NANOPARTICLE EXPOSURE AND SYSTEMIC/CARDIOVASCULAR EFFECTS – EXPERIMENTAL DATA[1]

PETIA P. SIMEONOVA*
Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA

Abstract: The most attractive properties of nanomaterials for medical and technological applications, including their small size, large surface area, and high reactivity, are also the main factors for their potential toxicity. Thus, some nanosized materials may induce not only a damage at the deposition site but also distant responses as a result of their transclocation and/or reactivity through the body. The exposure to low doses of these materials may modify the progression of existing pathophysiological conditions including cardiovascular diseases (CVD). In this respect, epidemiological and experimental studies have suggested an association between respiratory exposure to ambient ultrafine particles and the progression of cardiovascular disease. Our research efforts are currently directed to evaluate the cardiovascular effects, including vascular inflammation, blood cell coagulation status, atherosclerosis, as well as the related molecular mechanisms associated with respiratory exposure to different types of nanosized materials using animal models. Recently, we demonstrate that lung instillation of single wall carbon nanotubes (SWCNTs) is associated with a dose-dependent increase in oxidative vascular damage manifested by heme oxygenase-(HO-1) gene activation and mitochondrial alterations. Since these types of oxidative modifications are considered to play a role in atherogenesis, we further evaluated the effects of SWCNT respiratory exposure on atherosclerosis progression in ApoE–/– transgenic mice, a widely used model of human atherosclerosis. The accumulation of toxicological data on engineered nanomaterials will allow for development of adequate risk assessment and regulations.

---

* To whom correspondence should be addressed.

[1] Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the view of the National Institute for Occupational Safety and Health.

P.P. Simeonova et al. (eds.), Nanotechnology – Toxicological Issues and Environmental Safety, 53–64.

Keywords: atherosclerosis, mitochondrial DNA damage, nanomaterials, nanotoxicology, oxidative stress

1. Introduction

Nanotechnologies will revolutionize our life including medicine, but they also pose important toxicological questions that are related to the unique nature of materials and processes at the nanometer scale. Human contact with nanomaterials can be related to targeted exposure through therapeutics and cosmetiscs or untargeted exposure through occupational and environmental contamination. The most attractive features of nanomaterials including their small size, large surface area, and reactivity might also be the main factors for their toxicity. In this regard, they may induce not only damage at the penetration site but also can lead to unexpected distant responses, involving the immune system, cardiovascular system, liver, kidney, and brain, as a result of their transclocation and reactivity through the body. Evidence exist that very small particles can partition into the blood or the central nerve system (rev. in Oberdörster et al. 2005). For example, ultrafine size air pollutants are thought to be involved not only in pulmonary toxicity but also cardiovascular dieases and mortality (rev. in Brook 2004).

Engineered nanosized particles are new materials of emerging technological importance in different industries (Colvin 2003; Hood 2004). The US National Science Foundation estimated that millions of workers would be needed to support nanotechnology industries worldwide within 15 years. One direction of the nanomaterial industries is developing new carbon nanomaterials. Carbon atoms can be arranged into diverse geometries, forming a number of stable nanostructures. For example, a graphene sheet can be folded, usually in the presence of metal catalyst, to form a long single wall carbon nanotube (SWNT) with a diameter of ~1 μm. Bare carbon atoms can also be organized into spherical structures as fullerenes (buckyballs). The most stable and readily available fullerene is C_{60} having an average diameter of 0.72 nm. In addition to these single layer structures, large nanotubes and fullerenes can also be synthesized forming multiwall nanotubes (MWNT) or onion-like clusters, respectively (Park et al. 2003). Fullerenes, because of their strong electronegativity, can be combined with metals and other molecules to form for example metallofullerens.

The current state of toxicologic science and knowlege provides opportunities to study the toxic effects of nanomaterials in parallel with their development and discovery of their potential use. In order to facilitate the hazard evaluation of new nanomaterials, complex and systemic studies, including pharmacokinetic, cellular, molecular, physiological tests, and mathematical modeling should be

conducted as a part of multidisciplinary integrative research. The accumulation of toxicologic data on engineered nanomaterials will allow for development of adequate risk assessment and regulations.

