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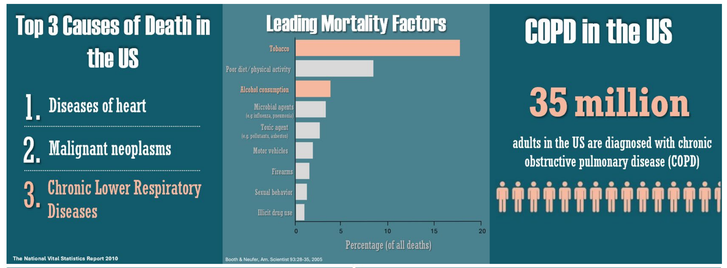
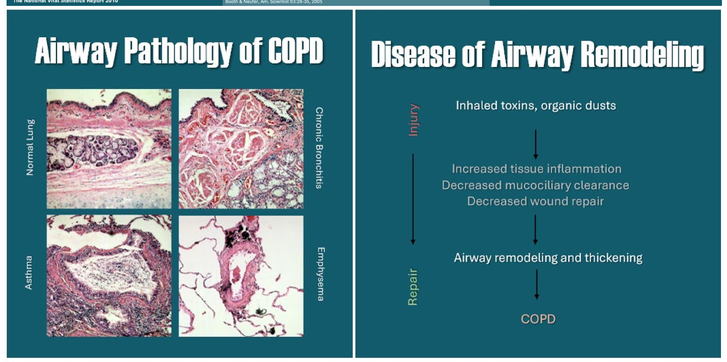
BACKGROUND

Significance of Alcohol Studies. In 2019, 14.5 million people ages 12 and older had an alcohol use disorder (AUD). Approximately 95,000 people die from alcohol-related causes annually. In 2010, alcohol misuse cost the United States $249.0 billion. [4]

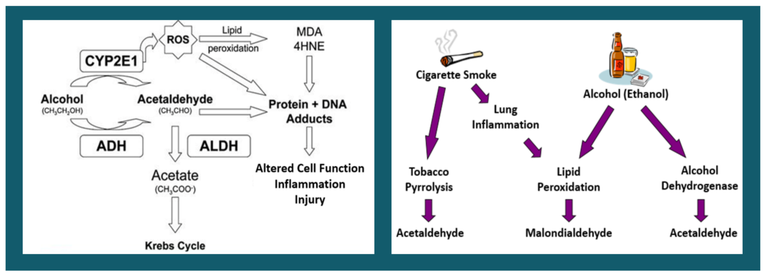
Polysubstance Misuse. >80% of persons with AUD also smoke. Nicotine dependence is more severe in smokers with a history of alcohol dependence. [3]

The Role of Alcohol in Lung Disease and Chronic Obstructive Pulmonary Disease (COPD) is Often Overlooked.





Effects of Smoke and Alcohol. The presence of cigarette smoke and alcohol in the lung leads to the build-up of reactive aldehydes, namely, malondialdehyde and acetaldehyde. One known consequence of aldehydes in smoke and alcohol co-exposure is the activation of Protein Kinase C epsilon. Activation of this enzyme can cause cilia slowing and increased inflammatory cytokine release.



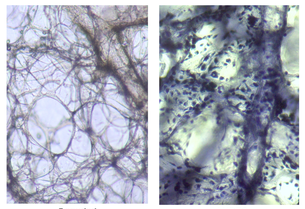
HYPOTHESIS

We hypothesize that cigarette smoke, alcohol, and the combination of the two will negatively impact epithelial cell proliferation on lung scaffolds. In the presence of ADX-102 , we hypothesize that the negative effect on cell proliferation will be mitigated.

MATERIALS & METHODS

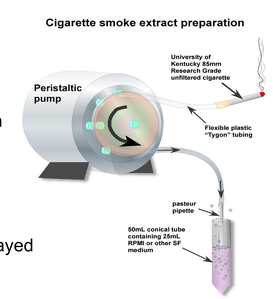
Cell Culture.
16 HBE wild type cells and cells expressing a dominant negative PKCε were cultured in DMEM with 10% serum. Cells were maintained in culture at 37°C. Primary mouse tracheal epithelial cells (MTEC) were grown on air-liquid interface for full differentiation.

Seeding Protocol on Lung Scaffolds.
Three different lung scaffolding types were used in this experiment. These scaffoldings were obtained from different human donors and were allowed to equilibrate in a 37°C incubator overnight. After equilibration of scaffolds, cells were seeded at 1×10^5 per scaffold and allowed to adhere onto scaffolds overnight in a 37°C incubator as previously outlined[2].

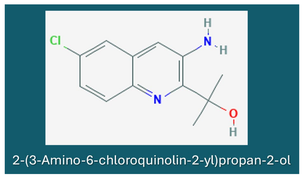


Scaffold Proliferation Protocol.
Cells were allowed to proliferate across lung scaffolds for 6 days. Cells were treated with the appropriate treatment group every day for the duration of the growth period. The cells were exposed to one of the following treatment groups: media control, 5% CSE, 100mM EtOH, and a combination of the CSE and EtOH.

