Interactions of Carbon Nanotubes with the Immune System: Focus on Mechanisms of Internalization and Biodegradation

Bengt Fadeel[a, b] · Anna A. Shvedova[c, d] · Valerian E. Kagan[e]

[a]Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, and [b]Childhood Cancer Research Unit, Department of Women's and Children's Health, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; [c]Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, [d]Department of Physiology and Pharmacology, West Virginia University, Morgantown, W. Va., and [e]Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, Pa., USA

Abstract

Carbon nanotubes (CNT) are cylinders of one or several coaxial graphite layer(s) with a diameter on the nanometer scale. CNT can be readily functionalized, and exciting studies on the use of CNT as excipients for drug delivery and imaging of disease processes have been reported. On the other hand, CNT were also shown to induce oxidative stress, inflammation, and fibrosis, and animal studies have suggested similarities between the pathogenic properties of certain multiwalled CNT and those of asbestos fibers. Recent studies have disclosed that CNT are susceptible to enzymatic biodegradation, and this observation could hold the key to the safe application of these nanomaterials in biomedicine. Here, we provide a brief overview of pertinent toxicological and biomedical investigations of CNT including recent work on the interaction of CNT with immune-competent cells, focusing on cellular recognition of nanotubes and their enzymatic degradation.

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Nanotechnology presents many opportunities and benefits for new materials with significantly improved properties as well as revolutionary applications in the fields of energy, environment, and medicine. Carbon nanotubes (CNT) are among the most studied nanomaterials to date and are currently of interest for a variety of uses in technological as well as biomedical applications, including as drug delivery devices and contrast agents in medical imaging. At the same time, there is considerable concern about the potential adverse effects of these materials, and several recent studies have pointed to asbestos-like properties of CNT. It is important to note that 'CNT' come in many flavors, and careful physicochemical characterization of each sample is required. Indeed, some CNT may very well be harmful to human health, and exposure should be avoided, whereas other CNT preparations could be harnessed for biomedical use.

Fig. 1. CNT – engineered nanomaterials with Janus-like properties. CNT have been reported to exert toxic effects in cell culture as well as in vivo. This schematic diagram summarizes some of the toxicities of CNT reported to date. On the other hand, CNT are readily functionalized and this may result in enhanced dispersibility and improved biocompatibility, making these materials versatile and attractive for various biomedical applications. Selected examples of pharmaceutical and biomedical applications of functionalized CNT (f-CNT) are depicted. Adapted from Shvedova et al. [3] with permission from Elsevier.
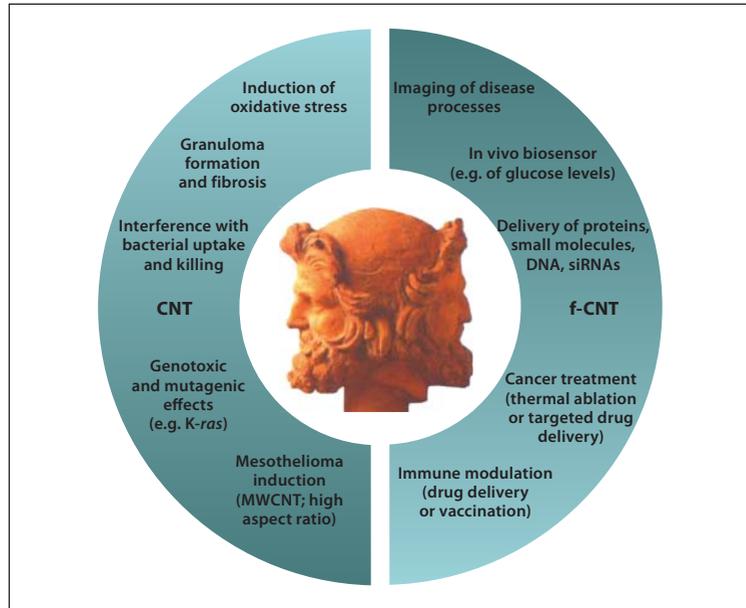
Paradoxically, the novel characteristics of nanomaterials that are essential for successful and innovative applications might also lead to negative health impacts [1, 2] (fig. 1). Therefore, toxicological investigations are needed to determine the relationship between the physicochemical properties of nanoparticles and their cellular interactions, reactivity, and biological/systemic consequences. In particular, it may be important to understand how nanoparticles including CNT interact with or interfere with immune-competent cells and whether the immune system is capable of handling nanomaterials or nanomaterials simply 'fly under the radar' and thus escape immune surveillance. This could have considerable implications for the biodistribution of nanomaterials in the body. Furthermore, studies on the biopersistence or biodegradability of nanomaterials, and in particular of CNT, are also of importance. Nanomaterials intended for biomedical use should be either excretable or degradable (or biologically inert, but few materials are inert in the long term) as they may otherwise accumulate in tissues and exert undesirable effects.

