HEI

STATEMENT

Synopsis of Research Report 215

HEALTH EFFECTS INSTITUTE

Role of Secondary Organic Aerosols Versus Macrophages in Formation of Reactive Oxygen Species in the Lung

BACKGROUND

Air pollution from particulate matter (PM) is associated with adverse health effects and is a leading risk factor for morbidity and mortality worldwide. PM is a complex mixture of microscopic particles and liquid droplets known as an aerosol; the mixture can contain various metals and carbon-containing particles. PM can originate from primary natural sources (e.g., windblown dust) and primary pollutant sources (e.g., fuel combustion), but a large fraction of PM is also formed by chemical reactions of gases in the atmosphere. PM formed in the atmosphere from gas-phase organic precursors is known as secondary organic aerosol (SOA). Inhalation of PM can damage tissues in the respiratory tract by chemically generating reactive oxygen species (ROS), which are highly reactive oxygen-containing chemicals, such as hydrogen peroxide (see **Statement Figure**). Inhalation exposure can also stimulate cells called macrophages to release ROS as part of the immune defense. Although ROS play a role in normal biological function, a buildup of ROS can induce oxidative stress and lead to the onset and progression of disease. Although research demonstrates that SOAs contribute to ROS formation in the respiratory tract, this pathway remains poorly understood.

To examine the effects of SOA exposure on the formation of ROS in lungs, HEI funded a study by Dr. Manabu Shiraiwa of the University of California, Irvine, titled "Formation of Reactive Oxygen Species by Organic Aerosols and Transition Metals in Epithelial Lining Fluid" in response to HEI's Request for Applications 17-3 Walter A. Rosenblith New Investigator Award. Dr. Shiraiwa proposed to investigate the kinetics and chemical mechanisms of ROS formation in the respiratory tract by different types of air pollutants and to quantify the relative importance of ROS formed by chemical reactions compared with ROS released as an immune response to pollution.

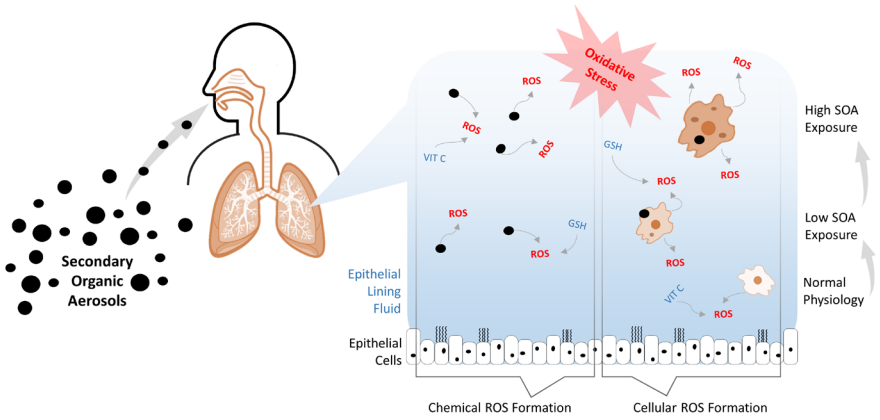
What This Study Adds

- This study evaluated the mechanisms of aerosol-induced reactive oxygen species formation by two pathways in the respiratory tract: by chemical reactions and by immune cells called macrophages.
- The investigators quantified aqueous reactive oxygen species formation under various experimental conditions and developed kinetic models of the respiratory tract. They also evaluated ambient air samples as a contrast to the laboratory-generated pollutants.
- Results showed that the quantity and composition of reactive oxygen species formation depends on the specific aerosol mixture, oxidation mechanisms, and other environmental conditions. Under certain exposure conditions, the reactive oxygen species released by macrophages far exceeded the reactive oxygen species formation by aqueous chemical reactions.
- Aerosol-induced respiratory health effects that are mediated by reactive oxygen species depend on a complex interplay between the aerosol mixture, chemical reactions, environmental conditions, and the influence of the immune cells.

APPROACH

Dr. Shiraiwa aimed to study ROS formation in the respiratory tract by designing a series of laboratory experiments and developing kinetic models. Because ROS formation in the respiratory tract occurs at the interface between air and tissue in a protective liquid layer called the epithelial lining fluid (ELF), the approach focused on aqueous reactions to approximate real-world conditions. The first objective of the study was to identify the mechanisms and kinetics of ROS formation by aqueous chemical reactions of SOAs. Specific tasks were to quantify ROS formation by laboratory-generated SOAs and by ambient PM samples. The second objective was to quantify the relative importance of ROS formed by chemical reactions compared with the cellular release of ROS by macrophages in synthetic ELF. Specific tasks

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Manabu Shiraiwa at the University of California, Irvine, and colleagues. Research Report 215 contains both the detailed Investigators' Report and a Commentary on the study prepared by the Institute's Review Committee.



Statement Figure. After inhalation of air pollutants, ROS are formed in lung fluid through chemical reactions and released by macrophages under normal physiological conditions. Excess ROS can lead to oxidative stress and inflammation, but the relative importance of the two pathways is unclear. GSH = glutathione; VIT C = vitamin C.

included using kinetic modeling to estimate ROS concentrations formed by chemical reactions within different lung regions and to quantify ROS released by macrophage cells when exposed to SOA.

Shiraiwa and colleagues first generated SOAs in a controlled laboratory environment by inducing oxidation of selected organic compounds in a reaction chamber. They also collected PM from a small number of ambient air samples from Los Angeles, California, that were used to represent urban, traffic, and wildfire locations. They conducted various in vitro experiments to quantify the chemical formation of several types of ROS in water and in synthetic ELF from SOAs generated by laboratory precursors or ambient air PM samples. The synthetic ELF contained the naturally occurring antioxidants vitamin C, citric acid, glutathione, and uric acid. They quantified several different ROS, including hydroxyl radical, superoxide radical, carbon- and oxygen-centered radicals, and hydrogen peroxide.

