Effect of Vaping Electronic-Cigarettes Aerosols on the Airway Barrier Function: A Pilot Study

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Electronic-cigarettes (E-cig) are battery powered devices (containing $\sim 7 \times 10^{11}$ free radicals per puff), vaporizes flavors with or without nicotine, which may aid smokers in quitting or attenuating their tobacco habits (Sussan et al., 2015; PMID: 25651083). While there has been an increase in the concern of the health effects of E-cig use, the mechanisms by which they alter lung function are not known. We aim to investigate if vaping E-cig aerosols alters the structure and barrier function of the airway epithelial cells. Primary human bronchial epithelial cells from healthy donors (Epithelix and MatTek Corporation) differentiated on an air-liquid interface were exposed to 10 puffs of tobacco flavored E-cig aerosols with or without nicotine (0% or 1.2%) and a reference cigarettes (3R4F) using a Vitrocell exposure system connected to a peristaltic pump according to Coresta Recommended Method No. 81 puffing regimen (55 mL puffs, 3 s duration, and 30 s puff interval) for 10 days. The control inserts were exposed to the humidified air in the exposure system using the same puffing regimen for 10 days. The barrier function of the epithelia was assessed by monolayer permeability (FITC-dextran flux assay and trans-epithelial electrical resistance), number of ciliated cells, and ciliary beat frequency (CBF). The effects on cell-cell adhesion were measured by quantifying junctional protein abundance (mRNA expression of E-cadherin, CDH1 relative to GAPD) and functional effects of altered adhesion were measured by computing cellular motion over time. We observed that vaping E-cig aerosols increased monolayer permeability, as well as decreased number of cilia and CBF of the airway epithelia (Figure 1). After vaping, the airway epithelial cells had lower expression of cell-cell adhesion junctional proteins. This was associated with increasing cellular motion in the monolayer over time. Our data suggests that vaping E-cig disrupts structural and functional integrity of airway epithelia, resulting in acute or chronic airway inflammation and lung diseases.

Figure 1: Disruption in barrier function of airway epithelia exposed to electronic-cigarette aerosols

\[ \text{A) Monolayer permeability} \]
\[ \text{FITC-dextran flux assay} \]
\[ \begin{array}{ccc}
\text{Air} & 3R4F & 0\% \text{ E-cig} \\
\text{Fluorescence (Fold change)} & * & \psi \\
\end{array} \]

\[ \text{Trans-epithelial electrical resistance} \]
\[ \begin{array}{ccc}
\text{Air} & 3R4F & 0\% \text{ E-cig} \\
\text{TEER (\text{\Omega} \cdot \text{cm}^2)} & * & \psi \\
\end{array} \]

\[ \text{B) Number of ciliated cells} \]
\[ \begin{array}{ccc}
\text{Air} & 3R4F & 0\% \text{ E-cig} \\
\text{% Pixels moving (Fold change)} & * & \psi \\
\end{array} \]

\[ \text{C) Ciliary beat frequency} \]
\[ \begin{array}{ccc}
\text{Air} & 3R4F & 0\% \text{ E-cig} \\
\text{CBF (Hz)} (\text{Fold change}) & * & \psi \\
\end{array} \]

\[ \text{D) mRNA expression of cell-cell adhesion junctional proteins} \]
\[ \begin{array}{ccc}
\text{Air} & 3R4F & 0\% \text{ E-cig} \\
\text{CDH1 mRNA expression (Relative to air)} & * & \psi \\
\end{array} \]

\[ \text{E) Cellular velocity} \]
\[ \begin{array}{ccc}
\text{Air} & 3R4F & 0\% \text{ E-cig} \\
\text{Cellular velocity (mm/hr)} & * & \psi \\
\end{array} \]

\* $P < 0.05$ compared to air. \# $P < 0.05$ compared to 3R4F. \$ $P < 0.05$ compared to E-cig with 0% nicotine.