At the National Institute of Occupational Safety and Health, we developed an integrative research program for evaluation of nanomaterial toxicity related to possible occupational exposure (http://www.cdc.gov/niosh/topics/nanotech). This collaborative multidisciplinary program provides unique possibilities for evaluation pulmonary and systemic toxicity of engineered nanomaterials. The methodological approaches, tested under this program, will help for the development of predictive tests for estimation of the toxicity of new nanomaterials based on their physicochemical characteristics, potential to induce oxidative stress, inflammation, specific pulmonary and systemic toxicity.

2. Respiratory Particulate Matter (PM) Exposure and Cardiovascular Toxicity

In addition to the well-established cardiovascular risk factors, such as high cholesterol levels, diabetes mellitus, and hypertension, many nontraditional risk factors, including concomitant infections, systemic autoimmune diseases, and chemical exposure, have been suggested to influence atherosclerotic process and precipitate disease complications (Libby 2000b; Ross 1999; Simeonova and Luster 2004). In this respect, epidemiological and experimental studies have recently found a positive association between air pollution and adverse cardiovascular outcomes (Brook et al. 2004; Kunzli et al. 2005; Peters et al. 2004; Pope et al. 2004). Although the putative biological mechanisms and factors linking air pollution to heart diseases remain not well understood, it is well accepted that PM respiratory exposure, specifically the smaller size PMs ("thoracic particles" PM_{10} <10 μm in aerodynamic diameter, "fine particles" $PM_{2.5}$ <2.5 mm, and "ultrafine particles" UFPs <0.1 μm), plays a significant role in the risk for cardiovascular disease and mortality. Short-term exposure to elevated PMs has been associated with increased acute cardiovascular mortality, particularly with at-risk subset of population, while pro-longed exposure has been considered a causative factor for atherosclerosis and reduced life expectancies (rev. in Brook et al. 2004). For example, recent epidemiological studies reported an association between $PM_{2.5}$ and overall cardiovascular mortality including death from ischemic heart disease (IHD), a consequence of atherosclerosis (Pope et al. 2004) as well as increased carotid intima-media thickness, a direct measure of atherosclerosis (Kunzli et al. 2005). Consistently, hyperlipidemic rabbits exposed to PM_{10} or ApoE–/– mice exposed to $PM_{2.5}$ develop advanced coronary and/or aortic atherosclerosis (Chen and Nadziejko 2005; Sun et al. 2005). The small size particles have been found to travel into the systemic circulation after pulmonary

experimental exposure (Nemmar et al. 2002; Oberdörster, et al. 2002). Evidence from human and toxicological exposure studies suggest that oxidative stress and inflammation is most likely involved in particle-mediated cardiovascular effects (Barclay et al. 2005; Brook et al. 2004; Sun et al. 2005).

Transition metals such as iron are considered to play a significant role in particle toxicity probably through "Fenton reactions" (Barchowsky and O'Hara 2003; Yuan and Brunk 1998). These reactions are involved in generation of exacerbated oxidative stress and related inflammatory responses. Transition metals may affect the cardiovascular system indirectly (through pulmonary inflammation or pulmonary neural reflexes) or directly (through penetration of particles or soluble metals into blood circulation). The plausibility for direct vascular effects of PM-associated metals is supported by the epidemiologically demonstrated association between increased blood cadmium levels as a result of cigarette smoking and accelerated peripheral atherosclerosis (Navas-Acien et al. 2004). Recently, it has been reported that long-term inhalation exposure to particles of combustion – derived fugitive emission induces myocardial injury in rats susceptible to spontaneous cardiomyopathy, Wistar-kyoto (WKY) rats, and water-leachable zinc has been suggested as involved in this cardiotoxicity (Kodavanti et al. 2003). Additionally, metals such as arsenic and lead have been related to cardiovascular effects (Nash et al. 2003; Simeonova and Luster 2004).

