Antioxidant response to episodic ozone exposure may be attenuated in neonatal rats

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Question

Why does early life ozone exposure in rats cause structural changes in the distal lung?

Background

Early life ozone exposure decreases lung function in humans [1]. These functional changes are thought to be related to structural changes in the distal lung. However, the mechanism by which early life ozone exposure causes lung structural changes is unknown [2-3].

Ozone is a powerful oxidant [4]. Glutathione (GSH) is a prevalent intracellular antioxidant and functions as a substrate for conjugation of endogenous byproducts of oxidative stress by glutathione s-transferases (GST) [5].

Animal and epidemiologic studies have implicated oxidant stress as an important mechanism in ozone-induced injury in children and juvenile animals [6-9]. Oxidant stress responses in the immature lung differ from those in the mature lung [10].

Neonatal rats exposed to particulate pollution are less able to mount GSH or GSH-related enzyme responses than adults [11-13]. Male neonatal rats had a more significant reduction in airway GSH than adults when subjected to oxidative stress from particulate matter inhalation [10-11]. GST has also been shown to be attenuated in neonatal antioxidant responses [10].

The rate limiting step of GSH de novo synthesis is catalyzed by glutamate cysteine ligase (GCL), which is comprised of a modulatory (GCLM) and catalytic (GCLC) subunit. While both have been shown to be upregulated following oxidative stress [14-15], both GCLC and GCLM gene expression have been found to decrease in neonates exposed to particulate matter [10].

Club cell secretory protein (CCSP or CC10) is a major secretory product of Club cells, an important metabolic and stem cell type within airways. Ciliated cells in the airways are the predominate target of ozone, and in response Club cells can participate in regeneration of the epithelium via de-differentiation [16]. Thus, decreased CC10 gene expression may correspond to Club cell de-differentiation and airway injury.

In the present study, we sought to identify mechanisms of ozone-induced change in the distal lung, investigating neonatal antioxidant capacity as a predisposing factor to airway remodeling.

Hypothesis

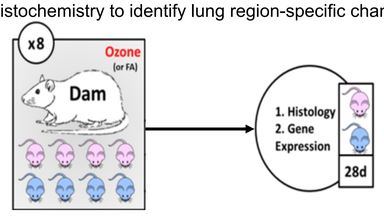
The distal neonatal lung is less able than mature lungs and the proximal lung to upregulate cellular antioxidant responses to ozone oxidative stress. This attenuated antioxidant response may predispose neonates to disrupted lung development due to ozone exposure.

Specific Aims

Aim 1: Quantify gene expression of key oxidant stress response enzymes Club cell secretory protein, glutathione s-transferase pi, and glutathione cysteine ligase catalytic and modulatory subunits to characterize neonatal antioxidant responses in lung sub compartments.

Aim 2: Characterize distribution of Club cell secretory protein, glutathione s-transferase pi, and glutathione cystine ligase in airways and alveoli using immunohistochemistry to identify lung region-specific changes.

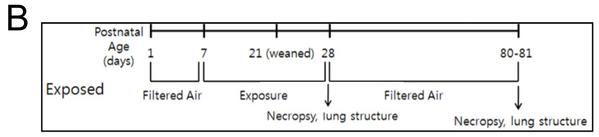
A



Approach

Sixteen adult lactating female rats Harlan Sprague-Dawley rats with litters of 8 pups were randomly divided into ozone (O3) and filtered air (FA) exposed groups (Specific Aims, Panel A). Half the dams with pups were exposed to ozone 5 days per week at 0.5 ppm in whole body chambers and the other half was exposed to FA (Approach, Panel B).

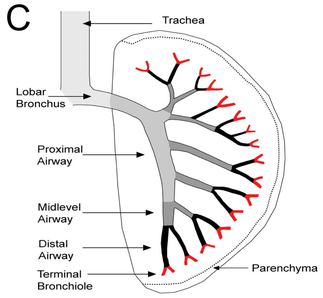
B



Animals were necropsied and lungs were examined immediately after the end of the exposure (28 days of age). We quantified CC10, GST-pi, GCLC, and GCLM using qRT-PCR. One lung lobe from rats in the above exposure study was inflated with stabilization solution and microdissected to distinguish airways and alveoli (Approach, Panel C). RNA from samples were isolated, reverse transcribed into DNA , and qRT-PCR was run using commercially available probes and primers.

One lung lobe from rats from the above exposure study was inflated with formaldehyde vapor and embedded in paraffin. Paraffin sections were stained with primary antibodies for CC10 and GST-pi. We then used a secondary antibody to stain primary antibodies in order to visualize site-specific enzyme distribution within the microdissected lung.