Toxicological Studies

Considerable attention has been focused on in vitro (cell culture) assessment of CNT toxicity. Several groups have reported that exposure to single-walled CNT (SWCNT) or multiwalled CNT (MWCNT) causes cytotoxicity in various cell lines [for a review, see 3]. Importantly, we and others have reported low cytotoxicity for purified CNT (low iron content) versus raw, nonpurified CNT which were found to induce oxidative stress [4, 5], thus leading to the conclusion that metal traces associated with the nanotube samples are responsible for the toxicological effects. Recently, several investigators have cautioned that CNT can interfere with commonly used dye-based viability assays [6, 7]. These findings strongly suggest verifying cytotoxicity data using independent test systems. Moreover, evidence has been provided that an increase in the agglomeration state of CNT appears to be related to the cytotoxicity of these materials [8]. Therefore, it may be prudent to conduct in vitro cytotoxicity testing of CNT using well-dispersed suspensions. In sum, if one consid-

ers interference with dye-based viability assays, agglomeration issues related to the method of dispersion, and oxidative stress due to metal impurities, the available data favor the conclusion that well-dispersed, purified CNT exhibit relatively low cytotoxicity in vitro. When it comes to in vivo studies, however, one has to also consider the context of exposure: needless to say, for any material that is intended for biomedical application (i.e. intentional exposure), safety issues are of utmost importance, and the materials to be administered should be well defined in terms of composition, dispersion, and so on. On the other hand, in the case of unintentional exposure in the occupational setting, the issue of whether CNT samples are purified or nonpurified, or well dispersed versus entangled, is somewhat academic: the material is what it is, and exposure may or may not occur. Hence, it may be argued that studies conducted on as-produced CNT are as important as studies on purified or functionalized samples. This distinction between safety assessment in the biomedical setting versus the assessment of the potential risk for workers or consumers accidentally exposed to CNT (or, for that matter, any other class of engineered nanomaterial) is often overlooked.

Pulmonary toxicity of SWCNT has been reported in several studies [9–12]. The inflammatory response to SWCNT delivered to the lung via pharyngeal aspiration is characterized by a brisk acute phase response followed by an early onset of lung fibrosis [12]. Some argue that intratracheal or pharyngeal aspiration of SWCNT in a single bolus dose is an artificial exposure. Moreover, aspiration/intratracheal studies reported thus far have utilized relatively high dose exposures, which may not be relevant to the chronic low dose exposure occurring in an occupational setting. Inhalation of SWCNT more closely mimics occupational and environmental scenarios. The recent application of a specially designed aerosolization technology resulted in the ability to obtain stable SWCNT aerosols and has enabled studies of the inhalation of nonpurified SWCNT (iron content