3. Nanoparticles – Hypothesis and Research Approaches

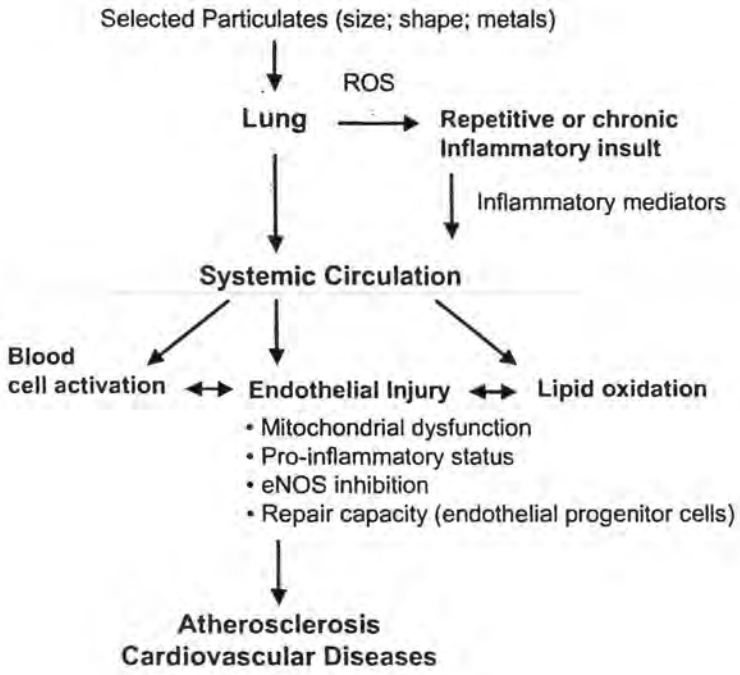
Long-term occupational respiratory exposure to nanoparticles, in addition to its pulmonary toxicity, may induce systemic effects of oxidative stress and inflammation which can modify the cardiovascular diseases (CVD) related to atherosclerosis. The unique physical characteristics (shape, size, surface area) and the metal constituents of these particles are major predictors for vascular toxicity. Smaller particles have greater deposition in the deep lung, a high surface area-to-mass ratio and prolonged clearance, potentially leading to lung inflammatory responses. The transition metals of the particles may accelerate the reactive oxygen species (ROS)-driven pulmonary toxicity. As a result, mediators might be released into the circulation leading to impaired endothelial as well as blood cell homeostasis (coagulation activation). Furthermore, nanoparticles may penetrate into the circulatory system and induce direct vascular toxicity. All stages of atherosclerosis can be modified by oxidative stress and inflammation. The primary underlying pathophysiological mechanisms of particle-mediated atherogenic effects might include the following: induction of oxidative-sensitive transcription factors and related inflammatory mediators (cytokines, chemokines, specifically monocyte chemoattractant protein

(MCP-1)-CCR2 axis, acute phase proteins); oxidative/inflammatory modifications in the endothelium (an altered ratio of oxidants/antioxidants, mitochondrial damage, apoptosis, eNOS inhibition; expression of adhesion molecules, specifically vascular cell adhesion molecule (VCAM-1); oxidative modifications of low-density lipoprotein (LDL); blood cell activation and coagulation abnormalities (increased blood levels of fibrinogen; tissue factor expression); and exhaustion of endothelial regenerative capacity. An early major event in atherogenesis is local recruitment of monocytes into the subendothelial space followed by phagocytosis of oxidized LDL, which is facilitated by the MCP-1 interaction with its receptor CCR2 and the endothelial expression of VCAM-1. The pathophysiological relevance of these mechanisms will vary dependent on the dose, duration of the exposure and physicochemical characteristics of the nanoparticles.

The observational human studies on the role of chemical exposure including particles and metals, in progression of atherosclerosis and related CVD may be influenced by numerous factors (e.g., characteristics of the exposure, personal, and life style confounders and population size). Understanding of the underlying biological mechanisms of the interaction between chemical exposure and atherogenesis can not be achieved without studies in relevant animal models. Recently, we characterized the application of an experimental mouse model for studying the role of metals in atherosclerosis (Simeonova et al. 2003). In these studies we were able to support the epidemiological findings linking arsenic exposure and atherosclerosis.

