Final Technical Report

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Center Director: Morton Lippmann
Title: Lung Hypoxia as Potential Mechanisms for PM-Induced Health Effects
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RFA: Airborne Particulate Matter (PM) Centers (1999)
Research Category: Particulate Matter

Objective(s) of the Research Project: Ambient particulate matter (PM) often contains relatively high levels of transition metals, predominantly iron (Fe), nickel (Ni), vanadium (V), aluminum (Al), and chromium (Cr), with levels of each varying depending on the particle source. At-risk individuals with pre-existing hypertensive disease or atherosclerosis, appear to have overtly negative responses to PM. However, both the mechanisms underlying these effects and the constituents that might be causing these outcomes remain unclear. As atherosclerosis has more recently been designated a chronic inflammatory process, it has been accepted that circulating levels of interleukin (IL)-6 may reflect the intensity of occult plaque inflammation and vulnerability to rupture. Both monocyte chemoattractant protein-1 (MCP-1) and IL-8 may also play a crucial role in initiating and promoting atherosclerosis by enhancing development of atherosclerotic lesions and plaque. Several atherogenic factors induce these cytokines in cardiovascular tissues, primarily through activation of transcription factors such as NF-κB or peroxisome proliferator-activated receptors. Hypertensive patients are at particular risk of cardiovascular complications, possibly related to endothelial damage or abnormal angiogenesis. These pathophysiologic processes correlate with plasma levels of vascular endothelial growth factor (VEGF); plasma VEGF levels are higher in hypertensive patients compared with controls and correlate significantly with age, systolic and diastolic BP, cardiovascular disease, and cerebrovascular accident risk scores.

We hypothesized here that select PM constituents (e.g., Al, V, Ni, Mn) act on lung epithelial cells and resident macrophages to stimulate release of proinflammatory cytokines/chemokines that may have a role in initiation or promotion of atherosclerosis. We further hypothesized that these metals contribute to the above noted deleterious responses in patients with pre-existing hypertensive disease/atherosclerosis by: 1) *a priori* altering the Fe status of these cells that, in turn, and 2) results in increased intracellular accumulation of hypoxia-inducible factor-1α (HIF-1α). As a result of the latter, lung epithelial cells and macrophages are stimulated to increase their formation/release of proinflammatory cytokines, such as IL-6, IL-8, tumor necrosis factor (TNF)-α, as well as MCP-1 and VEGF, that are then transported to the heart in relatively high (undiluted) concentrations. The underpinning of our hypotheses was our earlier studies that showed that exposure to soluble and insoluble Ni agents strongly activated hypoxia-inducible

pathways. These findings were not surprising in that: 1) HIF-1α induction and activation depends on the level of oxygen and activity of Fe-containing hydroxylases; 2) Fe regulation is critical in hypoxia-inducible gene expression; and 3) rat lung macrophage treatment with Ni ions caused significant shifts in cellular Fe levels. To validate our hypotheses, lung macrophage and airway epithelium cell lines were exposed to varying amounts of Fe alone or in combination with Al, V, Mn, or Ni (at levels relevant to those found in ambient urban PM from New York City [NYC]) and levels of each of the above-cited cytokines/chemokines are measured by ELISA, Northern, and Western analyses (the latter to increase the degree of detectability of effects from the treatments). These results could then be used as a baseline of effects for later comparison against the actual formation of these proteins in the lungs of rodents exposed to the "parent" NYC $PM_{2.5}$ samples.

Summary of Findings:

Technical Aspects

Since it was shown earlier that water soluble Ni was responsible in large part for the pulmonary injury caused by residual oil fly ash (ROFA) and that the local inflammation induced was reproducible with instilled soluble forms of Ni (i.e., nickel chloride, sulfate, or sulfide) (Benson, et al., 1986; Dreher, et al., 1997; Kodavanti, et al., 1997, 1998), our initial studies examined the potential effects of Fe and Ni (alone and in combination) on hypoxic stress induction and IL-8 production in the human lung epithelial 1HAEo- cell line. Exposure to Ni produced hypoxic stress as measured by the induction of inducible NDRG-1 and IL-8 production. Similar degrees of hypoxic stress and IL-8 production were induced by the Fe chelator desferroxamine (DFX), suggesting that Ni interfered with cell Fe status. Interestingly and unexpectedly, IL-1β, IL-6 and TNFα secretions were not affected. It is possible that the Ni may have become substituted for Fe in Fe-bearing enzymes/proteins, leading to inhibition of their activity and, subsequently, to activation or induction of HIF-1 transcription factors. It is also possible that Ni caused Fe entry into cells to be impacted, much in the manner seen with other PM-associated metals in Project 8 (R827351C008). Those studies using the IRP activation parameter indicated that treatment of the rat lung macrophage cell line NR8383 (NR) with PM-relevant levels of Ni in conjunction with Fe caused a cellular Fe deficit.

