[Frontiers in Bioscience 12, 1238-1246, January 1, 2007]

Impairment of mitochondrial function by particulate matter (PM) and their toxic components: implications for PM-induced cardiovascular and lung disease

Tian Xia, Michael Kovochich, and Andre E. Nel

Division of Clinical Immunology and Allergy, Department of Medicine; Southern California Particle Center and Supersite, University of California, Los Angeles, CA

TABLE OF CONTENTS

1. ABSTRACT

Increasing evidence suggests that reactive oxygen species (ROS) and oxidative stress are involved in PM-mediated lung and cardiovascular injury. The physical characteristics and the chemical composition of particulate matter (PM) play a key role in ROS generation *in vitro* and *in vivo*. The mitochondria are major subcellular targets for PM as well as a source of ROS production. ROS production is due to interference in mitochondrial electron transfer and PT pore opening by pro-oxidative PM components. Another possible mechanism is direct physical targeting by ambient ultrafine particles that lodge in and destroy mitochondrial structure. An understanding of the mitochondrial effects of PM is key in understanding the mechanisms of PM-induced adverse health effects.

2. INTRODUCTION

Recent epidemiological studies have shown an association between increased exposure of ambient particulate matter (PM) and adverse health effects in both the respiratory and cardiovascular systems (1,2). Increased morbidity and mortality due to PM exposure has been observed in both long and short-term studies. Susceptible human subsets include people suffering from asthma, COPD, pneumonia, and other respiratory diseases as well as patients with cardiovascular disease and diabetes (3). There is increasing toxicological evidence, both *in vivo* and *in vitro*, that PM-induced ROS production is an important mechanism contributing to PM-induced adverse health effects (4,5). In Southern California, PM that has been collected in the ultrafine range (< 100nm) have been shown

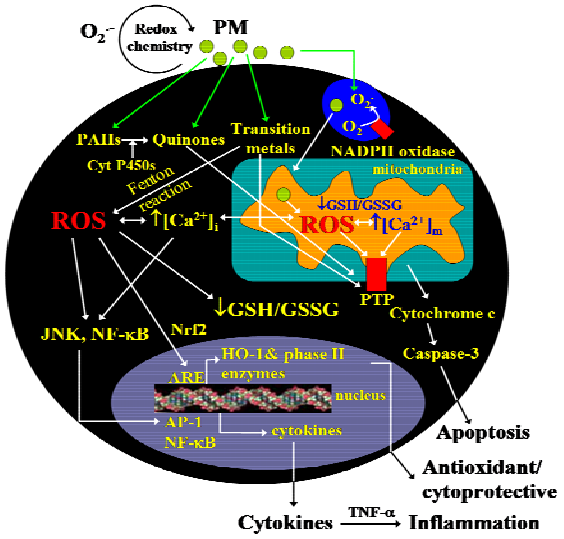

Figure 1. Sources of PM-induced ROS production and their cellular effects. Quinones, under the catalytic influence of NADPH-cytochrome P450 reductase, can redox cycle to produce ROS in the endoplasmic reticulum. Phagocytosis can induce the assembly and activation of NADPH oxidase to produce superoxide. PM can interfere in electron transduction in the mitochondrial inner membrane as well as perturb the PT pore to generate ROS. ROS induce lipid peroxidation in the cell membrane, crosslinking of protein SH groups, and DNA damage. ROS can also deplete GSH, resulting in redox dysequilibrium in the cell. Depending on the level of oxidative stress this could induce Nrf2 release to the nucleus, activation of MAPK and NF-kappaB signaling cascades or cytotoxicity. According to the hierarchical oxidative stress hypothesis, Nrf2 interaction with the ARE leads to heme oxygenase 1 and other phase II enzyme expression at lower levels of oxidative stress (Tier 1), while at a higher level of oxidative stress, activation of the MAPK and NF-kappaB signaling cascades can induce pro-inflammatory responses (e.g., cytokine and chemokine production) (Tier 2). At the highest oxidative stress level (Tier 3), ROS can induce the opening of the mitochondrial PT pore, followed by cytochrome c release, caspase-3 activation and induction of programmed cell death.

to be potentially more toxic than ambient particles of larger size, e.g. particles in the coarse (2.5-10 μm) or fine (0.1-2.5 μm) ranges. Particle-induced ROS production originates from the particle themselves as well as the chemicals coated on the particle surface. Additional ROS are being generated by the interactions of the particles and their components with cellular enzymes and organelles. The mitochondrion is a key subcellular target that contributes to ROS production and sheds new light on the mechanism of PM toxicity.

3. BIOLOGICAL PATHWAYS OF PM-INDUCED ROS GENERATION

PM-induced ROS production in biological systems and target cells originate from a variety of subcellular sources (Figure 1). These include: (i) catalytic conversion of polycyclic aromatic hydrocarbons (PAHs) to quinones by cytochrome P450 1A1 in the endoplasmic reticulum (6); (ii) quinone redox cycling by NADPH-dependent P450 reductase in microsomes (7); (iii) mitochondrial perturbation leading to electron leakage in the inner membrane (8,9); and (iv) NADPH oxidase activity in the macrophage surface membrane and associated phagosomes (10). Both the particles as well the chemicals coated on their surface play a role in these biological events. This includes evidence for the involvement of transition metals as well as redox cycling organic chemicals.

Redox cycling organic chemicals, such as quinones, are capable of generating ROS in cellular targets such as bronchial epithelial cells, macrophages, and endothelial cells (11,12). Quinones are byproducts of fossil fuel combustion, as well as the enzymatic conversion of PAH in the lung (7). Redox cycling quinones undergo one-electron reductions by NADPH cytochrome P450 reductase to form semiquinones (7). These semiquinones are meta-stable and donate electrons to O_2, leading to the formation of $O_2^{\cdot-}$ (7). In the process, the original quinone may be regenerated with the possibility of more $O_2^{\cdot-}$ generation (i.e., redox cycling). Due to their high content of organic chemicals, ambient UFP contribute proportionally more redox cycling chemicals than larger particles, as demonstrated by the increased ability of ultrafine particles (UFP) to generate O_2.– in the dithiothreitol assay (DTT) (13,14). This assay has been developed to quantitatively assess the content of redox cycling chemicals in ambient PM samples collected by ambient particle concentrators (13,14).