We designed studies to evaluate oxidative damage, inflammation, and coagulation status of plasma and heart/aorta in mice exposed through pharyngeal aspiration/inhalation to nanoparticles. Furthermore, we evaluate the effects of nanoparticle exposure on the genesis/progression of atherosclerosis using ApoE–/– mice or LDLR–/–, susceptible to atherosclerosis. If our findings link nanoparticle exposure to atherosclerosis, studies, using *in vivo* (mouse models) and *in vitro* (endothelial, smooth muscle, endothelial-progenitor cells), will be conducted to elucidate the putative role of several factors and mechanisms, e.g., vascular mitochondrial dysfunction, in these pathophysiological processes. In general, the conceptual objective of our studies is that nanoparticle pulmonary exposure may induce systemic effects which are relevant to atherogenesis or atherosclerosis progression. The systemic effects are related to ROS/inflammation – dependent cascades of signaling pathways which trigger endothelial dysfunction and impaired vascular repair mechanisms (as presented on the Diagram 1).

Diagram 1. Proposed processes by which certain materials influence CVD



4. SWCNT – Experimental Data

From the carbon nanomaterials, SWCNT recently elicited a great deal of interest due to their unique electronic and mechanical properties. SWCNT can be metallic or semiconducting thus offering amazing possibilities to create a broad spectrum of nanoelectronic devices as well as composite materials with extraordinary features (Sinnott and Andrews 2001; Subramoney 1998). Global revenues from carbon nanotubes (CNT) in 2006 are estimated at – $230 million with a growth rate of – 170%, which provides potential for workplace and even eventual general exposure (Donaldson et al. 2006). Concerns have been raised over occupational CNT exposure because they have several properties associated with potential adverse effects.

The extreme small size of SWCNT renders their chemical and physical properties fundamentally different from other particles with similar composition (Hood 2004). Several studies evaluated the potential pulmonary and cellular toxicity of SWCNTs. Lam et al. (2004) demonstrated that a single intratracheal

instillation in mice with three different types of SWCNT resulted in dose-dependent granulomas and some evidence of interstitial inflammation. Quartz and carbon NP were used as controls, and the author concluded that, on an equal mass basis, SWCNT in the lungs were far more toxic than carbon black and quartz. Histological images of the lungs in these studies showed that nanotubes induce significant granuloma formation and fibrous tissue accumulation compared to the control exposure. Based on the histological evaluation the inflammation was not a consistent finding. Warheit et al. (2004) evaluated the acute toxicity of intratracheally instilled SWCNT in rats. In this study, the authors showed that SWCNT exposure induced a transient inflammation and a nondose accumulation of multifocal granulomas. Shvedova et al. (2005) studied mice exposed to SWCNT of 99.7% weight elemental carbon and 0.23% weight iron. The primary nanotubes were 1–4 nm in diameter, but, as delivered by pharyngeal aspiration, two distinct particle morphologies were observed: aggregates and dispersed material. These two lung accumulations were associated with two types of responses – granulomas around the aggregates and diffuse fibrosis around the more dispersed SWCNT. Control mice were exposed to ultrafine carbon black or quatz and these exposures did not cause the response observed with SWCNT. All three studies demonstrated that SWCNT induce pulmonary toxicity which is different than this induced by graphite.

In the light of the pulmonary toxicological studies, we evaluated the cardiovascular responses in SWCNT-exposed mice (Li et al. 2006). To screen for systemic oxidative effects of SWCNT exposure, *Ho1-luc* reporter mice or C57BL/6 mice (at least 4 mice per treatment) were exposed to SWCNT in doses raging between 10 and 40 μg/mouse by single intrapharyngeal instillation and were sacrificed at time points including 1, 7, 28, and 56 days after exposure. These experimental settings were selected to correspond to the pulmonary toxicity studies by Shvedova et al. (2005). To evaluate SWCNT effects on atherosclerosis progression, a chronic process, ApoE–/– mice (10 mice per treatment) were exposed by pharyngeal aspiration to a medium dose SWCNT (20 μg/mouse) via multiple exposures (once every other week for 8 weeks). In all studies the control animals were exposed by pharyngeal aspiration to vehicle – sterile phosphate-buffered saline (PBS).

