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Respiratory toxicity of carbon nanotubes: How worried should we be?

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Abstract

The synthesis of carbon nanotubes (CNTs) and their actual and potential industrial uses have attracted the attention of many scientists world wide but relatively little attention has been paid, so far, to their potential detrimental effects on human health and the environment. Here, we briefly sketch the main determinants of the lung toxicity of inhaled particles and outline the existing experimental methods to explore their toxic potential to the lung. We then summarize the first available studies that have examined the respiratory toxicity of CNTs in experimental animals. Although data are still fragmentary and subject to criticisms, e.g. because of the non-physiological mode of administration used, the results indicate that if CNTs reach the lungs they can exert serious toxicity, manifested in experimental animals as inflammatory and fibrotic reactions. These reports represent a cause of concern for human health and indicate that strict preventive and protective measures should be taken to limit inhalation exposure to CNTs in occupational settings.

Keywords: Carbon nanotubes; Grinding; Bioactivity; Reactivity

1. Introduction

Since the discovery of carbon nanotubes (CNTs) in 1991 [1], there has been an explosive growth of interest in this material because of its huge potential for industrial and commercial applications. The unique physico-chemical, electr(on)ical and mechanical properties of CNTs make them suitable for many industrial developments including carbon nanotubes composites, electrochemical devices or biomedical applications [2–4]. As a consequence, the global market for CNTs reached approximately $12 million in 2002 and is expected to grow to $700 million in 2005.[1] However, as for most other nanomaterials, information concerning their possible impacts on human health and the environment in general remains limited.

Individual CNTs have structural characteristics resembling fibers and their extreme aspect ratio (length:diameter ratio) and low solubility in aqueous media suggest toxic properties similar to those observed with other fibrous particles such as asbestos [5].

The respiratory toxicity of inhaled dusts and fibers has been recognized for centuries and related lung diseases (pneumoconioses) are still commonly diagnosed all over the world [6,7]. The history of asbestos fiber toxicity is one of the most striking examples highlighting the necessity of rapidly identifying and recognizing the potential hazards of industrial materials. Indeed, the respiratory toxicity of asbestos was not recognized for a long time after its generalized industrial uses, leading to a disquieting increase of asbestos-related lung disorders (asbestosis, lung cancers and mesothelioma) [8]. To prevent similar catastrophes and to allow the recommendation of effective preventive measures, toxicologists have developed and validated bioassays to identify the potential lung toxicity of newly proposed replacement materials. Knowledge about the determinants of the respiratory toxicity of asbestos fibers was applied to explore the potential toxicity of proposed

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asbestos substitutes including man-made mineral vitreous fibers (MMVF) or refractory ceramic fibers (RCF) [9].

In this review, we briefly summarize the current concepts on the pulmonary toxicity of inhaled particles, and outline the available experimental models to characterize the toxic potential(s) of a given particle or fiber. We then critically review existing data on the toxicity of CNTs, including the work that was performed in our laboratory, and attempt to identify critical issues and data gaps.

2. Pulmonary response to inhaled dusts

2.1. Consequences of chronic dust inhalation

Because of its large surface area in contact with the environment, the lung is a common target of many toxicants [6]. Chronic inhalation of high concentrations of dusts may lead to the development of interstitial lung diseases, among which pneumoconioses are caused by the accumulation of inorganic particles in the lung tissue. The three main types of particles involved in the development of malignant pneumoconiosis are crystalline silica (silicosis), asbestos (asbestosis) and coal mine dust (coal workers' pneumoconiosis) [6]. These pneumoconioses are characterized by a persistent alveolar inflammation (alveolitis) and by an accumulation of fibroblasts and of collagen in the lung (fibrosis). These pulmonary responses can lead to impairment, sometimes severe, of the pulmonary function and ultimately to a fatal outcome [10]. In addition, malignant pneumoconioses predispose to the development of lung cancer and pulmonary infections (tuberculosis and bronchitis) [11,12].

2.2. Pathogenesis

The pathogenesis of pneumoconiosis is generally depicted as a exaggeration of the normal lung repair processes triggered by the initial injury induced by the accumulation of toxic particles. Although the exact biological processes implicated in the development of particle-induced lung injury are not completely understood, we know that a complex interplay of immune cell types and related mediators such as cytokines, growth factors, and chemokines are involved in the development of the pneumoconiotic disease.

