

Quinones and Aromatic Chemical Compounds in Particulate Matter Induce Mitochondrial Dysfunction: Implications for Ultrafine Particle Toxicity

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Particulate pollutants cause adverse health effects through the generation of oxidative stress. A key question is whether these effects are mediated by the particles or their chemical compounds. In this article we show that aliphatic, aromatic, and polar organic compounds, fractionated from diesel exhaust particles (DEPs), exert differential toxic effects in RAW 264.7 cells. Cellular analyses showed that the quinone-enriched polar fraction was more potent than the polycyclic aromatic hydrocarbon (PAH)-enriched aromatic fraction in $O_2^{>-}$ generation, decrease of membrane potential ($\Delta\Psi_m$), loss of mitochondrial membrane mass, and induction of apoptosis. A major effect of the polar fraction was to promote cyclosporin A (CsA)-sensitive permeability transition pore (PTP) opening in isolated liver mitochondria. This opening effect is dependent on a direct effect on the PTP at low doses as well as on an effect on $\Delta\Psi_m$ at high doses in calcium (Ca^{2+})-loaded mitochondria. The direct PTP effect was mimicked by redox-cycling DEP quinones. Although the aliphatic fraction failed to perturb mitochondrial function, the aromatic fraction increased the Ca^{2+} retention capacity at low doses and induced mitochondrial swelling and a decrease in $\Delta\Psi_m$ at high doses. This swelling effect was mostly CsA insensitive and could be reproduced by a mixture of PAHs present in DEPs. These chemical effects on isolated mitochondria could be reproduced by intact DEPs as well as ambient ultrafine particles (UFPs). In contrast, commercial polystyrene nanoparticles failed to exert mitochondrial effects. These results suggest that DEP and UFP effects on the PTP and $\Delta\Psi_m$ are mediated by adsorbed chemicals rather than the particles themselves. **Key words:** apoptosis, DEPs, diesel exhaust particles, PAHs, permeability transition pore, polycyclic aromatic hydrocarbons, quinones, ultrafine particles. *Environ Health Perspect* 112:1347–1358 (2004). doi:10.1289/ehp.7167 available via <http://dx.doi.org/> [Online 7 July 2004]

There is increasing evidence that particulate pollutants induce inflammatory responses in the cardiorespiratory system (Nel et al. 1998; Nightingale et al. 2000; Saldíva et al. 2002). These proinflammatory effects have been linked to the ability of particulate matter (PM), such as diesel exhaust particles (DEPs), to generate reactive oxygen species (ROSs) and oxidative stress in macrophages, bronchial epithelial cells, and lung microsomes (Gurgueira et al. 2002; Hiura et al. 1999; Kumagai et al. 1997; Nel et al. 2001). The pro-oxidative effects of the intact particles can be mimicked by organic chemical components extracted from these particles (Hiura et al. 1999; Kumagai et al. 1997; Li et al. 2002). The PM-induced oxidative stress response is a hierarchical event, which is characterized by the induction of antioxidant and cytoprotective responses at lower tiers of oxidative stress and by proinflammatory and cytotoxic responses at higher levels of oxidative stress (Li et al. 2002; Xiao et al. 2003).

Mitochondrial damage is a key event in PM-induced cytotoxicity (Hiura et al. 1999, 2000). The initial response to PM is a decrease in mitochondrial membrane potential ($\Delta\Psi_m$) and increased $O_2^{>-}$ production, followed by cytochrome *c* release and inner

mitochondrial membrane damage (Hiura et al. 1999, 2000; Upadhyay et al. 2003). It is also of interest that the smallest and potentially most toxic ambient particles, ultrafine particles (UFPs), lodge inside damaged mitochondria (Li et al. 2003). UFPs have a physical diameter < 0.1 μm , which allows them to penetrate deep into the lung as well as into systemic circulation (Nemmar et al. 2002). Although it is still a matter of debate whether UFPs target the mitochondrion directly or enter the organelle secondary to oxidative damage (Li et al. 2003), PM-induced mitochondrial perturbation has important biologic effects, which include the initiation of apoptosis and decreased ATP production (Hiura et al. 2000). Although the particles themselves may play a role in mitochondrial damage, it has been demonstrated that the organic chemicals adsorbed on the particle surface mimic the effects of the intact particles (Hiura et al. 1999). These effects can also be reproduced by functionalized aromatic and polar chemical groups fractionated from DEPs by silica gel chromatography (Alsberg et al. 1985; Li et al. 2000). These compounds are toxicologically relevant because the aromatic fraction is enriched in polycyclic aromatic hydrocarbons (PAHs), whereas the polar fraction contains

several oxy-PAH compounds, including quinones (Alsberg et al. 1985; Li et al. 2000). Quinones are able to redox cycle and to produce ROSs, whereas PAHs can be converted to quinones by cytochrome P450, epoxide hydrolase, and dihydrodiol dehydrogenase (Penning et al. 1999).

A key mitochondrial target for oxidizing chemicals is the permeability transition pore (PTP) (Jajte 1997; Susin et al. 1998; Zoratti and Szabo 1995). This calcium (Ca^{2+})-, voltage-, and pH-sensitive pore is permeant to molecules of < 1.5 kDa and opens in the mitochondrial inner membrane when matrix Ca^{2+} levels are increased, especially when accompanied by oxidative stress (Bernardi 1999; Kushnareva and Sokolove 2000; Zoratti and Szabo 1995). PTP opening causes massive *in vitro* mitochondrial swelling, outer membrane rupture, and release of proapoptotic factors such as cytochrome *c* (Susin et al. 1998). In addition, mitochondria become depolarized, causing inhibition of oxidative phosphorylation and stimulation of ATP hydrolysis. PTP opening is inhibited by cyclosporin A (CsA), which inhibits the peptidyl-prolyl *cis-trans* isomerase activity of cyclophilin D (Bernardi 1999). This has led to the proposal that PTP transition is mediated by a Ca^{2+} -triggered conformational change of inner membrane proteins (Woodfield et al. 1998). However, although this model may explain the action of some PTP modulators, PTP open–close transitions are also regulated by physiologic factors, drugs, and chemicals (Jajte 1997; Kushnareva and Sokolove 2000). Walter et al. (2000) characterized endogenous ubiquinones that stimulate or inhibit pore

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This work was supported by U.S. Public Health Service grants PO1 AI50495, RO1 ES10553, and RO1 ES10253 and by a U.S. Environmental Protection Agency (EPA) STAR award to the Southern California Particle Center and Supersite.

This work has not been subjected to the U.S. EPA for peer and policy review.

The authors declare they have no competing financial interests.

Received 8 April 2004; accepted 7 July 2004.