Our studies demonstrated that SWCNTs, under the described conditions, have the potential to influence CVD. A single intrapharyngeal instillation of SWCNTs induced heme oxygenase-1 (HO-1) activation, a marker of oxidative insults, in lung, aorta, and heart tissue in HO-1 reporter transgenic mice. Furthermore, we found that C57BL/6 mice, exposed to SWCNT (10 and 40 μg/mouse), developed aortic mtDNA damage at 7, 28, and 60 days after exposure. The SWCNT exposure also induced mitochondrial glutathione

depletion, and increased mitochondrial protein carbonyl formation in aortic tissue. Mitochondrial components have been reported to be highly susceptible to oxidative stress, mediated by metabolic defects and environmental insults (Madamanchi et al. 2005) and mitochondrial dysfunction is emerging as an important pathophysiological factor in a number of CVD including atherosclerosis (Binkova et al. 2001; Ballinger et al. 2002, 2005). Oxidative alterations of mitochondria result in compromised metabolic processes, such as oxidative phosphorylation, which can trigger endothelial dysfunction, a leading mechanism in atherosclerosis progression (Choksi et al. 2004). Altered endothelial activities lead to a series of events including vasoconstriction, increased adhesiveness resulting in inflammatory cell infiltration and platelet-thrombus formation (Cai and Harrison 2000; Libby 2000a, b). Combination of multiple cardiovascular risk factors which work through similar processes, such as mitochondrial dysfunction, may lead to synergistic acceleration of atherosclerosis progression and precipitation of its complications. Although the role of mitochondrial distress in atherosclerosis related to respiratory particle exposure has not been well explored, it is clearly demonstrated that cigarette smoke and hypercholesterolemia result in mtDNA damage and greater plaque formation (Knight-Lozano et al. 2002). Consistently, atherosclerosis was accelerated in SWCNT exposed ApoE–/– mice primed with a high-fat diet as measured by plaque morphometric analysis (Fig. 1). Although SWCNT exposed ApoE–/– mice did not have altered lipid profiles, they had exacerbated plaque development in the aorta and brachiocephalic arteries. The histopathology, including granulomas around the agglomerated SWCNT, fibrotic tissue in the granulomas and along the small SWCNT depositions (more dispersed material), in the lung of these mice was similar to the previously described pulmonary alterations in C57BL/6 mice after a single exposure to SWCNT.

Pulmonary exposure to SWCNT may induce cardiovascular effects either directly or indirectly through mitochondrial oxidative perturbations which can result in altered vessel homeostasis. SWCNT, although with a very low content of iron – induced lung pathophysiological responses, associated with deposition of particle agglomerates and histopathological alterations in the lung. Hypothetically, it is possible that individual SWCNTs can translocate from the lung into the systemic circulation causing direct cardiovascular endothelial dysfunction. It has been reported that nanoparticles treated with albumin and/or surfactant proteins cross the alveolo-capillary barrier to gain access to the systemic circulation (Kato et al. 2003; Oberdörster et al. 2005). The proximity between epithelial type I and endothelial cell alveolar membrane structures might play a role in the particle translocation mechanisms. Since the SWCNT

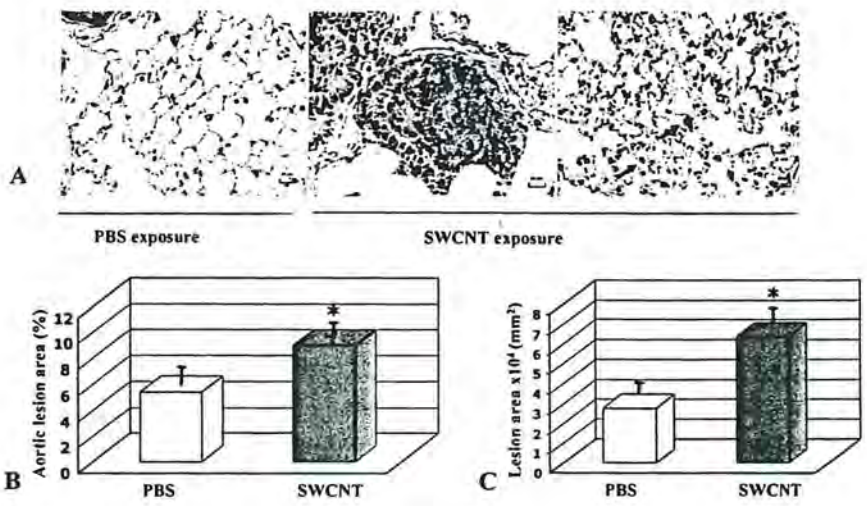


Figure 1. Lung histopathology and atheroma formation in ApoE–/– mice repeatedly exposed to SWCNTs (20 μg/ mouse every other week in two months) and fed a fat chow diet for four weeks followed by a regular chow for four weeks (adapted from Li et al 2006). A Representative images of lung (A) and morphometric analysis of the plaque size in the aortas (B) and the brachiocephalic arteries (C).

