Bioavailable Transition Metals in Particulate Matter Mediate Cardiopulmonary Injury in Healthy and Compromised Animal Models

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Many epidemiologic reports associate ambient levels of particulate matter (PM) with human mortality and morbidity, particularly in people with preexisting cardiopulmonary disease (e.g., chronic obstructive pulmonary disease, infection, asthma). Because much ambient PM is derived from combustion sources, we tested the hypothesis that the health effects of PM arise from anthropogenic PM that contains bioavailable transition metals. The PM samples studied derived from three emission sources (two oil and one coal fly ash) and four ambient airsheds (St. Louis, MO; Washington; Dusseldorf, Germany; and Ottawa, Canada). PM was administered to rats by intratracheal instillation in equimass or equimetal doses to address directly the influence of PM mass versus metal content on acute lung injury and inflammation. Our results indicated that the lung dose of bioavailable transition metal, not instilled PM mass, was the primary determinant of the acute inflammatory response for both the combustion source and ambient PM samples. Residual oil fly ash, a combustion PM rich in bioavailable metal, was evaluated in a rat model of cardiopulmonary disease (pulmonary vasculitis/hypertension) to ascertain whether the disease state augmented sensitivity to that PM. Significant mortality and enhanced airway responsiveness were observed. Analysis of the lavaged lung fluids suggested that the milieu of the inflamed lung amplified metal-mediated oxidant chemistry to jeopardize the compromised cardiopulmonary system. We propose that soluble metals from PM mediate the array of PM-associated injuries to the cardiopulmonary system of the healthy and at-risk compromised host. — *Environ Health Perspect* 105(Suppl 5):1053–1060 (1997)

Key words: particulate matter, metals, air pollution, lung injury, animal models, pulmonary hypertension

Introduction

Over the last several years, a robust database has emerged from a series of epidemiology studies demonstrating a statistical association between ambient air pollution particulate matter (PM) and mortality/morbidity among the exposed human population (*1–4*). These studies revealed remarkably consistent unit risk estimates across diverse urban airsheds, based primarily on outdoor mass metrics of exposure, i.e., total suspended particulates (TSP) and PM ≤ 10 μm in aerodynamic diameter (PM_{10}) (*3,4*). Indeed, as the mass metric for PM is restricted to smaller size ranges, the strength of the statistical correlation increases (i.e., $PM_{2.5} > PM_{10} > TSP$) (*3,5,6*). Subsequent studies have suggested that certain individuals may be at higher risk for adverse effects of PM (*3,7–10*). The elderly with chronic cardiopulmonary diseases, those with pneumonias, and those with asthma at any age appear to be at higher risk. However, considerable uncertainty remains about specific biomarkers or biological mechanism(s) that might underlie the higher risk.

Although the epidemiology of ambient air PM is compelling, these findings suffer from the limited ability of epidemiology to address the issue of causality. However, animal toxicology studies, though encumbered with other limitations that relate to extrapolation, are capable of directly addressing questions regarding the mechanisms of response to PM with controlled empirical study designs. We used a toxicologic approach to address two critical questions that have evolved from the epidemiology database:

- Are there constituents common to PM that could account for the consistency in epidemiologic findings across such diverse exposure environments?
- Can animal models of cardiopulmonary disease elucidate the etiology of apparent subpopulation susceptibilities to PM as identified by epidemiology?

These two questions transcend the PM issue and focus attention on the underlying causation and toxicologic potency of ambient air PM and how the physicochemical properties of PM link to the apparent potentiation of effects in predisposed individuals.

Ambient air PM_{10} is comprised of a complex mixture of crustal and anthropogenic matter that are naturally separated by size. The crustal material, consisting largely of insoluble silicates derived from abrasive processes, is typically larger in size (≥ 2.5 μm) and often is referred to as coarse mode PM. The PM fraction below 2.5 μm is considered fine mode; it arises from combustion processes or atmospheric transformation of combustion emissions. The PM fine mode is of particular concern as a potential health threat. As this fraction remains airborne for long periods of time, it penetrates the indoor air environment and enters the respiratory tract to deposit in the airways and deep lung.

Fly ash from fossil and waste fuel combustion contributes $> 2.5 \times 10^5$ tons annually to the U.S. ambient PM burden (*11*). Fly ash is concentrated mostly in urban areas and can be considered a fine

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Abbreviations used: BAL, bronchoalveolar lavage; CFA, coal fly ash; DC, Washington; DOFA, fly ash from a domestic oil-burning furnace; Dus, Dusseldorf, Germany; IT, intratracheal; LDH, lactate dehydrogenase; MCT, monocrotaline; NIST, National Institute of Standards and Technology; Ott, Ottawa, Ontario, Canada; PM, particulate matter; PM_{10}, particulate matter ≤ 10μm in aerodynamic diameter; PMN, polymorphonuclear neutrophil; ROFA, residual oil fly ash; St.L, St. Louis, MO; TSP, total suspended particulates; U.S. EPA, U.S. Environmental Protection Agency.

