Lipid-Laden Macrophages and Inflammation in the Lungs of Chronically-Vaped Mice

Researchers: Katlyn Langston, Alyson Stanger; **Mentors**: Todd Hillhouse, Scott Moore; **Senior Author and Mentor**: Matthew Nicholaou
Weber State University Medical Laboratory Sciences

Abstract

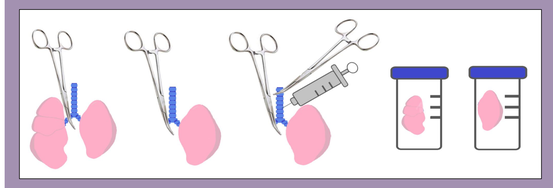
The CDC has tracked 2,807 cases of vaping-related illness in 48 states, the District of Columbia, Puerto Rico, and the U.S. Virgin Islands. There have been 68 deaths in 29 states (Outbreak of Lung Injury Associated with the Use of E-Cigarette, or Vaping, Products, 2020). Individuals who use vaping substances have been found to have lipid-laden macrophages, which are unusual to see in healthy lung tissue. This study determines whether the lung tissue from chronically-vaped mice shows pathologies associated with histological abnormalities and characterizes the cytokine immune profile of the vaped mouse lungs. Mice were sacrificed at the end of a 28-day vaping cycle and the respiratory tract was obtained during necropsy. Left lungs were used homogenization and cytokine testing. Right lungs were used to create histology slides for staining procedures.

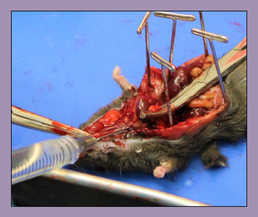
Introduction

Vaping usage has been on a steady incline since the introduction of electronic cigarettes in 2003. e-cigarettes work by heating a liquid to produce an aerosol that users inhale. This liquid can be a vector of several different compounds such as: nicotine, tetrahydrocannabinol, flavorings, and other additives. Out of 2022 hospitalized patients admitted for vaping related lung injury 57% of Patients who reported using nicotine-containing products. (Outbreak of Lung Injury Associated with the Use of E-Cigarette, or Vaping, Products, 2020). Individuals who use vaping produces have shown several pulmonary reactions such as: chemical irritation, allergic/immune reactions, shortness off breath and/or chest pain.

Methods

For this study 24 C57BL/6 female mice were used; the mice were divided evenly into 3 groups: one group received 30mg of nicotine a day, the second received vaping fluid with no nicotine, and the third was not exposed to vaping fluid. At the end of a 28-day vaping cycle mice were sacrificed by cervical dislocation. Respiratory tissue was obtained during necropsy using isolation and separation. The right lung tissue was immediately placed on dry ice after dissection and then stored at -80 degrees Celsius to preserve tissue for homogenization for cytokine analysis. The left lung, still attached to the mouse, was fixed with 10% formalin by inserting a needle through the trachea and filling the lung with fluid. It was then clamped off, removed from body cavity, and placed in a tube filled with 10% formalin to be fixed overnight.

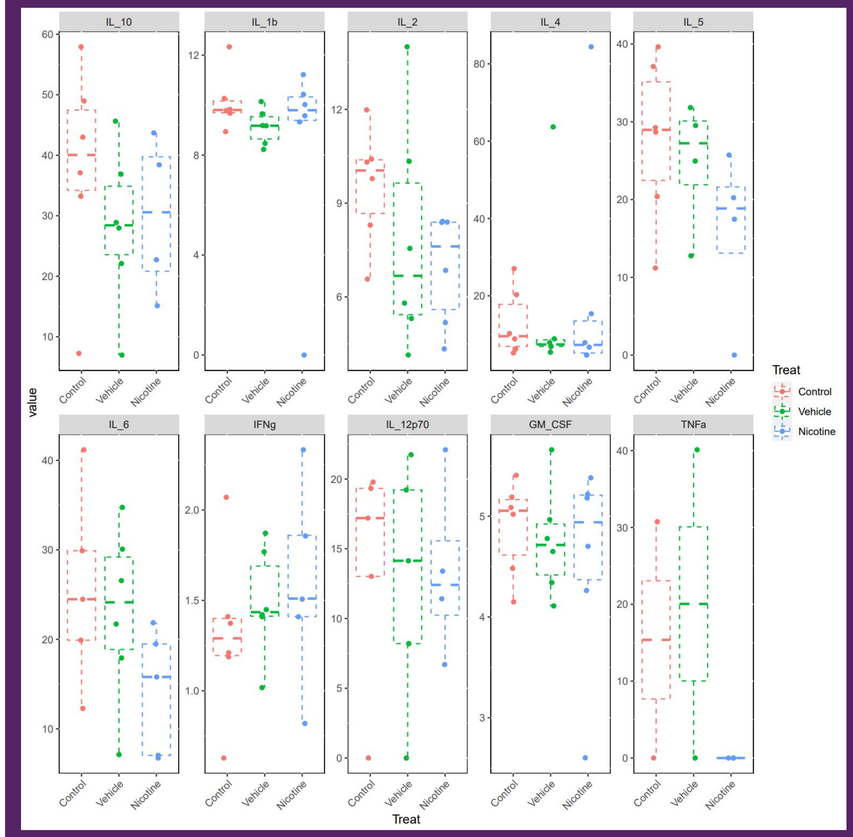




Hematoxylin and eosin (H&E) was used for this study. H&E stain is used for medical diagnosis for standard biopsies of suspected histological damage. Hematoxylin stains cell nuclei blue and eosin stains the extracellular matrix and cytoplasm pink. This stain was used to visualize any physical signs of inflammation including lipid-laden macrophages, lipoid pneumonia, and lymphocytes. The use of H&E stains were performed on the right lungs to mark the histological aspects of the tissue. H&E lungs that were processed in 10% formalin were paraffinized, cut, and placed on glass slides at Utah State University. Slides were then sent to Weber State for staining.
Deparaffinization of the slides was done by 2 sets (5 minutes each) of Safe Clear Xylene substitute then 2 (3 minutes each) of 100% ethanol. After, slides were stained with Vector Laboratories H&E stain per their procedure. Slides were then analyzed by a clinical pathologist for pathological identification.