PM also contains a number of transition metals (coarse > fine > UFP) that can contribute to ROS production. Catalytically active metals present on the PM surface have been shown to contribute to oxidative stress *in vitro* and *in vivo* (15,16). Among the transition metals, Fe, Al, Cu, Ni, Mn, Zn, Cr, Ba, and Sr are the most abundant. In the presence of hydrogen peroxide some of these metals, including Fe^{2+}, has the ability to generate the hydroxyl radical ($^{\cdot}OH$) through catalysis of a Fenton reaction:

$$Fe^{2+} + H_2O_2 \Rightarrow Fe^{3+} + {}^{\cdot}OH + OH^-$$

The $^{\cdot}OH$ radical is more reactive than $O_2^{\cdot-}$ and hydrogen peroxide by several orders of magnitude. Besides ROS generation, transition metals also have the ability to directly perturb the function of the mitochondrial permeability transition (PT) pore. For instance, Cu and Al can induce PT pore opening (17,18), while Sr, Ba, and Mn inhibit PT pore opening by interfering in Ca^{2+} binding (19). Transition metals may also act synergistically with other PM components in impacting mitochondrial function, ROS generation, ATP production and cell viability (20).

4. PM PERTURBATION OF MITOCHONDRIAL FUNCTION

4.1. Mechanism 1: Effects on the mitochondrial electron transfer chain

Mitochondria are the main subcellular source of ROS production under physiological conditions (21-23)