are not well recognized and cleared by lung macrophages (Kagan et al. 2006; Shvedova et al. 2005), nanotubes, dispersed or disintegrated from the agglomerates, may persist in the alveolar space which will facilitate their access into the systemic circulation. In contrast to SWCNTs, similar dose of UfCB agglomerated particles which are readily phagocytized by alveolar macrophages, did not produce lung fibrosis or granulomas (Shvedova et al. 2005), nor induce cardiovascular mtDNA damage. It is also possible that indirect processes are responsible for the cardiovascular effects induced by SWCNT exposure. This could occur if either mediators, released from the lung into the systemic circulation, or hypoxemia, associated with altered pulmonary function seen after SWCNT exposure (Shvedova et al. 2005), lead to oxidative modifications and low-level systemic inflammation. Several inflamematory mediators known to play a role in atherosclerosis were measured in the ApoE–/– mice evaluated for the atherosclerotic lesions. Although SWCNT exposure was associated with atherosclerosis acceleration, significant differences in the plasma levels of IL-6, IL-10, MCP-1, TNF-α, and IFN-γ were not observed. Furthermore,

SWCNT exposure was not related to increased inflammation in the vascular wall prior formation of the plaques. If SWCNTs with higher content of iron are deposited in the lung, chronic inflammation in the lung as well as systemic circulation might be induced. This will be tested in future studies. A third hypothesis for SWCNT exposure mediated cardiovascular effects is through platelet activation in the lung circulation. The pulmonary circulation is considered a site for platelet formation (Martin et al. 1983; O'Sullivan and Michelson 2006) and recently, it has been demonstrated that SWCNT can directly activate platelet aggregation *in vitro* (Radomski et al. 2005). Furthermore, transforming growth factor β 1(TGF β1), which is involved in platelet activation (Hoying et al. 1999), was found to be significantly increased in the lung of SWCNT-treated mice (Shvedova et al. 2005) and the time course of its increase paralleled the occurrence of cardiovascular mitochondrial dysfunction. Although, a link between activated platelets and cardiovascular mitochondrial distress has not been clearly established, it is well understood that that both platelet activation and mitochondrial damage lead to endothelial dysfunction and atherosclerosis (Ballinger 2005; Ross 1999).

5. Conclusions

Overall, these initial studies demonstrate that respiratory exposure to high concentrations, mostly agglomerated, SWCNT provokes not only pulmonary toxicity but vascular effects related to mitochondrial oxidative modifications and accelerated atheroma formation. Taken together, the findings are of sufficient significance to warrant further studies which should evaluate the systemic effects of SWCNT under inhalation exposure paradigms more likely to occur in the workplace or environment, such as low-level chronic inhalation exposure. Studies in progress, involving labeled SWCNT as well as detailed analysis of the role of lung platelet activation, will provide more insight into the mechanisms of the cardiovascular mitochondrial dysfunction in SWCNT-treated animals.

These studies also demonstrate that evaluation for systemic effects in parallel with pulmonary toxicity studies provides more complete toxicological information which will help in predicting the risk and development of safety regulations for the nanomaterial production and use.

References

Ballinger, S.W., Patterson, C., Knight-Lozano, C.A., Burow, D.L., Conklin, C.A., Hu, Z., et al., 2002, Mitochondrial integrity and function in atherogenesis, *Circulation* **106**:544–549.

Ballinger, S.W., 2005, Mitochondrial dysfunction in cardiovascular disease, *Free Radic. Biol. Med.* **38**:1278–1295.

Barclay, J., Hillis, G., and Ayres, J., 2005, Air pollution and the heart: cardiovascular effects and mechanisms, *Toxicol. Rev.* **24**:115–123.