of 17.7% by weight) at 5 mg/m^3, 5 h/day, for 4 days versus pharyngeal aspiration of varying doses (5–20 μg/mouse) of the same SWCNT. The chain of pathological events in both exposure routes was realized through interactions of an early inflammatory response and oxidative stress culminating in the development of multifocal granulomatous pneumonia and interstitial fibrosis [13]. SWCNT inhalation was more effective than aspiration in causing inflammatory response, oxidative stress, collagen deposition, and fibrosis as well as mutagenic effects in the lungs of C57BL/6 mice [13]. The observed toxic outcomes of exposure of C57BL/6 mice to aerosolized respirable SWCNT (5 mg/m^3, 5 h/day, 4 days) resulted in a calculated lung burden of 5 μg/mouse. This lung burden would be achieved by workers exposed for less than 1 year at the peak airborne concentrations measured in an occupational setting [14].

The pathogenic fiber paradigm predicts that fibers that are long and thin (high aspect ratio) and biopersistent are more pathogenic than short fibers [15]. Interestingly, in a recent study so-called nanoscale dispersions of SWCNT were produced using a biocompatible block copolymer, Pluronic F 108NF (Pluronic) [16]. The well-dispersed CNT did not induce granulomas or fibrosis in the airways of mice, and the authors concluded that the toxicity of SWCNT is attributable to aggregation of the nanomaterial rather than the high aspect ratio of the individual nanotubes.

Animal studies of MWCNT have not produced consistent results. The pulmonary toxicity of MWCNT in rats exposed by intratracheal instillation to intact or ground (short) MWCNT showed that both MWCNT samples caused acute pulmonary inflammation at 3 and 15 days postexposure and pulmonary fibrosis at 60 days postexposure [17]. Others have reported that inhalation exposure to MWCNT does not induce significant lung toxicity but does cause immunosuppression [18]. These discrepancies may be attributed to differences in the CNT samples tested and rodent species used and differences in observation period

in the two different studies. Even more controversial is the question of whether CNT may possess asbestos-like pathogenic properties [15]. Poland et al. [19] reported that abdominal injection of long but not short MWCNT caused an inflammatory reaction of the abdominal lining in mice which was similar to that seen after exposure of mice to asbestos. Takagi et al. [20] reported that MWCNT trigger mesothelioma formation when administered intraperitoneally to p53 heterozygous mice that have been reported to be sensitive to asbestos. However, high (nonrealistic) doses were used. Others have reported that MWCNT do not elicit any carcinogenic response in a 2-year bioassay in the peritoneal cavity of the rat [21]. The length of the CNT samples tested was suggested to be an important factor in determining the pathogenic potential of these materials.

Recent, important studies have provided evidence that MWCNT reach the subpleura in mice after a single inhalation exposure of 30 mg/m^3 for 6 h. Nanotubes were embedded in the subpleural wall and within subpleural macrophages. Subpleural fibrosis increased after 2 and 6 weeks following inhalation. None of these effects was seen in mice that inhaled carbon black nanoparticles or a lower dose of CNT (1 mg/m^3) [22]. Notwithstanding, an issue which remains is whether the acute responses to MWCNT would persist and progress to mesothelioma (there is a lag time of up to 30 years for mesothelioma formation in humans exposed to pathogenic asbestos fibers).

Cellular Uptake of Carbon Nanotubes

Cellular uptake is an important factor in determining the toxicity of engineered nanomaterials. Long CNT may induce 'frustrated phagocytosis' in macrophages, while short CNT appear to be handled much more effectively by the immune system [19]. Internalization of CNT by cells is also a critical determinant in different biomedical applications such as the delivery of therapeutical-