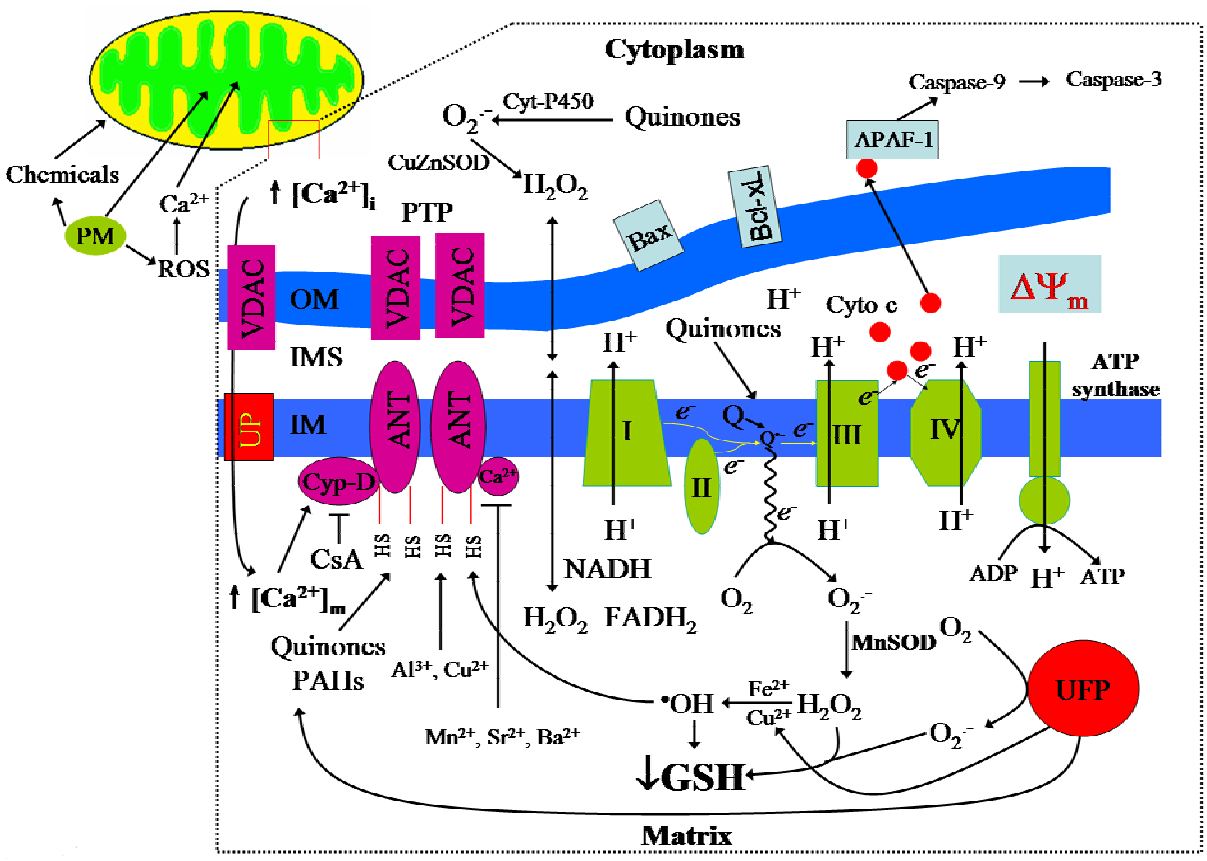

Figure 2. Putative structure of the PT pore and the electron transfer chain in the mitochondrial inner membrane. The PT pore is comprised of VDAC in the outer membrane, ANT in the inner membrane, and cyclophilin D (Cyp-D) in the matrix. Apart from the Ca^{2+}-dependent function of Cyp-D, the pore also contains putative quinone binding sites and vicinal thiol groups on ANT protein which can regulate the open/close status of the pore. Bcl-2 family proteins such as Bcl-2, Bax, Bak, and Bcl-xL also regulate PT pore function. PT pore opening leads to cytochrome c release from inter membrane space. Cytochrome c binding to APAF-1 leads to the formation of an apoptosome that leads to the sequential caspase 9 and caspase 3 activation, which can induce apoptosis. The electron transfer chain is composed of 4 complexes, along which electrons donated by NADH and $FADH_2$ are transferred along a decreasing redox potential gradient from complex I to complex IV. Energy dissipation along this gradient is used to pump protons from the matrix into the intermembrane space, thereby leading to the formation of the mitochondrial membrane potential. ATP synthase utilizes the proton motive force to produce ATP from ADP. During electron transfer, ubiquinone (Q) can accept an electron to form ubisemiquinone ($Q^{\cdot-}$), which can transfer an electron to O_2 to form superoxide. Mn-SOD in the matrix catalyzes $O_2^{\cdot-}$ dismutation to H_2O_2. H_2O_2 freely diffuses through the mitochondrial inner and outer membranes, which could lead to mitochondrial ROS to exert wider spread effects in the cell or cellular ROS to affect mitochondrial function. Transition metals can also catalyze $^{\cdot}OH$ production through Fenton reaction and could also regulate the open/close status of the PT pore. ROS react and deplete GSH pool in the matrix. Ambient UFP can lodge in mitochondria with the possibility of releasing their chemical components to promote ROS production, PT pore opening and mitochondrial destruction. It is also possible, however, that the mitochondria can be damaged because of chemical release and ROS production elsewhere in the cell and that the particles enter the already damaged mitochondria. One mechanism by which this takes place is the increase in $[Ca^{2+}]_i$ that occurs as a result of ROS generation extra-mitochondrially. This increase in $[Ca^{2+}]_i$ is buffered by Ca^{2+} uptake into the mitochondria. If this uptake leads to saturation of the mitochondrial Ca^{2+} retention capacity, large scale PT transition leads to structural mitochondrial damage. The extent to which Ca^{2+}-dependent and –independent processes contribute to PM-induced mitochondrial perturbation is difficult to quantify at this stage. It is apparent that the effects of aromatic PM chemicals proceed Ca^{2+}-independently and that the effects of polar compounds and redox cycling quinones are mostly Ca^{2+}-dependent.

(Figure 2). Mitochondria catalyze ATP production, which is linked to the activity of an electron transduction chain that operates in the inner membrane. The mitochondrial respiratory chain, which derives the electrons from NADH and succinate to O_2 consists of 4 multiprotein complexes in the inner membrane: the NADH dehydrogenase complex (complex I, NADH-ubiquinone reductase), the succinate dehydrogenase (complex II; succinate-ubiquinone reducrtase); the ubiquinol-cytochrome c reductase complex (complex III), and the cytochrome c oxidase (complex IV).

Table 1. PM-induced mitochondrial effects

PM components	Mitochondrial effects	Cellular consequences
Quinones	Disrupt the electron transfer chain	↑ROS, ↓ATP
	↑ROS generation	Oxidative stress
	↑Ca^{2+}-regulated PT pore opening	CsA-sensitive mitochondrial swelling and apoptosis
	↓Calcium Retention Capacity	Apoptosis
Aromatic compounds	Non-Ca^{2+} regulated PT pore opening	CsA-insensitive mitochondrial swelling and apoptosis
	↓Calcium Retention Capacity	↓PT pore threshold to PT
Transition metals	↓Psi_m	↓ATP, energy crisis, necrosis
	↑ROS generation	Oxidative stress
	PT pore opening	Apoptosis
Ultrafine Particles	Mitochondrial localization	Mitochondrial structural damage
		ROS generation
Chemical/Particle induced ROS	Oxidative stress	Phase II enzyme expression (Tier1),
		Inflammation (Tier2)
		Apoptosis (Tier3)
	mtDNA damage	Dysfunctional mitochondria
	Lipid, protein peroxidation	↓Oxidative phosphorylation
PM induced $[Ca^{2+}]_i$ ↑	Ca^{2+}-regulated PT pore opening	Apoptosis

This chain also includes two mobile or diffusible molecules, ubiquinone and cytochrome c, which function as electron transporters between complexes I, II and III, and between complexes III and IV, respectively. Electrons are transferred in a stepwise fashion along this chain, moving from a high to a low redox potential (21,22,23). This ultimately leads to the formation of H_2O (21,22,23). The dissipation of electron energy during this "downhill" flow is used by respiratory complexes I, III, and IV to pump protons (H^+) from the matrix into the intermembrane space against a concentration gradient. This event leads to the formation of the mitochondrial membrane potential (delta-Psi_m) (21,22,23). While efficient, this electron transfer process is not perfect. For instance, during the Q cycle, a two-step oxidation occurs in which ubiquinol is transformed into ubiquinone via an intermediary ubisemiquinone. The ubisemiquinone is capable of transferring electrons to molecular O_2, leading to the formation of $O_2^{\cdot -}$ (21,22,23).

Organic chemicals in the diesel exhaust particles (DEP) are capable of generating ROS by their ability to interfere in these electron transfer events (9,11,24) (Table 1). This includes the effect of polar DEP chemicals, such as redox cycling quinones to disrupt electron transfer in the inner membrane. We have demonstrated that this interference takes place between complexes I-III (9), suggesting that redox cycling quinones such as 9,10-phenanthaquinone may disrupt the Q cycle that operates between complexes I and III. This disruption in electron flow could favor the formation of ubisemiquinones, thereby contributing to mitochondrial $O_2^{\cdot -}$ production (9,25,26).

