Promotion of cardiovascular disease by exposure to the air pollutant ozone

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In the decades following the discovery of the chemical nature of environmental air pollutants and the biochemical reactions they could undergo, it was viewed that the primary tissue target of air pollution would be our lungs. This made sense, as the principal constituents of photochemical air pollution are highly reactive oxidizing and free radical gases frequently generated upon the solar radiation-induced "excitation" of primary pollutants. These gases arise from the myriad industrial and automobile combustion-derived precursor gases in the atmosphere [e.g., nitrogen oxides, volatile organic compounds (VOCs), carbon monoxide, aldehydes, and peroxyacetyl nitrates] that ultimately generate both particulate matter (PM) and ground-level ozone (O3). Ozone is formed by the UV-induced homolytic scission of nitrogen dioxide (\(\cdot NO_2\)) to products that react with molecular oxygen (O2) in a reaction stimulated by VOCs (Fig. 1).

There is both a broad target molecule population and a kinetically rapid reaction of O3 upon inhalation, resulting in an evanescent lifetime for this oxidant. Both computer simulation studies of pulmonary O3 reactions and experimental observations bear this out, since there is not a particularly "deep" penetration of O3 and O3-mediated cell injury into the lung. The primary anatomic sites of O3 reaction in the lungs (at environmentally relevant concentrations of 0.5 ppm or less) are the airway and nasal epithelium, with small conducting airways and bronchioles a preferred reaction site, rather than more distal parenchymal alveolar epithelial cell reactions (18, 20, 25). Even in still-developing primate infants, episodic O3 exposure primarily affects the morphogenesis of tracheobronchial airways and damages airway epithelial cells in terminal and respiratory bronchioles (8). Consequently, both acute and chronic exposure to environmental O3 results in relatively modest airway hyperplasia and hyperreactivity, with persistent accumulation of activated intraluminal macrophages (5). This latter response to ozone provides us with an important clue for how the actions of O3 may be transduced beyond the lung to induce adverse cardiovascular responses.

A substantial body of evidence links environmental O3 exposure with morbidities such as increased susceptibility to inhaled allergens, increased hospital admissions for asthma, and impaired lung function in children. Recent analysis of a large, U.S.-based, 96-metropolitan area cohort study showed tropospheric O3 and PM levels were also linked with risk of death from cardiopulmonary disease (11). When data was subjected to single-pollutant analyses, elevated O3 levels were clearly associated with risk for death from respiratory causes, whereas the association between O3 and cardiovascular end points was less clear because the data was sensitive to adjustment for concurrent PM levels. Other epidemiological evidence associating ozone-induced airway inflammation to cardiovasculardisease has demonstrated that elevated PM is robustly linked to cardiovascular morbidity and mortality (2).

There has been a number of reports over the past two decades suggesting that O3 exposure might result in extrapulmonary actions. This includes the observation of elevated indices of serum lipid oxidation, altered liver cytochrome P-450 metabolism, and chromosomal alterations in peripheral blood lymphocytes (17). We are now presented with a landmark observation by Chuang et al. (4) that lends precise and highly credible insight into the occurrence of adverse cardiovascular responses following O3 reaction in the lungs. This study utilized three different animal models (wild-type C57Bl/6 mice, apoE-/- mice on a C57Bl/6 background that are prone to atherosclerosis, and infant macaque monkeys) that were epidermically exposed to environmentally relevant 0.5 ppm concentrations of O3. These investigators report a profound impact of O3 exposure on the systemic vasculature, including altered physiological function, enhanced gene expression of proinflammatory enzymes, elevated indices of oxidative injury, oxidant-induced damage to mitochondrial DNA, impairment of antioxidant defenses in mitochondria, and, in the apoE-/- mice, an acceleration of atherogenesis. As for all high impact observations of a scientific nature, this seminal report is destined to redefine the boundaries of inhaled oxidant actions, motivate further laboratory and clinical studies, and should stimulate both the redesign and the reanalysis of epidemiological studies of photochemical air pollution exposure and cardiovascular consequences in urban populations.

Some of the most provocative observations in the report of Chuang et al. (4) are their identification of the biochemical intermediates and cellular events responsible for transducing the airway epithelial reactions of O3 into the proinflammatory responses that are manifested in the extrapulmonary vasculature. One must immediately discount the possibility of direct diffusion of O3 across the air-blood barrier of the lung and the occurrence of more remote subsequent intravascular oxidative reactions, since this highly reactive gas does not even significantly reach or impact the alveolar compartment where gas exchange occurs. Of significance, however, is the recent appreciation that tissue injury induced by O3 can be further propagated by endogenously produced O2-derived species and oxides of nitrogen. Notably, O3 reacts within the lung to initiate airway epithelial and inflammatory cell activation, events that can amplify the primary oxidant injury by increasing the endogenous pulmonary generation of reactive oxygen species [e.g., superoxide (O2-) and hydrogen peroxide (H2O2)] and nitric oxide (\(-NO\))-derived oxidizing and nitrating intermediates. Evidence for this latter event comes from studies showing elevated inducible nitric oxide synthase (iNOS) expression and the nitration of tyrosine to 3-nitro-tyrosine in alveolar macrophages and activated intraluminal macrophages (22).