mode PM that is relatively rich in metal contaminants (*12*). To address the hypothesis that PM-related health effects arise from anthropogenic combustion-related PM with bioavailable transition metals, we elected to use representative emission-source PM. The emission-source fly ash selected for study derives from the combustion of domestic and industrial grade fuel oil and coal, and represents a range of bioavailable constitutive metals. An isometric approach was used (equal dose–mass or equal dose of metal) in an attempt to characterize the constituents from these sources, which are important determinants of the inflammatory response. In addition, we used several collected TSP samples to ascertain the involvement of metals in ambient PM-induced acute lung injury. Previous work with various types of PM suggested that ionizable metals in PM correlate with indicators of inflammation, altered host resistance, macrophage dysfunction, and airway hyperreactivity (*13–16*), but these studies have not attempted to manipulate mass and metal dosing to directly address this concept. Concomitantly, we established a model of cardiopulmonary disease in the form of advanced pulmonary vasculitis/hypertension to explore the question of susceptibility and the possible mechanisms by which PM effects are potentiated.

Methods

Animals

Male Sprague-Dawley rats (60 days of age) were obtained from Charles River Breeding Laboratory (Raleigh, NC) and housed in an American Association for Assessment of Laboratory Animal Care International approved animal facility for at least 1 week before use. They were maintained at 22 to 23°C, 50 to 60% relative humidity, and a 0600- to 1800-hr light cycle. Purina rat chow (Purina Mills, Richmond, IN) and water were available *ad libitum*. Study group sizes varied.

Model Particulate Matter

Three types of fly ash were used as emission-source particulates to model the combustion component of fine mode PM. Residual oil fly ash (ROFA) was collected as a fugitive ash downstream of an air-cleaning cyclone from a power plant burning industrial grade #6 low-S residual oil (*13*). Fly ash from a domestic oil-burning furnace (DOFA) that provided heat for a large apartment complex was collected as distal flue-deposited ash. It may be considered a mix of both fugitive and settled emission PM, and was provided by A. Ghio of the U.S. Environmental Protection Agency (U.S. EPA). The coal fly ash ([CFA] #4213.3) was collected by the Energy Assessment and Control Division, National Exposure Research Laboratory, U.S. EPA, from an experimental boiler furnace at the Research Triangle Park, NC research facility. The source coal was Pittsburgh seam coal, burned at 825°C using Grove limestone absorbent (Ca/S ratio = 3/1).

St. Louis, MO (St.L) and Washington (DC) PM were purchased from the National Institute of Standards and Technology ([NIST] Gaithersburg, MD) as SRM #1648 and SRM #1649, respectively. Dusseldorf, Germany (Dus), and Ottawa, Ontario, Canada (Ott), PM samples were donated by G. Hatch, U.S. EPA, and R. Vincent, Health Ministry of Canada, respectively. By their collection mode, bagged house TSP samples are less than ideal. There is little selectivity in the collection process except for the screening of relatively large debris and vegetative fragments, and collection occurs usually over long periods of time with wide-ranging ambient humidity and temperature. Thus, it is likely that any losses or changes in PM chemistry due to inherent sample instability would have already taken place, even if the sample was subsequently stored under controlled conditions designed for preservation. In this sense, the samples used here must also be considered less than ideal. They were not of similar age, nor were they stored under similar conditions. Their age ranged from about 15 years (St.L, DC, Dus) to 3 years (Ott), and they were either stored frozen (DC, St.L) or at room temperature (Dus, Ott) in sealed bottles. However, each sample was sieved through a 30-100 mesh to remove large extraneous debris. As such, though there were likely to be differences in PM organic character, we felt that the metal content would be relatively stable for the purpose of hypothesis testing. Indeed, comparisons of the metal content of the St.L (SRM #1648) and DC (SRM # 1649) dust in our laboratory with that published by the NIST indicated no change. Similarly, the ROFA dust analysis that we conducted for metals (*16*) was quite similar to that done in 1985 (*13*). We recently showed that low levels of PM-associated endotoxin did not contribute to the *in vivo* toxicity of these ambient PM samples (*17*).

The basic physicochemical properties of each emission and urban PM studied are provided in Table 1. The samples were prepared similarly for characterization of soluble metal and S (presented as $SO_4^=$) content. To determine the acid-extractable metal content, PM samples were suspended in 1 M HCl in polypropylene tubes at a concentration of 6.4 to 7.9 mg/ml, followed by mixing end-over-end for 1 to 2 hr at room temperature. The acid hydrolysates were then centrifuged at 17,000 × *g* for 20 min, then the supernates were transferred to fresh polypropylene tubes for metal analysis. Analogously, double-distilled H_2O was used

Table 1. Particulate matter physicochemical characterization.

