CHEST

Effect of e-Cigarette Use on Cough Reflex Sensitivity

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BACKGROUND: E-cigarettes (e-cigs) have attained widespread popularity, yet knowledge of their physiologic effects remains minimal. The aim of this study was to evaluate the effect of a single exposure to e-cig vapor on cough reflex sensitivity.

METHODS: Thirty healthy nonsmokers underwent cough reflex sensitivity measurement using capsaicin cough challenge at baseline, 15 min, and 24 h after e-cig exposure (30 puffs 30 s apart). The end point of cough challenge is the concentration of capsaicin inducing five or more coughs (C_5). The number of coughs induced by each e-cig inhalation was counted. A subgroup of subjects (n = 8) subsequently underwent an identical protocol with a non-nicotine-containing e-cig.

RESULTS: Cough reflex sensitivity was significantly inhibited (C_5 increased) 15 min after e-cig use (−0.29; 95% CI, −0.43 to −0.15; $P < .0001$); 24 h later, C_5 returned to baseline (0.24; 95% CI, 0.10-0.38; $P = .0002$ vs post-15-min value). A subgroup of eight subjects demonstrating the largest degree of cough reflex inhibition had no suppression after exposure to a non-nicotine-containing e-cig ($P = .0078$ for comparison of ΔC_5 after nicotine vs non-nicotine device). Furthermore, more coughing was induced by the nicotine-containing vs non-nicotine-containing device ($P = .0156$).

CONCLUSIONS: A single session of e-cig use, approximating nicotine exposure of one tobacco cigarette, induces significant inhibition of cough reflex sensitivity. Exploratory analysis of a subgroup of subjects suggests that nicotine is responsible for this observation. Our data, consistent with previous studies of nicotine effect, suggest a dual action of nicotine: an immediate, peripheral protussive effect and a delayed central antitussive effect.

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ABBREVIATIONS: C_5 = concentration of capsaicin inducing five or more coughs; e-cig = e-cigarette

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e-Cigarettes (e-cigs) have rapidly attained common usage worldwide, prompting significant discussion and speculation in the medical and lay media regarding potential harms and benefits of these devices,[1-6] their role as smoking cessation aids,[7-10] and how their sale and distribution should be regulated, if at all.[11,12] Despite the popularity of e-cigs, remarkably little scientific data have been generated exploring the physiologic effects of e-cig use or "vaping." Few studies have evaluated the effect of e-cig use on the respiratory tract[13-16] and on pulmonary function[17,18] and none, to our knowledge, has evaluated its effect on cough reflex sensitivity. Indeed, there has been a call for increased medical research efforts in the field of health effects of e-cigs.[1,19,20]

Previous studies have shown that otherwise healthy smokers of tobacco cigarettes have a diminished cough reflex sensitivity relative to nonsmokers,[21] presumably on the basis of chronic, cigarette smoke-induced desensitization of airway cough receptors. This hypothesis is supported by the demonstration, in chronic tobacco cigarette smokers, of enhancement of cough reflex sensitivity within 2 weeks of smoking cessation.[22] These studies suggest that cough reflex sensitivity is a dynamic phenomenon, able to be modulated by the presence or absence of stimuli such as cigarette smoke, even after prolonged exposure.[23] Thus, the aim of the present study was to evaluate the effect of a single exposure to e-cig vapor on cough reflex sensitivity in healthy nonsmokers.

e-cigs are electronic nicotine delivery devices. A cartridge within the e-cig contains nicotine in a vehicle of distilled water, as well as either vegetable glycerin or propylene glycol. A lithium battery within the e-cig generates heat, thus vaporizing the nicotine solution. No combustion is involved in the creation of the nicotine-containing vapor that is inhaled by the user and promptly absorbed from the respiratory tract into the bloodstream.[24]

Capsaicin, the pungent extract of red peppers, has been shown in over 3 decades of clinical experience to experimentally induce cough in a safe, dose-dependent, and reproducible manner.[25] Thus, capsaicin cough challenge testing has become an important tool in clinical research, allowing for the accurate measurement of the effect of a pharmacologic or other intervention on the sensitivity of the cough reflex.[25,26] The standard end point measured in capsaicin cough challenge testing is the concentration of capsaicin inducing five or more coughs (C_5). In healthy volunteers, this end point has been demonstrated to be highly reproducible, in the short-term (20 min to 14 days) and long-term (months to years).[27] Standard capsaicin challenge methodology was used in this study to assess the effect of e-cig vapor exposure on cough reflex sensitivity.

