Effects of fine Particulate Matter ($PM_{2.5}$) composition on ROS production in alveolar macrophages

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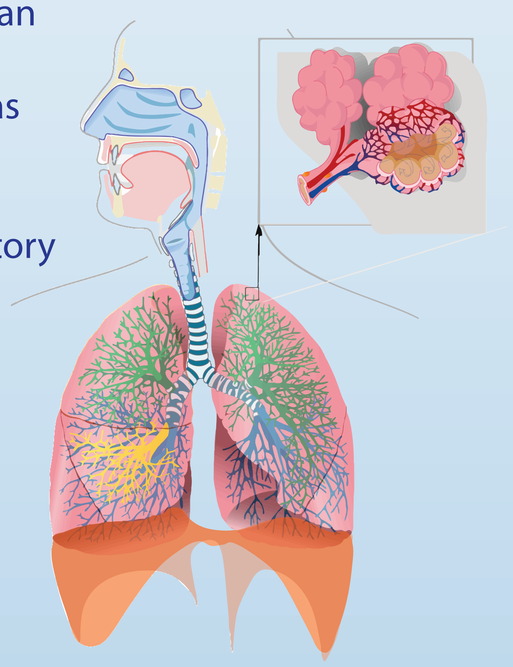
Introduction

How can we explain the molecular basis of inflammatory responses to fine particulate matter pollution?

In recent years, small airborne particulate matter (suspended solid and liquid particles with an aerodynamic diameter less than 2.5 microns; $PM_{2.5}$) has been identified as a leading cause of respiratory affliction in heavily-polluted regions[1]. Despite its pervasive impact on the health of urban communities, the molecular basis of pollutant exposure in the respiratory microenvironment has not been thoroughly characterized.

Innate defense mechanisms of the upper respiratory tract are ill-equipped to capture particulate matter (PM) within this size range. It has been suggested that these particles may trigger an acute stress response at the level of the alveolar microenvironment by perturbing local inflammatory features (e.g. alveolar macrophages).



Credit: National Institutes of Health

Methods

Parameter	Fresno F15	Fresno F46	Claremont C12	Claremont C24
Composition				
Cu(II) (ng/ug $PM_{2.5}$)	19.2446	85.9092	19.6338	48.2538
9,10 phenanthrenequinone (ng/ug $PM_{2.5}$)	0.2389	0.0100	0.0000	0.2211
Collection season	Winter	Winter	Summer	Summer
Time of collection (hours)	12	12	12	12

Fig 1. Geography and parameters of filter sampling. Ambient $PM_{2.5}$ samples were collected during the winter months of 2013 and summer months of 2012 in Fresno and Claremont to assess PM saturation during periods which have historically coincided with peak levels of air pollution in each respective city. Samples were characterized for their chemical composition using spectrophotometric and spectrometric techniques in collaboration with Alam Hasson's atmospheric chemistry laboratory.