4.2. Mechanism 2: Effects on permeability transition pore opening

In addition to depolarizing effects on the inner membrane, organic DEP chemicals such as quinones and PAHs also have the capability to perturb the mitochondrial permeability transition (PT) pore (9) (Figure 2). The PT pore is a redox-, pH-, Ca^{2+}-, and delta-Psi_m –dependent protein complex, which plays a pivotal role in regulating mitochondrial function and controlling cellular apoptosis (19,27,28). Opening of the PT pore allows the non-elective passage of molecules of less than 1.5 kDa across the inner membrane, thereby leading to mitochondrial depolarization and uncoupling of oxidative phosphorylation (19). In addition, the redistribution of small solutes across the inner membrane and of cytosolic proteins leads to extensive mitochondrial swelling (19). Following the rupture of the mitochondrial outer membrane, various pro-apoptotic proteins such as cytochrome c, SMAC, AIF, etc., are released into the cytosol where they activate a number of apoptotic pathways, ultimately leading to programmed cell death (29,30).

Although PT pore composition remains controversial, its core components are generally believed to include the adenine nucleotide translocator (ANT) in the inner membrane, voltage-dependent anion channel (VDAC) in the outer membrane, and cyclophilin D (Cyp-D) in the matrix (19,30). The PT pore serves as a sensor for small molecules by displaying binding sites for divalent cations (e.g., Ca^{2+} and Mg^{2+}) and ubiquinones (19,25,26). The PT pore is also sensitive to thiol oxidation, particularly a number of closely spaced thiols on ANT that projects towards the matrix, namely cysteine C56, C159, and C256 (19). ROS crosslinking of C56 to C159, or C159 to C256, increases the probability of PT pore opening (19). It is therefore not a surprise that the redox status of the ANT thiol groups is tightly regulated by changes in the GSH content of the matrix (19).

In addition to its perturbation by thiol crosslinking, it has also been suggested that the PT pore may be regulated by a putative ubiquinone binding site, according to which ubiquinones can be classified into 3 functional classes, namely PT pore inducing, inhibitory or neutral ubiquinones (25). It has also been suggested that this ubiquinone binding site regulates open/close transitions of the PT through effects on the Ca^{2+} binding affinity of the pore (25,26). Our own studies have demonstrated, however, that redox cycling quinones presence in PM can regulate mitochondrial swelling in a cyclosporin A (CsA) dependent fashion (9). Whether the putative ubiquinone binding site is also accessable to exogenous quinones, such as the 9,10-phenanthroquinone or 1,2-naphthoquinone, needs to be confirmed.

4.3. Mechanism 3: Indirect PM effects on mitochondrial function

Besides direct effects of PM components on mitochondria, the function of this organelle can also be indirectly perturbed by ROS generation and Ca^{2+} flux in the cell. Intracellular free calcium $[Ca^{2+}]_i$, plays an important role in regulating the open/close state of the mitochondrial PT pore (28,31). Redox cycling chemicals, such as quinones, also regulate the PT indirectly through their ability to induce ROS production (7). ROS, such as H_2O_2, can induce an elevation of $[Ca^{2+}]_i$ in a variety of cell types by either inhibiting sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) or plasma membrane Ca^{2+}-ATPase (PMCA) (32,33), as well activating 1,3,5-trisphosphate (IP_3) receptors (33). SERCA inhibition by H_2O_2 is mediated by oxidation of sulphydryl groups in that protein (32). Use of the antioxidant, t-butylhydroxytoluene, can improve SERCA function (34).

A rise in $[Ca^{2+}]_m$ induced by increased $[Ca^{2+}]_i$ affects PT activity through the involvement of cyclophilin D (Cyp-D) (35). The route of entry into mitochondrial matrix for Ca^{2+} is through the uniporter. Cyp-D is a soluble matrix protein which is considered an integral PT pore component (19,30). When triggered by a rise in $[Ca^{2+}]_m$, Cyp-D binds to ANT and induces a conformational change that leads to PT pore opening. CsA, a fungal metabolite, prevents this Cyp-D interaction with ANT, thereby interfering in PT pore opening. While this regulatory mechanism works well under physiological conditions, the inhibitory effect of CsA may disappear under high Ca^{2+} concentrations, presence of heavy metals (e.g. Hg) and high levels of oxidative stress (35). Under these conditions, it is possible that mitochondrial swelling can proceed independently of Cyp-D (35). In order to explain this phenomenon, Lemasters *et al.* proposed that the mitochondrial PT pore exhibits a Ca^{2+}-regulated as well as Ca^{2+}-nonregulated states (35). Under physiological conditions, where the cis-trans proline peptidyl isomerase activity of Cyp-D is maintained, the protein effectively functions as a chaperone that maintains its ability to interact with ANT, thereby imparting on the PT pore a CsA-inhibitable function. However, under conditions of oxidative stress and high $[Ca^{2+}]_i$, considerable protein misfolding and clustering of native membrane proteins may disrupt the chaperone function of Cyp-D. This could lead to Ca^{2+}-unregulated PT pore opening (35). This could explain why isolated mitochondria undergo CsA-independent mitochondrial swelling when exposed to aromatic PM chemicals. These aromatic chemicals, including PAHs, are present in UFP, which also contain quinines. This chemical mixture may explain why mitochondrial swelling by UFP exhibit both Ca^{2+}-dependent and –independent activities (9).

H_2O_2 generated in the cytosol freely diffuses across the mitochondrial outer and inner membranes, and could participate in $^{\cdot}OH$ generation in the matrix through Fenton chemistry (33). These ROS induce damage to mitochondrial lipids, proteins, and mtDNA. Mitochondria serve as an important intracellular calcium store that readily takes up Ca^{2+} that is released into the cytosol from the endoplasmic reticulum, as described above. This is compatible with our observations that ambient ultrafine particles induce an increase in cytoplasmic and mitochondrial Ca^{2+} levels in macrophages, in association with effects on mitochondrial depolarization (manuscript in preparation). Ca^{2+} taken up by the mitochondria can be released by periodic flicking of the PT pore, thereby helping to maintain cellular Ca^{2+} homeostasis as well as generating Ca^{2+} propagation waves that are important in cell signaling. If, however, the PM-induced Ca^{2+} increase in the cytoplasm of sufficient magnitude exceeds the Ca^{2+} retention capacity of the mitochondria, irreversible PT pore opening may occur, with secondary consequences as described.