Materials and Methods

Subjects

Thirty adult lifetime nonsmokers were enrolled after providing written, informed consent for this study, which was approved by the institutional review board of the Albert Einstein College of Medicine (institutional review board No. 2014-3288). Subjects were without history of asthma, gastroesophageal reflux disease, or symptoms suggestive of acute viral upper respiratory tract infection (common cold) or allergies within 4 weeks of enrollment. Subjects were not receiving medication known to affect cough reflex sensitivity.

Study Design

Upon enrollment, subjects underwent capsaicin challenge testing on day 1 to establish their baseline cough reflex sensitivity. On study day 2, subjects underwent an e-cig vaping session. While in a relaxed, seated position, subjects inhaled a total of 30 puffs (one puff every 30 s) from a disposable e-cig (Blu, Classic Tobacco flavor; Lorillard Technologies [Blu is now made by Fontem US, Inc]). The Blu e-cig is among the most, if not the most, commonly used e-cig in the United States. A disposable Blu e-cig contains 20 to 24 mg of nicotine and delivers approximately 400 puffs of nicotine-containing vapor. The ingredients of the vapor include distilled water, nicotine, vegetable glycerin, natural flavors, artificial flavors, and citric acid.[28] Thus, 30 puffs of the e-cig delivered approximately 1.5 to 1.8 mg of nicotine. In comparison, the estimated nicotine intake from a tobacco cigarette is in the range of 1.07 to 2.6 mg, depending on the brand.[29,30] Fifteen minutes after the conclusion of the e-cig session, subjects underwent capsaicin cough challenge. On study day 3, approximately 24 h after the vaping session, subjects underwent repeat capsaicin challenge. In addition, the number of coughs induced by each of the 30 puffs of the e-cig was tabulated. A cough number of five was assigned for at least five coughs.

A subgroup of eight subjects who demonstrated large degrees of cough reflex sensitivity inhibition after e-cig exposure (defined as at least a two doubling-concentration increase in C_5) underwent a repeat protocol identical to the above but with a disposable non-nicotine-containing e-cig with similar vehicle (Full Tobacco flavor; Blue Star). Subjects were unaware that the e-cig used in this portion of the study was nicotine free.

Capsaicin Cough Challenge

Capsaicin cough challenge testing was performed as previously described.[25,27] Briefly, subjects inhaled single, vital-capacity breaths of ascending, doubling concentrations (range, 0.49-1,000 μM) of aerosolized capsaicin solution, administered via a compressed air-driven nebulizer controlled by a dosimeter, with 1-min intervals between inhalations, until five or more coughs resulted in the 15 s following an inhalation. Placebo saline breaths were randomly interspersed between capsaicin doses to increase challenge blindness. The end point of capsaicin challenge testing is C_5.

Statistical Analysis

Cough reflex sensitivity (C_5) was analyzed using mixed-effects modeling, with subsequent post hoc analysis correcting for multiple comparisons using the Tukey-Kramer approach. Pre-e-cig and post-e-cig exposure differences in C_5 response and number of coughs between nicotine and non-nicotine-containing e-cigs were compared using the Wilcoxon signed-rank test. Statistical analyses were performed using SAS version 9.3 software (SAS Institute, Inc).

Results

Thirty subjects (15 women; age, 29.8 ± 4.5 years [SD]) were enrolled and completed the study. After e-cig exposure, cough reflex sensitivity was significantly diminished (ie, C_5 was significantly increased) compared with baseline. This effect was transient, as demonstrated by the enhancement of cough reflex sensitivity back to baseline levels 24 h after the e-cig exposure. Mean log C_5 at baseline was 0.50 ± 0.09 (SEM); 15 min after electronic cigarette exposure it was 0.79 ± 0.11; and 24 h subsequently it was 0.55 ± 0.10. Using mixed-effects modeling, with subsequent post hoc analysis correcting for multiple comparisons using the Tukey-Kramer approach, the difference between log C_5 at baseline and post-e-cig exposure was significant (difference in mean log C_5, −0.29; 95% CI, −0.43 to −0.15; $P < .0001$) as was the difference between post-e-cig use and 24 h later (difference in mean log C_5, 0.24; 95% CI, 0.10-0.38; $P = .0002$) (Fig 1). In terms of individual responses, 23 of 30 subjects demonstrated an inhibition of cough reflex sensitivity (increased C_5) after e-cig exposure; five subjects had no change, and two subjects had a one-doubling concentration decrease in C_5.