C



Gene Expression

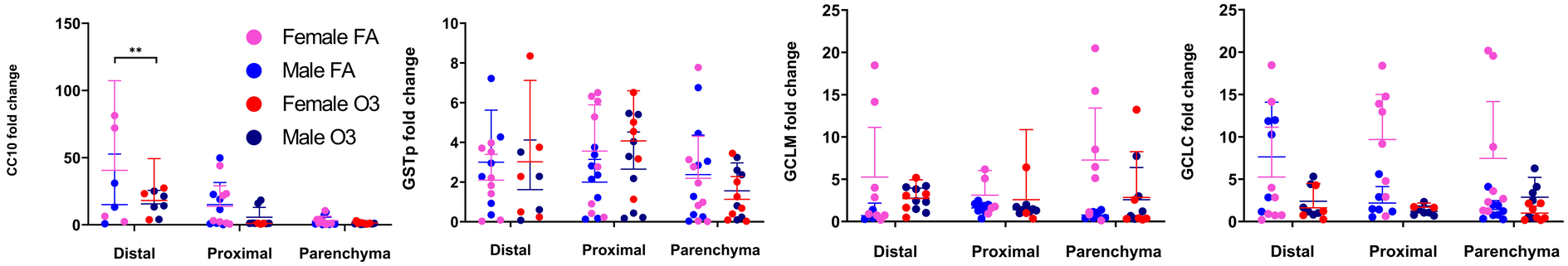


Figure 1. Relative gene expression of ozone and filtered air-treated rats. Panel A: CC10 gene expression. All values are given as fold change relative to housekeeper gene (HPRT), relative to male filtered air parenchyma. Error bars on all plots represent mean +/- SEM. O3 exposure decreased expression of CC10 in distal and proximal airways. CC10 expression in distal airways in O3 treated rats relative to FA, p=0.0018. **Panel B:** GST-pi gene expression. While no differences are statistically significant, a trend suggests that O3 exposure increased expression of GST-pi in distal and proximal airways. **Panel C:** GCLM gene expression. While no differences are statistically significant, a trend suggests that O3 exposure increased expression of GCLM in male distal airways and decreased expression in female distal airways. **Panel D:** GCLC gene expression. While no differences are statistically significant, a trend suggests that O3 exposure decreased expression of GCLC in all lung sub compartments.

Immunohistochemistry

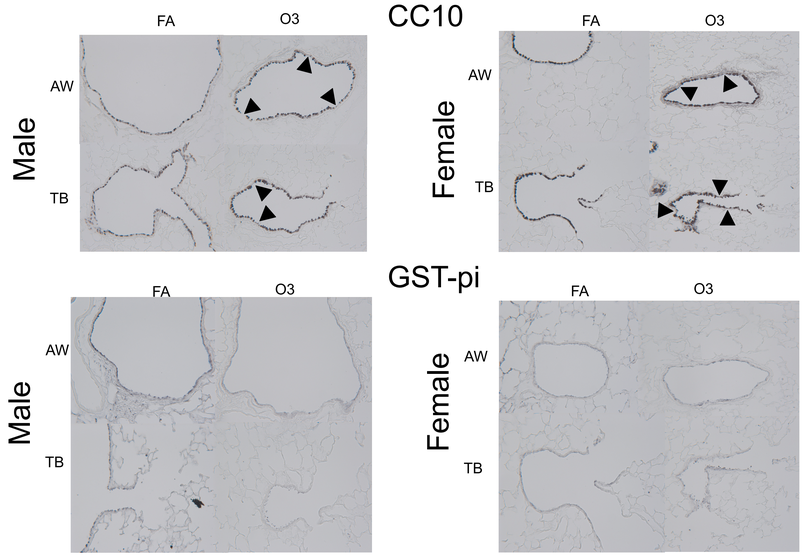


Figure 2: In situ distribution and abundance of CC10 and GST-pi in airways (AW) and terminal bronchioles (TB) of FA and O3 treated rats. CC10 distribution is altered in TB of O3 treated rats, indicating Club cell injury or de-differentiation. While FA-treated rats display regularly distributed Club cells, O3 treated rats have gaps (black arrowheads). GST-pi abundance is increased in TB of O3 treated rats, especially females.

Conclusions

CC10 gene downregulation in the distal airways demonstrates ozone's expected site-specific effects and emphasizes the need for site-specific investigation of gene expression in the lung.

GST-pi may be upregulated on O3 treated rats, especially in the distal lung. This upregulation represents an appropriate antioxidant response in neonates to ozone. Given GST-pi's detoxifying role via direct conjugation of GSH to oxidants, detoxification of ozone may be functional in neonates, contrary to our hypothesis. This functionality may instead implicate mechanisms other than antioxidant response (e.g. immature immune response, alveolar development) to early life ozone exposure-induced lung structural changes.

However, gene expression of enzymes involved in the rate-limiting step of GSH de novo synthesis (GCLM and GCLC) was not upregulated, as would be expected given the oxidant challenge. Failure to upregulate GCL and thus GSH de novo synthesis may indicate an attenuated antioxidant response, as hypothesized, and may result in GSH depletion. These results emphasize the importance of direct measurements of GSH in further studies.

Our results suggest that neonates may not be able to upregulate GSH de novo synthesis and may implicate neonatal antioxidant response in ozone-induced damage in the distal lung.

References and Acknowledgments

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