4.4. Mechanism 4: Direct physical targeting of mitochondria with structural damage

Mitochondria have been shown to be a direct subcellular target for ambient UFP (19,14,24). Ambient UFP lodge in the mitochondria of target cells such as macrophages and epithelial cells after exposure to an aqueous UFP suspension (8,14). Morphologically, this manifests as disruption of the mitochondrial integrity, with disappearance of the cristae. Functionally, these changes are accompanied by a loss of mitochondrial membrane potential, decrease in mitochondrial mass, opening of the PT pore, ROS production and cell death (9,14). In contrast, ambient fine and coarse particles do not gain direct access to mitochondria but are still able to affect mitochondrial function indirectly through chemical release, ROS generation and $[Ca^{2+}]_i$ flux (5,14,36). We do not know whether structural mitochondrial damage by UFP is secondary to the UFP entering this organelle or whether the organelle is damaged prior to the particle intake into this subcellular site.

Although the mechanism of mitochondrial localization is still elusive, we hypothesize that particle size, hydrophobicity and presence of organic chemicals may play a role in the process. In this regard, we have observed that positively charged amino-modified polystyrene microsphere particles can decrease the calcium retention capacity of isolated mitochondria in a dose-dependent fashion, while uncharged or carboxylate-modified microspheres do not have the same effect. It is possible that charge distribution across the mitochondrial inner membrane plays a role in mitochondrial targeting.

In addition to ambient ultrafine particles, other nano-sized materials (<100 nm) have also been demonstrated to localize in mitochondria. As early as 1970, de Lorenzo found that after intranasal instillation of colloidal gold particles (50 nm) in squirrel monkey, the particles crossed the olfactory nerve/mitral cell synapse and translocated to mitochondria localized in the mitral cells of the olfactory bulb (37). Fullerenes are a new class of compounds with potential uses in biology and medicine. A fullerene derivative, $C_{61}(CO_2H)_2$, has been found to be able to cross the external cellular membrane and localize preferentially in mitochondria (38). Another example is block copolymer micelles, which are water-soluble biocompatible nanocontainers with great potential for delivering hydrophobic drugs . Fluorescent-labeled block

copolymer micelles have been revealed to localize in several cytoplasmic organelles, including mitochondria, but not in the nucleus. These micelles may thus be worth exploring for their potential to selectively deliver drugs to specified subcellular targets (39). In summary, mitochondrial targeting and damage could constitute an important mechanism of nanomaterial-induced toxicity, including engineered nanomaterials.

5. DISEASE AND SECONDARY CONSEQUENCES OF PM-INDUCED MITOCHONDRIAL DAMAGE

In addition to serving as one of the major sources of ROS production, mitochondria are highly susceptible to oxidative damage. ROS can damage mitochondrial enzymes directly, which further contributes to decreasing the effectiveness of the electron transfer chain and changing the mitochondrial membrane potential (delta-Psi_m). This leads to a vicious cycle in which more ROS can be generated. Other secondary consequences of mitochondrial damage include decreased ATP production, leading to an energy crisis, as well as ROS leakage to the extracellular environment with damage to the extracellular matrix and surrounding cells.

Large scale mitochondrial permeability transition causes dissipation of delta-Psi_m, uncoupling of oxidative phosphorylation, cessation of ATP synthesis, matrix Ca^{2+} outflow, $O_2^{\cdot -}$ generation, and release of intermembrane proteins (19,24). Irreversible PT pore opening can lead to apoptosis or necrosis. Apoptosis leads to the formation of apoptotic bodies that are intended for "silent" removal by bystander cells so as to avert an inflammatory reaction. It is possible, however, that the toxic chemicals present in apoptotic bodies may be disseminated to surrounding cells, which can be subsequently damaged by the release of those chemicals. Interference in ATP production could induce cellular necrosis as an event that supercedes programmed cell death. Necrosis is characterized by cellular lysis and release of the intracellular content, leading to an inflammatory response, with edema and possible damage to the surrounding cells.

5.1. Airway disease

While there is a paucity of data showing that PM can induce mitochondrial function *in vivo*, several *in vitro* studies from our group and other laboratories have shown that PM exert toxic effects on epithelial cells and macrophages (8,11,14). PM chemical components play an important role in inducing ROS production from several cellular sources, as discussed before. If the level of oxidative stress exceeds the natural or inducible antioxidant defense of the cell, the response could transition to pro-inflammatory cytokine and chemokine production as well as the induction of a mitochondrion-mediated programmed cell death response (8,11). There is some *in vivo* evidence showing that acute PM exposure leads to degeneration of type I alveolar pneumocytes and epithelial cells, that could be instrumental in alveolar destruction with pulmonary edema, inflammation, destruction of alveoli, and even mortality (40). Damage and shedding of bronchial epithelial cells can lead to airway hyperactivity in asthmatics. Other than ambient pollutant particles, it has also been demonstrated that particulates present in occupational exposure, e.g. fine silicon dioxide powder (41) or crocidolite asbestos fibers can induce mitochondrial ROS production and apoptosis in alveolar epithelial cells (42,43). It was also reported that the silicon dioxide particles can permeate the cytoplasm, mitochondria and nuclei of alveolar cells at the air-blood barrier in rat lungs (41). Respiratory diseases, such as asthma, COPD, and interstitial fibrosis, are all considered to be inflammatory ailments that are linked to PM or occupational dust exposures (4). There is a suggestion, therefore, that particulate and fiber-induced effects on the respiratory tract may be mediated, in part, by mitochondrial damage.