ly active molecules, including proteins, peptides, and genes. Functionalization of SWCNT and MWCNT using the 1,3-dipolar cycloaddition reaction, thus rendering the CNT water soluble, allows for the internalization of CNT by a wide range of cell types [23]. Porter et al. [24] reported that human monocyte-derived macrophages are able to internalize SWCNT; however, the process apparently took between 2 and 4 days, a long time for a macrophage to ingest a meal. Nevertheless, careful imaging revealed that the majority of SWCNT were located within phagosomes and lysosomes, suggestive of active, phagocytic uptake of the nanotubes. Some SWCNT were also found to translocate across the membrane into the cytoplasm [24]. Indeed, it has been proposed that CNT could penetrate the lipid bilayer by virtue of their 'needle-like' structure. The notion of spontaneous piercing through the membrane induced only by thermal motion was tested in a recent study in which the energy cost associated with the insertion of a CNT into a model phospholipid bilayer was calculated using the single-chain mean field theory [25]. The authors arrived at the conclusion that the energy cost of the bilayer rupture is high compared to that of the energy of thermal motion, which may support other energy-dependent translocation mechanisms, such as endocytosis. In a recent study, Neves et al. [26] reported that oxidized double-walled CNTs wrapped in RNA are taken up by prostate and cervical cancer cell lines in vitro but are then released after 24 h without discernable cellular stress. The mechanism of exocytosis of CNT remains to be understood.

Cells that are programmed to die by apoptosis typically expose the anionic phospholipid, phosphatidylserine (PS) on their surface and this serves as a specific recognition signal for neighboring phagocytes. Utilizing the same principle, we have recently shown that coating of SWCNT with PS makes the nanotubes recognizable in vitro by primary monocyte-derived human macrophages and dendritic cells [27]. Cellular uptake was suppressed by the PS-binding protein An-

nexin V, demonstrating that uptake was specific. In line with this, in vivo aspiration of PS-coated SWCNT stimulated their uptake by lung alveolar macrophages in mice, while SWCNT coated with the phospholipid phosphatidylcholine, which is not specifically recognized by phagocytes, were not as effectively taken up. Furthermore, Dutta et al. [28] reported that CNT avidly adsorb albumin when suspended in serum-containing medium. Upon binding to the surface of CNT, albumin is structurally altered and is recognized by scavenger receptors, which could facilitate the cellular uptake of CNT. Taken together, it seems apparent that the presence or absence of specialized signals determines the recognition and subsequent interactions of CNT with cells. Overall, pristine CNT are poorly recognized, whereas chemically modified CNT or CNT with a corona of adsorbed macromolecules (e.g. proteins and lipids) are more readily recognized and engulfed by cells.

Biodegradation of Carbon Nanotubes

Nondegradable nanomaterials can accumulate in cells and tissues where they can exert detrimental effects. Indeed, it has been shown that intravenously injected pristine (nonfunctionalized) SWCNT are highly enriched in the liver, lung, and spleen in mice and remain in the body over an extended period of time [29]. Controlling the biodistribution of CNT through surface functionalization will be instrumental in transforming these materials into candidates for drug delivery and in vivo imaging. Enzymatic degradation of SWCNT was recently demonstrated by incubating SWCNT in a cell-free system with horseradish peroxidase (HRP) and low amounts of H_2O_2 [30]. Others have reported that SWCNT with carboxylated surfaces undergo a 90-day degradation in a phagolysosomal simulant [31]. Unmodified, ozone-treated, and aryl-sulfonated nanotubes did not degrade under the same conditions. Oxidative HRP-dependent degradation was also report-

ed recently for MWCNT after 2 months, although not to completeness [32]. In contrast, we have demonstrated complete biodegradation of CNT by human myeloperoxidase (hMPO) [33]. Biodegradation was shown in a cell-free system but we also provided evidence for hMPO-driven biodegradation in neutrophils isolated from normal healthy donors (fig. 2). Degradation occurred within 12 h. Macrophages were less proficient at biodegrading SWCNT, in line with the fact that these cells express much lower amounts of hMPO when compared to neutrophils. Importantly, SWCNT fully biodegraded by hMPO in vitro did not elicit typical inflammatory and oxidative stress responses characteristic of CNT after pharyngeal aspiration in mice [33]. These results open new opportunities for controlled regulation of CNT in an occupational or biomedical setting through natural (enzymatic) degradation by immune-competent cells (neutrophils).