To assess if treatment with PM-associated metals caused an altered Fe homeostasis that should, in turn, induce HIF-1α expression, cultures of 1HAEo- or NR were treated overnight with various amounts of soluble forms of Ni, V, or Al in combination with a fixed amount of Fe. Each level of metal tested was equivalent to that amount that would have been present in a representative 500 μg sample of NYC $PM_{2.5}$ during the month of October in 2001). Western analysis of cellular products indicated that increases in the ratio of V:Fe caused significant increases in HIF-1α expression (to values similar to those from DFX). Exposure to increasing amounts of Ni also caused increased HIF-1α expression, but less so than with V. Al treatments seemed to have no effect on HIF-1α expression; however, as these exposures caused more cell death and proteolytic damage to materials isolated from viable cells, these measurements may have been biased from those that would be seen at more realistic levels of exposure.

Analyses of HIF-1α-inducible cytokines/chemokines indicated that Al, Ni, and V each induced VEGF formation in NR cells. Induction levels were all equal-to-greater than that from DFX and the strength of effect was Al ≥ Ni > V. Western analyses of MIP-2 levels were inconclusive, primarily due to difficulties in trying to adequately resolve the 8 Kd protein in the cell lysates. ELISAs yielded indications that Al had the strongest impact among the metals on MIP-2 release. With the 1HAEo- line, ELISAs of culture supernatants from treated cells suggested that at the higher metal:Fe levels tested, each metal caused a strong induction in IL-8 formation/release. In contrast, any stimulation of VEGF production appeared to only occur with increasing levels of Ni. Western blot analyses were attempted to confirm these patterns, but problems of poor specificity by the antibodies precluded adequate determinations. Because of these technical problems, NR mRNA levels of respective HIF-1α-inducible genes were measured to obtain an indication of any induced shift in cytokine/chemokine formation. RT-PCR analyses showed that Al and Ni induced significant levels of VEGF mRNA and that the effect was ratio (i.e., Al:Fe, Ni:Fe)-related; oddly, the effect of V treatment was nominal. Similar results were also noted for IL-6 gene induction. With MCP-1, MIP-2, and TNFα, none of the metals tested had any effect.

The results with the rat macrophage line (as compared to the human lung epithelial line) suggest that there may be potentially species-/cell type-related differences in responses to PM-metal-induced alterations in Fe homeostasis. The data also suggest that the relationship between metal-induced HIF-1α expression and levels of select cytokine/chemokine products may not necessarily be linear. However, this latter finding needs to remain tentative as effects of Al, Ni, and V on several relevant processes in cells need to be refined, i.e., differential effects of each metal on the timeframe of induction/maintenance of elevated gene expression in response to increased HIF-1α levels need to be examined.

Conclusions

These studies showed that:

- Select metals within a given sample of $PM_{2.5}$ can cause altered cellular Fe homeostasis and this effect appears to be governed by the relative content relationships between Fe and at least three co-constituent metals, e.g., Ni, Al, and V.

- Among these metals (that alter Fe homeostasis), Ni and Al (more so than V) also cause changes in the release of select cytokine/chemokine factors that could impact initiation or promotion of atherosclerosis. This effect also is related to relative content relationships between Fe and these metals.

- The observed effects on the inducible release of these factors seem, for now, to be both species- and cell type-dependent.

Except for the above-noted problems encountered during some of the Western blot analyses, the project was found to be technically feasible to conduct.

References:

Benson JM, Henderson RF, McClellan RO, Hanson RL, Rebar AH. Comparative acute toxicity of four nickel compounds to F344 rat lung. *Fundamental and Applied Toxicology* 1986;7:340-347.

Dreher KL, Jaskot RH, Lehmann JR, Richards JH, McGee JK, Ghio AJ, Costa DL. Soluble transition metals mediate residual oil fly ash induced acute lung injury. *Journal of Toxicology and Environmental Health* 1997;50:285-305.

Kodavanti UP, Jaskot RH, Costa DL, Dreher KL. Pulmonary proinflammatory gene induction following acute exposure to residual oil fly ash: Roles of particle-associated metals. *Inhalation Toxicology* 1997;9:679-701.

Kodavanti UP, Hauser R, Christiani DC, Meng ZH, McGee J, Ledbetter A, Richards J, Costa DL. Pulmonary responses to oil fly ash particles in the rat differ by virtue of their specific soluble metals. *Toxicological Sciences* 1998;43:204-212.

Supplemental Keywords: NA

Relevant Web Sites: http://www.med.nyu.edu/environmental/
http://es.epa.gov/ncer/science/pm/centers.html