Sample	Transition metal content[a] Fe	Cu	Ni	V	Zn	Total metal	Total sulfate	Size[b]/ geometric mean	pH[c]
Emission PM									
DOFA	154.51 (85)	2.07 (80)	0.12 (100)	0.00	9.00 (100)	165.70 (86)	358.74 (95)	1.78/2.02	2.59
ROFA	23.31 (68)	0.23 (97)	37.51 (92)	41.71 (84)	1.01 (95)	103.77 (83)	560.52 (100)	1.95/2.19	2.99
CFA	14.57 (0)	0.03 (0)	0.70 (8.2)	0.37 (0)	0.08 (0)	15.75 (0.4)	49.36 (58)	4.17/5.00	9.72
Ambient air PM									
StL	8.91 (0.4)	0.37 (30)	0.11 (35)	0.11 (30)	3.56 (73)	13.06 (22)	142.66 (93)	3.72/4.42	5.22
Dus	9.65 (0.5)	0.42 (21)	0.24 (49)	0.18 (31)	3.70 (68)	14.19 (20)	137.28 (100)	3.58/4.44	5.28
Ott	6.33 (2.3)	0.82 (18)	0.27 (30)	0.21 (0.9)	10.63 (57)	18.26 (35)	70.41 (90)	4.09/4.90	6.0
DC	6.57 (4.7)	0.14 (33)	0.02 (100)	0.20 (76)	1.49 (91)	8.42 (23)	93.48 (91)	3.27/3.88	3.65

[a]Transition metal content obtained from 1 M HCl hydrolysis of PM and expressed in μg/mg PM. Values in parenthesis represent percent of the metal that is water soluble. [b]Mass median aerodynamic diameter of PM. [c]pH of a 10- to 11-mg/ml aqueous suspension.

to extract the water-soluble metals, starting with a concentration of 10 to 11 mg/ml. The recovered supernate from this procedure was then acidified to pH < 2.0 with 1 M HCl for metal analysis. The concentrations of Fe, V, Ni, Cu, Zn, and $SO_4^=$ in each extraction were quantified against specific standards (Sigma Chemical, St. Louis, MO) using a PE Model P40 inductively coupled plasma emission spectrophotometer (Perkin Elmer, Norwalk, CT). PM aerodynamic size distributions were determined using a TSI 3310A aerodynamic particle sizer (TSI, St. Paul, MN). The acid-soluble transition metals (Cu, Fe, V, Fe, Zn) common to all PM were used as the basis for total metal comparisons.

Particulate Matter Exposure and Bronchoalveolar Lavage

Each PM was administered by intratracheal (IT) instillation as previously described by Dreher et al. (*16*). Briefly, a predetermined mass of PM was diluted with sterile, injection-grade saline (0.9%) to provide sufficient dosing mixture to dose all the animals in a group. The rats were anesthetized lightly (to lose gagging reflex and response to pinch) with halothane and instilled IT with 0.3 ml containing the desired PM sample, via a transoral tube placed under laryngoscopic view (*18*). The animals were then returned to their cages; they regained consciousness within approximately 10 min. The treatment doses were based on either *a*) administration of equal dose by mass (nominal 2.5 mg/rat) of each PM or *b*) normalization of each PM mass to a total metal content of 46 µg/dose for the emission PM comparison (see Table 1 for the appropriate metal content) and 35.5 µg of total metal (as Cu, Fe, V, Fe, Zn) for the ambient PM and ROFA comparison.

At 24 and/or 96 hr following IT instillation, the rats were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, IL) (50 mg/kg body weight, ip), then exsanguinated via the abdominal aorta. Rat tracheas were cannulated and bronchoalveolar lavage (BAL) performed with phosphate-buffered saline (Mg^{+2}- and Ca^{+2}-free) pH 7, using a volume equivalent to 28 ml/kg body weight, infused and withdrawn three times.

BAL cell counts were determined using a Coulter Counter (Coulter, Miami, FL). Cell differential analyses were performed on BAL cytospin samples prepared using a Shandon model cytospin 3 (Shandon Instruments, Pittsburgh, PA) and stained with LeuKoStat (Fisher Chemical, Pittsburgh, PA). BAL samples were centrifuged at $850 \times g$ for 10 min at 4°C. The recovered cell-free supernates were used for the following biochemical analyses: total protein was determined using the Coomassie Plus Protein Assay (Pierce, Rockland, IL); albumin was determined using the MALB SPQ kit (INCSTAR, Stillwater, MN); and lactate dehydrogenase (LDH) activity was determined using kit #228 (Sigma Chemical). All assays were adapted for automated analysis performed on a Hoffmann-La Roche Cobas Fara II clinical analyzer (Roche Diagnostics, Branchburg, NJ).

Animal Model of Pulmonary Hypertension

An animal model was established to assess the role of preexisting lung disease as a predisposing factor in PM responses. Pulmonary vasculitis/hypertension was induced in male Sprague Dawley rats 60 days of age, using a single ip injection of monocrotaline ([MCT] Aldrich Chemical, Milwaukee, WI, 60 mg/kg), as previously described (*19*). After 10 to 12 days vascular remodeling was well under way, and pulmonary arterial pressure was just be beginning to increase (*19*). Pulmonary artery pressure was measured in a limited number of rats ($n = 3$/group) by catheterizing the pulmonary artery via the jugular vein in the anesthetized intact rat (*20*). In a similar cohort of rats, BAL was conducted to ascertain the degree of lung inflammation as described above as well as H_2O_2 content (assayed by chemiluminescent reaction with isoluminol and microperoxidase). Control and MCT groups ($n = 6$) were treated IT with ROFA at doses of 0, 0.25, 1.0, and 2.5 mg/rat. At 96 hr post-IT ROFA, the rats underwent BAL as above.

