What is the Role of Oxidative Stress in the Mechanism of Particulate Matter Toxicity?

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research and development

Importance of Research

Epidemiological studies have shown an increase of adverse health effects from exposure to ambient PM, but the underlying basis for toxicity is not established.

Mechanistic research can help identify the most toxic PM for regulation

- Airborne particles are highly variable and complex mixtures.
- The toxicity of “real world” PM is not likely to be a simple sum of the toxicity of the diverse chemical components.
- The ability of PM to participate in chemical and biochemical reactions that produce health effects could be used to group and rank the importance of PM sources in health outcomes.
- This data will enable EPA to effectively develop policy approaches to control important sources of PM emissions.

Oxidative stress and inflammatory responses are involved in PM toxicity

- Oxidative stress is considered an important molecular mechanism of PM-mediated toxicity.
- Oxidative stress can produce pro-inflammatory effects that help explain PM-related asthma and atherosclerosis.
- Assays that quantify the capacity of PM from different sources to induce oxidative stress may be useful indicators of the potential of PM to induce pulmonary and cardiovascular inflammatory illnesses.
- Such measures have applications in exposure assessment and may facilitate development of science based regulations.

The chemical reactivity of PM can be measured

- The chemical species in PM can be organic or inorganic and are likely to act via two general chemical mechanisms, redox and electrophilic reactions.
- In the cell, the most common functional group associated with redox and electrophilic reactions are thiols and oxidation of these thiols by reactive species can lead to adverse cellular events.
- Chemical assays have been used to demonstrate that PM can generate reactive oxygen species.
- This reactivity is quantitatively assessed with chemical assays based on thiol reactivity and oxidation of the cellular antioxidant, ascorbate

HYPTOHESIS: Organic chemical components and transition metals associated with PM contribute to adverse cardiorespiratory effects based on their ability to induce oxidative stress. We propose that oxidative stress is responsible for the development of inflammation in the lung and cardiovascular system, and that a failure in antioxidant defense plays a role in the susceptibility to PM-induced adverse health impact.

Chemical basis for oxidative stress

•Formation of reactive oxygen species (ROS) (Superoxide, peroxide, hydroxyl) by metal, organic catalyzed reduction of oxygen.

•Oxidation of intracellular antioxidants (GSH, ascorbate) by ROS and redox active metals and organics.

•Depletion of intracellular thiols (GSH, RSH) by covalent attachment of metals and organics.

Hierarchical model of oxidative stress

High GSH/GSSG Ratio — Low GSH/GSSG Ratio
Level of oxidative stress
Normal — Anti-oxidant Defense — Inflammation — Toxicity

Cell response pathway:	Anti-oxidant Defense	Inflammation	Toxicity
Signaling pathway:	Nrf-2	NF-κB & MAPK	Mitochondrial perturbation
Genetic response:	ARE	NF-κB AP-1	PT pore

Tier 1 (lower levels of oxidative stress) - induction of phase II enzymes by a genetic response (which protect against Tier 2/3 responses). If Tier 1 protection fails…

Tier 2 (further increase in oxidative stress) - generation of pro-inflammatory responses, dependent on the activation of intracellular signaling pathways. Then…

Tier 3 - cytotoxic effects. Responses involve mitochondrial perturbation by pro-oxidative chemicals, leading to disruption of permeability transition (PT) pore opening.