Cytokines that were analyzed for this study (interleukins -1β, -2, -4, -5, -6, -10, -12 [p70], IFNγ, GM-CSF, and TNFα) are specific markers for inflammation. A specific ProcartaPlex Multiplex Immunoassay cytokine panel was run over the course of two days. On day 1, the cytokine panel reagents were prepared for mixing via thermo Fisher scientific procartaplex multiplex immunoassays user guide. It was incubated for 24 hours. . On day 2, detection antibody and streptavidin PE were added, alternating between several wash steps that were performed per the Thermo Fisher Scientific procartaplex multiplex immunoassay user guide. After the cells were resuspended, the panel was read on the Magpix system per the Magpix machine protocol.

Results



With an alpha level of 0.05, none of the cytokines showed clinical significance after an ANOVA test was performed. However, IL-6 and IL-5 showed the most significance of the cytokines overall with a p-value of 0.17 and 0.24 respectively.

interleukin-2: Average concentration of for each mouse group. Orange: Nicotine, Yellow: Vape, Green: control. values are median +/- 1 standard deviation. Following a 28-day vaping cycle, IL-2 promotes the growth of regulatory T-cells.

interleukin-1β: Average concentration of for each mouse group. Orange: Nicotine, Yellow: Vape, Green: control. Values are median +/- 1 standard deviation. After a 28-day vaping cycle, IL-1β is a proinflammatory cytokine that represents inflammation.

interleukin-6 : Average concentration of for each mouse group. Orange: Nicotine, Yellow: Vape, Green: control. Values are median +/- 1 standard deviation. After a 28-day vaping cycle, IL-6 stimulates acute-phase protein production.

interleukin-10: Average concentration of for each mouse group. Orange: Nicotine, Yellow: Vape, Green: control. Values are median +/- 1 standard deviation. After a 28-day vaping cycle, IL-10

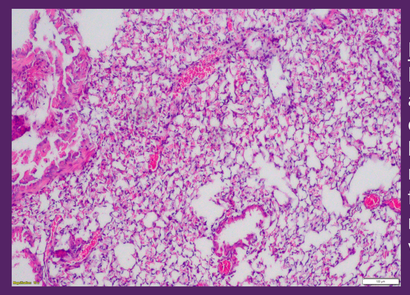


Figure A1: Diffuse alveolar damage, hyaline membranes from nicotine-vaped group

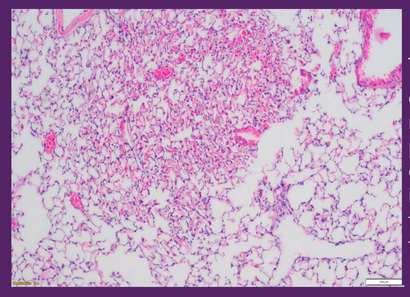


Figure A2: Fibrinous exudates, myxoid plugs, and chronic inflammation from vape fluid group

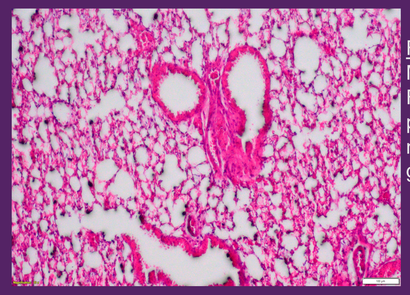


Figure A3: Myxoid Fibroblast plug from nicotine group

Discussion

- H&E stains showed that the 30mg of nicotine a day and vaping fluid with no nicotine groups showed nonspecific histological pathologies including chronic inflammation, hyaline membranes, diffuse alveolar damage, and fibrinous exudates. However, the control mice showed overall healthy lung tissue. This supports our hypothesis of the link between vaping and inflammation of the lungs.
- The cytokine panel somewhat supports this hypothesis. With an alpha level of 0.05, Interleukin-6 (IL-1β) had a p-value of 0.17, and interleukin 5 had a p-value of 0.24. Neither were enough to reject the null hypothesis that this situation increases the inflammatory state, however there is more work that can be done in this area with potentially larger groups and the addition of Vitamin E acetate, as is mentioned in the limitations section.
- Of the cytokines that were the most significant, IL-6 and IL-5, the nicotine group had lower levels of both.
  - IL-6 is a cytokine that stimulates acute-phase protein production and is released from macrophages. This could potentially mean that the macrophages are dysfunctional if they are secreting lower amounts of IL-6 than the control mice.
  - IL-5 serves to promote growth and differentiation in B-cells and eosinophils. It is secreted by T-cells. Low levels of eosinophils and T-cells are seen in heart failure and coronary death, which could be related.

Limitations

- Due to the slide preparation of the fixed mouse lungs, Oil Red O was not performed due to a requirement of frozen tissue sections. The H&E stain only shows if there was tissue damage to the lung before fixation and does not show if there were any lipid-filled macrophages within the lung tissue.
- Two mice in the control group showed mild chronic inflammation with no correlation to this project.
- After starting this project new research came out correlating Vitamin E as being the most likely cause of the lipid-macrophages and correlated Vitamin E to severe lung disease, which was not used as part of this project.
- There were only 6 mice used per group which limits the data produced. For future experiments, larger groups should be used.

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