Statistical Analysis

Data were analyzed using one- or two-factor analysis of variance and assessed for overall differences. Pairwise testing (*t* test) was used, with adjustment for multiple comparison. The hypothesis tested was that each PM sample would differ from control and that specifically for metal-based comparison the PM samples would not differ among themselves. Significance was assigned when the *p* value was ≤ 0.05.

Results and Discussion

Metal-based Particulate Matter Toxicity

This study attempted to evaluate further the relative importance of bioavailable metal to that of the mass dose of PM. By analogy, this approach was applied to ambient urban PM. To this end, three emission-source PM, representing a wide range of varied metal composition and bioavailability, were evaluated and compared in the context of four urban TSP from domestic and international sites. The findings were consistent with other studies, which indicated that the toxicity of PM generally correlated with total acid-soluble metal content, and that the oxidant potential of the variously solubilized metal extracts might be a contributing mechanism (*13–15*). The variability in correlations both here and in the noted studies may reflect the varied oxidant potential of transition metals, their multiplicity of actions, or potential interactions between the metals (*16*).

Table 1 displays the physicochemical characterization of the fly ash and urban PM we examined. Among the fly ash, there was considerable variation in constituent acid-soluble metal; the DOFA contained about 50% more total metal than ROFA and nearly 10 times that of the CFA. However, while the metal content of ROFA was largely distributed among Fe, Ni, and V, DOFA-associated metal was primarily Fe, exceeding that of ROFA by 7- to 10-fold. The Zn content of DOFA was also relatively high compared to ROFA, and like much of the acid-available metal in oil fly ash, the majority was water soluble and thus easily bioavailable. This contrasted with the CFA, which contained a relatively small amount of acid-extractable Fe and even less V, Ni, Cu, and Zn; thus no metal was readily bioavailable. The pH values for oil fly ash were low compared to the CFA, which probably reflects the relatively high $SO_4^=$ content of the former. Oil fly ash is known to associate with the metals during combustion cooling (K Olin, personal communication).

The ambient PM samples represented in Table 1 contained about 10% of the acid-available metal of the oil fly ash, of which only a small amount was water soluble. Therefore, fly ash appears to be a reasonable, if significantly more potent, surrogate for the study of PM mechanisms. However, it should be noted that because TSP comprises nearly the entire size range of ambient PM, the indicated metal bioavailablity likely underrepresents the relatively metal-rich respirable fine mode PM that would be of most health concern (*19*). With regard to bioavailable metal, the TSP may be considered a mass-diluted form of fine mode PM. In PM $SO_4^=$, the

difference was less dramatic (< 50%) than that of oil fly ash. This probably reflects the contributions of other sources of $SO_4^=$ such as that from photochemical transformation of SO_2 and perhaps stabilized by NH_4^+ (*21*). This would account for the lower acidity of TSP relative to that of oil fly ash.

A wide range of metals was represented in the ambient TSP samples, but we elected to focus on the predominant five that also were predominant in fly ash. The particle size of the ambient TSP samples likely does not represent that which exists in the atmosphere but rather is the product of agglomerations that occurred during collection. However, their size and relative metal insolubility compared to oil fly ash prompted us to include the CFA to make comparisons on the basis of dose mass of the PM challenge.

Figure 1A to C illustrates the impact of IT-instilled fly ash on BAL parameters of altered permeability (total protein, albumin) and cellular injury (LDH) at 24 and 96 hr after administration. The instillation of saline had no significant effect on these parameters when compared to caged controls (K Dreher, unpublished data); similarly, acidic instillates have no effect on these parameters (*16*). With an instillate dose of approximately 2.5 mg fly ash, DOFA had the greatest effect, with an acute (24 hr) and dramatic alteration in permeability and clear evidence of cellular injury (Figure 1D–F). By 96 hr, permeability alterations reversed considerably, although the indicators of cell injury remained high. ROFA also induced considerable injury but clearly less than that of DOFA; their relative patterns of reversal, however, were similar. The degree of response exceeded that predicted solely on the basis of total metal. As DOFA's metal mix (a ratio of 75:1:17 for Fe:Cu:Zn) differed from that of ROFA (1:1.5:2 for Fe:Ni:V) and as there are known interactions among some transition metals (*16*), varied mechanisms may have been operant. Additionally, it appears that Zn may act differently (alone or interactively) in high concentrations, as shown in another fly ash that also contained V and Ni, albeit in lower concentrations (*22*). When compared to oil fly ash samples, CFA with most of its metal tightly bound induced little, if any, injury, and none of its parameters differed from those of the saline control. Clearly, the differential bioavailability of metal content was reflected in the dramatically different responses obtained with DOFA, ROFA, and CFA when administered in an equimass manner.