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Ozone-induced lung inflammation is thought to result from formation of nitrogen dioxide ($\text{NO}_2$), forming nitric oxide ($\text{NO}$) and atomic oxygen ($\text{O}_2^-$) that then undergoes reaction with molecular oxygen ($\text{O}_2$) to produce ozone ($\text{O}_3$) through a homolytic cleavage of the primary pollutant, nitrogen dioxide. 

This leads to the formation of reactive oxygen species (ROS), such as superoxide ($\text{O}_2^-$), hydrogen peroxide ($\text{H}_2\text{O}_2$), and singlet oxygen ($\text{O}_2^*$), which can be scavenged by antioxidants like ascorbate and glutathione. The production of ROS can also lead to the generation of reactive nitrogen species (RNS), such as nitric oxide ($\text{NO}$) and nitrous oxide ($\text{N}_2\text{O}$), which can interact with ROS to form more reactive nitrogen and oxygen species.

The lipid-rich epithelial lining fluid (ELF) provides a microenvironment where ROS and RNS can interact with cellular components, including proteins and lipids. These interactions can lead to posttranslational modifications (PTMs) of proteins, such as nitration, nitrosylation, and oxidation, which can alter protein structure and function. For example, nitration of tyrosine residues in proteins can increase their reactivity with electrophilic molecules, leading to further modifications.

ROS and RNS can also lead to the formation of reactive oxygen and nitrogen species that can propagate oxidative stress, leading to the formation of reactive intermediates that can amplify secondary reactions. For example, hydrogen peroxide can react with nitric oxide to form peroxynitrite ($\text{ONOO}^-\text{H}_2\text{O}_2$), which can be toxic to cells.

It is likely that the posttranslational modification (PTM) of proteins by $\text{O}_3$ and the secondary reactive species that it gives rise to will be of importance to the extrapulmonary manifestation of ozone-induced cardiovascular injury. The increased endogastic rates of production of oxidizing and nitrating species following $\text{O}_3$ exposure are expected to target redox-sensitive amino acids such as cysteine, histidine, methionine, and tryptophan, thereby altering protein structure and function. Also, electrophilic byproducts of unsaturated fatty acid ozonolysis will induce protein PTM upon their adduction of nucleophilic amino acid targets. In this regard, the inflammatory milieu initiated by $\text{O}_3$ in the airway sets the stage for PTMs to occur, inducing a cascade of signaling actions that span from the relatively constrained sites of initial $\text{O}_3$ reaction to inducing a more global cardiovascular response.

From the aforementioned, one can hypothesize the following scenario for how $\text{O}_3$ exposure might induce atherogenesis. The primary reactions of $\text{O}_3$ will first occur, inducing direct chemical oxidation of susceptible amino acids, unsaturated fatty acids, and low-molecular-weight antioxidants. Redox-induced PTMs of oxidant-susceptible sites on key inflammatory-related transcription factors [e.g., thiol residues of NF-$\kappa$B (3)] would represent a prime target. This latter reaction could then lead to the increased expression of enzymes and proteins that amplify pulmonary redox reactions, such as the iNOS, cell surface adhesion molecules, and cytokines that promote inflammatory cell accumulation and trafficking. The activated macrophages and oxidatively injured epithelial cells that are “primed” by $\text{O}_3$ and its byproducts will express and release a spectrum of proinflammatory cytokines and growth factors, including TNF-α, IL-1, IL-6, IL-10, TGF-β, MCP-1, and PDGF (1, 13, 16). This can result in a cascading proinflammatory state and the extrapulmonary release of diffusible mediators that can initiate and propagate inflammatory responses in the vascular compartment (7).

Atherosclerosis is a progressive disease characterized by deposition of macrophages engorged with lipid within the vessel wall and is accompanied by altered vascular function. Macrophage activation by lung-derived circulating mediators leads to vessel wall infiltration of macrophages that, when faced with elevated vascular cell expression of adhesion molecules and oxidatively modified low-density lipoproteins, results in foam cell formation. These cells can release growth and chemotactic factors that further induce the propagation of inflammatory responses within the vessel wall of systemic vascular beds (10) (Fig. 2).