After inhalation of inorganic dusts, particles can either be translocated to extrapulmonary organs across epithelial layers [13], or be cleared from the respiratory tract by different processes such as dissolution, and active mucociliary or passive transport along surface liquids [14]. Particles that reach the alveolar epithelial surface can initiate an inflammatory process (alveolitis) in which alveolar macrophages and epithelial cells are activated by the deposited particles. Particles are phagocytozed mostly by macrophages which release a broad array of inflammatory mediators such as cytokines (IL-1, TNF-α) [15,16], arachidonic acid metabolites (LTB4) [17–21], chemokines (IL-8, MCP-1, MIP-2) [22], reactive oxygen species (ROS e.g. superoxide anion, hydrogen peroxide, hydroxyl radical, singlet oxygen), nitrogen species (RNS e.g. nitric oxide) [23–26] and also proteases (elastase, collagenases, matrix metalloproteases or MMP) [27,28].

All these factors can injure the pulmonary architecture and promote the recruitment of macrophages, neutrophils [29] and lymphocytes [30] into the lung. Activated leukocytes will contribute to enhance the production of growth factors (PDGF, EGF, IGF, FGF) and cytokines (TNF-α, IL-1, IL-10, TGF-β) that will then stimulate the recruitment and the proliferation of fibroblasts [6,31–33]. The resulting disorganization and remodeling of the insterstitium accompanied by an excessive production of extracellular matrix proteins such as type I and type III collagen, fibronectin, proteoglycans and laminin [34] leads, in turn, to the establishment of lung fibrosis.

In addition, reactive species (ROS and/or RNS) that may derive from the surface properties of particles themselves, the presence of transition metals, intracellular iron mobilization or lipid peroxidation are believed to induce cellular and DNA damages (genotoxicity), a prerequisite for mutagenicity and, in turn, the development of lung cancer [35–37].

The hypothetical mechanisms explaining the main biological processes implicated in the development of particle-induced lung injuries are depicted in Fig. 1.

2.3. Characteristics of toxic particles

2.3.1. Dose and dimensions

The particle dose and dimensions are key parameters with respect to the induction of adverse pulmonary effects. Both are intimately linked because the dose reaching the respiratory tract is dependent on particle dimensions [9] (Fig. 2).

Mathematical models are available for estimating the *aerodynamic diameter* of a given particle which is defined as the diameter of a virtual sphere of unit density and which would have the same penetration in the human respiratory tract than the particle under consideration. The aerodynamic diameter predicts if and where a particle may deposit in the respiratory tract; this parameter depends on particle density, size and form.

For a fibrous particle, two equations [38,39] are generally used to calculate the aerodynamic diameter (ad):

$$\text{Stober} \quad \text{ad} = 2.2 * d * \beta^{0.12},$$

$$\text{Harris} \quad \text{ad} = \frac{3d}{2} * \sqrt{\frac{\rho}{\left(\frac{0.385}{\ln(2\beta)-0.5} + \frac{1.23}{\ln(2\beta)+0.5}\right)}},$$

where $\rho =$ density (g/cm^3), $d =$ diameter (μm) and $\beta =$ density/length.

These equations indicate that the outer diameter of a fiber is the main determinant of the ad, and hence markedly influences the deposition of a fiber in the respiratory tract.