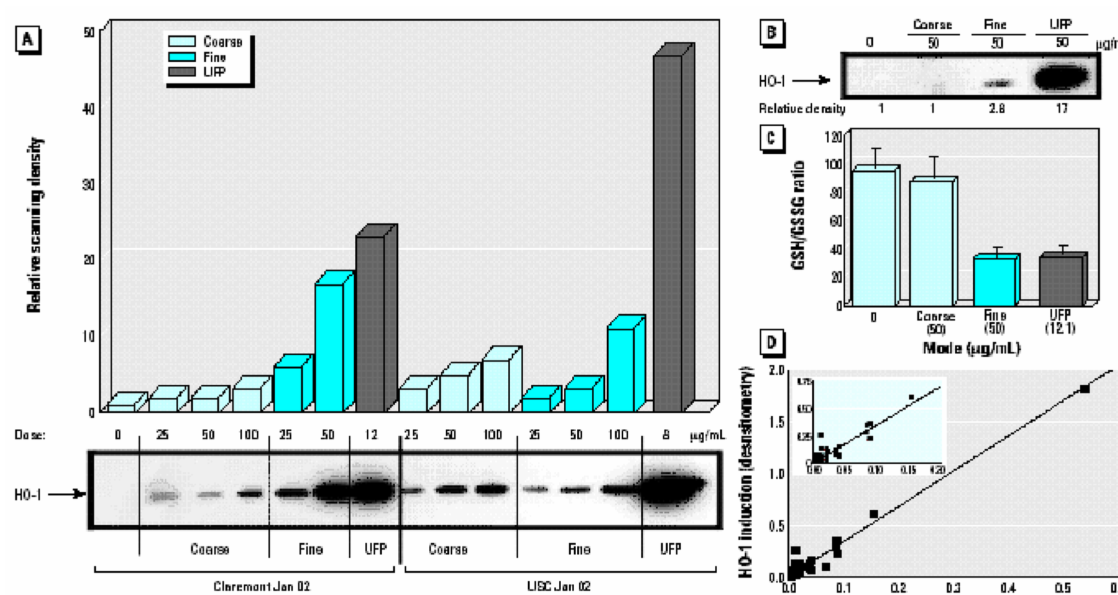
Testing the hierarchical model

•Model predicts different levels of cellular responses will occur depending on the **GSH/GSSG levels**.

•Responses will be **dose dependent**, related to the potency of the PM's ability to elicit oxidative stress.

•**Exposure** to PM will be **related** to both the **potency** of the PM and the **quantity** in the ambient air.

Ambient PM causes oxidative stress

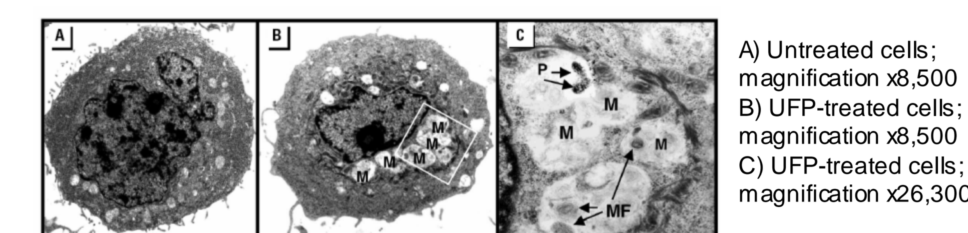


(A) **Dose dependent** induction of the antioxidant enzyme, hemeoxygenase-1 by ambient PM. **Ultrafine PM most active.**

(C) Change in GSH/GSSG ratios with different size fractions; **ultrafine most potent.**

(D) Correlation of HO-1 induction with PM redox activity, assayed by consumption of the dithiol, DTT, a measure of redox activity.

Uptake of ultrafine PM by mitochondria



A) Untreated cells; magnification x8,500
B) UFP-treated cells; magnification x8,500
C) UFP-treated cells; magnification x26,300

Figure shows formation of myelin figures (MF), and presence of particles (P) inside mitochondria (M).

Table 4. Comparison of DEP and UFP effects on isolated mitochondria.

Assay	DEP particle	Ambient UFPs
ΔΨm	Dose-dependent delayed or rapid depolarization CsA insensitive	Rapid depolarization CsA insensitive
Mitochondrial Ca^{2+} retention capacity	Decreased retention capacity CsA sensitive	Decreased retention capacity CsA sensitive
Mitochondrial swelling	Dose-dependent inhibition of Ca^{2+}-induced swelling No spontaneous swelling effects at any dose	Inhibition of Ca^{2+}-induced swelling at low doses (1 μg/mL) Spontaneous swelling at doses > 1.9 μg/mL Partially CsA sensitive

All assays were performed as described in “Materials and Methods”; DEPs were sonicated and tested in the dose range 1–50 μg/mL.

Diesel exhaust particles and ultrafines change membrane potential, calcium fluxes and the permeability of transition pores in mitochondria.

Metal Containing Fly Ash Oxidizes Lung Lining Fluid

Lung lining fluid normally contains the antioxidants glutathione and ascorbic acid. Oxidation of this fluid was increased by exposure to residual oil fly ash and this oxidation was enhanced by both ascorbate and glutathione. During the oxidation ascorbic acid becomes depleted.

IMPLICATION: Fly ash oxidized lung lining fluid. Dietary or disease induced changes in ascorbate or glutathione affect oxidation.