To test critical elements of the above scenario, fallow grounds have been plowed by Chuang et al. (4) that will inevitably germinate more detailed future investigations. These investigators utilized apoE-/- mice as harbingers of extrapulmonary $\text{O}_3$ signaling, because these hypolipidemic mice characteristically display increased susceptibility for development of atherosclerotic lesions. Following ozone exposure, the systemic vascular compartment of the accompanying murine model (C57Bl/6 mice) is also clearly affected in an adverse manner, with elevated indices of oxidative stress (3-nitrotyrosine adducts of proteins and mitochondrial DNA damage in the aortic wall and increased plasma isoprostane levels) attesting to the transmittal of inflammatory responses from the lung to the vascular compartment (4). Of particular relevance, the PTM of mitochondrial MnSOD by nitration of a crucial catalytic tyrosine occurs in aortic tissue, setting the stage for the amplification of oxidant reactions in vascular cell mitochondria (14, 15). Because both superoxide ($\text{O}_2^-$) and hydrogen peroxide ($\text{H}_2\text{O}_2$) support the catalytic scavenging of $\text{NO}$, multiple downstream responses would be expected as a result of decreased MnSOD activity. This includes the impairment of vascular function, manifested by an inhibition of endothelial-dependent relaxation, diminution in eNOS protein levels and activity, an increased diastolic pressure, and an increased heart rate. These results are particularly impressive, since with the

**Fig. 1.** Photochemically induced ground level ozone generation. UV radiation from the sun induces homolytic cleavage of the primary pollutant nitrogen dioxide ($\text{NO}_2$), forming nitric oxide ($\text{NO}$) and atomic oxygen ($\text{O}_2^-$) that then undergoes reaction with molecular oxygen ($\text{O}_2$) to produce ozone ($\text{O}_3$).
exception of plaque formation studies in the apoE−/− mice, they all occurred in an animal model that is not compromised by an underlying pathology such as hyperlipidemia. Clearly, the combination of oxidative and nitratative responses observed in the systemic vasculature of O₃-exposed mice does not bode well for their (or our) cardiovascular health!

It is tempting to speculate that there might even be amplified rates of inflammatory cell-derived endogenous O₃ generation occurring in the systemic vascular compartment, based on recent reports of amino acid, antibody, and leukocyte-dependent oxidation of water to O₃ by singlet oxygen (24, 26). This speculation, while elevated by the attractive symmetry wherein O₃-induced responses could beget endogenous generation of O₃, lacks traction. Closer inspection of the analytical approaches used by investigators proposing the concept of endogenous O₃ formation and actions casts doubts on their conclusions. Extents of oxidant generation and identification of individual species are likely confused by a combination of promiscuous detector molecules (such as indigo carmine and vinylbenzoic acid) and nonbiological reaction conditions, thus laying a shaky foundation for the concept that endogenous O₃ generation might occur to any significant extent during basal metabolic or inflammatory conditions (12, 23).

The compelling biochemical measurements made by Chuang et al. (4) of an elevated vascular inflammatory and redox state are nicely summarized by their measurement of increased deposition of atherosclerotic plaque on the aortic wall of apoE−/− mice. Voluminous model system-based studies and clinical observations reveal there is a strong causal connection between oxidative inflammatory reactions in the vasculature and atherogenesis, with very few previous studies providing the extensive and incisive measurements of the impact of a xenobiotic on vascular redox status, as the present report has done. Of particular note in this regard is their observation that a single ozone challenge in infant primates produces a downstream series of vascular inflammatory events, as evidenced by the significant mitochondrial DNA damage that had a pattern of distribution that mimics the distribution of plaque in previous reports of atherosclerotic lesion formation in primates.

In aggregate, this study has provided new inspiration for future pursuits, including the identification of specific cells and cell signaling mediators involved in transducing airway oxidative reactions that then mediate the activation of vascular inflammatory responses and metabolic perturbation. In this regard, the identification of specific PTMs of proteins by oxidized lipids, oxides of nitrogen, and reactive oxygen species will add important perspective as to how redox reactions alter pulmonary protein structure and function in such a way as to transduce primary inflammatory signaling events in the lung to the systemic vasculature.

Since clean air standards are presently being reevaluated on the basis of scientific observations, rather than being victimized by ideology, and urban populations of the industrialized regions of the world are subjected to ever-increasing photochemical air pollution exposure, this study is particularly timely. Importantly, current understanding of the redox-related basis for the pathogenesis of not only atherogenesis, but also myocardial infarction, atrial fibrillation, and heart failure, suggest that both retrospective and prospective epidemiological analysis of an array of adverse cardiovascular events in urban regions having high levels of air pollution is warranted.

“Healthy citizens are the greatest asset any country can have.” - Winston Churchill.

**GRANTS**

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REFERENCES


