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E-Cigarettes: Mucus Measurements Make Marks

Cigarette smoking is a leading cause of morbidity and mortality in the United States. It is the principal cause of chronic obstructive pulmonary disease (COPD) and lung cancer, and it is a major risk for the development of pulmonary fibrosis. Additional effects of tobacco smoke on lung health include triggering asthma exacerbations and increasing the risk of respiratory infections. The effects of smoking tobacco extend beyond the lungs, leading to the development of atherosclerotic cardiovascular disease and cerebral vascular disease, as well as cancers in nonrespiratory sites such as the bladder, blood, and bowel. Cigarette smoking is thus a significant factor driving the high costs of health care, as well as the associated effects of missed work at best and patient mortality at worst (1).

Quitting smoking improves health, slows disease progression, and reduces mortality. Because of the public-health need, various substitutes, such as nicotine patches and gum, are widely used, and these have proven effective and safe in helping with smoking cessation. E-cigarettes have been proposed to be a safe alternative to traditional tobacco cigarettes, as well as a potentially useful means of assisting smoking-cessation efforts (2). However, there is debate about the safety of e-cigarettes (3, 4). In this issue of the *Journal*, Reidel and colleagues (pp. 492–501) address this important emerging issue in respiratory medicine and pathobiology (5). Conventional cigarette and e-cigarette users were recruited, and the two groups were compared with each other and with nonsmoking controls. The authors examined whether e-cigarettes differ from traditional tobacco cigarettes in their ability to affect markers of tissue injury and inflammation.

A proteomic analysis of induced sputum showed that compared with nonsmoking controls, both traditional tobacco cigarette and e-cigarette users demonstrated significantly altered protein populations. A total of 110 proteins were increased in the cigarette smoker and e-cigarette smoker groups, with 66 of these proteins being unique to e-cigarette users and 15 overlapping between the user groups. This shared group contained known smoke-associated markers that were upregulated by ~1.5- to >10-fold. Included among these markers were aldehyde dehydrogenase 3A1, thioredoxin, and glutathione S-transferase, suggesting that similar detoxification programs are elicited irrespective of the mode of delivery. Likewise, both modes of tobacco product inhalation were

associated with increases in the levels of the airway mucin MUC5AC (approximately four- to ninefold).

In the clusters of differentially expressed proteins, the authors also identified proteins that are related to inflammatory and antimicrobial activities. One group is associated with the formation of neutrophil extracellular traps (NETs). NETs are fibrous networks of DNA that tether secretory granule enzymes (including neutrophil elastase and matrix metalloproteinase 9) along with DNA-binding proteins (including histones modified by peptidyl arginine deiminase 4). These NET-related proteins were increased in both tobacco user groups, but they occurred to a greater extent in e-cigarette users. Accordingly, the authors tested the functional significance of the relationship between neutrophil NET formation (NETosis) and tobacco intake using purified peripheral blood neutrophils. Strikingly, they observed heightened sensitivities to NET activation in neutrophils isolated from e-cigarette users. The apparent reprogramming of circulating cells raises a number of questions about the nature of systemic responses in traditional cigarette and e-cigarette user groups.

Based on the above findings, there is a clear need to expand these lines of study. The study by Reidel and colleagues was conducted in a small subject population, the subjects were relatively young (late 20s to early 30s), and the majority of the e-cigarette smoking group was classified as former traditional cigarette smokers. With these caveats in mind, it is important to consider that the sputum proteomes of former smokers are strikingly similar to those of never-smokers (6), and the demographics of the study groups (new and former smokers) are groups currently targeted by e-cigarette company marketing (7).

Taken together, these data provide clinically relevant biochemical evidence that the promotion of e-cigarettes as safe alternatives to traditional cigarettes should be viewed with concern. On the one hand, it may seem logical to assume that using a version of inhaled nicotine that has fewer particulates that could directly injure pulmonary surfaces or diffuse into the circulation would cause fewer detrimental effects. However, in airway epithelial cells *in vitro*, tests of particulate versus vapor phases of traditional cigarette smoke have shown that many cellular stress responses are induced to greater degrees in vapor-phase- than in particulate-phase-treated cells (8). Furthermore, findings from animals *in vivo* have shown that e-cigarette vapors contain many of the same toxic agents as tobacco (9), and that e-cigarette exposures induce inflammatory and COPD-like changes in the lungs (10, 11). In a recent mouse study in which the effects of e-cigarettes were tested during pregnancy, adverse inflammatory effects on both maternal and offspring health were observed whether the animals were exposed to nicotine-containing or nicotine-free e-cigarette vapors (12). Thus, it is unclear whether nicotine itself,

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Figure 1. Unlike traditional cigarettes, which use flame combustion to generate tobacco smoke and vapors, e-cigarettes use an electricity-powered heating element to vaporize liquid containing excipient (propylene glycol), flavorings, and nicotine (e-liquid). An e-cigarette atomizer unit has electric coils that heat e-liquid to create an inhalable vapor. (A) E-liquids are supplied in reservoirs or cartridges that are attached to a heating coil and wick or other absorptive filament. (B) With repeated use, burnt material accumulates on wicks and coils that can be changed as the user experiences changes in atomizer performance or e-liquid taste. Images supplied by Wikimedia Commons: (A) Creative Commons CC0 1.0 Universal Public Domain Dedication user OGREBot (https://commons.wikimedia.org/wiki/File:Offene_Wicklung.jpg) and (B) Creative Commons Attribution 4.0 International license user QuackGuru (https://commons.wikimedia.org/wiki/File:Thermally_decomposed_material_on_electronic_cigarette_wick.jpg).

an excipient, or a flavoring is responsible for the effects of the e-cigarettes.