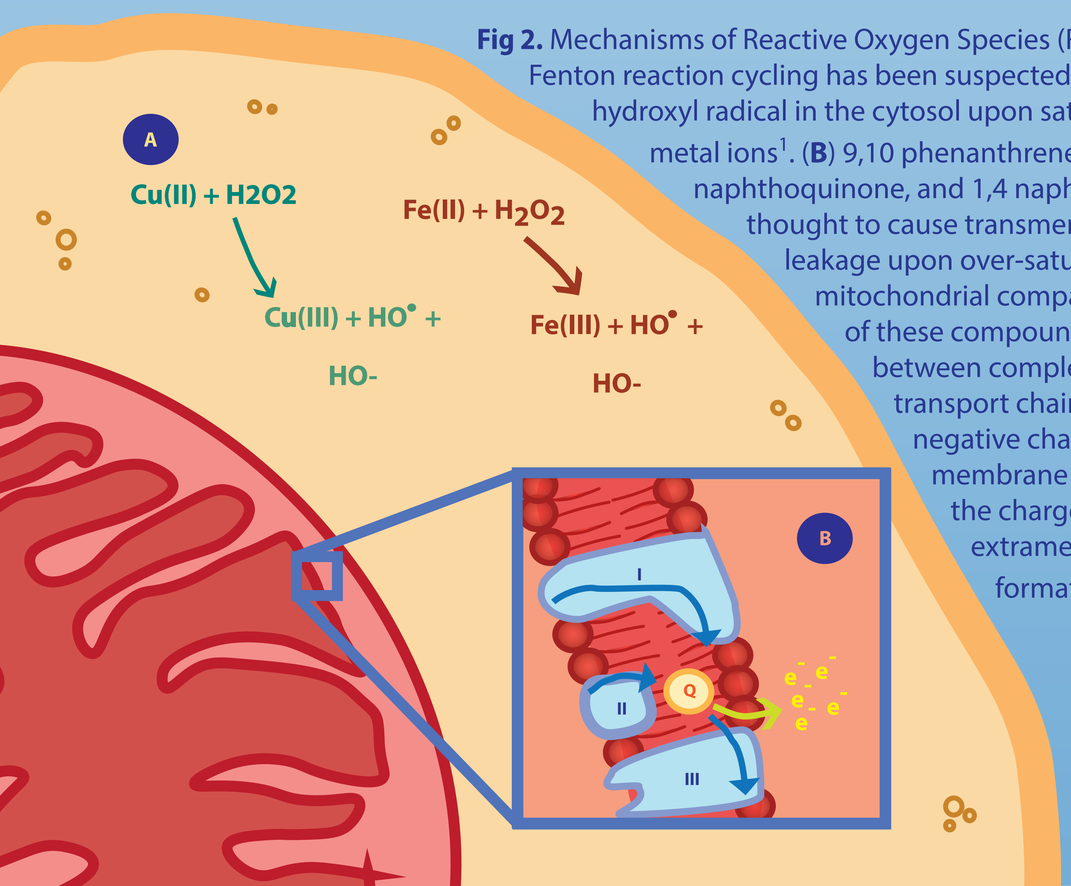


Fig 2. Mechanisms of Reactive Oxygen Species (ROS) Production. (**A**) Fenton reaction cycling has been suspected to produce hydroxyl radical in the cytosol upon saturation with transition metal ions[1]. (**B**) 9,10 phenanthrenequinone (PQ), 1,2 naphthoquinone, and 1,4 naphthoquinone are thought to cause transmembrane electron leakage upon over-saturation of the inner mitochondrial compartment. The propensity of these compounds to shuttle electrons between complexes of the electron transport chain generates a high negative charge within the membrane and, upon leakage of the charge into the extramembrane space, the formation of free radicals[2].

Outcomes

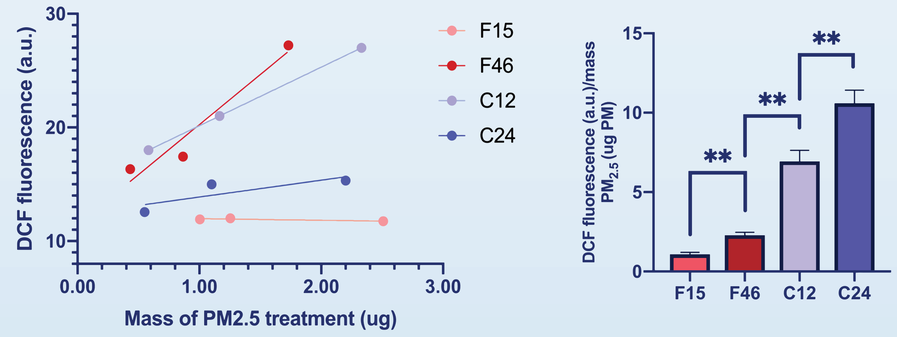


Fig 3. Treatment of NR8383 with $PM_{2.5}$ extracts. Populations of NR8383 (rat alveolar macrophage) cells were treated with filter elutions from four unique sampling campaigns to identify both regional and temporal specificity in the stress potential of pollutants within the alveolar macrophage model. Dichlorofluorescein-dihydrate (DCFH) probe was administered to sense ROS production; DCF flurorescence was recorded using a Biotek Synergy HT single-channel spectrophotometer at 485 ± 20 nm excitation and 528 ± 20 nm emission. Slopes obtained from linear regression of trends in DCF fluorescence were shown to be statistically significant by way of student's t-test ($p<0.05$). Error bars denote SEM.

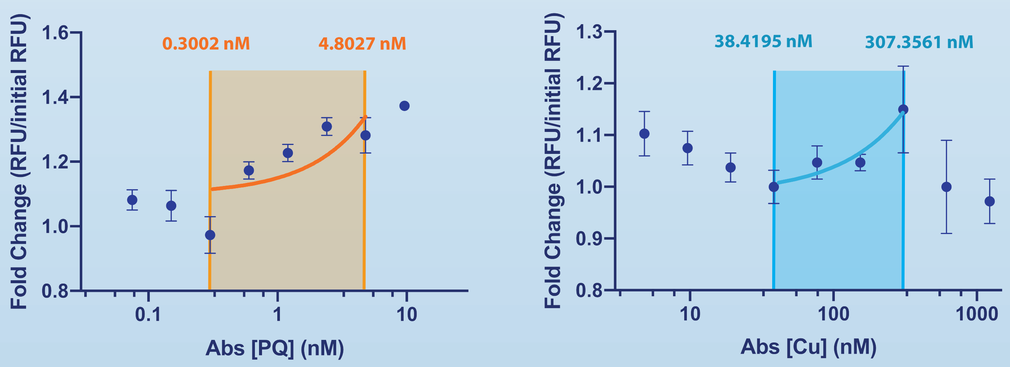
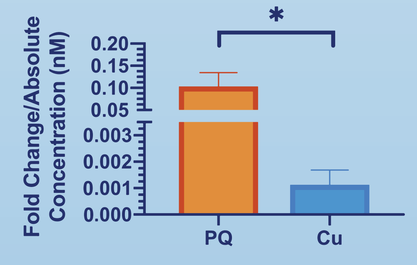


Fig 4 (above). Dose response curves from experimentally-determined atmospheric concentration ranges. Alveolar macrophages were treated with aqueous solutions of 9,10 phenanthrenequinone and $CuSO_4$ to identify ROS production trends in reponse to isolated chemical components of the sampled PM. Each graph represents one experiment with three replicate trials per treatment (n=3), wherein error bars denote SEM. The shaded regions denote the respective absolute concentration ranges of chemicals derived from atmospheric analysis.

Fig 5 (right). Comparison of slopes identified above. Linear regression of three experiments each of 9,10 phenanthrenequinone and $CuSO_4$ (n=3) revealed stark differences in the fold change of RFU per absolute concentration of relative treatment. Student's t-test revealed a statistically significant difference between cellular responses to PQ and Cu treatment ($p<0.05$) within experimentally-identified concentration ranges of either compound. Error bars denote SEM.



Implications and Future Directions

- PM-mediated ROS production in alveolar macrophages varies by location of PM collection, suggesting a variation in the reactivity of chemical constituents within alveolar macrophages
- Dose response curves of 9,10 phenanthrenequinone and copper ion suggest a significant difference in alveolar stress capacity of transition metals and quinones within ambient concentrations representing experimental levels of atmospheric chemical abundance
- Forthcoming assays will aim to identify the subcellular localization of PM-mediated ROS production upon treatment with selected comounds

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