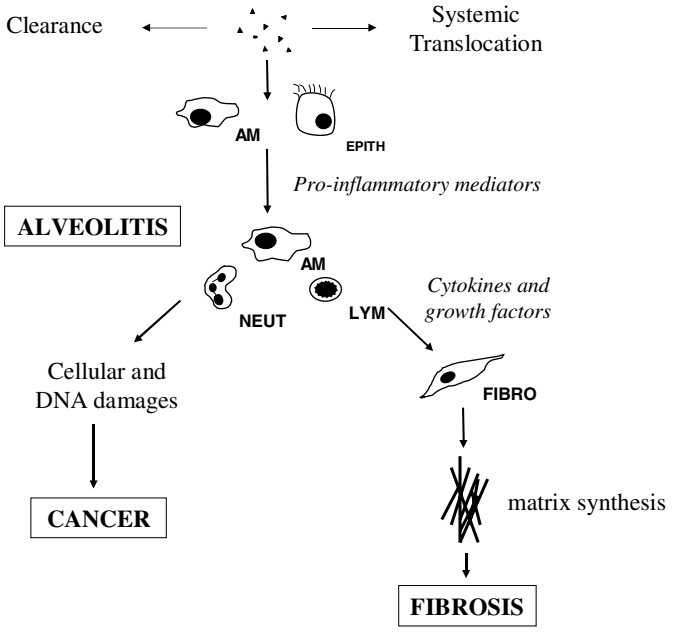

Fig. 1. Major biological processes associated with particles-induced lung injuries. After toxic dust (▲) inhalation, they can be either translocated or be cleared. Particles that reach the alveolar epithelial surface can stimulate alveolar macrophages (AM) but also epithelial cells (EPITH) to release several factors attracting leukocytes such as neutrophils (NEUT) and lymphocytes (LYM) into the lung. The activated alveolar macrophages and neutrophils produce a burden of oxidants and proteases that injure the alveolar wall. Oxidants can also induce cellular and DNA damages leading to cancer. Leukocytes also release growth factors and cytokines that stimulate lung fibroblast to proliferate with a subsequent accumulation of extracellular matrix. The net result is an expansion of mesenchymal cells and an accumulation of connective tissue matrix which characterize lung fibrosis.

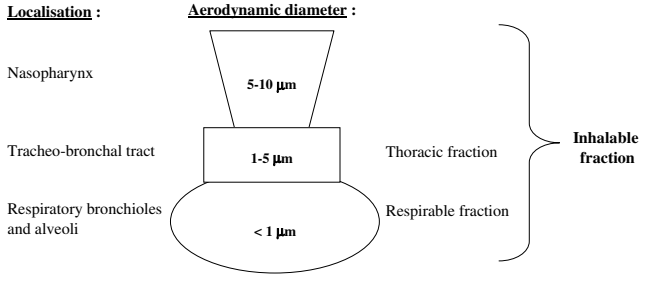

Fig. 2. Penetration of inhaled particles in the respiratory tract.

2.3.2. Biopersistence

The biopersistence of an inhaled particle also strongly determines its toxicity [9]. The study of the pulmonary clearance of MMVF (man-made vitreous fibers) has indicated that the occurrence of pulmonary disease (fibrosis or cancer) is closely linked with the biopersistence of those particles in the lung [40]. It was shown that fibers which are rapidly cleared from the lung did not induce pulmonary adverse effects, whereas those that remain in the lung can cause fibrosis and/or cancer in the rat (Table 1).

The lung possesses multiple clearance mechanisms, including phagocytosis, muco-ciliary transport and dissolution that contribute to the elimination of inhaled particles from the lung [41]. The retention of a particle in the lung is in part dependent on the efficiency of these clearance mechanisms. For fibers, length plays an important role in determining potential toxicity because it influences the persistence of the fiber in the respiratory tract. Studies have demonstrated that longer fiber (>20 μm) have the greatest toxic and carcinogenic potential in comparison with shorter fibers. Shorter fibrous particles can be phagocytozed by alveolar macrophages and are consequently more rapidly and easily cleared from the lung [41]. The mean diameter of the alveolar macrophage represents a limit above which fibers cannot be phagocytozed. This macrophage diameter is 10.5–13 μm for the rat and 14–21 μm for humans [42–46]. As the elimination efficiency of a particle contributes to its pulmonary toxicity, biopersistence of a particle must be considered in order to assess its potential toxic properties.

Table 1
Correlation between biopersistence and lung pathology (adapted from [40])

Type of fibers	Fibrosis	Tumors	Retention time (half-life in days)
MMVF 34	–	–	6
MMVF 21	+	–	53
RCF-1[a]	+	+	88
Crocidolite	+	+	±1000
Amosite	+	+	>1000
Chrysotile	+	+	>100

[a] RCF = refractory ceramic fibers.

2.4. Particular case: toxicity of ultrafine (nanometric) particles

Compared with larger respirable particles at the same mass dose, ultrafine particles (<0.1 μm in diameter) have a higher deposition efficiency in the pulmonary regions [47]. Epidemiological studies have reported associations between increases in ambient ultrafine particles concentration and increases in morbidity and mortality, particularly in susceptible populations [48–50]. Typically, ultrafine particles are associated with exacerbations of airway diseases (asthma, chronic obstructive pulmonary disease or COPD) [51] and cardiovascular effects such as ischemic episodes or myocardial infarction [52,53].

