Proteomic, Metabolomic, and Lipidomic Analyses of Lung Tissue Exposed to Mustard Gas

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Abstract

Sulfur mustard (HD) was developed as a chemical warfare agent for use in World War I, but remains a valid concern for the Warfighter today. This is due in part to the large stockpiles of HD still in existence, as well as the relative ease of obtaining materials for production and the simplicity of the production process itself. Exposure to HD, while lethal at high doses, can result in a number of non-lethal symptoms with often long-term and debilitating effects, including respiratory inflammation, cellular necrosis, blister formation, and permanent DNA damage. Here we utilize EpiAirway Lung Tissue to investigate the pathological effects of HD using a multiomic approach.

Methods

A viability test was first done on EpiAirway Lung Tissues to determine the appropriate dosage and time requirements for multiomic analyses. EpiAirway Lung Tissues were exposed to 0.01 and 0.1 mg/mL HD and were analyzed at 3, 6, 18, and 24 hours post-exposure. Several different analyses were preformed on the tissue samples including histology, cytokine panel screenings, and proteomic, metabolomic, and lipidomic mass spectrometry. The 0.01 mg/mL exposure resulted in little change, and so the experiment was repeated only using 0.1 mg/mL HD at 3 and 24 hours post-exposure.

Samples were analyzed for proteins, lipids and metabolites via mass spectrometry.

Scan for publication

Results

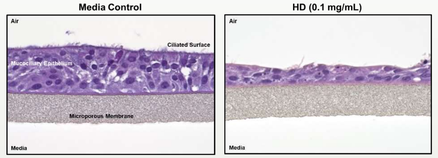


Figure 1. Histology changes associated with HD exposure in EpiAirway™ tissues. Paraffin-embedded sections from EpiAirway™ tissues exposed to media or HD (0.1 mg/mL; 0.629 mM) were rehydrated and stained with hematoxylin and eosin.

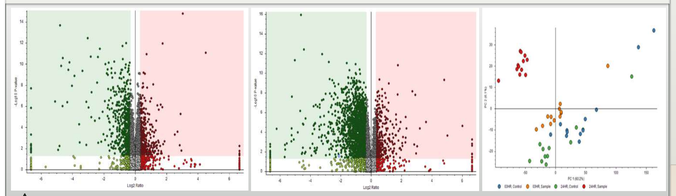


Figure 2. (A) At 3 hours post exposure, 724 proteins were down-regulated and 655 were up-regulated significantly. (B) At 24 hours post exposure, 2045 proteins were down-regulated and 527 were up-regulated significantly. (C) Principal component analysis (PCA) of metabolite samples at various time points; the greatest separation is between the 24-hour exposed samples and all the other samples.

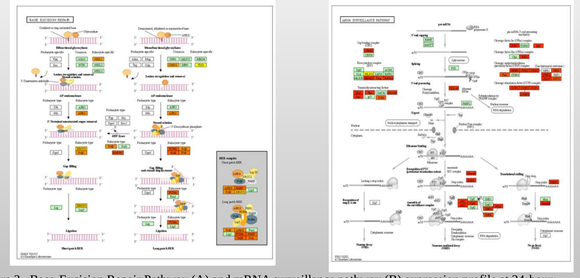
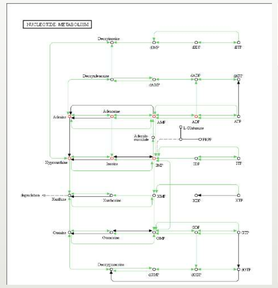


Figure 3. Base-Excision Repair Pathway (A) and mRNA surveillance pathway (B) expression profile at 24-hour post exposure.



Feature	Log_2 FC – 24 hour	p-value – 24 hour
Adenine	0.31	0.05
Adenosine	-1.33	0.00099
AMP	-1.41	0.00290
ADP	-2.51	0.00041
IMP	-5.94	2.1804×10^{-8}
Inosine	-3.42	3.3710×10^{-8}
Hypoxanthine	-2.69	1.2348×10^{-5}

Figure 4. Identified and mapped to the nucleotide metabolism pathway, particularly those focused around adenosine metabolism. Several metabolites are seen to be down-regulated at 24 hours post-exposure.

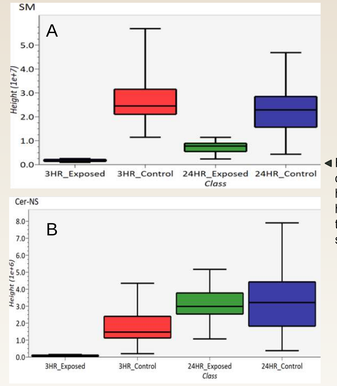


Figure 5. (A) Compares the abundance of sphingomyelins of 3 hour exposed, 3 hour control, 24 hour exposed and 24 hour control samples, (B) compares the abundance of ceramides in these samples

Discussion and Conclusion

After lung tissues were exposed to HD, histology and multiomic mass spectrometry were performed – this includes analysis of proteins, metabolites, and lipids. **Figure 1** compares the control tissues to HD-exposed, and the right image shows much of the ciliated surface of the lung tissue has been sloughed off and the mucociliary epithelium has collapsed.

Proteomic and metabolic analysis shows significant disruption, especially at the 24 hour mark. **Figure 2C** shows that the greatest change in the metabolic profile occurs at the 24 hour in HD exposed samples. When the significantly changing proteins and metabolites are mapped to biochemical pathways, paths related to DNA and RNA function and repair are significantly changed. **Figure 3** shows two such pathways – base excision repair and mRNA surveillance. Proteins shaded orange to red in the figure are upregulated; in **Figure 3A**, the upregulated proteins are related to recognition of damaged DNA, specifically that done by mutagenic agents. The mRNA surveillance pathway is a quality control mechanism that detects and degrades abnormal mRNA.

Figure 4 is the nucleotide metabolism pathway, and several metabolites associated with adenosine metabolism are highlighted in red. Metabolites in this pathway are down-regulated at 24 hours post-exposure. Adenosine levels fluctuate based upon stress and can act as a feedback regulator of inflammation. Figure 5 are box-and-whisker plots of two lipid classes – (A) ceramides and (B) sphingomyleins. These are both utilized in signaling, and whenever sphingomyelin breaks down, ceramide is generated.

Initial multiomic analysis of lung tissues exposed to sulfur mustard show proteins and metabolites related to nucleotide metabolism and damage to genetic material. The pathways related to base excision repair are upregulated, indicating these pathways are in overdrive trying to replace the alkylated nucleotides.

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