Hybrid Aldehyde Protein Adducts Form in a Mouse Model of Cigarette Smoke and Alcohol Exposure

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Most alcohol abusers smoke cigarettes and approximately half of all cigarette smokers consume alcohol. However, no animal models of cigarette and alcohol co-exposure exist to examine reactive aldehydes in the lungs. Cigarette smoking results in elevated lung acetaldehyde (AA) and malondialdehyde (MDA) levels. Likewise, alcohol metabolism produces AA via the action of alcohol dehydrogenase and MDA via lipid peroxidation. A high concentration of AA and MDA form stable hybrid protein adducts known as malondialdehyde-acetaldehyde (MAA) adducts.

METHODS: We hypothesized that chronic cigarette smoke and alcohol exposure in an in vivo mouse model would result in the in vivo formation of MAA adducts. We fed C57BL/6 mice ad libitum ethanol (20%) in drinking water and exposed them to whole body cigarette smoke 2 hour/d 5d/week for 6 weeks. Bronchoalveolar lavage fluid and lung homogenates were assayed for AA, MDA, and MAA adduct concentrations. MAA adducted proteins were identified by Western blot and ELISA.

RESULTS: Smoke and alcohol exposure alone elevated both AA and MDA, but only the combination of smoke+alcohol generated protein-adducting concentrations of AA and MDA. MAA adducted protein (~500ng/ml) was only detected by ELISA in the smoke+alcohol-exposed mice. Of the five MAA adducted proteins identified by Western blot, a major protein band immunoprecipitated with antibodies to surfactant protein D. Similar to in vitro PKC stimulation by purified MAA adducted protein, PKC epsilon was activated only in tracheal epithelial extracts from smoke+alcohol -exposed mice.

CONCLUSION: Only the combination of cigarette smoke exposure and alcohol feeding in mice resulted in the generation of significant AA and MDA concentrations, the formation of MAA adducted protein, and the activation of airway epithelial PKC epsilon in the lung.

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Effect of TMA and DNCB on In Vivo and Ex Vivo Lung Function in a Mouse Allergy Model

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Aim of this study was the comparison between in vivo and ex vivo techniques (Precision cut-lung slices, PCLS) lung function parameters early airway response (EAR) and airway hyperresposiveness (AHR) after sensitisation with respiratory allergen trimellitic anhydride (TMA) and contact allergen 2,4-dinitrochlorobenzene (DNCB).

Female BALB/c mice were sensitized epicutaneously on flanks and ears with TMA and DNCB. The EAR to TMA and DNCB was registered in vivo and ex vivo on day 20 after inhalational challenge with dry aerosols or after exposure of PCLS with allergen. Twenty-four hours after allergen challenge AHR to increasing doses of methacholine was measured in vivo and ex vivo. For investigation of inflammation of respiratory tract IL-5 and eotaxin-2 concentration in BAL fluid, number of eosinophils in BAL and increase in serum IgE were determined.

TMA and DNCB sensitized mice showed increase in serum IgE concentration (TMA: 24 ng/ml vs. 200 ng/ml and DNCB: 21 ng/ml vs. 70 ng/ml). Mice dermally sensitized with TMA and then inhalationally challenged with TMA aerosol had significant increases in number of eosinophils in BAL and increased eotaxin-2 concentration (167 pg/ml vs. 994 pg/ml) in BAL fluid 24 h after allergen challenge. TMA-sensitized mice and lung slices showed increased methacholine responsiveness in comparison to negative control group (in vivo ED100: 0.48 µg vs. 0.13 µg and ex vivo EC50: 4*10-7 M vs. 2,4*10-7 M) but not any significant differences in EAR. No significant changes of these parameters were detected after sensitization with contact allergen DNCB.

Compared to in vivo lung function measurement, PCLS provide an suitable ex vivo method to detect lung function parameter AHR.

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Pulmonary Toxicity of Size-Classified Coal Fly Ash Particles of Varying Carbon Content

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Epidemiological studies have shown that morbidity and mortality increase along with concentration of particulate matter (PM) in many different countries and regions despite great variations in the chemical makeup of the PM. In this study, Illinois bituminous coal with high sulfur and iron was burned to produce fly ash particles with no detectible carbon, and medium and high carbon content. Particles were then separated into coarse (>2.5 µm), fine (0.5-2.5 µm) and ultrafine (<0.5 µm) fractions, and the samples were analyzed for elemental composition. Pulmonary inflammation in mice was assessed at 4 or 18 h after oropharyngeal aspiration of 100 µg of particles. In the medium and high carbon samples, iron was detected in various oxidation states while sulfur was in the reduced form. The ultrafine fractions of these samples had higher carbon, but a similar iron and sulfur chemistry profile to the fine and coarse fractions for each combustion condition. These ultrafine samples induced a higher degree of neutrophil influx and IL-6 release in the lung. For the carbon-free samples, inflammation was highest in the fine fraction, which was dominated by oxidized iron and alumino-silicates. We conclude that either carbon, or iron combined with alumino-silicates can cause pulmonary inflammation in different size fractions, and may provide an explanation for the relatively non-specific effects that different sizes of ambient PM appear to cause, regardless of geographic location and chemistry. (This abstract does not reflect EPA policy.)

