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Review Article

Nanomedicine and nanotoxicology: two sides of the same coin

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Abstract Current advances in nanotechnology have led to the development of the new field of nanomedicine, which includes many applications of nanomaterials and nanodevices for diagnostic and therapeutic purposes. The same unique physical and chemical properties that make nanomaterials so attractive may be associated with their potentially calamitous effects on cells and tissues. Our recent study demonstrated that aspiration of single-walled carbon nanotubes elicited an unusual inflammatory response in the lungs of exposed mice with a very early switch from the acute inflammatory phase to fibrogenic events resulting in pulmonary deposition of collagen and elastin. This was accompanied by a characteristic change in the production and release of proinflammatory to anti-inflammatory profibrogenic cytokines, decline in pulmonary function, and enhanced susceptibility to infection. Chemically unmodified (nonfunctionalized) carbon nanotubes are not effectively recognized by macrophages. Functionalization of nanotubes results in their increased recognition by macrophages and is thus used for the delivery of nanoparticles to macrophages and other immune cells to improve the quality of diagnostic and imaging techniques as well as for enhancement of the therapeutic effectiveness of drugs. These observations on differences in recognition of nanoparticles by macrophages have important implications in the relationship between the potentially toxic health effects of nanomaterials and their applications in the field of nanomedicine.

Key words: Nanotoxicology; Macrophages; Carbon nanotubes; Inflammatory response; Phosphatidylserine

Current advances in nanotechnology have led to the development of the new field of nanomedicine, which includes many applications of nanomaterials and nanodevices for diagnostic and therapeutic purposes. In particular, new approaches to site-specific drug targeting using nanoparticle drug carrier systems have been developed. Nanoparticle-based molecular imaging techniques have been introduced as informative adjuncts in personalized treatment of patients. The extraordinary sensitivity of the physical characteristics of nanoparticle complexes with biomolecules has initiated a significant interest in the design of new sensors making use of a strong dependence of electron transfer and energy transfer on donor-acceptor distances. The distance-dependent devices provide the opportunity to make use of the optical and electronic signals thus obtained for extremely sensitive and specific biomolecular recognition of bioanalytes of interest—that is, the creation of nanosensors. As another example, complexes of single-walled carbon nanotubes (SWCNTs) with single-stranded DNA exert conductivity properties dependent on the presence of the complementary DNA strand. If this complementarity is disturbed by even a single nucleotide alteration, the DNA association with the nanotube and consequently the conductivity is changed markedly. This phenomenon can be used for very sensitive and specific detection of single-nucleotide polymorphisms [1,2]. The use

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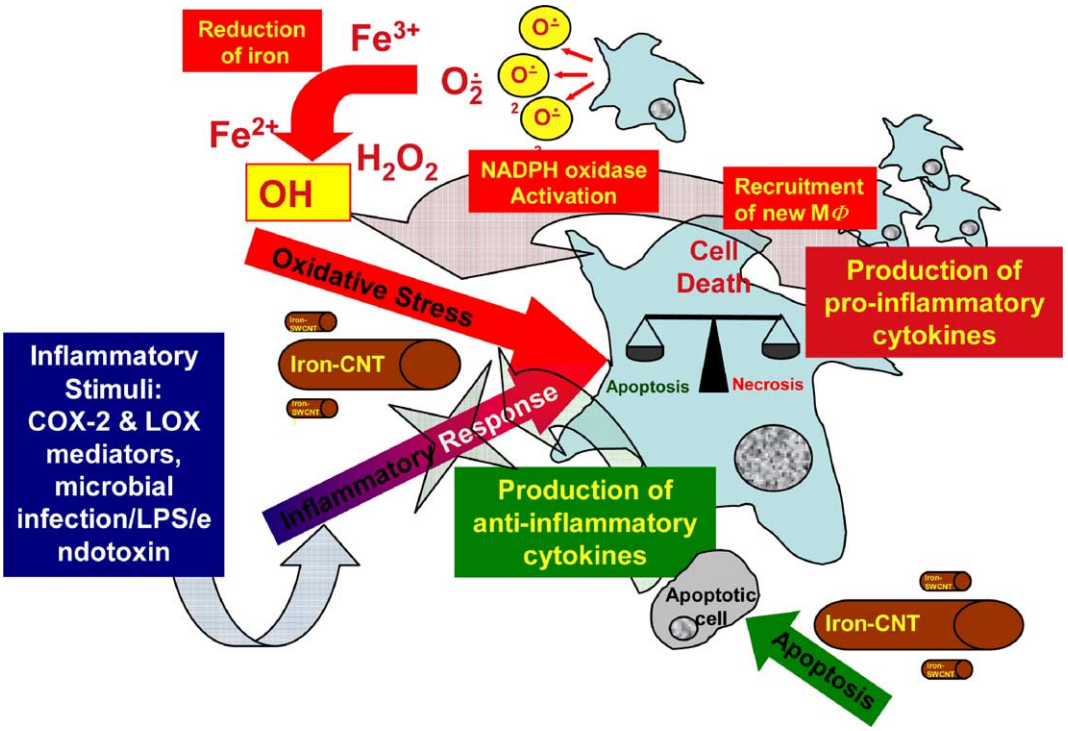