Guidelines to regulate the manufacturing of e-cigarettes, the marketing of e-cigarettes to the public, and the consumption of e-cigarettes in public spaces are new but incomplete. Because various forms of tobacco, such as orally used smokeless versions, are known to cause morbidities (e.g., periodontitis and oral cancer), it should not be assumed that the inhalation of tobacco extracts via e-cigarettes is a safe choice. E-cigarettes themselves induce markers of toxicity and injury in the airways, and these effects may be driven in part by synthetic components that are independent of tobacco extracts or nicotine (9, 13, 14). Studies to characterize the sources of nicotine, the nature of the excipients and flavorings, and differences in dose responses and user demographics are ongoing. These studies will confirm which agents of flame combustion or electric heat-element activation, including tar, are present in these vehicles (Figure 1). Although toxicological findings show that several of the potentially harmful chemicals found in traditional cigarette smoke are also present in e-cigarette smoke, it is quite likely that these chemicals will differ between traditional cigarettes and e-cigarettes, and among e-cigarette varieties and delivery mechanisms. Thus, a strength of the study by Reidel and colleagues is that it focuses on the responses of the hosts.

Results from cessation studies measuring the efficacy of switching from traditional cigarettes to e-cigarettes as a means of maintaining abstinence are conflicting, and long-term studies of chronic use by former smokers who transition to e-cigarettes, or e-cigarette users who have never smoked traditional cigarettes, are lacking or incomplete (2–4). Recent data have begun to provide snapshots of useful clinical endpoints. In a study of two independent observational COPD study populations (smokers who were at risk of COPD and smokers who already had COPD), the use of e-cigarettes did not improve health outcomes or cessation of traditional cigarette smoking (15). Rather, e-cigarette use was linked to worsened lung-function outcomes. In addition, e-cigarette use is increasing in adolescents and young adults, and it may increase the risk of initiating traditional cigarette use (14, 16).

In summary, like traditional tobacco cigarettes, e-cigarettes are vehicles for delivering nicotine, an addictive agent, into the lungs. The reports published here and elsewhere regarding the effects of e-cigarettes do not show sufficient evidence to support the use of e-cigarettes as safe substitutes for traditional tobacco cigarette smoking. ■

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Is There a TIME and Place for Thrombolytics in Malignant Pleural Effusions?

The use of intrapleural thrombolytics as an adjunct therapy to promote pleural drainage is probably best elucidated in patients with pleural infections (1), but has also been studied for other indications including traumatic or nontraumatic retained hemothorax (2) and multiloculated malignant pleural effusions (MPE). Considering that MPEs have increased procoagulant properties and depressed fibrinolytic activity, similar to other causes of exudative pleuritis (3), it is not surprising that over time, many MPEs form fibrinous strands and multiple loculations. This presumably leads to difficulty with management, as fibrinous material may preclude adequate drainage and lung expansion before pleurodesis, or impede tunneled pleural catheter function. A natural clinical question then is, “can improved drainage with the use of fibrinolytics improve dyspnea and pleurodesis in the management of MPE?”

Nearly 20 years ago, Davies and colleagues (4) showed that intrapleural thrombolytics could be used safely in patients with multiloculated MPEs resistant to conventional standard chest tube drainage. In this small (but novel) uncontrolled study of 10 patients, all patients with loculated MPEs received intrapleural streptokinase and reported symptomatic benefit afterward, with an increased volume of pleural fluid drained and radiographic improvement without significant adverse effects. Only a small number of these patients received subsequent pleurodesis after thrombolysis, and pleurodesis success rates were not reported. Subsequently, additional studies (5–7) examined the role of thrombolytics in MPEs before pleurodesis with a number of different outcomes. All showed an increase in pleural fluid drainage

in the thrombolytic group, although care must be taken in the interpretation of this, as thrombolytics alone are known to cause increased pleural fluid production, possibly through mesothelial cell-secreted monocyte chemotactic protein-1 (8). In addition, patients who received intrapleural thrombolytics were universally more likely to have improvement radiographically with better lung expansion, which allowed in one study for more frequent pleurodesis attempts. However, more clinically relevant outcomes such as differences in symptomatic improvement was only addressed in one study, and in all studies, in those patients who did ultimately receive a pleurodesis, pretreatment with thrombolytics did not significantly influence the rates of recurrent effusion. Importantly, all the studies reported that intrapleural treatment of MPEs was a safe intervention for patients, with minimal complications, which alone represented a change in the dogma at the time.

In this issue of the *Journal*, Mishra and colleagues (pp. 502–508) report on the third Therapeutic Intervention in Malignant Effusion trial (TIME3) (9). The TIME3 trial is the largest study to date that seeks to clarify the role of intrapleural thrombolytic therapy before pleurodesis in loculated MPEs, using highly clinically relevant outcomes out to a year. The design of the study was ideal, using a multicenter double-blind randomized controlled trial of patients with nondraining malignant effusions randomly allocated 1:1 to intrapleural urokinase versus placebo and analyzed in an intent-to-treat manner. Patients were only included if drainage via conventional tube was incomplete. The authors found no significant difference in the primary outcomes of dyspnea score and pleurodesis efficacy, with failure rates of 37% and 32% in the urokinase and control group, respectively. Many secondary outcomes, however, did reach significance, with improved radiographic appearance, shorter

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