Nitrogen Mustard Exposure Upregulates PCNA Expression in Mouse Precision Cut Lung Slices

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Nitrogen mustard (NM) is a bifunctional alkylating agent that causes acute injury to the respiratory tract leading to fibrosis. In these studies, precision cut lung slices (PCLS) were used to assess initiating events in NM lung toxicity. PCLS are comprised of parenchyma and all resident cells within the lung. Proliferating cell nuclear antigen (PCNA) is a marker of DNA replication and repair. Increases in expression of PCNA is considered an indicator of DNA damage caused by toxicant exposure. In this study, PCNA expression was assessed in PCLS after NM exposure. C57BL/6 mice were euthanized, tracheotomized and the lungs filled with agarose. Lung lobes were isolated and 300 µm thickness PCLS prepared using a Krumdieck Tissue Slicer. PCLS were washed twice with culture media and incubated for 24 hours. PCLS were then exposed to 30 µM NM or culture media (control) for 1 hour. After 24 hours PCLS were fixed with formalin, embedded in paraffin, and sectioned for immunohistochemistry. Non-specific antigen binding sites in the tissue was blocked by incubation of the PCLS with serum for 2 hours; this was followed by overnight incubation at 4ºC with anti-PCNA antibody. Visualization of antibody binding was performed using a Vectastain kit and diaminobenzidine. Qualitative analysis of images revealed greater expression of PCNA in airways of lung slices exposed to NM compared to control. Increased levels of PCNA expression indicate initiation of tissue repair mechanisms after NM-induced DNA damage. Further studies will investigate signaling pathways regulating tissue repair. Supported by NIH Grants AR055073, R25ES020721, and the ASPET SURF Program.