5.2. Cardiovascular disease

Compared with airway disease, there is more evidence linking cardiovascular disease with mitochondrial damage (44,45,46). For instance, there is an association between mtDNA damage and coronary atherosclerotic hearth disease (47), while a deficiency of mitochondrial antioxidants can promote the onset of cardiovascular disease *in vivo* (48). PM is now considered a cardiovascular risk factor, and could be contributing to atherogenesis and cardiac rhythm disturbances either by direct effects on the heart or blood vessels, or through the impact on the lung. Direct effects are possible through the ability of ambient ultrafine particles to gain direct access to the systemic circulation, although the effect is still controversial. Through its ability to generate oxidative stress and inflammation in the lung, PM can impact the cardiovascular system by inducing systemic inflammation, including increased circulatory levels of IL-1, IL-6, TNF-alpha, fibrinogen, factor VII, C-reactive protein, blood viscosity and oxidized LDL (oxLDL) (49). Both oxLDL and free cholesterol can alter mitochondrial function (50,51). oxLDL has been shown to increase mitochondrial complex I activity and mitochondrial oxidative stress in endothelial cells (50). In human macrophages, oxLDL can increase mitochondrial ROS production, decrease mitochondrial membrane potential, and induce apoptosis (52,53). Mitochondrial ROS production can further contribute to oxLDL formation (54). oxLDL, together with other factors can promote the formation of atheromatous plaques in the arterial wall. It is also possible that through its effects on the cardiovascular system, the effects of oxLDL can synergize with the effects of pro-oxidative PM chemicals and ultrafine particles. This is in keeping with the data of Chen *et al.* demonstrating that concentrated ambient particles can promote atherogenesis in a mouse ApoE-KO model (55).

6. SUMMARY

Epidemiology has shown an association between PM exposure and increased respiratory and cardiovascular morbidity and mortality. There is increasing evidence that ROS production and oxidative stress at the cellular level may play a major role in the mechanism of PM-induced adverse health effects. Different PM components can interfere in mitochondrial function, including a possible direct effect of ambient UFP on mitochondrial function and

localization. Mitochondrial damage leads to cellular energy failure, and the initiation of apoptosis. Inflammation and epithelial shedding in the lung could compromise lung function or lead to asthma exacerbation. Enhanced mononuclear cell infiltrates and apoptosis could also play a role in atherogenesis.

7. ACKNOWLEDGEMENTS

Support was obtained from US Public Health Service Grants, PO1 AI50495 (funded by the National Institute of Allergy and Infectious Diseases and National Institute of Environmental and Health Science), RO1 ES10553, RO1 ES10253 and RO1 ES13432 (funded by the National Institute of Environmental and Health Science) and the U.S. Environmental Protection Agency STAR award to the Southern California Particle Center and Supersite. This work has not been subjected to the Environmental Protection Agency for peer and policy review and therefore does not necessarily reflect the views of the agency; no official endorsement should be inferred.

9. REFERENCES

1. Dockery D W, C. A. Pope, III, X. Xu, J. D. Spengler, J. H. Ware, M. E. Fay, B. G. Ferris, Jr. & F. E. Speizer: An association between air pollution and mortality in six U.S. cities. *N. Engl. J. Med.* 329, 1753-1759 (1993)

2. Samet J M, F. Dominici, F. C. Curriero, I. Coursac & S. L. Zeger: Fine particulate air pollution and mortality in 20 U.S. cities, 1987-1994. *N. Engl. J. Med.* 343, 1742-1749 (2000)

3. Kappos A D, P. Bruckmann, T. Eikmann, N. Englert, U. Heinrich, P. Hoppe, E. Koch, G. H. Krause, W. G. Kreyling, K. Rauchfuss, P. Rombout, V. Schulz-Klemp, W. R. Thiel & H. E. Wichmann: Health effects of particles in ambient air. *Int. J. Hyg. Environ. Health* 207, 399-407 (2004)

4. Li N, M. Hao, R. F. Phalen, W. C. Hinds & A. E. Nel: Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin. Immunol.* 109, 250-265 (2003)

5. Nel A: Atmosphere. Air pollution-related illness: effects of particles. *Science* 308, 804-806 (2005)

6. Penning T M, M. E. Burczynski, C. F. Hung, K. D. McCoull, N. T. Palackal & L. S. Tsuruda: Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones. *Chem. Res. Toxicol.* 12, 1-18 (1999)

7. Kumagai Y, T. Arimoto, M. Shinyashiki, N. Shimojo, Y. Nakai, T. Yoshikawa & M. Sagai: Generation of reactive oxygen species during interaction of diesel exhaust particle components with NADPH-cytochrome P450 reductase and involvement of the bioactivation in the DNA damage. *Free Radic. Biol. Med.* 22, 479-487 (1997)

8. Upadhyay D, V. Panduri, A. Ghio & D. W. Kamp: Particulate matter induces alveolar epithelial cell DNA damage and apoptosis: role of free radicals and the mitochondria. *Am. J. Respir. Cell Mol Biol* 29, 180-187 (2003)

9. Xia T, P. Korge, J. N. Weiss, N. Li, M. I. Venkatesen, C. Sioutas & A. Nel: Quinones and aromatic chemical compounds in particulate matter induce mitochondrial dysfunction: implications for ultrafine particle toxicity. *Environ. Health Perspect.* 112, 1347-1358 (2004)

10. Block M L, X. Wu, Z. Pei, G. Li, T. Wang, L. Qin, B. Wilson, J. Yang, J. S. Hong & B. Veronesi: Nanometer size diesel exhaust particles are selectively toxic to dopaminergic neurons: the role of microglia, phagocytosis, and NADPH oxidase. *FASEB J.* 18, 1618-1620 (2004)

11. Li N, M. Wang, T. D. Oberley, J. M. Sempf & A. E. Nel: Comparison of the pro-oxidative and proinflammatory effects of organic diesel exhaust particle chemicals in bronchial epithelial cells and macrophages. *J. Immunol.* 169, 4531-4541 (2002)

12. Sagai M, h. saito, T. Ichinose, m. kodama & y. mory: Biological effects of diesel exhaust particles. I. *In vitro* production of superoxide and *in vivo* toxicity in mouse. *Free Radic Biol Med.* 14, 37-47 (1993)