To address the role of metals as the primary determinant of injury, rats were treated with each emission fly ash on an equimetal basis (~ 46 µg per rat); mass dose varied. As predicted, the initial injuries induced by these emission PMs did not differ appreciably from each other at the 24-hr time point (Figure 1D–F). However, by 96 hr, some significant differences became apparent. In DOFA and CFA, where the predominant metal was Fe, the equimetal comparison shows similar responses for all BAL inflammatory parameters at 24 and 96 hr. In contrast, the ROFA effect on the BAL parameters at 96 hr persisted, which suggested either that one of the metals not in the other fly ash had a sustained impact (perhaps Ni) (*16*) or that metal mixture interactions in the equimetal dose of ROFA prolonged or altered the longevity of the response, as reported previously (*16*). Thus, caution is necessary in interpreting bioavailable metal effects when multiple metals may be involved. However, a primary role of the bioavailable transition metals in the magnitude of the responses is clear.

The impact of fly ash on BAL cellular profiles (Figure 1A–C) when administered in accordance with the equimass protocol yielded results in general agreement with the BAL injury parameters. With the metal-rich oil fly ash, the macrophage and polymorphonuclear neutrophil (PMN) influxes approached an order of magnitude higher cell number when compared to the CFA, which had only a small amount of bioavailable metal. When administered on an equimetal basis (Figure 1D–F), the inflammatory cell responses are surprisingly similar in spite of minor fluctuations that are common to BAL cell data. The eosinophils, normally considered in the context of allergic or parasitic inflammation, respond to the instilled PM and similarly appear to be metal mediated. This eosinophil response to metals is in many ways a curiosity that is characteristic of metal exposure in the context of the PM response, and suggests a means of indicting metal-based activity and anthropogenic combustion sources in the assessment of ambient PM.

Examination of Figure 2A to F reveals that the inflammatory responses to the urban PM are coherent with the oil fly ash samples when linked by the hypothesis that bioavailable metals drive the response. Therefore, ROFA studies could serve as the positive control and, using the extensive database developed in this laboratory, further insights could be developed. The equimass dosing with urban PM samples and ROFA clearly demonstrated the relative potency of fly ash compared to urban PM samples. With some variability in response, all four of the urban PM samples displayed about 10% of the ROFA activity in terms of BAL injury markers. On the other hand, when all the PMs including ROFA were administered in equimetal doses, the magnitude of the change in the BAL parameters was similar. Closer scrutiny of the data reveals that the Ott PM induces a small but consistently greater response for all inflammatory markers. Lacking an obvious explanation, one might argue that the excess response relates to the somewhat higher content of water-soluble metal in this PM (Table 1) or perhaps a proinflammatory role of Zn, as suggested by Gavett et al. (*22*). Alternatively, the Ott was also the freshest (most recently collected) PM of the group studied, although a loss of metal potency with age of the PM seems unlikely. The potency of ROFA in this study is virtually identical to that previously tested (*13*).

As with oil fly ash, there was an appreciable eosinophil response associated with the urban PM samples (Figure 2F). What is striking is that even with the dilution effect of coarse mode PM mass on that of the fine mode within TSP, the response persists. If one assumes that eosinophilia in BAL is a marker of metal associated with fly ash as noted above, then this cell type possibly could serve as a potential marker of fine mode PM exposure. Recent studies (K Dreher, unpublished data) utilizing selectively collected fine mode urban PM appear to support the contention that eosinophils can serve as such a marker. Likewise, the equimetal studies demonstrated the remarkable consistency of eosinophilia across ambient PM samples as well as with ROFA itself, which lends further support to the notion that combustion source metals in PM play a contributory role in the biologic responses to ambient PM.

In conclusion, it appears that the induction of injury by emission and ambient PM samples is determined primarily by constituent metals and their bioavailability. Based on studies with fly ash, the earliest phases of the response appear to be driven by the individual metals (*16*), but the persistence of the response perhaps reflects the complexity of the bioavailable metal mix or the