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Pulmonary Effects and Tissue Distribution of Metals after Inhalation of Mild Steel Welding Fume

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Rationale: Many welders experience bronchitis, metal fume fever, and lung function changes. The objective was to assess the effect of mild steel welding fume (MSWF) inhalation on lung injury, inflammation, defense responses, and fate of inhaled metals. **Methods:** Sprague-Dawley rats were exposed to MSWF at a concentration of 40 mg/m^3 x 3 hr/d x 3 or 10 d using a novel robotic welding fume generator. Controls were exposed to air. To assess lung defense responses, a group of animals were intratracheally inoculated with 5 x 10^4 *Listeria monocytogenes* 1 d after the last daily exposure. Welding particles were collected during exposure, and chemical composition and particle size were determined. After exposure, parameters of lung injury (lactate dehydrogenase and albumin), inflammation (PMN influx), and host defense (bacterial clearance) were measured. Also, multiple organs were recovered for metal analysis to assess fate of inhaled particles. **Results:** The particles were composed primarily of Fe (80.6 %) and Mn (14.7 %) with a mass median aerodynamic diameter of 0.31 µm. No significant difference was observed in lung injury or inflammation after MSWF inhalation at 1, 4, and 11 d after the last exposure. However, there were significantly more bacteria at 3 d after infection in the lungs of the animals exposed to MSWF compared to air controls. Significant elevations in lung Fe and Mn, liver Fe, and kidney Mn were observed after 10 d of exposure to MSWF compared to air. **Conclusions:** Acute exposure of rats to MSWF had no effect on injury and inflammation, but suppressed lung defense responses after infection. Also, metals in MSWF may translocate from the lungs to other organs, possibly causing systemic effects. Chronic inhalation studies are needed to further examine the health effects and fate of inhaled welding fume.

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Exposure to Concentrated Coarse Air Pollution Particles Causes Mild Cardiopulmonary Effects in Young, Healthy Adults

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Air pollution particulate matter (PM) exposure has been linked to adverse health outcomes, yet the specific causes of morbidity and mortality remain elusive. It has been proposed that the size of particles might play an integral role in stimulating detrimental physiological effects. To evaluate the influence of air pollution particle size on human health, we exposed young, healthy volunteers to concentrated ambient coarse PM ($PM_{10-2.5}$) (CAPS) and filtered air to assess the potential physiologic effects of acute coarse PM exposure. Volunteers were exposed to coarse CAPS (concentrated from ambient Chapel Hill, NC air on the day of the study) and filtered air for two hours while undergoing intermittent exercise in a single-blind, cross-over study. We measured pulmonary, cardiac, and hematological endpoints before, immediately post, and 20 hrs post exposure. Compared to filtered air exposure, coarse CAPS exposure produced a small increase in polymorphonuclear neutrophils (PMNs) in the bronchoalveolar lavage (BAL) fluid approximately 20 hrs post-exposure, indicating the ability to induce mild pulmonary inflammation. However, no changes were observed in pulmonary function. Other changes at 20 hrs post-exposure included decreases in the percent of monocytes in the bronchial lavage (BL) fluid, a decrease in tissue plasminogen activator (tPA) in the blood, and a slight decrease in heart rate variability (SDNN). No changes were noted in the immediate post-exposure measurements. This proposed abstract does not necessarily reflect EPA policy.

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Exposure to Sudbury Particulate Matter (SPaM) Elicits Pro-Inflammatory Cytokine Expression in Airway Epithelial Cells