Twenty-six of the 30 subjects coughed to some degree in response to inhalation of the 30 puffs of the e-cig. The median number of coughs for the study group was 15.5 with a range of 0 to 114 coughs. There was no correlation between the number of coughs induced by e-cig inhalation and subsequent change in cough reflex sensitivity (C_5), as demonstrated by computation of the Spearman correlation coefficient, with Fisher z-transformation. The point estimate of this correlation was −0.20 with 95% CI (−0.62, 0.23) and was not significantly different from zero ($P = .453$).

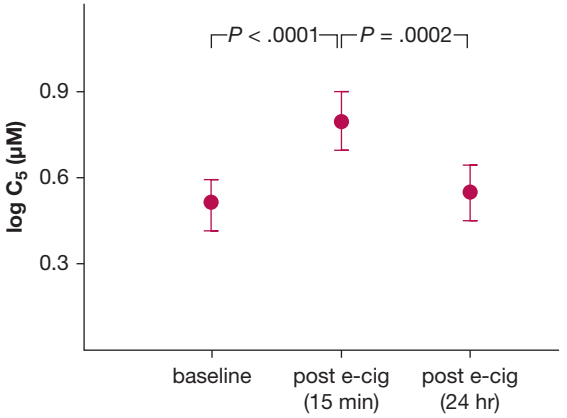

Figure 1 – *Change in cough reflex sensitivity (C_5) from baseline after e-cig exposure (30 puffs delivered 30 s apart) in 30 healthy adult nonsmokers. Significant inhibition of cough reflex sensitivity (increase in C_5) occurred 15 min after exposure (P < .0001). This effect was transient, as C_5 returned to baseline 24 h after exposure (P = .0002 vs post-15-min value). C_5 = concentration of capsaicin inducing five or more coughs; e-cig = e-cigarette.*

To further investigate the role of nicotine in our observations, we performed an additional exploratory analysis by repeating an identical protocol of cough reflex sensitivity measurement before and after exposure to a non-nicotine-containing disposable e-cig in a subgroup of subjects. All eight subjects who had demonstrated large degrees of inhibition of cough reflex sensitivity after exposure to the nicotine-containing e-cig, defined as an at least two doubling-concentration increase in C_5, agreed to participate in a follow-up study of a different brand of e-cig. Subjects were not aware that the e-cig being evaluated in the second phase of the study did not contain nicotine.

No inhibition of cough reflex sensitivity was observed after exposure to the non-nicotine-containing e-cig, in contrast to the change in C_5 after use of the nicotine-containing e-cig (median difference in ΔC_5, 0.6; range, 0.6-0.9; $P = .0078$, Wilcoxon signed-rank test) (Fig 2). In addition, significantly less coughing was observed after 30 puffs of the non-nicotine-containing e-cig compared with the nicotine-containing product (median difference in Δ number of coughs, six; range, 0-21; $P = .0156$).

Discussion

We have demonstrated in a group of healthy adult nonsmokers that a single exposure to e-cig vapor, approximating the nicotine delivery of one tobacco cigarette, significantly inhibits cough reflex sensitivity as measured by capsaicin inhalation cough challenge testing. We noted the effect to be transient, as cough reflex sensitivity returned to baseline 24 h after e-cig use.

These findings are consistent with observations in healthy smokers of tobacco cigarettes, whose cough reflex sensitivity is suppressed relative to nonsmokers.[21] The demonstration that cough reflex sensitivity is

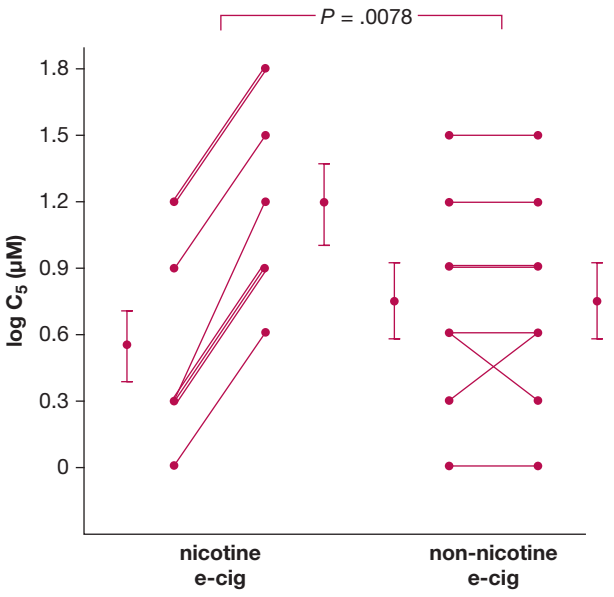

Figure 2 – *Comparison of the effect of nicotine-containing and non-nicotine-containing e-cig exposure on cough reflex sensitivity (C_5) in a subgroup of eight subjects who had demonstrated the largest increments in C_5 (greatest degree of inhibition of cough reflex sensitivity) after nicotine-containing e-cig use. The non-nicotine-containing e-cig exposure did not affect cough reflex sensitivity as did the nicotine-containing product (P = .0078 for difference in change in C_5). See Figure 1 legend for expansion of abbreviations.*