Fig 1. In the lung, the initial target for cytotoxicity by SWCNTs is probably type I epithelial cells whose necrotic death stimulates a proinflammatory response and recruitment of inflammatory cells. This schema describes the major pathways involved in interactions of nanoparticles (unrefined SWCNTs) with macrophages. These interactions include oxidative burst due to activation of NADPH oxidase, catalytic reactions of transition metals with oxygen radicals, and possible interactions of nanoparticles with microbial pathogens. NADPH oxidase complex is activated in macrophages during inflammation and acts as the major source for generation of reactive oxygen species, such as superoxide $O_2^{\cdot-}$ radicals that disproportionate to form hydrogen peroxide (H_2O_2). Transition metals, through their interactions with $O_2^{\cdot-}$ and H_2O_2, act as catalysts for the formation of highly reactive hydroxyl ($OH^{\cdot}$) radicals. Oxidatively modified lipids generated by cyclooxygenase (COX-2) and lipooxygenase (LOX) participate in amplification of the inflammatory response via recruitment of new inflammatory cells.

of nanodevices to facilitate molecular repair mechanisms of damage to macromolecules, especially DNA [3], is another innovative venue of nanomedicine. Finally, recently created muscle-powered "Biobots" and nanosized excitable vesicles not only provide the possibility of crafting and manufacturing devices with the potential to replace lost biological functions [4] but also advance our understanding of the fundamentals of life and disease.

Whereas these exciting areas of research and technology are indisputably important, the same unique physical and chemical properties that make nanomaterials so attractive may be associated with their potentially calamitous effects on cells and tissues. Biomedical and health professionals do not yet understand the toxicity of nanomaterials in relation to environmental and occupational diseases or the full extent of the growing use of nanomaterials in the environment and workplace. There is emerging concern that nanosized particles merit a more rigorous assessment of their potential effects on health and the associated control requirements, because their surface area and toxicity may be significantly greater than those of larger particles. However, specific mechanisms and pathways through which nanomaterials may exert their toxic effects remain unknown. A recent report described unusual redox features of SWCNTs in the presence of physiologically relevant redox agents [5]. Most importantly, at the time of this writing it remains to be elucidated whether unique electronic and redox properties of nanostructures translate into their unusual or even unknown effects on cells and tissues.

Pulmonary toxicity of nanoparticles

Although several laboratories have reported potential toxic effects of nanoparticles on different types of cells in vitro [6-12], there are only a few publications on in vivo toxicity of nanomaterials [13,14]. Our recent study demonstrated that aspiration of SWCNTs elicited an unusual inflammatory response in the lungs of exposed mice [15]. The initial acute inflammatory reaction is probably triggered by damage to pulmonary epithelial type I cells. The response includes a robust neutrophilic pneumonia followed by recruitment and activation of macrophages. The unusual feature of the response is a very early switch from the acute phase of the response to fibrogenic events resulting in significant pulmonary deposition of collagen and elastin. This is accompanied by a characteristic change in the

production and release of proinflammatory (tumor necrosis factor-α, interleukin-1β) to anti-inflammatory profibrogenic cytokines (transforming growth factor-β, interleukin-10). The inflammatory and fibrogenic responses were accompanied by a detrimental decline in pulmonary function and enhanced susceptibility to infection (Listeria monocytogenes) [15].

