Another Warning Sign: High Nicotine Content in Electronic Cigarettes Disrupts Mucociliary Clearance, the Essential Defense Mechanism of the Lung

Electronic cigarette (e-cigarette) usage has become popular at an alarming rate and continues to rise, especially among younger populations in the United States. In 2018 alone, there was an ~40% increase in usage (from 12% to 21%) compared with 2017 among high school students, according to a recent Centers for Disease Control and Prevention report (1). Many incorrectly believe that smoking e-cigarettes (vaping) is not harmful to health. The general misconception by the public that "e-cigarettes are safe," however, has been challenged or overturned by many recent studies revealing the association between e-cigarettes and adverse cardiovascular, pulmonary, and systemic health effects.

As a nicotine delivery system, e-cigarette liquids typically contain *1*) a vehicle (propylene glycol/vegetable glycerin [PG/VG]), *2*) a chemical that gives an appealing flavor, and *3*) various

Supported by the NIH/NHLBI/FDA (P50HL120100).

Originally Published in Press as DOI: 10.1164/rccm.201905-1080ED on June 14, 2019

concentrations of nicotine. Previous studies have focused on the toxic effects of these ingredients either as a whole or as separate components of e-cigarettes. Using airway epithelial cell lines, Sassano and colleagues showed that PG/VG itself adversely affected cell viability, and that certain e-liquids, including the most common ones on the market, are more toxic than PG/VG alone (2). White blood cells in the airways, such as neutrophils and macrophages, are crucial for the innate defense of the airways. However, when they are exposed to constant stimulation, such as from cigarette smoke, they can contribute to the initiation and progression of chronic lung diseases such as chronic obstructive pulmonary disease. It has been shown that e-cigarettes can also trigger neutrophils and macrophages to release their enzymes such as elastase and MMP9 (matrix metalloproteinase 9) (3, 4), which are known to cause tissue damage in the long run. Furthermore, e-liquids, even without nicotine, can trigger an inflammatory and oxidative response and cytotoxicity on the human monocytes (5). Moreover, *in vivo* human unbiased proteomics studies using human induced sputum and epithelial cells derived from human airways from smokers and e-cigarette users revealed that vaping causes marked adverse changes in the airways, including altered epithelial and sputum proteomes and mucus/mucin composition (3, 6) in both a similar and a unique way relative to cigarette smokers.

Nicotine is a highly addictive substance, and the high level of nicotine delivered by the current generation of e-cigarette devices can cause addiction in never-smokers (7) and could subsequently provide a foundation to start traditional cigarette smoking (8). The nicotine content of e-cigarettes typically varies between 3 and 36 mg/ml. Most recent generations of e-cigarettes contain much more nicotine (up to 60 mg/ml), typically in a salt form, to speed up and increase the delivery of nicotine to the brain at rates comparable to those found with cigarette smoking. The adverse effect of nicotine on the airways and its consequences, however, is an understudied area. As reported in this issue of the *Journal*, Chung and colleagues (pp. 1134–1145) addressed this critical issue by performing a comprehensive mechanistic study using *in vivo* (sheep) and *in vitro* models (human primary bronchial epithelial cell cultures) to observe lung hemostasis and pathobiology in response to nicotine (9). The authors previously showed that chronic e-cigarette exposure caused chronic obstructive lung disease in mice in a nicotine-dependent manner (10). In this work, they used primary airway epithelial cells derived from nonsmoking healthy individuals for *in vitro* exposure experiments. They then assessed the effects of e-cigarette vapor on airway cells via sophisticated mucociliary transport (MCT), mRNA and protein expression, calcium imaging, mucus concentration, and complex viscosity assays. For *in vivo* animal (sheep) studies, tracheal mucus velocity (TMV), a surrogate marker for mucociliary clearance, was measured after nebulized e-cigarette exposure.

The relative viscosity analysis of cell secretions showed that compared with an air-only control and e-cigarette vapor with no nicotine, e-cigarette vapor with nicotine increased mucus viscosity and decreased the airway surface liquid height in a dose-dependent fashion. Also, human primary bronchial epithelial cell mucus concentrations, measured by percent solids, increased approximately 1.5- to 2-fold. Given the fact that increased mucus concentration and viscosity are inversely related to mucociliary clearance rates, they measured the MCT in cell cultures. As predicted, MCT was significantly reduced, by approximately sixfold on average, in the cultures exposed to nicotine. To understand the mechanism of the adverse effect of nicotine on the cells, the authors focused on a Ca^{2+}-selective ion channel, TRPA1 (transient receptor potential ankyrin 1), as a possible nicotine receptor. In fact, using known inhibitors of TRPA1, the authors averted the adverse effects of the nicotine on airway surface liquid, mucus concentration, and viscosity, which strongly suggests that the effects of nicotine were transmitted through TRPA1 and not the nicotinic acetylcholine receptor.

To validate their *in vitro* observations, the authors used an *in vivo* (sheep) exposure model. They found that TMV diminished by approximately half after exposure to e-cigarette liquid with 15–20 mg/ml nicotine as compared with e-cigarette liquid with 10 mg nicotine or e-cigarette vapor with no nicotine. This effect was prevented when a TRPA1 inhibitor was added to the mixture, which is consistent with the cell culture results and confirms that the nicotine effect occurs via the TRIPA1 receptor. However, this important *in vivo* observation (i.e., impaired mucociliary clearance via nicotine and its consequences) needs to be tested and replicated in the future in *in vivo* human studies involving e-cigarette users with no history of smoking cigarettes. Although this study focused on certain e-cigarette liquids with nicotine up to 36 mg/ml, some new e-cigarette devices and some e-liquids contain a much higher nicotine content and can potentially deliver more nicotine to the airways (10). Given the nicotine concentration–dependent nature of the impaired TMV shown here, it can therefore be speculated that these new devices could be more harmful to the airways.

Mucus abnormalities, such as increased viscoelasticity, elevated mucus/mucin concentrations, and impaired MCT, are commonly seen in cigarette smokers with and without chronic bronchitis, and are closely related to chronic inflammatory muco-obstructive lung diseases such as chronic obstructive pulmonary disease (11). Altogether, this important report published by Chung and colleagues provides novel and unique data showing the harmful effect of e-cigarettes on mucociliary clearance, a crucial part of the lungs' first line of defense. Along with previously reported adverse effects of e-cigarettes, such as increased oxidative stress, neutrophil and macrophage activation, impaired and altered innate defense, and inflammatory response and cytotoxicity, the current findings provide further convincing evidence that e-cigarette smoking is harmful to the airways. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Mehmet Kesimer, Ph.D.
Marsico Lung Institute
and
Department of Pathology and Laboratory Medicine
University of North Carolina
Chapel Hill, North Carolina

ORCID ID: 0000-0003-3867-1873 (M.K.).

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