Ambient particulate matter: Is there a toxic role for constitutive transition metals?

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1. INTRODUCTION

A core of epidemiology studies have demonstrated consistent statistical associations between daily concentration profiles of ambient particulate matter (PM) and mortality / morbidity rates in exposed human populations [reviewed in 1]. That the impact of ambient PM on human health can be observed at low, heretofore thought to be "safe" levels has attracted considerable attention within the environmental health and political communities, and has raised the question of whether the observations are "biologically plausible" [2]. In other words, is there an adverse biologic mechanism(s) of clinical significance that could account for the impact of such low levels of PM on human health? Indeed, if the observations can be substantiated, the question arises as to what subpopulations are most at risk and what are their risk factors?

Until recently, there has been little formal coordination between the epidemiological and toxicological assessments of PM-related health issues. The epidemiologists had, for many years, been without sufficiently sensitive statistical tools to clearly dissect the impact of pollutants like PM from other covariates, while the toxicologists had, for their part, largely focused attention on the effects on healthy laboratory animals of protracted exposures to single acidic aerosols occasionally co-mingled with ozone. However, with

the advent and relatively recent application of time-series data analyses to the air pollution issue [3], unperceived, yet significant, associations between PM and human health have been unveiled. The result has been a plea for relevant laboratory studies that might elucidate a "biologically plausible" explanation for the recent epidemiologic observations. This impetus has prompted most current empirical PM research to become more "hypothesis-driven", conceptually integrating aerosol monitoring and chemistry data, animal and clinical toxicology, with existent epidemiology.

Currently, several hypotheses proposed by laboratory investigators to explain the epidemiological findings are receiving particular attention. These hypotheses include: the acidic properties of PM [4], the ultrafine fraction (<0.01 (m) of the PM distribution [5], altered intrapulmonary distribution of the lung PM dose [6], the organic fraction of PM [7, focusing on cancer], the presence of biological materials in PM [8], and the transition metal content of PM [9,10]. Each hypothesis has a sufficient database to merit support and consideration in the PM research arena, but it is the "transition metal" hypothesis which has captured particular attention in our laboratory at EPA. This is not to imply that other explanatory theories have been dismissed or are being ignored off-hand; rather we feel that the "metal hypothesis" merits special attention because the concept of PM-associated metals provides reasonable answers to the following generic questions pertinent to any such theory.

- Are there environmental sources for exposure to the putative toxicant(s)?
- Is there evidence of personal exposure to the toxicant(s)?
- Does the putative toxicant(s) possess sufficient toxicity?
- Are the suspected mechanisms of toxicity able to be extrapolated to the human?
- Is there evidence of an exposure-response relationship with the toxicant(s), especially at low concentrations?
- How well does the theory generalize from one PM sample or locale to another?

2. THE "METAL HYPOTHESIS"

In brief, it is our opinion that PM-associated transition metals can reasonably satisfy each of the criteria as stated. Firstly, metals are ubiquitous in ambient PM, though they vary widely in concentration and in type. Since the early 1970's, it has been appreciated that the fine respirable PM fraction is most enriched with metal [11], which would agree with the apparent stronger associations of health effects with the fine PM (<2.5 (m) that deposits deep in the lung [1]. It is in the deep lung of urban dwellers that metals are retained and readily detected, particularly among inhabitants of air-sheds surrounding heavy industry [12]. Finally, studies in laboratory animals have shown that PM-associated metals can be toxic to the lung, inducing inflammation, altering normal physiologic function, and impairing host defenses [9,10,13].

The bioavailability of the metal (mostly as water or acid soluble cations) appears to govern its acute proinflammatory toxicity [14]. Recently, we demonstrated that a metal-rich emission source PM (a fugitive residual oil fly ash - ROFA) and ambient PM from various urban environments (St. Louis, MO; Washington, DC; Ottawa, Canada; and Düsseldorf, Germany) having only 10% the metal of the ROFA, induced qualitatively and quantitatively similar toxicologic effects when the samples were delivered to the lung in terms of bioavailable metal content, irrespective of PM mass. Likewise, extractable metal from PM samples in the industrial regions of Mexico City were found to be of equal potency as the ROFA when assayed *in vitro* for cytotoxicity, while the samples from the lesser polluted regions of the city were substantially less toxic (J. Bonner and K. Dreher, personal communication). In related studies, Ghio and coworkers [15] have shown that metal associated with humus-like organic material derived from fossil fuel combustion products, as well as ambient PM from several regions in the U.S., induced inflammation in the lungs of rodents in apparent proportion to the amount of extractable metal. Thus, bioavailable metal regardless of the carrier appears to be requisite for the PM toxicity [16]. Other studies suggest that some metals in a complex mixture like ROFA can interact, indeed in an antagonistic fashion, but that total bioavailable metal can be a reasonable metric for PM dose [10,14]. Not only does the theory relate to ambient PM, recent preliminary data from our laboratory indicates the

toxicity of domestic indoor air PM in rodents is similarly defined by its bioavailable metal content (unpublished data).