Cigarette Smoke Extract.
Cigarette smoke was extracted as previously described[1] using 1R6F reference cigarettes. One 85mm research grade unfiltered cigarette prepares 25mL of CSE solution (100%) in serum-free medium. Solution is filter-sterilized and diluted to desired target concentration (5%).

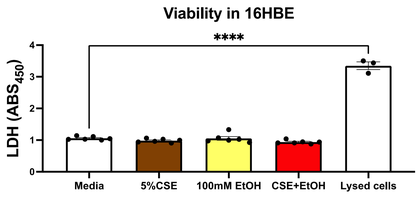


Proliferation Assay.
After duration of the growth period, proliferation was assayed using the MTT Assay to determine cell growth using the protocol previously described[2]



ADX Treatment.
ADX-102 (Reproxalap) is a novel inhibitor of reactive aldehyde species. This drug acts as an aldehyde trapping/scavenging drug. Following the protocol above, 16 HBE WT cells were seeded onto lung scaffolds then pretreated with or without 10 μM ADX-102 for 1 hr. Cells were then allowed to proliferate for 4 days and exposed to media, 5% CSE, 100 mM EtOH, or the combination of both CSE and EtOH).

Viability Assay.
Cell viability was determined by lactate dehydrogenase (LDH) release using a commercial kit (Sigma). No loss of cell viability was determined under smoke and alcohol treatments.



REFERENCES

1. Allen-Gipson D. S., et al. (2005). Cigarette Smoke Extract Increases C5a Receptor Expression in Human Bronchial Epithelial Cells. JPET, 314 (1) 476-482.
2. Nordgren, T. M., et al. (2018). DHA enhances amphiregulin-mediated bronchial epithelial cell repair processes following organic dust exposure. Am J Phys, 314(3), L421–L431.
3. Romberger, D. J., & Grant, K. (2004). Alcohol consumption and smoking status: the role of smoking cessation. Biomedicine & pharmacotherapy, 58(2), 77-83.
4. “Alcohol Facts and Statistics.” NIAAA, (www.niaaa.nih.gov/publications/alcohol-facts).

RESULTS

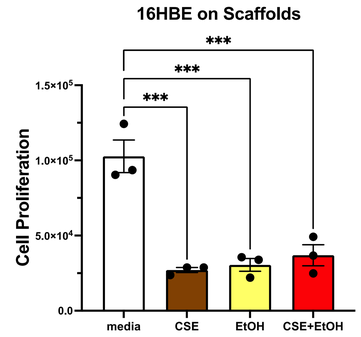


Figure 1. 16 HBE WT cells that were treated with CSE and EtOH show a significant decrease in cell proliferation. ***P<0.0006 vs media.

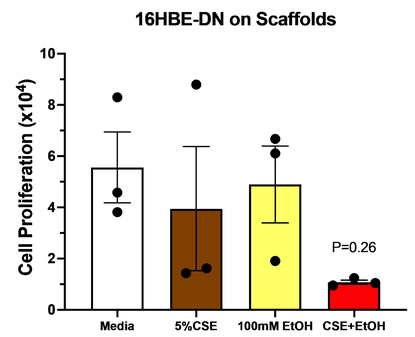


Figure 2. 16 HBE DN cells (lacking PKCε activity) treated with CSE and EtOH show no significant decrease in cell proliferation.

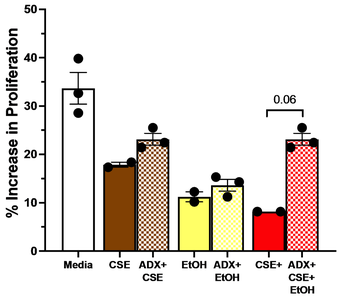


Figure 3. 16 HBE WT cells pre-treated with ADX-102 show a non-significant trend toward protection from smoke and alcohol effect on proliferation.

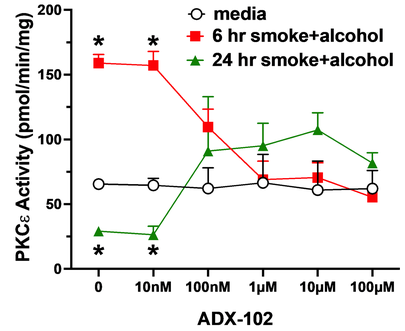


Figure 4. Primary ciliated mouse tracheal epithelial cells (MTEC) pre-treated with ADX-102 show no changes in PKCε activity in response to smoke and alcohol. *P<0.05 vs media control.

CONCLUSION

1. Alcohol and cigarette smoke can slow the proliferation of bronchial epithelial cells on a lung scaffold.
2. Cells lacking Protein kinase C epsilon do not significantly respond to smoke and alcohol with this injury to proliferation.
3. The aldehyde trapping drug, ADX-102, appears to have a potential protective role in cell proliferation by preventing smoke and alcohol activated PKC epsilon.
4. ADX-102 may have future potential therapeutic applications to lung injury and repair.

ACKNOWLEDGEMENTS

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