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Air pollution is of serious concern for many people, but it is of special interest in communities where there are prominent local sources of aerosol emissions. In Sudbury, Ontario, Canada, local smelting processes release substantial amounts of airborne contaminants, both particulate and gaseous. In this study, we collected ambient Sudbury Particulate Matter (SPaM) from HVAC air intake filters and sieved the material to remove all particles larger than 38 µm. SPaM was then subjected to preliminary physical, chemical and biological characterization. SPaM was determined to have a mean diameter of 31.1 µm (± 2.62%), a carbon composition of 64.8% (± 1.66%) and a sulfur composition of 1.08% (± 0.10%) by weight. To compare the pro-inflammatory effects of SPaM to other forms of ambient particulates we cultured airway epithelial cells (A549 and BEAS-2B) with varying concentrations (0-25 µg/mL) of SPaM, diesel exhaust particles (DEP), or ambient Ottawa particles (EHC-93) for 24h, and collected culture supernatants to measure inflammatory cytokines by ELISA. SPaM exposure induced IL-8 production by BEAS-2B and A549 cells in a dose-responsive fashion. Expression of IL-8 by both cell lines was significantly increased by exposure to 25 µg/mL of any of the particle types compared to untreated controls. Treatment of A549 cells with 25 µg/mL of SPaM elicited significantly higher levels of IL-8 production than treatment with the same concentration of DEP (p=0.025) or EHC-93 (p=0.017); however, there were no significant differences in IL-8 production by BEAS-2B cells exposed to 25 µg/mL of SPaM, DEP, or EHC-93. These studies suggest that SPaM exposure may be a more potent stimulus for inflammation than other types of airborne particulates. Identifying the components of SPaM that are responsible for these effects will require further study.

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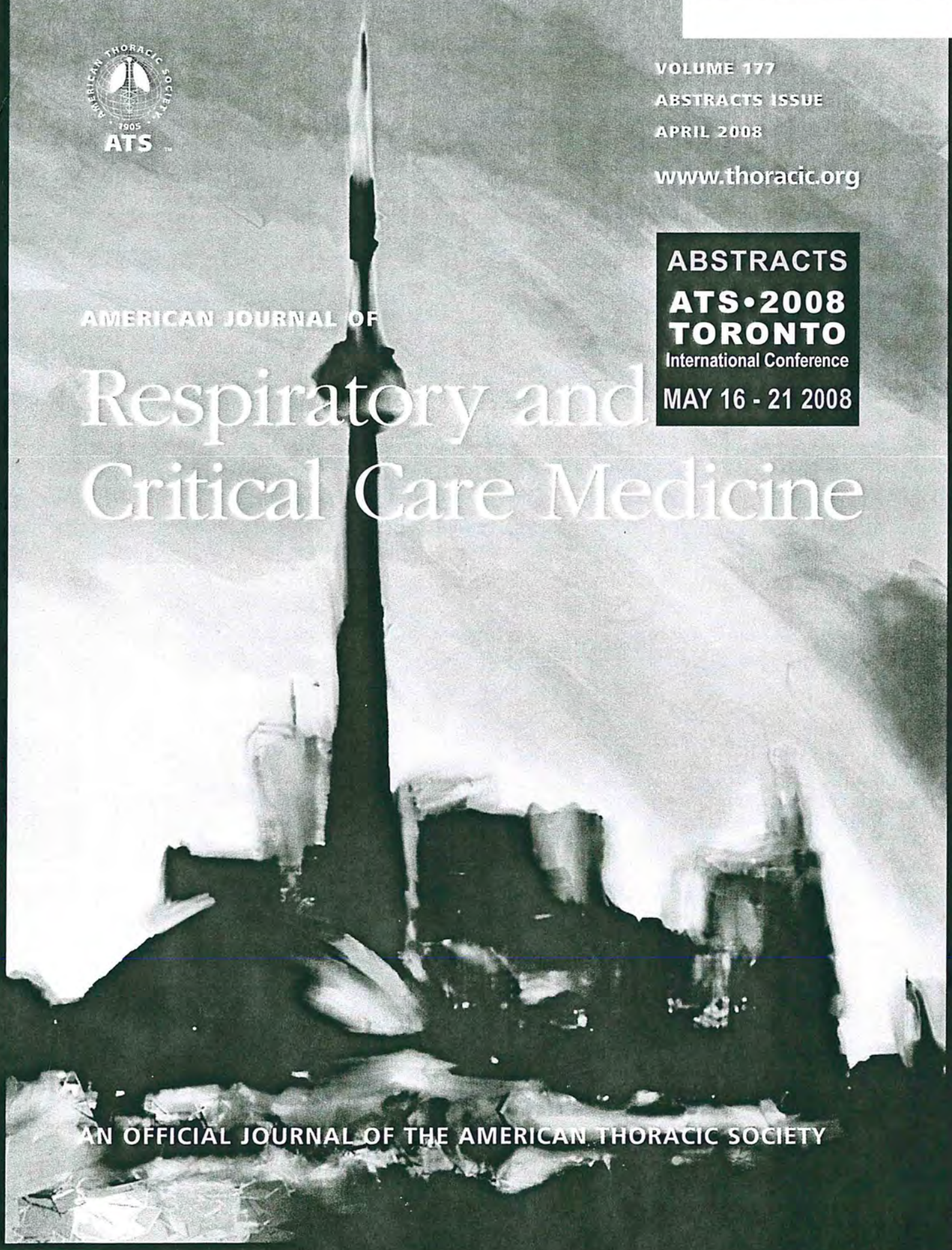

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