Biomedical Applications of Carbon Nanotubes

Several recent developments, particularly in the synthesis of chemically modified CNT resulting in their enhanced 'solubilization' (dispersibility) and improved biocompatibility, make them more versatile and show potential pathways for their utilization in medicine [34]. Due to their strong optical absorbance, CNT have become candidates for biophysical approaches to destroy cancer cells using, for instance, hyperthermia. In principle, functionalization of CNT with tumor-specific epitopes followed by uptake only by cancerous cells affords an opportunity to locally heat the CNT using infrared laser radiation, resulting in thermal destruction of cancer cells without harm to surrounding cells. Using this approach, Kam et al. [35] showed that SWCNT functionalized with folic acid were specifically internalized by cancer cells expressing folic acid receptors on their surface. Hyperthermic treatment resulted in the killing of cancer cells in vitro without harm to by-
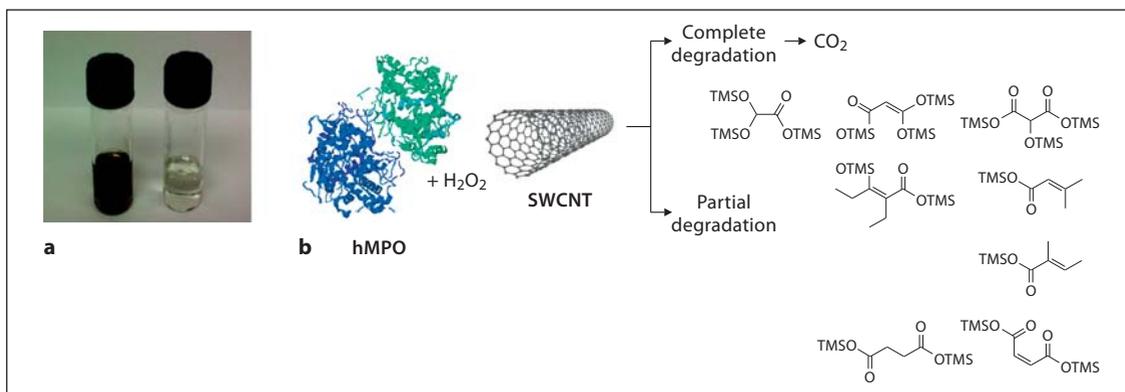
Fig. 2. Biodegradation of CNT. Recent studies have provided evidence of enzymatic degradation of SWCNT following incubation with HRP or hMPO. Detailed analysis of the biodegradation products showed that biodegradation produces CO_2. **a** Photograph showing carboxylated SWCNT (left) which were biodegraded upon 10 days of incubation with HRP and H_2O_2 (right). **b** Schematic of the biodegradation of SWCNT by neutrophil-derived hMPO. The chemical formulas depict short-chained tri-, di-, and monocarboxylated alkanes and alkenes. Reprinted from Kagan et al. [40] with permission from the American College of Occupational and Environmental Medicine.

stander cells. Similarly, Chakravarty et al. [36] used a combination of antibody-directed targeting and thermal ablation to demonstrate that SWCNT can be used for selective killing of human B lymphoma cells in vitro. Podesta et al. [37] reported that amino-functionalized MWCNT could be used to deliver therapeutic siRNA sequences in mice bearing a human lung carcinoma xenograft.

CNT have a tunable near-infrared emission that responds to changes in the local dielectric function but remains stable to permanent photobleaching. Barone et al. [38] reported the synthesis of solution phase, near-infrared sensors, with glucose sensing as a model system, using SWCNT that modulate their emission in response to the adsorption of specific biomolecules. By inserting a capillary tube containing such functionalized CNT into human epidermal tissue ex vivo, they could show that the CNT senses glucose concentrations in the range commonly found in the blood of diabetic patients. CNT could thus potentially serve as biosensors as they emit fluorescence in the near-infrared, a region of the spectrum not occupied by other organic molecules and where tissue is transparent.