13. Cho A K, C. Sioutas, A. H. Miguel, Y. Kumagai, D. A. Schmitz, M. Singh, A. Eiguren-Fernandez & J. R. Froines: Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. *Environ. Res.* 99, 40-47 (2005)

14. Li N, C. Sioutas, A. Cho, D. Schmitz, C. Misra, J. Sempf, M. Wang, T. Oberley, J. Froines & A. Nel: Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ. Health Perspect.* 111, 455-460 (2003)

15. Ghio A J, A. Hall, M. A. Bassett, W. E. Cascio & R. B. Devlin: Exposure to concentrated ambient air particles alters hematologic indices in humans. *Inhal. Toxicol.* 15, 1465-1478 (2003)

16. Lay J C, W. D. Bennett, C. S. Kim, R. B. Devlin & P. A. Bromberg: Retention and intracellular distribution of instilled iron oxide particles in human alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* 18, 687-695 (1998)

17. Zazueta C, H. Reyes-Vivas, G. Zafra, C. A. Sanchez, G. Vera & E. Chavez: Mitochondrial permeability transition as induced by cross-linking of the adenine nucleotide translocase. *Int. J. Biochem. Cell Biol* 30, 517-527 (1998)

18. Toninello A, G. Clari, M. Mancon, G. Tognon & P. Zatta: Aluminum as an inducer of the mitochondrial permeability transition. *J. Biol Inorg. Chem.* 5, 612-623 (2000)

19. Halestrap A P, G. P. McStay & S. J. Clarke: The permeability transition pore complex: another view. *Biochimie* 84, 153-166 (2002)

20. Valavanidis A, K. Fiotakis, E. Bakeas & T. Vlahogianni: Electron paramagnetic resonance study of the generation of reactive oxygen species catalysed by transition metals and quinoid redox cycling by inhalable ambient particulate matter. *Redox. Rep.* 10, 37-51 (2005)

21. Andreyev A Y, Y. E. Kushnareva & A. A. Starkov: Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Mosc).* 70, 200-214 (2005)

22. Papa S & V. P. Skulachev: Reactive oxygen species, mitochondria, apoptosis and aging. *Mol Cell Biochem.* 174, 305-319 (1997)

23. Raha S & B. H. Robinson: Mitochondria, oxygen free radicals, disease and ageing. *Trends in Biochemical Sciences* 25, 502-508 (2000)

24. Hiura T S, N. Li, R. Kaplan, M. Horwitz, J. C. Seagrave & A. E. Nel: The role of a mitochondrial pathway in the induction of apoptosis by chemicals extracted from diesel exhaust particles. *J. Immunol.* 165, 2703-2711 (2000)

25. Walter L, V. Nogueira, X. Leverve, M. P. Heitz, P. Bernardi & E. Fontaine: Three classes of ubiquinone analogs regulate the mitochondrial permeability transition

pore through a common site. *J. Biol Chem.* 275, 29521-29527 (2000)
26. Fontaine E, F. Ichas & P. Bernardi: A ubiquinone-binding site regulates the mitochondrial permeability transition pore. *J. Biol Chem.* 273, 25734-25740 (1998)
27. Bernardi P, V. Petronilli, F. Di Lisa & M. Forte: A mitochondrial perspective on cell death. *Trends in Biochemical Sciences* 26, 112-117 (2001)
28. Zamzami N & G. Kroemer: The mitochondrion in apoptosis: how Pandora's box opens. *Nat. Rev. Mol Cell Biol* 2, 67-71 (2001)
29. Jiang X & X. Wang: Cytochrome C-mediated apoptosis. *Annu. Rev. Biochem.* 73, 87-106 (2004)
30. Zamzami n & g. Kroemer: The mitochondrion in apoptosis: how pandora's box opens. *Nature Reviews Molecular Cell Biology* 2, 67-71 (2001)
31. Ichas F & J. P. Mazat: From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state. *Biochim. Biophys. Acta* 1366, 33-50 (1998)
32. Redondo P C, G. M. Salido, J. A. Rosado & J. A. Pariente: Effect of hydrogen peroxide on Ca2+ mobilisation in human platelets through sulphydryl oxidation dependent and independent mechanisms. *Biochemical Pharmacology* 67, 491-502 (2004)
33. Brookes P S, Y. Yoon, J. L. Robotham, M. W. Anders & S. S. Sheu: Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol* 287, C817-C833 (2004)
34. Adachi T, R. Matsui, S. Xu, M. Kirber, H. L. Lazar, V. S. Sharov, C. Schoneich & R. A. Cohen: Antioxidant improves smooth muscle sarco/endoplasmic reticulum Ca(2+)-ATPase function and lowers tyrosine nitration in hypercholesterolemia and improves nitric oxide-induced relaxation. *Circ. Res.* 90, 1114-1121 (2002)
35. He L & J. J. Lemasters: Regulated and unregulated mitochondrial permeability transition pores: a new paradigm of pore structure and function? *FEBS Lett.* 512, 1-7 (2002)
36. Donaldson K, V. Stone, P. J. Borm, L. A. Jimenez, P. S. Gilmour, R. P. Schins, A. M. Knaapen, I. Rahman, S. P. Faux, D. M. Brown & W. MacNee: Oxidative stress and calcium signaling in the adverse effects of environmental particles (PM10). *Free Radic. Biol. Med.* 34, 1369-1382 (2003)
37. de Lorenzo A J, The olfactory neuron and the blood-brain barrier: Taste and smell in verterbrates (Wolstenholme G, Knight J, eds). London: Churchill.. 151-76. (1970)
38. Foley S, C. Crowley, M. Smaihi, C. Bonfils, B. F. Erlanger, P. Seta & C. Larroque: Cellular localisation of a water-soluble fullerene derivative. *Biochemical and Biophysical Research Communications* 294, 116-119 (2002)
39. Savic R, L. Luo, A. Eisenberg & D. Maysinger: Micellar Nanocontainers Distribute to Defined Cytoplasmic Organelles. *Science* 300, 615-618 (2003)
40. Ichinose T, A. Furuyama & M. Sagai: Biological effects of diesel exhaust particles (DEP). II. Acute toxicity of DEP introduced into lung by intratracheal instillation. *Toxicology* 99, 153-167 (1995)
41. Lebkova N P & V. I. Baranov: Ultrastructural and cytochemical changes in the respiratory compartment of the lungs in rats after combined treatment with fine silicon dioxide powder and uridine. *Bull. Exp. Biol Med.* 137, 556-559 (2004)
42. Upadhyay D, V. Panduri & D. W. Kamp: Fibroblast growth factor-10 prevents asbestos-induced alveolar epithelial cell apoptosis by a mitogen-activated protein kinase-dependent mechanism. *Am J Respir. Cell Mol Biol* 32, 232-238 (2005)
43. Panduri V, S. Surapureddi, S. Soberanes, S. A. Weitzman, N. Chandel & D. W. Kamp: P53 Mediates Amosite Asbestos Induced Alveolar Epithelial Cell Mitochondria-regulated Apoptosis. *Am. J. Respir. Cell Mol. Biol.* 2005-0352OC (2005)