PM-derived ROS generation of antioxidant enzymes

Table 7. Activity of antioxidant enzymes in the lung and heart of rats exposed to CAPs or filtered air.

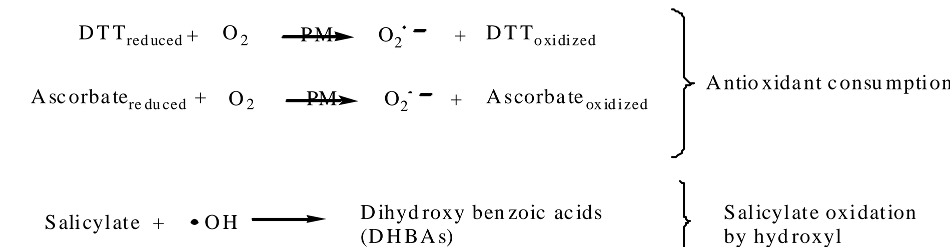
Activity	Lung: Filtered air	Lung: CAPs	Heart: Filtered air	Heart: CAPs
Cu/ZnSOD (U/mg protein)	38 ± 7	38 ± 10	170 ± 20	340 ± 60*
MnSOD (U/mg protein)	6 ± 3	10 ± 2*	11 ± 1	15 ± 2 *
Catalase (mU/mg protein)	43 ± 4	55 ± 5*	0.28 ± 0.03	0.34 ± 0.02
Fumarase (U/mg protein)	0.17 ± 0.01	0.19 ± 0.01	1.7 ± 0.1	1.8 ± 0.1

Values indicate mean ± SE ($n = 10$).
*$p < 0.05$ compared to control values.

•Animals were exposed to CAPs for 5 hours and lung/heart tissue content of proteins examined.

•Results show a rapid increase in these proteins after short exposure as a result of ROS generation.

Antioxidant consumption redox assays



$$DTT_{reduced} + O_2 \xrightarrow{PM} O_2^{\cdot -} + DTT_{oxidized}$$

$$Ascorbate_{reduced} + O_2 \xrightarrow{PM} O_2^{\cdot -} + Ascorbate_{oxidized}$$

} Antioxidant consumption

$$Salicylate + \cdot OH \rightarrow \text{Dihydroxy benzoic acids (DHBAs)}$$

} Salicylate oxidation by hydroxyl

•PM contains substances that accept **electrons** from DTT or ascorbate and transfer them to oxygen to generate **superoxide**.

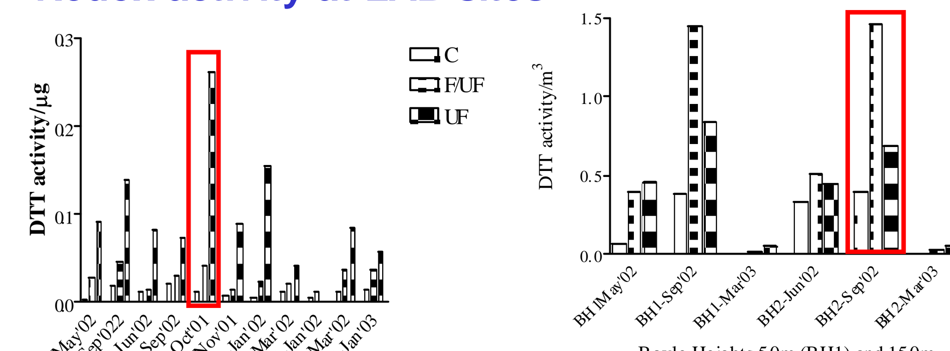
•Transition metals reduce hydrogen peroxide to hydroxyl which will oxidize salicylate to dihydroxybenzoic acids.

Application of antioxidant consumption assays

Assays provide **quantitative** measurements of the of **capacity of PM** to carry out reactions involved in ROS formation and ultimately, **oxidative stress**. DTT and ascorbate can be quantitatively determined and are complementary.

- •**DTT** reflects **redox** active **organic** compounds
- •**Ascorbate** reflects **metals and organics**
- •**Salicylate/DHBA** assay is a measure transition metal-based reactivity.

Redox activity at LAB sites



Toxicity when **mass** is standardized. Toxicity based on actual **exposure.**

•DTT measures superoxide radical anion formation, not Fenton chemistry.

•When activity is expressed **per mass**, the results reflect the **potency** of the sample. **Total exposure** measures the **quantity** of material to which an individual is exposed.