Photoacoustic imaging has the potential to image organs in vivo with simultaneous high contrast and high spatial resolution, and De la Zerda et al. [39] have provided recent evidence that SWCNT conjugated with arginine-glycine-aspartic acid-containing peptides can be used as a contrast agent for photoacoustic imaging in tumor-bearing mice. Importantly, a greater spatial resolution was achieved by photoacoustic imaging using CNT as compared with fluorescence imaging using tumor-targeted quantum dots. These elegant preclinical studies suggest new approaches to noninvasive imaging and monitoring of disease processes, taking advantage of the specific physicochemical properties of CNT.

Concluding Remarks

CNT have been ascribed various toxic effects in vitro and in vivo, and some studies have even indicated similarities between certain CNT (multiwalled, high aspect ratio) and asbestos fibers. On the other hand, recent studies have also demonstrated that CNT could potentially be harnessed

for biomedical purposes, as vectors for drug delivery, and for imaging. The important task that lies ahead will thus be to determine which physicochemical characteristics of CNT drive the toxic responses and to utilize this knowledge in the design of CNT that are biocompatible and safe. Furthermore, some of the unwanted responses of CNT may, in fact, lead to desirable outcomes depending on the specific context. For instance, the induction of CNT-dependent cellular toxicity could serve as a novel and effective approach to combat cancer cells, provided that the excretion or biodegradation of CNT can be controlled. Overall, a detailed understanding of the pharmacological and toxicological properties of CNT and a critical and balanced evaluation of risk/benefit ratios is required when considering the medical applications of these novel materials.

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The authors have nothing to disclose.

Disclaimer

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References

1 Shvedova AA, Kagan VE, Fadeel B: Close encounters of the small kind: adverse effects of man-made materials interfacing with the nano-cosmos of biological systems. Annu Rev Pharmacol Toxicol 2010;50:63–88.

2 Feliu N, Fadeel B: Nanotoxicology: no small matter. Nanoscale 2010;2:2514–2520.

3 Shvedova AA, Kisin ER, Porter D, Schulte P, Kagan VE, Fadeel B, Castranova V: Mechanisms of pulmonary toxicity and medical applications of carbon nanotubes: two faces of Janus? Pharmacol Ther 2009;121:192–204.

4 Kagan VE, Tyurina YY, Tyurin VA, Konduru NV, Potapovich AI, Osipov AN, Kisin ER, Schwegler-Berry D, Mercer R, Castranova V, Shvedova AA: Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: role of iron. Toxicol Lett 2006;165:88–100.

5 Pulskump K, Drabate S, Krug HF: Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. Toxicol Lett 2007;168:58–74.

6 Monteiro-Riviere NA, Inman AO: Challenges for assessing carbon nanomaterial toxicity to skin. Carbon NY 2006; 44:1070–1078.

7 Wörle-Knirsch JM, Pulskamp K, Krug HF: Oops they did it again! Carbon nanotubes hoax scientists in viability assays. Nano Lett 2006;6:1261–1268.

8 Wick P, Manser P, Limbach LK, Dettlaff -Weglikowska U, Krumeich F, Roth S, Stark WJ, Bruinink A: The degree and kind of agglomeration affect carbon nanotube toxicity. Toxicol Lett 2007;168:121–131.

9 Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GA, Webb TR: Comparative pulmonary toxicity assessment of single wall carbon nanotubes in rats. Toxicol Sci 2003;26:2281–2200.

10 Lam CW, James JT, McCluskey R, Hunter RL: Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol Sci 2004;77:126–134.

11 Li Z, Hulderman T, Salmen R, Chapman R, Leonard SS, Young SH, Shvedova A, Luster MI, Simeonova PP: Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Environ Health Perspect 2007; 115:377–382.