Binkova, B., Strejc, P., Boubelik, O., Stavkova, Z., Chvatalova, I., and Sram, R.J., 2001, DNA adducts and human atherosclerotic lesions, *Int. J. Hyg. Environ. Health* **204**:49–54.

Barchowsky, A., and O'Hara, K.A., 2003, Metal-induced cell signaling and gene activation in lung diseases, *Free Radic. Biol. Med.* **34**(9):1130–1135.

Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., and Lipsett, M., et al., 2004, Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association, *Circulation* **109**:2655–2671.

Cai, H. and Harrison, D.G., 2000, Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress, *Circ. Res.* **87**:840–844.

Chen, L.C. and Nadziejko, C., 2005, Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice, V. CAPs exacerbate aortic plaque development in hyperlipidemic mice, *Inhal. Toxicol.* **17**:217–224.

Choksi, K.B., Boylston, W.H., Rabek, J.P., Widger, W.R., and Papaconstantinou, J., 2004, Oxidatively damaged proteins of heart mitochondrial electron transport complexes, *Biochim. Biophys. Acta* **1688**:95–101.

Colvin, V.L., 2003, The potential environmental impact of engineered nanomaterials, *Nat. Biotechnol.* **21**(10):1166–1170.

Donaldson, K., Aitken, R., Tran, L., Stone, V., Duffin, R., Forrest, G., and Alexander, A., 2006, Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety, *Toxicol. Sci.* **92**:5–22.

Hood, E., 2004, Nanotechnology: looking as we leap, *Environ. Health Perspect.* **112**(13):A740–749.

Hoying, J.B., Yin, M., Diebold, R., Ormsby, I., Becker, A., and Doetschman, T., 1999, Transforming growth factor beta1 enhances platelet aggregation through a non-transcriptional effect on the fibrinogen receptor, *J. Biol. Chem.* **274**:31008–31013; http://www.cdc.gov/niosh/topics/nanotech.

Kagan, V.E., Tyurina, Y.Y., Tyurin, V.A., Konduru, N.V., Potapovich, A.I., and Osipov, A.N., et al., 2006, Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: role of iron, *Toxicol. Lett.* **165**:88–100.

Kato, T., Yashiro, T., Murata, Y., Herbert, D.C., Oshikawa, K., and Bando, M., et al., 2003, Evidence that exogenous substances can be phagocytized by alveolar epithelial cells and transported into blood capillaries, *Cell Tissue Res.* **311**:47–51.

Knight-Lozano, C.A., Young, C.G., Burow, D.L., Hu, Z.Y., Uyeminami, D., and Pinkerton, K.E., et al., 2002, Cigarette smoke exposure and hypercholesterolemia increase mitochondrial damage in cardiovascular tissues, *Circulation* **105**:849–854.

Kodavanti, U.P., Moyer, C.F., Ledbetter, A.D., Schladweiler, M.C., Costa, D.L., Hauser, R., Christiani, D.C., and Nyska, A., 2003, Inhaled environmental combustion particles cause myocardial injury in the Wistar Kyoto rat, *Toxicol. Sci.* **71**(2):237–245.

Kunzli, N., Jerrett, M., Mack, W.J., Beckerman, B., LaBree, L., and Gilliland, F., et al., 2005, Ambient air pollution and atherosclerosis in Los Angeles, *Environ. Health Perspect.* **113**:201–206.

Lam, C.W., James, J.T., McCluskey, R., and Hunter, R.L., 2004, Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation, *Toxicol. Sci.* **77**:126–134.

Li, Z., Hulderman, T., Salmen, R., Chapman, C., Leonard, S.S., Young, S.H., Shvedova, A., Luster, M.I., and Simeonova, P.P., 2006, Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes, *Environ. Health Perspect.* December (online).

Libby, P., 2000a, Coronary artery injury and the biology of atherosclerosis: inflammation, thrombosis, and stabilization, *Am. J. Cardiol.* **86**:3J–8J.

Libby, P., 2000b, Multiple mechanisms of thrombosis complicating atherosclerotic plaques, *Clin. Cardiol.* **23**(Suppl. 6):VI–7.

