STATEMENT

Synopsis of Research Report 201

HEALTH EFFECTS INSTITUTE

Effects of a VOC–Ozone Mixture on Human Lung Epithelial Cells

BACKGROUND

In this study, Dr. Lydia Contreras, a recipient of HEI's 2014 Walter A. Rosenblith New Investigator Award, and her colleagues evaluated how exposure to components of air pollution affected the oxidation of ribonucleic acid (RNA) inside lung cells. The role of RNA oxidation in cellular responses is drawing increased attention, because several species of RNA — including messenger, transfer, and micro RNA — play key roles inside cells, particularly in the regulation of protein synthesis. The investigators also studied how the oxidation of RNA might affect pathways inside the cell.

APPROACH

The investigators mixed 790 ppb acrolein, 670 ppb methacrolein, and 4 ppm ozone in a dark chamber at 37°C. After 10 minutes, the aged VOC–ozone mixture was introduced into a module containing the human lung epithelial cell line BEAS-2B in an air–liquid interface system, in which the gases were passed over the top surface of the cells grown on a membrane inserted in a cell culture plate. Exposures lasted 90 minutes. The investigators monitored the composition of gas-phase compounds as well as particle size distribution and particle chemical composition.

Contreras and colleagues identified specific RNA transcripts that were either up- or downregulated by the exposures. They also identified specific transcripts that were oxidized by the exposure by using an antibody specific to a particularly sensitive RNA oxidation product, 8-oxo-7,8-dihydroguanine (8-oxoG).

Using this information, the investigators performed analyses to identify the biological pathways inside the cells that were most associated with these changes in transcription. Having identified specific pathways, they then performed biological assays to see whether the oxidation of transcripts also had effects on levels of specific proteins or lipids in the exposed cells. Based on preliminary data suggesting that genes involved in the cytoskeleton structure were both oxidized and downregulated by the VOC–ozone exposure, they used microscopy to evaluate effects on the actin cytoskeleton. They also evaluated markers of cell injury and death — lactate dehydrogenase (LDH) and adherence to the culture plate.

What This Study Adds

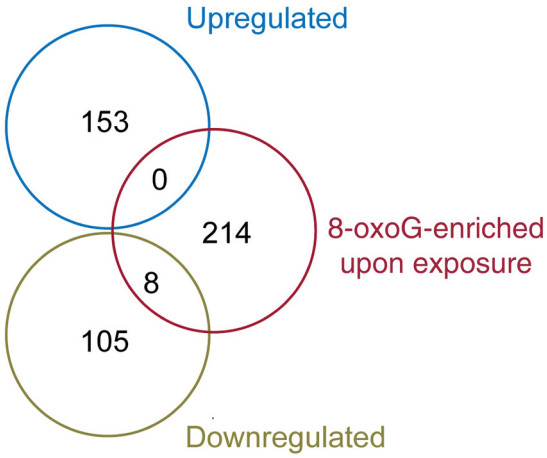
- The study evaluated how exposure of lung cells to volatile organic compounds (VOCs) plus ozone affects oxidation of ribonucleic acid (RNA), a key component of cells.
- VOC–ozone exposure resulted in multiple oxidized transcripts, as well as up- or downregulated transcripts. Pathways associated with the oxidation of specific transcripts included cholesterol synthesis and organization of the cell's structure (the cytoskeleton).
- However, VOC–ozone exposure also increased markers of cell injury and death, so questions remain about the potential cytotoxicity of the VOC–ozone mixture used. Nonetheless, this approach provides a powerful and logical template for future research on the effects of air pollution on RNA oxidation.

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Lydia M. Contreras at the University of Texas, Austin, and colleagues. The complete report, *Understanding the Functional Impact of VOC–Ozone Mixtures on the Chemistry of RNA in Epithelial Lung Cells* (© 2020 Health Effects Institute), can be obtained from HEI or our website (see next page).

MAIN RESULTS AND INTERPRETATION

Exposure to the VOC–ozone mixture for 90 minutes resulted in 153 upregulated transcripts, 113 downregulated transcripts, and 222 8-oxo-G-enriched transcripts that were preferentially oxidized in the VOC–ozone exposed cells (see Statement Figure). Eight transcripts were in the overlapping set of oxidized and downregulated transcripts. One of the eight was farnesyl diphosphate farnesyltransferase 1, an enzyme involved in cholesterol biosynthesis.

Preliminary analysis of the cellular pathways suggested that oxidation was associated with changes in the cytoskeleton, which was confirmed by microscopy analysis of the organization of the cytoskeleton. However, the VOC–ozone mixture also increased markers of cell injury and death: there were large increases in LDH concentrations and loss of adherence to the culture membrane, suggesting that exposure to the VOC–ozone mixture was cytotoxic.



Statement Figure. Number of upregulated, downregulated, and 8-oxoG-enriched transcripts after exposure of lung epithelial cells to a VOC–ozone mixture.

REVIEW OF THE REPORT

In its independent review, the HEI Review Committee considered the study to be an exciting new approach to the toxicology of air pollution and an important initial attempt to understand an understudied area: the role of messenger RNA regulation, modification, and oxidation in the effects of exposure to air pollutants. The investigators used a logical combination of powerful approaches — including transcriptome analysis, biochemistry, and cell biology — to identify candidate genes and pathways for further evaluation of the effects of exposure to pollutants.

Exposing cells to a mixture of gases — ozone plus the VOCs acrolein and methacrolein — was potentially relevant to assessing the effects of reactants that may be found in some urban atmospheres. The exposure generation and physicochemical characterization were state of the art. The air–liquid interface in vitro system was more physiologically representative than many in vitro methods because cells are directly exposed to the pollutant gas mixture instead of mixing pollutants into the culture medium.

However, the Committee noted several important limitations in the study design that reduced confidence in the generalizability of the results toward understanding the role of RNA oxidation after exposure to air pollution. One major limitation was that the concentrations of VOCs and ozone used were much higher than would be found even in heavily polluted urban environments and appeared to be cytotoxic. In addition, the results were based on exposure of cells to a single mixture, at one high exposure level, and measured at only one timepoint. Also, the study focused on a single RNA oxidation product, 8-oxoG. This focus was understandable given the availability of a specific monoclonal antibody that recognized this molecule but was not well justified from a theoretical perspective. In future studies, other potentially more biologically relevant RNA oxidation products need to be identified using more sensitive techniques.

Nonetheless, the Committee considered the study to be an important preliminary demonstration of RNA oxidation in lung cells exposed to a VOC–ozone mixture. This powerful combination of techniques — including transcriptome analysis, biochemistry, and cell biology — offers a template to be applied to more exact and comprehensive studies in the future.

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