12 Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, Tyurina YY, Gorelik O, Arepalli S, Schwegler-Berry D, Hubbs AF, Antonini J, Evans DE, Ku BK, Ramsey D, Maynard A, Kagan VE, Castranova V, Baron P: Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. Am J Physiol 2005;289:L698–L708.

13 Shvedova AA, Kisin E, Murray AR, Johnson VJ, Gorelik O, Arepalli S, Hubbs AF, Mercer RR, Keohavong P, Sussman N, Jin J, Yin J, Stone S, Chen BT, Deye G, Maynard A, Castranova V, Baron PA, Kagan VE: Inhalation versus aspiration of single walled carbon nanotubes in C57BL/6 mice: inflammation, fibrosis, oxidative stress and mutagenesis. Am J Physiol Lung Cell Mol Physiol 2008;295:L552–L565.

14 Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER, Castranova V: Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material. J Toxicol Environ Health A 2004;67:87–107.

15 Donaldson K, Murphy FA, Duffin R, Poland CA: Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Part Fibre Toxicol 2010;7:5.

16 Mutlu GM, Budinger GR, Green AA, Urich D, Soberanes S, Chiarella SE, Alheid GF, McCrimmon DR, Szleifer I, Hersam MC: Biocompatible nanoscale dispersion of single-walled carbon nanotubes minimizes in vivo pulmonary toxicity. Nano Lett 2010;10:1664–1670.

17 Muller J, Huaux F, Moreau N, Misson P, Heilier JF, Delos M, Arras M, Fonseca A, Nagy JB, Lison D: Respiratory toxicity of multi-wall carbon nanotubes. Toxicol Appl Pharmacol 2005;207:221–231.

18 Mitchell LA, Lauer FT, Burchiel SW, McDonald JD: Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice. Nat Nanotechnol 2009;4:451–456.

19 Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, Stone V, Brown S, Macnee W, Donaldson K: Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol 2008;3:423–428.

20 Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, Kitajima S, Kanno J: Induction of mesothelioma in p53+/– mouse by intraperitoneal application of multiwall carbon nanotube. J Toxicol Sci 2008;33:105–116.

21 Muller J, Delos M, Panin N, Rabolli V, Huaux F, Lison D: Absence of carcinogenic response to multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat. Toxicol Sci 2009;110:442–448.

22 Ryman-Rasmussen JP, Cesta MF, Brody AR, Shipley-Phillips JK, Everitt JI, Tewksbury EW, Moss OR, Wong BA, Dodd DE, Andersen ME, Bonner JC: Inhaled carbon nanotubes reach the subpleural tissue in mice. Nat Nanotechnol 2009;4:747–751.

23 Kostarelos K, Lacerda L, Pastorin G, Wu W, Wieckowski S, Luangsivilay J, Godefroy S, Pantarotto D, Briand JP, Muller S, Prato M, Bianco A: Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. Nat Nanotechnol 2007;2:108–113.

24 Porter AE, Gass M, Muller K, Skepper JN, Midgley PA, Welland M: Direct imaging of single-walled carbon nanotubes in cells. Nat Nanotechnol 2007;2:713–717.

25 Pogodin S, Baulin VA. Can a carbon nanotube pierce through a phospholipid bilayer? ACS Nano 2010;4:5293–5300.

26 Neves V, Heister E, Costa S, Tilmaciu C, Borowiak-Palen E, Ciusca CE, Flahaut E, Soula B, Coley HM, McFadeen J, Silva SR: Uptake and release of double-walled carbon nanotubes by mammalian cells. Adv Funct Mater 2010;20:3272–3279.

27 Konduru NV, Tyurina YY, Feng W, Basova LV, Belikova NA, Bayir H, Clark K, Rubin M, Stolz D, Vallhov H, Scheynius A, Witasp E, Fadeel B, Kichambare PD, Star A, Kisin ER, Murray AR, Shvedova AA, Kagan VE: Phosphatidylserine targets single-walled carbon nanotubes to professional phagocytes in vitro and in vivo. PLoS One 2009;4:e4398.