The underlying mechanisms by which ultrafine particles cause toxicity are not completely understood but different pathways have been suggested. An inflammatory process following exposure to ultrafine particles is largely implicated in the pulmonary disorders such as asthma and COPD [54–56]. Experimental in vitro studies suggest that the large surface area of ultrafine particles magnifies the amplitude of oxidative stress [57] and induces intracellular calcium variations in macrophages and epithelial cells [58] that could be important in priming and activating cells for inflammation. The mediators produced in the lung during

Table 2
Experimental approaches for characterizing the toxicity of inhaled particles

Physico-chemical investigations
Morphology and surface properties (size, length, d iameter, specific surface charge and area, hydrophobicity)
Purity and contaminants
Presence of transition metals
Solubility, durability
Capacity to form reactive species in vitro
Interaction with biological macromolecules (lipids, proteins, DNA)
In vitro studies: macrophage and/or epithelial cell cultures
Cytotoxicity, apoptosis
Oxidative stress and responses: 4-hydroxynonenal, intracellular glutathione, calcium, NFκ-B activation
Production of pro-inflammatory mediators: TNF-α, IL-1, MIP-2α, ROS, nitric oxide and derivatives
Production of pro-and anti-fibrotic mediators: TGF-β, PGE2, IL-10, IFN-γ
Genotoxicity: DNA breaks, 8-hydroxydeoxyguanosine, chromosomal aberrations, micronuclei, gene mutations
In vivo studies (intratracheal or inhalation in rats, mice or hamsters)
Biopersistence, clearance kinetics
Inflammation: histology, cell proliferation, apoptosis, broncho-alveolar lavage (BAL) analysis
Fibrosis: histology, biochemical measurements (e.g. hydroxyproline content)
Genotoxicity: mutations in epithelial cells (e.g. micronuclei)
Cancer: long-term bioassays, intrap leural injection

the inflammatory stage can be released in the bloodstream and can exert extrapulmonary effects such as exacerbation of clotting and thrombosis. Animal studies show that intratracheal administration of 60 nm positively, but not negatively, charged polystyrene particles can induce lung inflammation and affect hemostasis by enhancing peripheral vascular thrombosis [59]. Moreover, other studies suggest that ultrafine particles can have a direct effect on the cardiovascular system because they can translocate directly into the bloodstream. Systemic translocation of ultrafine particles has been demonstrated in the hamster [60] and in humans [61] using radioactively labeled ultrafine particles.

Therefore, when considering particles with nanometric dimensions, specific surface properties are likely to contribute greatly to the mechanisms involved in the toxic processes.

In conclusion, to explore the toxic potential of a particulate material, a large array of information, summarized in Table 2, needs to be collected through physico-chemical investigations, in vitro and in vivo experiments [62] (Table 2).

3. Carbon nanotubes

3.1. Structure of carbon nanotubes

Carbon nanotubes can be defined as tubular molecules with an axial symmetry and a diameter in the nanometer range. They can be synthetized by several methods including electric arc discharge [63], laser ablation [64] or catalytic decomposition of hydrocarbons [65]. Two main types of CNTs can be differentiated by their structure: single-walled carbon nanotubes (SWNTs) consist of singular graphene cylindrical walls [66,67] while multiwalled carbon nanotubes (MWNTs) have thicker walls of several coaxial graphene cylinders [1]. While the diameter of SWNT is only a few nanometers, that of MWNTs is about ten nanometers or more depending on the number of layers. The length of CNTs can reach several micrometers for both types of nanotubes [2]. Catalytic material (iron, cobalt, nickel) is often present inside and/or at the extremity of carbon nanotubes together with variable amounts of inert synthesis support (silica, alumina). The purification processes are not all of equal efficiency, often leading to varying final chemical compositions of nanotubes. These contaminants may play a role in the (biological) activity of carbon nanotubes and represent a significant source of variation across samples.

Carbon nanotubes may exist as singlet particles or as aggregates or agglomerates (nanoropes). As a consequence, their lung deposition characteristics can change as the aggregates will have a much greater aerodynamic diameter than the singlet particles. Because of this propensity of CNTs to form clumps sometimes with an aerodynamic diameter beyond the range of respirability (>5 μm), inhalation exposure is expected to be very low in industrial settings. One should, however, not conclude that the risk of respiratory toxicity is negligible, especially if this material is expected to be processed, and certainly if the intrinsic toxicity of the material is very high [68].

