The Effects of Ozone and SP-D on Epithelial Barrier Integrity and Inflammation in the Colon

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Ozone is a reactive air pollutant that induces lung injury and systemic inflammation, resulting in intestinal oxidative stress and epithelial barrier disruption. Surfactant protein D (SP-D), a protein associated with macrophage efferocytosis, may contribute to intestinal inflammation. Thus, the purpose of this study was to examine the effects of prolonged ozone exposure on the colonic epithelium and the onset of inflammation, and to explore the role of SP-D in the colon. Adult C57/BL6 mice were exposed to filtered air or ozone for 2h, 2x/wk for 6wk; humanized wild-type (WT), SP-D homozygous, and SP-D heterozygous mice generated using CRISPR-cas9 were acutely exposed to filtered air for 3h. Colon samples were collected 24h after final exposures for western blotting. Tissues were homogenized and protein measured using a BCA assay. For western blotting, loaded protein volumes were normalized and membranes probed for expression of COX-2, an inflammatory marker, and claudin-3, a tight junction protein. Densitometry was performed using ImageJ Fiji and protein expression normalized to GAPDH with $p<0.05$ considered statistically significant. No significant differences in claudin-3 expression were detected in acute or prolonged exposure groups. Expression of COX-2 was significantly increased in female mice following ozone exposure compared to air-exposed controls, and in SP-D homozygous mice compared to WT mice. In conclusion, increased COX-2 expression was observed in female, but not male mice following prolonged ozone exposure. Mutation of the SP-D gene in mice increases basal COX-2 levels. Future studies will determine whether ozone exposure in SP-D humanized mice exacerbates colonic inflammation.

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Methods