In their experiments, the investigators evaluated the effects of different experimental conditions on ROS formation, including the addition of nitrogen oxides (NO_x), the addition of ferrous iron (Fe^{2+}), and changes in pH levels. Both NO_x and Fe^{2+} are important atmospheric oxidizing agents. They used a relatively new and sensitive method called continuous wave electron paramagnetic resonance spectroscopy with a spin trapping technique to capture the formation of various ROS species. Shiraiwa and colleagues then used computer modeling to determine the ROS chemical reaction kinetics and to estimate ROS burden in different respiratory tract regions. Finally, they compared the chemical formation of ROS by SOAs with the ROS released by macrophage cells when exposed to SOAs.

KEY RESULTS

Shiraiwa and colleagues found that the formation of various ROS and their reaction kinetics strongly depended on the method of chemical oxidation and the chemical precursor used to generate SOAs. Different experimental conditions, including the presence of NO_x or Fe^{2+} and changes in pH levels, altered ROS formation. For example, the introduction of high concentrations of NO_x to laboratory-generated SOAs decreased the formation of hydroxyl and superoxide radicals. In contrast, the introduction of Fe^{2+} to SOA generally increased ROS formation. The type of ROS formed, and the kinetics of that formation, differed when the experiment was carried out in water versus the synthetic ELF — an effect attributed to the presence of antioxidants in the synthetic ELF. Overall, chemical ROS formation from SOA was highly complex and variable.

The investigators found that the quantity and composition of ROS formed by ambient PM varied by sampling site in Los Angeles, with marked differences between wildfire and nonwildfire samples. ROS formed per air volume were highest in highway and lowest in wildfire samples, and the ROS formed per mass were higher in urban compared with wildfire samples. On average, PM from urban and highway sampling sites formed primarily hydroxyl radicals (>84%) with the remaining proportion being carbon-centered radicals. PM from wildfire samples formed mostly carbon-centered radicals (~50%).

Modeling results indicated that ROS formation in the human respiratory tract depended on the size composition of PM exposure and on ELF volume and particle deposition in the different respiratory tract regions. ROS formation was highest in the

extrathoracic region and lowest in the alveolar region. The estimated formation of hydrogen peroxide was highest, and hydroxyl radical was lowest. The investigators noted that their model assumed a uniform particle size, which would somewhat misrepresent real-world conditions because larger particles are generally deposited in the upper respiratory tract and only smaller particles reach deep into the lungs.

Shiraiwa and colleagues found that certain SOAs activated macrophage cells to release higher concentrations of superoxide than what was formed through chemical reactions in the synthetic ELF. Superoxide formation depended on the concentration of SOA precursor and duration of the exposure. At low concentrations, cellular formation of superoxide was 10 times higher than chemical formation, particularly at the beginning of the experiments. The investigators reported that the decreased superoxide formation by macrophages over longer exposure times was due to the activation of antioxidant processes, oxidative stress, and cell death. The chemical superoxide formation modeled by the investigators was similar to the experimental results, providing reassurance that the kinetic modeling approach accurately captured the processes.

INTERPRETATION AND CONCLUSIONS

Dr. Shiraiwa and colleagues demonstrated that the quantity and composition of ROS formed by aqueous reactions highly depends on such factors as the specific aerosol mixture, oxidation mechanisms, and other environmental conditions. They also found that for certain exposure conditions, the ROS released by macrophages far outweighed the ROS formation by aqueous chemical reactions. This result implies that aerosol-induced respiratory health effects mediated by ROS might not depend on the aerosol composition and associated chemical reactions alone.

In its independent evaluation of the report, the HEI Review Committee members noted that the investigators made a valuable contribution to the study of the health effects of air pollution mediated through ROS. They thought that the work comparing chemical versus cellular ROS formation was novel, and they appreciated the inclusion of ambient aerosol samples from field measurements in addition to the lab-generated aerosols. However, they thought that it was difficult to translate the results of the experiments to real-world exposure conditions in the human lung and that the full implications of the results were therefore difficult to determine.

The Committee noted that the experimental methods enabled the investigators to establish their proof of concept but that the next steps were to design experiments under more realistic conditions. For example, the investigators used relatively high SOA precursor concentrations in their laboratory studies to ensure sufficient ROS yield for quantitative comparisons and selected a macrophage cell line that is extensively used to study oxidative stress, although not resident to the respiratory tract. Testing SOA generated from precursor concentrations relevant to the ambient air and lung-derived macrophages would advance future research. The investigators also simulated ELF by including naturally occurring antioxidants and compounds, but other key immune cells such as neutrophils would be valuable to include next. It would also be helpful in future work to use more robust negative-control scenarios, for example, by varying the experimental timeframes and by using nonchemical stimuli. Furthermore, the Committee thought that the evaluation of ambient PM samples was preliminary; it included only a few samples from three locations in Los Angeles. Thus, any conclusions regarding wildfire versus nonwildfire PM should be regarded with caution until a much larger set of samples has been analyzed. The Committee noted that the kinetic modeling of ROS formation in different respiratory tract regions demonstrated that the approach was possible. However, as with any modeling efforts, the results hinged on the model inputs, which can change under various realistic conditions and should be interpreted cautiously.

In conclusion, the Committee commended Dr. Shiraiwa and colleagues for the novelty and thoughtfulness in the study approach. This work is an important first step in understanding the relative importance of chemical versus biological ROS formation in the lung. Further work is recommended to evaluate ROS formation using additional PM samples from various ambient sources and including an evaluation of responses from other key immune cells.

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