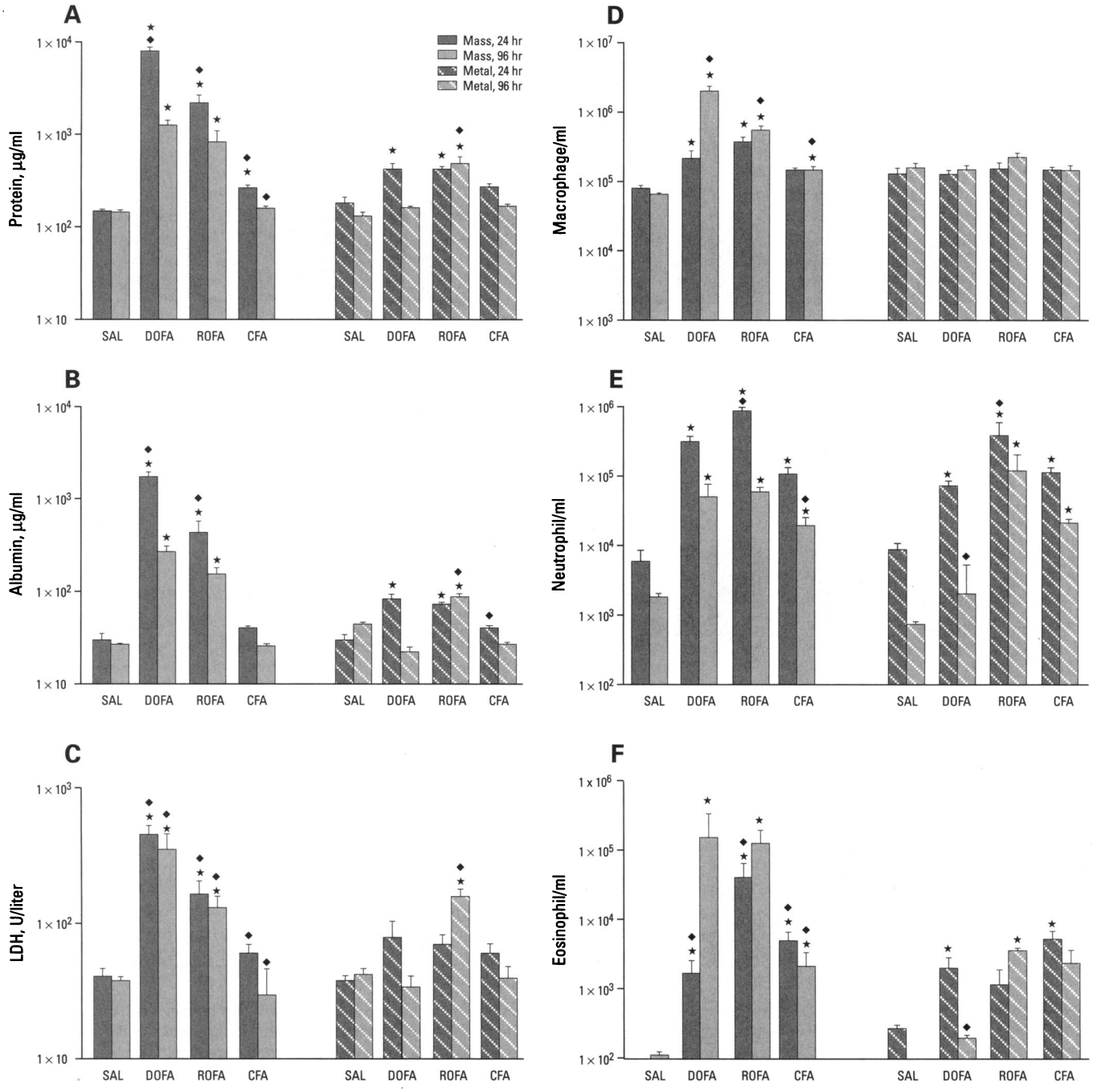

Figure 1. Emission source PM-induced acute pulmonary injury. Results were obtained from analyses performed on BAL samples at 24 and 96 hr postexposure. Rats were exposed by IT instillation to saline (SAL), ROFA, DOFA, or CFA. Mass refers to responses obtained from animals exposed to an equivalent mass (~2.5 mg/rat) of each PM. Metal refers to responses obtained from animals exposed to an equivalent amount of total transition metal of each PM (46 µg/rat). This dose of metal translates to the following mass dose of PM: ROFA, 0.450 mg/rat; DOFA, 0.282 mg/rat; and CFA, 2.97 mg/rat. Values are expressed as means + SEM. Animals per group, $n = 3$ to 6 at 24 and 96 hr postexposure. ★, significant difference from saline control. ◆, significant difference from the other emission samples, excluding SAL.

unique qualities of one metal compared to another. How the metals are solubilized *in vivo* and interact to induce toxicity requires further investigation, as redox potential is not the same for all the transition metals and may depend in part on its coordination state (*23*). The PM challenges described herein involve complex mixtures of metals and other PM-associated compounds (i.e., small molecular weight or acidic water-soluble organics) for which further study is also needed. Clearly, these conclusions need to be confirmed using actual inhalation exposures, which involves both temporal and dosimetric relationships in need of examination.