3.2. Pulmonary toxicity of carbon nanotubes

The current knowledge about the lung toxicity of CNTs is fragmentary, sometimes contradictory and still inconclusive (Table 3). This is partly due to the fact that the few studies that have explored lung toxicity have used different types of CNTs (SWCNT or MWCNT, intact or ground MWCNT, purified or not) in varying animals species (rat, mouse or guinea pig). In all these studies, investigators

Table 3

	Type of CNT	Species/dosis/vehicle	Route	Findings	Interpretation
Huczko et al. [70]	Soot with high CNT content containing Co and Ni (not otherwise specified) compared to soot w/o CNTs	Guinea pigs 25 mg/guinea pigs suspended in saline + Tween Number of animals not specified	i.t.	No pulmonary changes after 4 weeks: pulmonary function testing (tidal volume, breathing frequency and lung resistance); broncho-laveolar lavage (cells, proteins content)	CNTs are unlikely to be associated with health risks
Lam et al. [71]	Unpurified SWCNT containing Fe, Ni and Y compared to carbon black and quartz particles	Mice 0.1 or 0.5 mg/mouse suspended in mouse serum + sonication 5 mice per group	i.t.	Persisting granulomas and interstitial inflammation (7 and 90 days after administration)	If CNTs reach the lung, they are much more toxic than carbon black and more toxic than quartz
Warheit et al. [73]	Soot containing SWCNT + 30–40% amorphous carbon + 5% each of Co and Ni compared to quartz, carbonyl iron particles	Rats 1 or 5 mg/kg suspended in PBS + 1% Tween + polytron dispersement Number of animals not specified	i.t.	Some animals died with the highest dosis suffocated within 24 h Transient inflammatory and cell injury (24 h, 1 week, 1 and 3 months); non-dose-dependent granulomas distributed unevenly in the lung	The pulmonary toxicity…may not have physiological relevance and may be related to the instillation of a bolus of agglomerated CNTs (nanoropes)
Muller et al. [74]	Purified MWCNT intact or ground containing Co (0.95%), Fe (0.47%) and Al (0.05%) compared to croccidolite and carbon black	Rats 0.5, 2 or 5 mg/rat suspended in saline + 1% Tween + sonication 4–6 rats per group	i.t.	Intact MWCNT agglomerated in the airways and induced collagen-rich granulomas + surrounding alveolitis Ground MWCNT were better dispersed in the lung parenchyma and also induced inflammation and fibrotic responses (granulomas) MWCNT persisted in the lung (80% and 40% of the dosis after 60 days for intact and ground MWCNT, respectively)	CNTs are potentially toxic to humans
Shvedova et al. [72]	Purified SWCNT containing Fe (0.23%) compared to ultrafine carbon black and crystalline silica	Mice 10, 20, 40 μg/mouse suspended in phosphate buffered saline PBS + sonication 6–12 mice per group	Pharyngeal aspiration	Dense SWCNT aggregates are associated with the formation of foci of granulomatous inflammation Diffuse interstitial fibrosis with alveolar wall thickening in the lung region Persistent changes in pulmonary functions and decreased bacterial clearance induced by SWCNT	SWCNT induces robust acute inflammatory reaction with the very early onset of a fibrogenic response and the formation of granulomas in C57BL/6 mice

administered a single dose of CNTs suspended in a vehicle (saline buffer with a detergent or mouse serum) directly into the trachea or pharynx of anesthetized animals. This route of exposure is obviously less realistic compared to inhalation exposure and has been regarded as an important limitation and a possible source of artifacts because of the non-physiologic rapid delivery of the particles, the possible delivery of aggregates and the bypass of the nose filtering mechanism [69]. The main limitation, which seems critical for CNTs, is that by injecting a bolus dose of aggregated material, investigators can induce the formation of "foreign-body granulomas" that are non-specific and may not reflect an intrinsic toxic potential of the tested material. Provided this pitfall can be avoided, intratracheal instillation is, however, generally regarded as a useful method to characterize the toxicity of a new material, and lung responses induced with this route of administration are very similar to those observed after inhalation exposure [69].