Transition metals and oxidative stress induced by nanoparticles

Manufacturing of nanoparticles, particularly of carbon nanotubes, is commonly associated with the use of different catalysts, often transition metals. In the case of carbon nanotubes, the so-called decorating iron or nickel levels in the product may make up as much as 40% and 25% by weight, respectively. Transition metals are particularly effective as catalysts of oxidative stress in cells, tissues, and biofluids. Therefore, the inflammatory responses caused by a combined presence of nanotubes with metals can be particularly damaging. As shown in Figure 1, oxidative species generated during inflammatory response can interact with transition metals to trigger redox-cycling cascades with a remarkable oxidizing potential to deplete endogenous reserves of antioxidants and induce oxidative damage to macromolecules. Our experiments with cells and cell-free systems demonstrated a significant potential of nonpurified iron-containing SWCNTs to generate radicals and cause accumulation of biomarkers of oxidative stress and cytotoxicity [16]. Therefore, toxic effects of nanomaterials should be assessed in conjunction with the presence of contaminants that may enhance, sometimes synergistically, their health effects and toxicity.

Macrophage recognition of nanoparticles

Another unusual property of the in vivo response is that carbon nanotubes are not effectively recognized by macrophages. In fact, typical macrophage responses, such as particle-dependent activation of oxidative burst and generation of nitric oxide, were not observed when macrophages were incubated with SWCNTs. Thus, neither engulfment nor oxidative/nitrosative activation of macrophages was triggered by carbon nanotubes. As a result, phagocytosis is probably not involved in elimination of nonfunctionalized carbon nanotubes from the lung. This "mishandling" of nanotubes may be relevant to their penetration into the systemic circulation, where they may continue exerting their extrapulmonary effects similarly to the effect demonstrated for ultrafine carbon particles [13].

Notably, the lack of recognition by macrophages is typical of nonfunctionalized carbon nanotubes, whereas their chemical modifications are consistently associated with increased recognition by macrophages. In fact, carbon nanotubes that have been chemically modified are readily recognized by macrophages to the extent that the functionalization is directed toward targeted delivery to macrophages of nanotubes with attached drugs. This approach has been used for delivery of nanoparticles to macrophages and other immune cells to improve the quality of diagnostic and imaging techniques as well as to enhance the therapeutic effectiveness of drugs [17,18].

Because macrophages play such a prominent role in regulation of inflammatory response, their interactions with functionalized nanoparticles can be used for preliminary toxicological assessments of nanomaterials. It is possible that functionalized nanoparticles may be toxic to some cells in vitro, yet the importance of this toxicity in vivo may not be realized in a pronounced inflammatory response. Therefore, detailed in vitro studies of macrophage cytokine and other inflammatory mediators along with the engulfment characteristics induced by their interactions with nanoparticles may be more instrumental and informative for preliminary in vitro toxicological screening than cytotoxicity to other types of cells.

Poor engulfment of nonfunctionalized SWCNTs by macrophages probably results from the lack of recognition signals on their surfaces. It has been established that phosphatidylserine (PS), which is normally confined to the cytosolic surface of the plasma membrane, is externalized during programmed cell death or apoptosis [19,20]. The externalized PS acts as a universal "engulf-me" signal for macrophages that is required for effective clearance of apoptotic cells [21]. Thus it is logical to expect that coating of carbon nanotubes with PS would confer a signal recognizable by macrophages. Our preliminary experiments indeed indicate that carbon nanotubes with adsorbed PS are effectively engulfed by macrophages. The observation that other phospholipids, such as phosphatidylcholine, when adsorbed to the surface of carbon nanotubes, do not confer a recognizable signaling moiety on carbon nanotubes and hence do not stimulate their phagocytosis, emphasizes the specificity of PS.

These observations on differences in recognition of carbon nanotubes by macrophages delineate a notable relationship between potentially toxic health effects of nanotubes and their applications in the field of nanomedicine. Note that the interactions of PS with macrophages are not limited to its role in recognition and tethering of target cells to macrophages but extend to further signaling events culminating in the complete reprogramming of the macrophage cytokine profile [22]. Indeed PS-driven mechanisms probably orchestrate the macrophage-dependent switch from acute proinflammatory response to anti-inflammatory cytokine repertoire [23]. If so, it is tempting to use PS-loaded nanoparticles to control the inflammatory response and prevent its exuberant development leading to tissue and organ damage, in several severe inflammatory disease conditions, including sepsis. Experiments are now underway to test this potentially important use of PS-containing nanoparticles.

Conclusions

Nanomaterials are widely used in research, industry, and medicine. The same unique physical and chemical properties that make nanomaterials so attractive may be associated with their potentially calamitous effects on cells and tissues. Our results indicate that inhalation of SWCNTs elicits robust and unusual inflammatory response in the lungs of exposed mice. These findings call for further toxicological studies and assessment of risk associated with the manufacturing and use of nanoparticles.

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