It is our view that transition metals provide the foundation for "biologic plausibility" and the theory appears to be garnering more and more attention [17], however, we remain far from accounting the epidemiologic observations. What is critical at this point is acquiring evidence demonstrating that metals associated with ambient PM can have effects at the low concentrations likely to be encountered in the ambient environment, and whether / how such PM-associated metal can induce effects in humans. *In vitro* studies with human airway epithelium show clearly that metals, such as vanadium, can induce toxicity by perhaps at least two mechanisms [18], but as with most *in vitro* studies, concerns remain regarding dose and extrapolation to the whole organism. Moreover, of incidental, but no less importance is the potential for cumulative metal toxicity, a mechanism yet to be explored.

3. ANIMAL MODEL OF PRE-EXISTENT CARDIOPULMONARY DISEASE

The epidemiology strongly suggests that the aged, particularly those with underlying cardiopulmonary diseases (e.g., COPD, infection) and children with asthma are more susceptible to the reputed PM-associated mortality and morbidity, respectively [1]. The difficulty in experimentally studying such persons has passed the gauntlet to toxicologists to attempt to study relevant animal models bearing analogous cardiopulmonary impairments. A number of models are currently being characterized and pursued in an effort to address this question, but one model recently emerged as having particular sensitivity to certain PM and may be working via metal-mediated mechanism.

When the alkaloid, monocrotaline (MCT), is injected into rodents, a progressive vasculitis appears after several days which results in pulmonary inflammation and vascular remodeling by ~10-12 days such that the animals go on to develop pulmonary hypertension, right cardiomegaly, and ultimately *cor pulmonale* ending in death after several weeks [19]. This injury has been exploited widely as a model to study the pathogenesis of pulmonary hypertension. This model has been shown to be sensitive to

ROFA when administered by intratracheal instillation [14] and more recently by inhalation [20]. The cause of death in the MCT-ROFA animals in the intratracheal studies appears to have been related to altered cardiac function [21], although it is unclear whether death was associated with direct cardiac injury or was secondary to pulmonary failure. Recent electrocardiographic studies in humans [22] suggest that heart rate changes and loss of beat variability with PM is consistent with the current clinical perception that loss of variability is a risk factor for cardiac arrhythmias and heart attack. The MCT model has not as yet been utilized with ambient PM in this laboratory, although Godleski and coworkers [17] have reported mortality in a similar model with inhalation of concentrated ambient Boston air (~350 mg/m^3 for 6 hr/d, 3 days). The significance of these findings demands that these studies be replicated both in Boston and elsewhere.

It is uncertain what the potential underlying mechanisms are determinant of the apparent susceptibility of the MCT treated animals. The induction of inflammation and associated interstitial edema reduces pulmonary diffusing capacity (unpublished data) could lead to ventilation-perfusion mismatches within the lung leading to secondary cardiac changes to an already damaged or stressed heart as suggested by the arrhythmias reported in the work of Watkinson et al. [21] and perhaps implicit in the recent human field study findings [22]. Our recent studies have suggested that the enhancement of the toxicity of intratracheally instilled ROFA in the MCT-treated rat may be mediated by augmentation of metal-catalyzed oxidant injury via the classic Fenton pathway due to the substrate rich milieu of the lung lining fluid during inflammation and the weakened state of the blood-air barrier (preliminary data). *In vitro* assessment of the feasibility of this reaction with ROFA is supportive [9], but direct evidence of this reaction in the *in vivo* model with ROFA, let alone ambient PM, is yet to be verified. More work is needed to assess this hypothesis in light of other pathophysiologic frailties of the impaired animal.

4. CONCLUSIONS

Recent epidemiologic studies indicate a significant association between ambient PM exposure and adverse health consequences, most apparent in susceptible subpopulations. The lack of a clear mechanism implying "biologic plausibility" has stimulated considerable speculation and research in the toxicologic community. Among the several hypotheses suggested, that involving bioavailable transition metals appears, thus far, to be consistent with the epidemiologic observations. However, results to date involve relatively high concentrations of metal in model emission and ambient PM samples. As results accumulate at much lower, and preferably inhalation exposure, concentrations the veracity of this hypothesis will be ascertained.

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