In a short report, non-purified CNTs did not induce significant signs of lung toxicity 4 weeks after a single intratracheal (i.t.) administration in guinea pigs [70]. Lam et al. [71] found that a single i.t. treatment with SWCNTs induced the formation of persistent granulomas and interstitial inflammation in the lungs of mice; the severity of the lesions was dose-dependent. Very similar data were observed by Shvedova et al. [72] who reported that purified SWCNTs induced a rapid, dose-dependent and progressive development of fibrotic granulomas at the site of SWCNT aggregates deposition in addition to interstitial fibrosis in pulmonary regions distant from these sites. In another study, SWCNT soot administered

in rats produced transient inflammation and cell injury and resulted in the formation of multifocal granulomas centered around nanotubes, similar to a foreign body reaction. These investigators questioned the toxicological relevance of these findings because of the absence of a dose/effect–response relationship and because the formation of granulomas was suspected to be the consequence of instilling a bolus of agglomerated nanotubes (nanoropes) [73,74]. The absence of a dose–effect/response relationship noted by these authors might, however, be the consequence of the clumping of SWCNTs in large airways which did not allow for adequate dispersion of the delivered dose in the lungs.

In order to circumvent these difficulties, we have attempted to reduce the agglomeration of CNTs by grinding the material. Thus, we compared the lung response to MWCNT that had been ground (mean length <1 μm) or not (mean length ~ 6 μm) and demonstrated that while intact MWCNTs formed large aggregates that remained trapped in large airways, ground MWCNTs were much better dispersed in the lung (Fig. 3).

We demonstrated that when MWCNT reach the lungs they are not rapidly cleared, with intact MWCNT being apparently retained longer than ground MWCNT [75]. Moreover, we showed that both types of MWCNT induced pulmonary inflammation (increased LDH activity, total protein, and neutrophils in broncho-alveolar lavage) and fibrosis (increased levels of hydroxyproline and type I soluble collagen in lung tissue). In line with previously reported data [71–73], we found collagen-rich granulomas in the lumen of airways where intact MWCNT aggregates had accumulated. Interestingly, the lesions produced by ground CNTs had a more uniform distribution (Fig. 3), persisted for at least 2 months and followed a dose–effect relationship. Since the nature of the material (outer diameter, specific surface area, surface oxidized forms, carbon content, see Table 2 in [74]) was not significantly affected by grinding, we concluded that carbon nanotubes, even in the absence of agglomerates, are intrinsically toxic to the lung [74].

While recognizing the limitations of the i.t. mode of administration, it is interesting to note that, when a reference particle was included in the experimental design to calibrate the bioassay, the lung toxicity of CNTs (inflammation, fibrosis) was of similar intensity or even more severe than well-recognized lung toxicants such as quartz or crocidolite fibers. This should be a source of concern when considering the toxic potential of CNTs.

The underlying mechanism(s) by which CNTs exert their toxicity to the lung is(are) not yet understood. Most materials that have been tested so far contained significant amounts of transition metals (Fe, Co) which are known to be important sources of ROS, contributing thereby to the toxicity of several toxic fibers and particles, including asbestos [75] and ambient particles [76]. The possible contribution of oxidative stress in the lung toxicity of CNTs was tested by Shvedova et al. [72] by measuring a series of biomarkers associated with oxidative stress. They found accumulation of 4-hydroxynonenal (a product of lipoperoxidation) in broncho-alveolar lavage fluid and a depletion