Animal Model of Pulmonary Hypertension

MCT is metabolized in the liver to the pyrrole that is specifically toxic to the

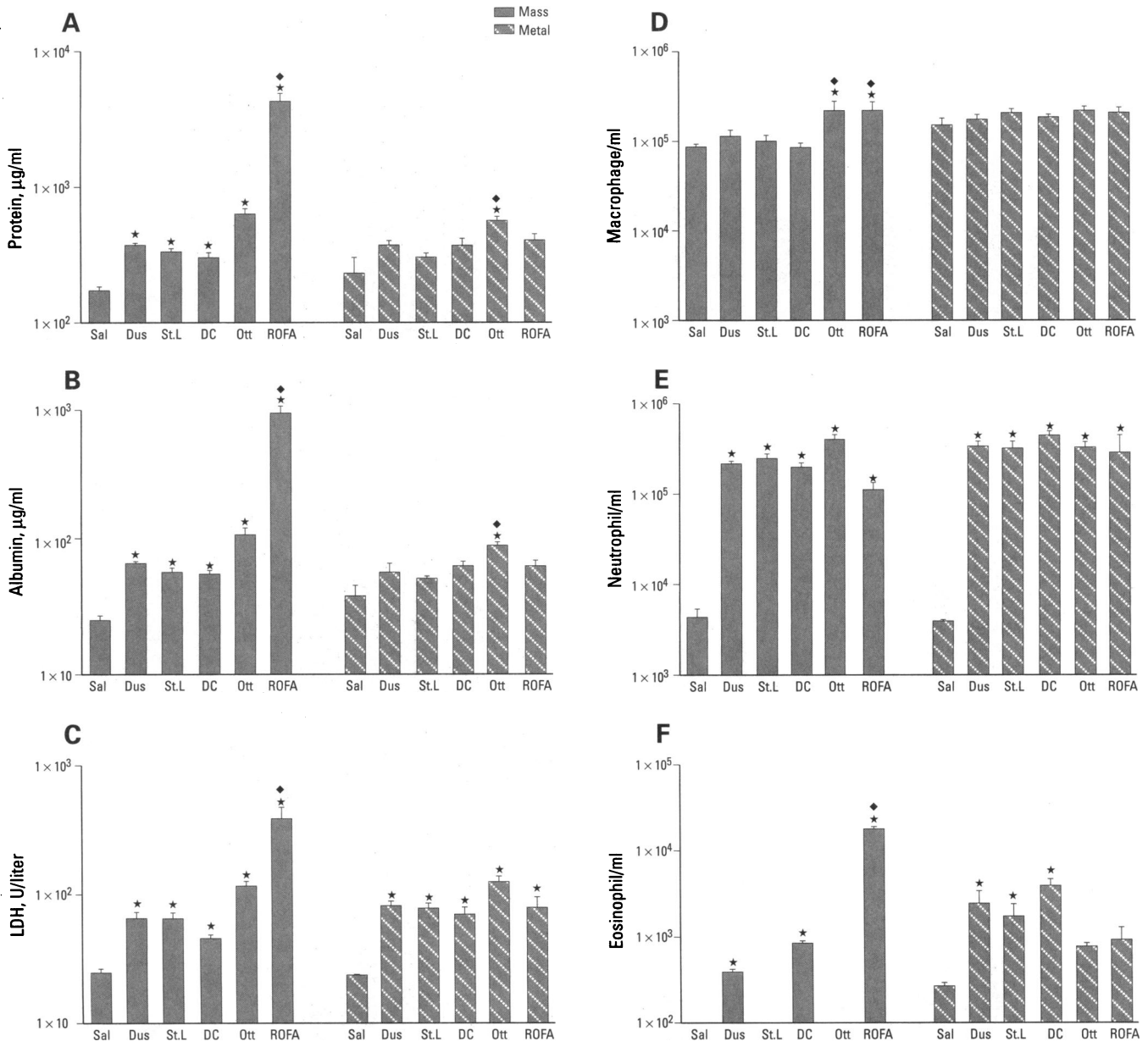

Figure 2. Urban ambient air PM-induced acute pulmonary injury. Results were obtained from analyses performed on BAL samples at 24 hr postexposure. Rats were exposed by IT instillation to SAL, Dus, St.L, DC, Ott, or ROFA. Mass refers to responses obtained from animals exposed to an equivalent mass (~2.5 mg/rat) of each PM. Metal refers to responses obtained from animals exposed to an equivalent amount of total transition metal of each PM (35.5 µg/rat). The specific mass doses of each PM are as follows: Dus, 2.50 mg/rat; St.L, 2.72 mg/rat; DC, 4.21 mg/rat; Ott, 1.94 mg/rat; and ROFA, 0.324 mg/rat. Values are expressed as mean + SEM. Animals per group, $n = 4$ to 6 at 24 hr postexposure. ★, significant difference from saline control; ◆, significant difference from the other ambient PM samples, excluding SAL.

pulmonary vascular endothelium (*24*). This injury has been exploited as a model to study the pathogenesis of pulmonary hypertension. Typically, this lesion progresses to induce pulmonary hypertension by 10 to 12 days, worsens to cause right cardiomegaly, then *cor pulmonale* and death over the ensuing 3 to 4 weeks (*19*). Our choice of the model was to establish a preexistent state of progressive inflammatory cardiopulmonary disease as a potential risk factor for PM toxicity. Initial studies focused on the enhancement of the toxicity of IT-instilled ROFA to assess the possible involvement of augmentation of metal-catalyzed oxidant injury via the Fenton pathway. This is due to the substrate-rich milieu of the lung lining fluid during inflammation and the weakened state of the blood–air barrier. The prototypic Fenton reaction is depicted below for Fe but can be considered applicable to most other transition metals (i.e., metals that exist in two or more valence states and are thus capable of shuttling electrons for redox purposes).

$$Fe^{+3} + e^- \leftrightarrow Fe^{+2}$$
$$Fe^{+2} + H_2O_2 \leftrightarrow OH^\bullet + Fe^{+3}$$

In vitro assessment of the feasibility of this reaction with ROFA has been positive

Table 2. Lung inflammation in the monocrotaline cardiopulmonary disease model after 10 to 12 days.