Antioxidant consumption by PM in a tunnel

Sample	DTT	Ascorbate	Salicylate (DHBA)
	nmoles consumed or formed /min*micrg		
Coarse	0.0200	0.0510	0.00049
Fine	0.0510	0.0410	0.00038
Ultrafine	0.1250	0.1168	0
DEP	0.1000	0.0430	0
Reactivity			
Metals (Cu, Fe)	No	Yes	Yes
Quinone (PQ, NQ)	Yes	Yes	Not detectible

•In the presence of ascorbate and salicylate, hydroxyl radical, generated by the Fenton reaction will form dihydroxy benzoic acids (DHBA). This reaction is catalyzed by metals.

•The results show that the coarse and fine fractions contain metals that catalyze the Fenton reaction whereas the contribution by metals to ascorbate consumption by the ultrafine fraction is minimal.

CONCLUSIONS:

Chemical/toxicological properties of PM

Contrasting features of coarse, fine, and ultrafine particles[a]

Parameters	Particle mode: Coarse (PM_{10})	Fine ($PM_{2.5}$)	Ultrafine
Size	2.5–10 μm	2.5–0.15 μm	<0.15 μm
Organic carbon content	+	++	+++
Elemental carbon content	+	++	+++
Metals as % of total elements	+++	++	+
PAH content	+	+	+++
Redox activity (DTT assay)	+	++	+++
HO-1 induction	+	++	+++
GSH depletion	+	+++	+++
Mitochondrial damage	None	Some	Extensive
Hydroxyl radical formation	++	+++	Not detectable

The chemical species of PM varies with size, source, and location of the particle.

Chemical reactivity vs. toxicity

The ability to induce oxidative stress is related to the redox activity of the PM sample.

•Exposure to PM results in the depletion of cellular antioxidants (GSH, ascorbate) and the induction of antioxidant proteins (HO-1, SOD).

•Cellular toxicity is related to changes in the redox status of the cell, i.e. thiol changes. Depending on the potency of the PM sample, cells as well as mitochondria exhibit different levels of toxicity. The ultrafine fractions of PM exhibit the greatest potency.

•Metals, organic compounds as well as the particle core itself are capable of catalyzing redox reactions that generate reactive oxygen.

Future Research

1. ***Development of highly sensitive biochemical assays for key thiol containing proteins:*** Assays for chemical reactivity and PM-induced biochemical alterations extend the ability to characterize PM from a range of sources in terms of potential for redox activity, oxidative stress, and cellular toxicity.

2. ***In vivo studies:*** New animal models are expected to be sensitive to the pro-inflammatory effects of PM exposure. Nrf2 knockout mice are an excellent model for studying the effects of defective phase II antioxidant response on the induction of airway inflammation. ApoE deficient mice can be used to study the role of PM-induced oxidative stress in arterial inflammation and apoptosis, and the development of early cardiovascular lesions following PM exposure.

3. ***Characterization of PM samples from varied sources, seasons, and photochemical conditions:*** Coordinated application of chemical assays, biochemical assays and in vivo studies will allow a wide range of PM to be tested and ultimately characterized in terms of potential to cause health harms. A battery of assays can be applied to compare PM from different emissions sources, atmospheric conditions, seasons, and co-pollutant concentrations.

Impact and Outcomes

While the adverse effects from PM have historically been associated with its airborne concentrations, it is necessary to develop chemical/biological approaches to **quantitatively** measure the ability of PM to catalyze the induction of oxidative stress to fully understand the resulting health effects. The **measurement** of redox and electrophilic activity represents **a first step** in better understanding the subsequent **downstream processes**.

This ability of PM to generate oxidative stress could be related to different sources, chemical composition, physical and spatial/temporal characteristics in the ambient environment. By linking the results of chemical/biological reactivity assays with in vivo based adverse responses to PM, it may be possible to identify the most harmful exposure sources and scenarios.

To be useful in setting standards and policies, quantitative information on source emissions, chemical/biological characteristics of PM, and exposure must be linked with the mechanistically-based assays and in vivo studies to identify the sources and characteristics of greatest health consequence. The work reported here addresses those issues and works to provide a **roadmap** for identification of the **most important characteristics of PM** that are **responsible for health effects** and form a scientific basis for **regulatory intervention**.

*The California Air Resources Board was a major contributor to this work.

Health and Exposure

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