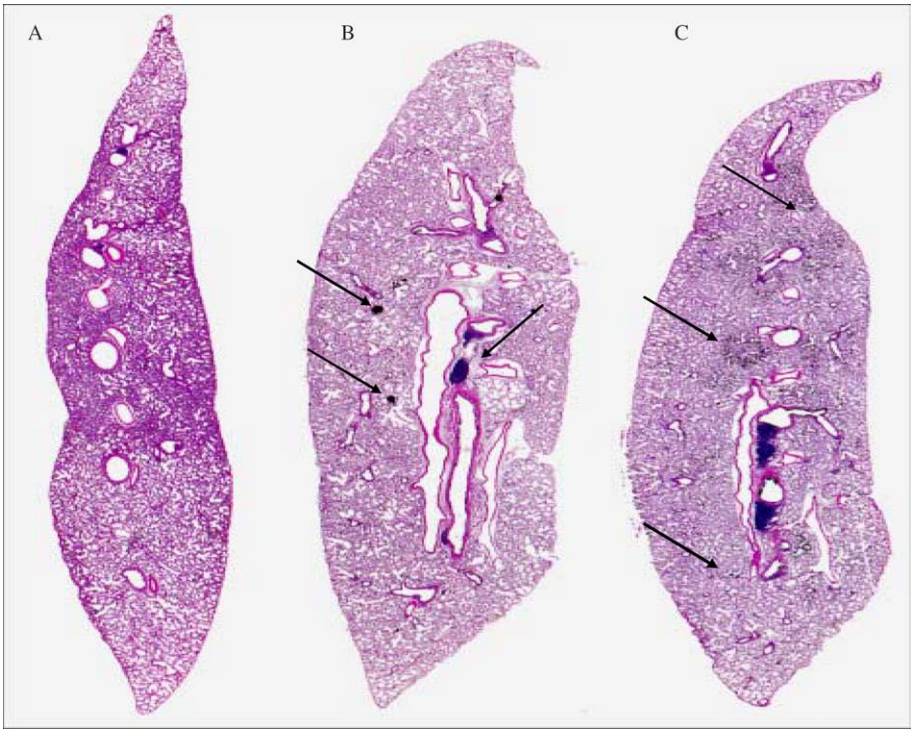

Fig. 3. Sections of control rat lung (A), and immediately after intratracheal instillation of 2 mg/animal ground MWCNTs (C, arrows) which are much better dispersed in the lung than intact MWCNTs which mainly remained clumped in large airways (B, arrows).

of the lung glutathione content, both consistent with a role for oxidative stress in the toxicity of SWCNTs. It is, however, not known whether this oxidative stress is triggered at the surface of CNTs or results from the burden of ROS generated by inflammatory cells accumulating in the lung. In this regard, it is interesting to note that CNTs have been reported to act as antioxidants rather than being a source of ROS [77].

The mediators involved in the lung response to CNTs also need to be further characterized. Administration of MWCNTs in rats was found to induce an increased production of TNF-α in the lung until 60 days after administration (fibrotic stage) [74]. In another study, proinflammatory cytokines such as TNF-α and IL-1β were rapidly increased (1 day) after administration of SWCNT in mice [72] but both parameters returned to control values as early as 3 days after exposure. The pro-fibrotic mediator TGF-β1 was only increased from 7 to 28 days post exposure. The study was complemented with in vitro experiments using a macrophagic murine cell line (RAW264.7) exposed to SWCNT [72]. They showed that TGF-β1 but not TNF-α or IL-1β was produced by macrophages. In contrast, we found in rat peritoneal macrophages an upregulation of TNF-α upon exposure to MWCNTs [74]. In vitro, using the murine RAW264.7 macrophages, no oxidative burst, nitric oxide production or apoptosis was observed in cells exposed to SWCNTs [72].

4. Conclusions and perspectives

Overall, the available studies indicate that, if they reach the lung, CNTs (SWCNTs and MWCNTs) have the potential to cause severe inflammatory and fibrotic reactions. While recognizing their limitations, these data suggest that if workers were exposed to respirable CNTs, they may be at risk of developing serious lung diseases.

The objective of the toxicologist is, however, not to simply identify the toxic potential of a new chemical or material with a view to recommend banning their production or uses. The ultimate goal of toxicological research is to identify the determinants of toxicity (dose, physical and chemical characteristics, susceptible populations) in order to make recommendations for the safe use of the chemical or material, e.g. to recommend acceptable exposure levels. It is clear that the picture of the toxic potential of CNTs is currently too fragmentary and that a number of gaps need to be filled to allow establishing sound science-based recommendations.

Efforts should be made to expose experimental animals under more realistic conditions (inhalation exposure) in order to verify the relevance of the lesions observed in studies conducted by instillation. When considering the possible adverse health effects of inhaled particles, it seems important for future studies to address the possible carcinogenic potential of CNTs, especially as these particles have proved to elicit an inflammatory reaction (alveolitis) which is considered as a source of genotoxic lesions that may be the forerunners of lung cancer [78]. The fibrotic activity of CNTs is a second reason for seriously considering the carcinogenic potential of these particles because we know that lung fibrosis and malignant pneumoconioses are associated with an increased risk of lung cancer [79].

Efforts should also be devoted to identifying the determinants of CNTs toxicity in order to direct effective control measures and possibly pave the way for the design of less toxic materials. In this respect, it is of utmost importance that investigators provide a detailed physico-chemical characterization (Table 2) of the material tested in toxicological studies. Meanwhile, it can be recommended that, in occupational settings where CNTs are produced or manipulated, efforts should be made to strictly control and limit inhalation exposure to CNTs.

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