Madamanchi, N.R., Vendrov, A., and Runge, M.S., 2005, Oxidative stress and vascular disease, *Arterioscler. Thromb. Vasc. Biol.* **25**:29–38.

Martin, J.F., Slater, D.N., and Trowbridge, E.A., 1983, Abnormal intrapulmonary platelet production: a possible cause of vascular and lung disease, *Lancet* **1**:793–796.

Nash, D., Magder, L., Lustberg, M., Sherwin, R.W., Rubin, R.J., Kaufmann, R.B., and Silbergeld, E.K., 2003, Blood lead, blood pressure, and hypertension in perimenopausal and postmenopausal women, *JAMA*, **289**(12):1523–1532.

Navas-Acien, A., Selvin, E., Sharrett, A.R., Calderon-Aranda, E., Silbergeld, E., and Guallar, E., 2004, Lead, cadmium, smoking, and increased risk of peripheral arterial disease, *Circulation* **109**(25):3196–3201.

Nemmar, A., Hoet, P.H., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M.F., Vanbilloen, H., Mortelmans, L., and Nemery, B., 2002, Passage of inhaled particles into the blood circulation in humans, *Circulation* **105**(4):411–414.

O'Sullivan, B.P. and Michelson, A.D., 2006, The inflammatory role of platelets in cystic fibrosis, *Am. J. Respir. Crit. Care Med.* **173**:483–490.

Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., and Cox, C., 2002, Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats, *J. Toxicol. Environ. Health A*, **65**(20):1531–43.

Oberdörster, G., Oberdörster, E., and Oberdörster, J., 2005, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, *Environ. Health Perspect.* **113**:823–839.

Park, K.H., Chhowalla, M., Iqbal, Z., and Sesti, F., 2003, Single-walled carbon nanotubes are a new class of ion channel blockers, *J. Biol. Chem.* **278**(50):50212–50216.

Peters, A., Von, K.S., Heier, M., Trentinaglia, I., Hormann, A., Wichmann, H.E., and Lowel, H., 2004, Exposure to traffic and the onset of myocardial infarction, *N. Engl. J. Med.* **351**:1721–1730.

Pope, C.A., III, Burnett, R.T., Thurston, G.D., Thun, M.J., Calle, E.E., Krewski, D., and Godleski, J.J., 2004, Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease, *Circulation* **109**:71–77.

Radomski, A., Jurasz, P., Onso-Escolano, D., Drews, M., Morandi, M., and Malinski, T., et al., 2005, Nanoparticle-induced platelet aggregation and vascular thrombosis, *Br. J. Pharmacol.* **146**:882–893.

Ross, R., 1999, Atherosclerosis is an inflammatory disease, *Am. Heart J.* **138**:S419–S420.

Shvedova, A.A., Kisin, E.R., Mercer, R., Murray, A.R., Johnson, V.J., and Potapovich, A.I., et al., 2005, Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice, *Am. J. Physiol. Lung Cell Mol. Physiol.* **289**:L698–L708.

Simeonova, P.P., Hulderman, T., Harki, D., and Luster, M.I., 2003, Arsenic exposure accelerates atherogenesis in apolipoprotein E(–/–) mice, *Environ. Health Perspect.* **111**:1744–1748.

Simeonova, P.P., Luster, M.I., 2004, Arsenic and atherosclerosis, *Toxicol. Appl. Pharmacol.* **198**:444–449.

Sinnott, S.B. and Andrews, R., 2001, Carbon nanotubes: synthesis, properties, and applications, *Crit. Rev. Solid State Mater. Sci.* **26**:145–249.

Subramoney, S., 1998, Novel nanocarbons – structure, properties, and potential applications, *Adv. Mater.* **10**:1157–1171.

Sun, Q., Wang, A., Jin, X., Natanzon, A., Duquaine, D., and Brook, R.D., et al., 2005, Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model, *JAMA* **294**:3003–3010.

Warheit, D.B., Laurence, B.R., Reed, K.L., Roach, D.H., Reynolds, G.A., and Webb, T.R., 2004, Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats, *Toxicol. Sci.* **77**(1):117–125.

Yuan, X.M. and Brunk, U.T., 1998, Iron and LDL-oxidation in atherogenesis, *APMIS* **106**(9):825–842.