BAL end points	Control	Monocrotaline
Protein, μg/ml	188.3 ± 30.0	3951.8 ± 423.5*
LDH, U/liter	28.3 ± 9.7	423.5 ± 33.5*
PMNs/ml × 10^3	45.9 ± 0.39	462.8 ± 120.0*
H_2O_2, V/0.4 × 10^6 cells	0.0063 ± 0.0011	0.0175 ± 0.002*

*p<0.01 compared to control.

(*14,25*), but the plausibility of this reaction in the *in vivo* model with PM has not been verified to date. Theoretically, lung inflammation can result in the release of the reductant superoxide and labile H_2O_2 from lung cells, which in the presence of lipids can react *in vitro* with ROFA to yield more oxidants (D Costa, unpublished data). More work is needed to assess this hypothesis in light of other pathophysiologic frailties of the impaired animal.

Over the 10- to 14-day incubation period after MCT injection, the rats lost little body weight (~10%); however, pulmonary artery pressure measured in a small cohort of similarly treated rats indicated a doubling of P_{pa} from 10 to 20 mm Hg. Baseline BAL values in Table 2 for control and MCT rats reflect the inflammatory state of the MCT model at the point of IT instillation of ROFA.

Ninety-six hours after ROFA instillation, there was clear evidence of enhanced neutrophilic inflammation (Figure 3), with the influx of PMNs appearing to plateau at 1 mg ROFA/rat. However, other BAL end points did not show a consistent picture of enhanced injury because of great variability in response (data not shown). Notably, by 96 hr, significant mortality had occurred in the MCT–ROFA groups (~50% across all doses of ROFA), with the remaining animals in a somewhat more impaired state than either MCT or ROFA alone. Thus, interpretation of any 96-hr data is representative of the survivor response. Table 3 sums our experience with the MCT model regarding mortality. To date, there does not appear to be a dose response over the range of doses evaluated; time to death also appears not to be dose dependent. Preliminary studies in our laboratory with Mt. St. Helens volcanic ash, which has virtually no bioavailable metal or significant toxicity in healthy animals, also was innocuous in the MCT animal. Mt. St. Helens volcanic ash was collected for the U.S. EPA by A McFarland, Texas A&M University, from an open field near Ritzville, WA 340 km NE of the volcano. Thus the PM-associated metal component seems to be an essential factor for amplification.

The cause of death in the MCT–ROFA animals appears related to altered cardiac function, although it is unclear whether death was associated with direct cardiac injury or was secondary to pulmonary failure (*26*). Electrocardiographic study revealed that ROFA alone in the healthy rat clearly induced a mild arrhythmic condition over the acute inflammatory time period (96 hr). However, animals pretreated with MCT exhibited significantly more and worsened dysrhythmias, among which bundle branch A-V block and S-T segment inversion were considered most severe. These alterations progressed with time and correlated with time of death. Though their origin is as yet uncertain, these cardiographic changes appear consistent with the formation of ectopic foci in heart muscle and/or hypoxic ischemia, possibly secondary to pulmonary diffusion abnormalities (S Gardner, unpublished data). This model has not yet been utilized with ambient PM in this laboratory; however, Godleski et al. (*27*) reported mortality in a similar model with inhalation of concentrated ambient Boston, MA air (~350 μg/M^3 for 6 hr/day for 3 days).

Conclusion

We propose that soluble metals from PM mediate the array of PM-associated injuries to the cardiopulmonary system of both the healthy and the at-risk compromised host. When adjusted for bioavailable metal content, combustion source and ambient PM (TSP) show remarkable similarity in the inflammatory response. The data in the compromised animals appear to parallel the recent epidemiology studies suggesting that those at enhanced risk possess chronic inflammatory lung conditions. The current need is to ascertain the relevance of these data and validate the hypothesis that metals mediate toxicity when applied to inhalation exposure scenarios that approximate similar doses in humans.

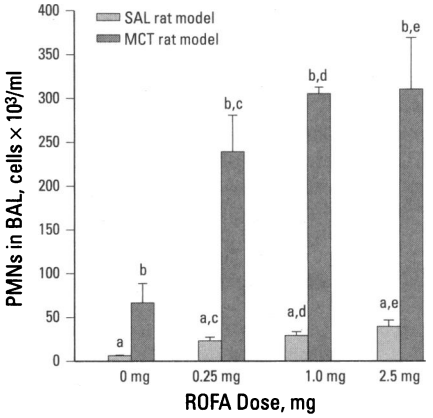

Figure 3. Neutrophilic response in the BAL fluid in a rat model of pulmonary vasculitis/hypertension induced by a single ip injection of MCT 96 hr after IT instillation of various doses of ROFA in 0.3 ml saline. Potentiation of the response occurs at each dose of ROFA; these animals represent the survivor cohort (~50%) at the time of analysis. Like-lettered superscripts indicate significantly different from the appropriate control or ROFA alone (– MCT) group.

Table 3. Mortality of monocrotaline rat model treated with intratracheal residual oil fly ash.

Treatment	Mortality by 96 hr
MCT + saline	4/46
MCT + 0.25 mg ROFA	8/16
MCT + 1.0 mg ROFA	10/16
MCT + 2.5 mg ROFA	25/46

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