28 Dutta D, Sundaram SK, Teeguarden JG, Riley BJ, Fifield LS, Jacobs JM, Addleman SR, Kaysen GA, Moudgil BM, Weber TJ: Adsorbed proteins influence the biological activity and molecular targeting of nanomaterials. Toxicol Sci 2007;100:303–315.

29 Yang S, Guo W, Lin Y, Deng X, Wang H, Sun H, Liu, YF, Wang X, Wang W, Chen M, Huang YP, Sun YP: Biodistribution of pristine single-walled carbon nanotubes in vivo. J Phys Chem C 2007;111:17761–17764.

30 Allen BL, Kichambare PD, Gou P, Vlasova II, Kapralov AA, Konduru N, Kagan VE, Star A: Biodegradation of single-walled carbon nanotubes through enzymatic catalysis. Nano Lett 2008;8:3899–3903.

31 Liu X, Hurt RH, Kane AB: Biodurability of single-walled carbon nanotubes depends on surface functionalization. Carbon NY 2010;48:1961–1969.

32 Russier J, Ménard-Moyon C, Venturelli E, Gravel E, Marcolongo G, Meneghetti M, Doris E, Bianco A: Oxidative biodegradation of single- and multi-walled carbon nanotubes. Nanoscale 2011;3:893–896.

33 Kagan VE, Konduru NV, Feng W, Allen BL, Conroy J, Volkov Y, Vlasova II, Belikova NA, Yanamala N, Kapralov A, Tyurina YY, Shi J, Kisin ER, Murray AR, Franks J, Stolz D, Gou P, Klein-Seetharaman J, Fadeel B, Star A, Shvedova AA: Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation. Nat Nanotechnol 2010;5:354–359.

34 Kostarelos K, Bianco A, Prato M: Promises, facts and challenges for carbon nanotubes in imaging and therapeutics. Nat Nanotechnol 2009;4:627–633.

35 Kam NW, O'Connell M, Wisdom JA, Dai H: Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. Proc Natl Acad Sci USA 2005;102:11600–11605.

36 Chakravarty P, Marches R, Zimmerman NS, Swafford AD, Bajaj P, Musselman IH, Pantano P, Draper RK, Vitetta ES: Thermal ablation of tumor cells with antibody-functionalized single-walled carbon nanotubes. Proc Natl Acad Sci USA 2008;105:8697–8702.

37 Podesta JE, Al-Jamal KT, Herrero MA, Tian B, Ali-Boucetta H, Hegde V, Bianco A, Prato M, Kostarelos K: Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model. Small 2009;5:1176–1185.

38 Barone PW, Baik S, Heller DA, Strano MS: Near-infrared optical sensors based on single-walled carbon nanotubes. Nat Mater 2005;4:86–92.

39 De la Zerda A, Zavaleta C, Keren S, Vaithilingam S, Bodapati S, Liu Z, Levi J, Smith BR, Ma TJ, Oralkan O, Cheng Z, Chen X, Dai H, Khuri-Yakub BT, Gambhir SS: Carbon nanotubes as photoacoustic molecular imaging agents in living mice. Nat Nanotechnol 2008;3:557–562.

40 Kagan VE, Shi J, Feng W, Shvedova AA, Fadeel B: Fantastic voyage and opportunities of engineered nanomaterials: what are the potential risks of occupational exposures? J Occup Environ Med 2010;52:943–946.

Prof. Bengt Fadeel
Division of Molecular Toxicology, Institute of Environmental Medicine
Nobels väg 13, Karolinska Institutet, SE–17177 Stockholm (Sweden)
Tel. +46 8 524 877 37, E-Mail bengt.fadeel@ki.se