelsus (1493–1541), “the right dose differentiates a poison and a remedy” [64]. The success of CNTs in drug delivery will be achieved by using the lowest possible dose combined with single tube dispersability, functionalization to target and/or carry a therapeutic agent and biodegradability to avoid long-term chronic side effects.

Five-year view

The literature on the toxicity of CNTs will no doubt continue to grow and it is anticipated that new information will emerge on the cellular and molecular mechanisms of action through which CNTs cause disease, especially in susceptible populations. It is possible that some of the first evidence of lung diseases from CNT exposure will be discovered in humans; probably as a result of occupational exposure. Diseases will most likely manifest as a pneumoconiosis (reaction to inhaled reactive particles or dust), as is the case for diseases such as asbestosis or silicosis. If this is the case, then onset might not be diagnosed for 5–20 years after exposure. If mesothelioma is an outcome of CNT exposure, then a longer time frame of onset – that is to say decades – would be anticipated. On the drug-discovery side of the coin, it is anticipated that new advances in nanoengineering will probably result in the design of less toxic CNTs with potential for therapeutic value. CNTs used for drug delivery will probably be less biopersistent than their more durable counterparts, have single-tube dispersability, and will be ‘cleaned’ of residual metals or perhaps synthesized with less toxic metals. Therefore, these highly functionalized CNTs will less likely pose chronic side effects in the lungs or other organs as compared with their crude original counterparts. It is not likely that CNTs will be used for chronic respiratory disease such as asthma, which requires repeated treatments over years, owing to growing evidence that CNTs exacerbate allergic disease, unless extensive functionalization can overcome this problem. Other biodegradable NP-based therapies will probably be very useful for the treatment of asthma and other chronic airway diseases. Since it has been shown that CNTs can kill cancer cells upon heating with infrared laser radiation, it is possible that promising advances will develop in the treatment of lung cancer through a CNT-based platform and this benefit may outweigh the adverse side effect of fibrosis, if minimized, and prolong the life span of patients with lung cancer. Along the same lines, it would be interesting to explore the utility of CNTs for the treatment of infectious diseases such as TB. Concerted efforts of multiple disciplines, including engineering and toxicology, will be required to realize a goal of safe nanotechnology in medicine.

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Key issues

- Nanoparticle delivery systems have great potential for the treatment of respiratory diseases, including asthma, chronic obstructive pulmonary disease and cancer.
- Numerous adverse effects have been documented in the toxicology literature for carbon nanotubes (CNTs), including pulmonary fibrosis, asthma exacerbation, suppressed immunity and genotoxicity.
- Susceptibility factors must be considered and it is essential to thoroughly examine the interactions between nanomaterial exposures and infectious agents or pre-existing diseases.
- Individuals with pre-existing respiratory diseases such as asthma or chronic obstructive pulmonary disease could have altered airway deposition pattern for CNTs or impaired clearance of CNTs.
- Evidence from studies with rodent models suggests that the health risks from commercially available CNTs outweigh many or most of the potential benefits for drug delivery or imaging in the lung.
- Functionalization could remedy some or all of the adverse effects by increasing biocompatibility, evading macrophage uptake and allowing for biodegradation of CNTs without compromising efficacy of drug delivery.
- Increased dispersion of CNTs will be necessary for drug development, but dispersion alone without other types of functionalization could represent a special hazard by increasing the severity of a lung fibrogenic response or altering focal lesions to more widespread interstitial fibrosis.
- CNTs are less likely to be viable as a potential drug-delivery platform for allergic diseases such as asthma, but will likely be more useful for targeting certain diseases such as lung cancer or possibly difficult-to-treat infectious diseases, such as TB.

Figure 1. Hypothetical outcomes of altering the physicochemical properties of carbon nanotubes for drug delivery

Inhaled pristine CNTs aggregate and are taken up by macrophages, where they stimulate ROS generation and activation of the inflammasome to produce IL-1 β , a proximal mediator of pulmonary fibrosis. Purifying CNTs to remove residual metal catalysts reduces ROS, macrophage activation and fibrosis. Functionalization of CNTs to increase dispersability and modify biodegradation would be needed for drug delivery and could result in evasion of macrophage uptake and increased bioavailability. Further functionalization could involve attachment of therapeutic agents and allowing for cell-specific targeting. The ultimate success of using CNTs as a drug-delivery platform would depend on the risk of side effects (fibrosis or cancer) in relation to effective therapy of a particular disease.

CNT: Carbon nanotube; ROS: Reactive oxygen species.

Figure 2. Postulated susceptibility factors to carbon nanotube exposure and associated pathologic or physiologic consequences

Mouse models of susceptibility have been used to demonstrate that pre-existing inflammation induced by allergens to induce experimental asthma, bacterial wall products or bacterial infection increase lung fibrogenic reactions to CNTs. Factors that influence CNT-induced exacerbation of pre-existing disease include reduced mucociliary clearance of CNTs in asthma or COPD, and enhanced inflammation by CNTs when combined with microbial infection. Susceptibility genes also probably play an important role in determining severity of disease outcome in response to CNTs.

CNT: Carbon nanotube; COPD: Chronic obstructive pulmonary disease.