44. Ballinger S W: Mitochondrial dysfunction in cardiovascular disease. *Free Radic Biol Med.* 38, 1278-1295 (2005)
45. Alberici L C, H. C. Oliveira, E. J. Bighetti, E. C. de Faria, G. R. Degaspari, C. T. Souza & A. E. Vercesi: Hypertriglyceridemia increases mitochondrial resting respiration and susceptibility to permeability transition. *J Bioenerg. Biomembr.* 35, 451-457 (2003)
46. Oliveira H C, R. G. Cosso, L. C. Alberici, E. N. Maciel, A. G. Salerno, G. G. Dorighello, J. A. Velho, E. C. de Faria & A. E. Vercesi: Oxidative stress in atherosclerosis-prone mouse is due to low antioxidant capacity of mitochondria. *FASEB J* 19, 278-280 (2005)
47. Corral-Debrinski M, J. M. Shoffner, M. T. Lott & D. C. Wallace: Association of mitochondrial DNA damage with aging and coronary atherosclerotic heart disease. *Mutat. Res.* 275, 169-180 (1992)
48. Ballinger S W, C. Patterson, C. A. Knight-Lozano, D. L. Burow, C. A. Conklin, Z. Hu, J. Reuf, C. Horaist, R. Lebovitz, G. C. Hunter, K. McIntyre & M. S. Runge: Mitochondrial integrity and function in atherogenesis. *Circulation* 106, 544-549 (2002)
49. Donaldson K, V. Stone, A. Seaton & W. MacNee: Ambient particle inhalation and the cardiovascular system: potential mechanisms. *Environ. Health Perspect.* 109 Suppl 4, 523-527 (2001)
50. Ceaser E K, A. Ramachandran, A. L. Levonen & V. M. rley-Usmar: Oxidized low-density lipoprotein and 15-deoxy-delta 12,14-PGJ2 increase mitochondrial complex I activity in endothelial cells. *Am J Physiol Heart Circ. Physiol* 285, H2298-H2308 (2003)
51. Yao P M & I. Tabas: Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway. *J Biol Chem.* 276, 42468-42476 (2001)
52. Asmis R & J. G. Begley: Oxidized LDL promotes peroxide-mediated mitochondrial dysfunction and cell death in human macrophages: a caspase-3-independent pathway. *Circ. Res.* 92, e20-e29 (2003)
53. Kinscherf R, H. P. Deigner, C. Usinger, J. Pill, M. Wagner, H. Kamencic, D. Hou, M. Chen, W. Schmiedt, M. Schrader, G. Kovacs, K. Kato & J. Metz: Induction of mitochondrial manganese superoxide dismutase in macrophages by oxidized LDL: its relevance in atherosclerosis of humans and heritable hyperlipidemic rabbits. *FASEB J* 11, 1317-1328 (1997)

54. Mabile L, O. Meilhac, I. Escargueil-Blanc, M. Troly, M. T. Pieraggi, R. Salvayre & A. Negre-Salvayre: Mitochondrial function is involved in LDL oxidation mediated by human cultured endothelial cells. *Arterioscler. Thromb. Vasc. Biol* 17, 1575-1582 (1997)
55. Chen L C & C. Nadziejko: 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 (2005)

Abbreviations: AQ, 9, 10-anthraquinone; ARE, antioxidant response element; DEP, diesel exhaust particles; GSH, glutathione; GSSG, glutathione disulfide; JNK, NH_2-terminal Jun kinase; HO-1, heme oxygenase 1; MAPK, mitogen-activated protein kinase; NAC, N-acetylcysteine; NQ, 1,2-naphthaquinone; PQ, 9, 10-phenanthraquinone; O_2^-, superoxide; ^-OH, hydroxyl radical; PAH, polycyclic aromatic hydrocarbons; PM, particle matter; PT, permeability transition; ROS, reactive oxygen species; SERCA, Sarco/endoplasmic reticulum Ca^{2+}-ATPase, SOD, superoxide dismutase; UFP, ultrafine particles; delta-Psi_m, mitochondrial membrane potential.

Key Words: Particulate matter (PM), ROS, apoptosis, oxidative stress, mitochondria, PT pore, Review

Send correspondence to: Andre E. Nel, PhD., M.D. Department of Medicine, Division of Clinical Immunology & Allergy, UCLA School of Medicine, 52-175 CHS, 10833 Le Conte Ave, Los Angeles, CA 90095-1680, Tel: 310-825-6620, Fax: 310-206-8107, E-mail: anel@mednet.ucla.edu

http://www.bioscience.org/current/vol12.htm