significantly enhanced as soon as 2 weeks after smoking cessation supports the hypothesis that inhibition of cough reflex sensitivity is due to desensitization of cough receptors within the airway epithelium caused by chronic exposure to tobacco smoke.[22] Furthermore, as this effect is promptly reversible even after years of tobacco smoking, cough reflex sensitivity is apparently a dynamic phenomenon, able to be modulated by the presence or absence of stimuli such as tobacco smoke.[23] Given that these previous studies were performed in chronic tobacco cigarette smokers, the observations of the present study are perhaps more remarkable in that significant inhibition of cough reflex sensitivity was demonstrated after a single brief exposure to an e-cig. Our findings therefore raise the question of the effect on cough reflex sensitivity, and other pulmonary consequences, of repeated or chronic use of e-cigs and thus support the need for further investigation in this field. Indeed, if chronic e-cig use led to a sustained diminution of the cough reflex, one could speculate that loss of this important defense mechanism might have adverse clinical consequences. Furthermore, our demonstration of the acute effect of a single e-cig exposure on cough reflex sensitivity refutes the opinion of some that e-cig vapor is a benign and physiologically inert substance. A study demonstrating diminished cough reflex sensitivity in children exposed to environmental tobacco smoke relative to children not so exposed[31] supports the need for further investigation on potential secondhand effects of e-cigs.[6,15]

In an attempt to gain insight as to the causative agent within the e-cig vapor that led to significant inhibition of cough reflex sensitivity, we performed an exploratory analysis of a subgroup of our 30 subjects. Eight of the 30 subjects with the greatest degree of cough reflex suppression (defined as an elevation of capsaicin C_5 at least two doubling concentrations) after nicotine-containing e-cig exposure were subsequently exposed in a similar manner to a non-nicotine-containing e-cig with similar flavoring and vehicle. The absence of an effect on cough reflex sensitivity implicates nicotine as the agent within the e-cig vapor causing the inhibition of cough reflex sensitivity that we observed. However, some degree of unintentional unblinding may have occurred in that the non-nicotine-containing e-cig vapor may have been perceived by subjects as qualitatively different from the nicotine-containing e-cig vapor, even though subjects were unaware that the second phase of the study used a non-nicotine-containing e-cig.

Several studies lend support to the hypothesis that nicotine is the main causative factor in our observations. In an in vivo model of e-cig exposure in mice, a chronic, 4-month exposure to nicotine-containing e-cig vapor caused airway hyperreactivity and emphysema, whereas the vehicle (50% propylene glycol/50% vegetable glycerin) had no effect.[32] Nicotine has been demonstrated in animals and humans to have a peripheral, rapid-onset, cough-inducing effect, probably through stimulation of nicotinic acetylcholine receptors expressed on sensory terminals of cough receptors within the airway mucosa[33]; but in cats, nicotine has been shown to have an antitussive effect when administered centrally, suggesting that nicotinic acetylcholine receptors modulate brainstem functions, particularly caudal ventral respiratory column neurons involved in expression of the tracheobronchial cough reflex.[34] These observations may be relevant to the findings of the present study, since most of our subjects did cough immediately and transiently in response to e-cig inhalation, yet demonstrated inhibition of cough reflex sensitivity when measured 15 min after completion of the e-cig vaping session. In the subgroup of subjects who also underwent an exposure to a non-nicotine-containing e-cig, less cough occurred during the vaping session, and inhibition of cough reflex sensitivity was absent. Thus, the results of our study may

be an illustration of a dual action of nicotine: an acute, peripheral tussive effect and a delayed, central antitussive effect. The putative action of nicotine as a centrally acting inhibitor of cough reflex sensitivity introduces the concept of nicotinic receptor agonists as potential therapeutic antitussive agents.

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Author contributions: P. V. D. had full access to all of the data in the study and takes responsibility for the integrity of the data, the content of the manuscript, and the accuracy of the data analysis, including and especially any adverse effects. A. L. C., A. J. D., and A. N. contributed substantially to the study design, data analysis and interpretation, and the writing of the manuscript.

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