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Studying the interactive effects of menthol and nicotine among youth: An examination using e-cigarettes

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ABSTRACT

Background: Tobacco products containing menthol are widely used by youth. We used e-cigarettes to conduct an experimental evaluation of the independent and interactive effects of menthol and nicotine among youth.

Procedures: Pilot chemosensory experiments with fourteen e-cigarette users identified low (barely perceptible, 0.5%) and high (similar to commercial e-liquid, 3.5%) menthol concentrations. Sixty e-cigarette users were randomized to a nicotine concentration (0 mg/ml, 6 mg/ml, 12 mg/ml) and participated in 3 laboratory sessions. During each session, they received their assigned nicotine concentration, along with one of three menthol concentrations in random counterbalanced order across sessions (0, 0.5%, 3.5%), and participated in three fixed-dose, and an ad-lib, puffing period. Urinary menthol glucuronide and salivary nicotine levels validated menthol and nicotine exposure. We examined changes in e-cigarette liking/wanting and taste, coolness, stimulant effects, nicotine withdrawal and ad-lib use.

Results: Overall, the high concentration of menthol (3.5%) significantly increased e-cigarette liking/wanting relative to no menthol ($p < 0.001$); there was marginal evidence of nicotine* menthol interactions ($p = 0.06$), with an increase in liking/wanting when 3.5% menthol was combined with 12 mg/ml nicotine, but not 6 mg/ml nicotine. Importantly, both 0.5% and 3.5% menthol concentrations significantly improved taste and increased coolness. We did not observe nicotine or menthol-related changes in stimulant effects, nicotine withdrawal symptoms or ad-lib use.

Conclusions: Menthol, even at very low doses, alters the appeal of e-cigarettes among youth. Further, menthol enhances positive rewarding effects of high nicotine-containing e-cigarettes among youth.

1. Introduction

Use of tobacco products remains a significant health concern worldwide. Regulatory measures need to focus on reducing the appeal of tobacco products (WHO-FCTC, 2015). Flavors may enhance the appeal of tobacco products, and facilitate progression from initiation to nicotine dependence (Samet et al., 2016). Scientific evidence about the influence of flavors on the appeal of tobacco products is critically needed to support regulatory efforts.

Menthol-containing tobacco products are very popular, especially among young users (Rock et al., 2010). Menthol is also a common flavor used in e-liquids (Yingst et al., 2017). Recent evidence suggests that while cigarette smoking rates are going down, use of menthol cigarettes is increasing, especially among young smokers (Villanti et al., 2016), suggesting that the presence of menthol cigarettes may be impeding decreases in cigarette use (Giovino et al., 2015).

Menthol is an organic compound that not only has a characterizing aroma but also has pharmacological effects which may interact with nicotine/tobacco. For example, menthol produces analgesic and cooling sensations via activation of the TRPM8 receptors (Hatem et al., 2006; Wasner et al., 2004), and alters irritant responses via the TRPA1 receptor, which also mediates irritant responses to nicotine (Fan et al., 2016; Karashima et al., 2007; Xiao et al., 2008). Menthol also modulates nicotinic receptor (nAChR) function (Hans et al., 2012; Ton et al., 2015). Up-regulation of nAChRs has been observed in mice following chronic exposure to menthol concentrations comparable to those in

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cigarette smoke (Henderson et al., 2016) and in human menthol cigarette smokers (Brody et al., 2013). Menthol may also slow nicotine metabolism (Alsharari et al., 2015; Benowitz et al., 2004; Fagan et al., 2016).

A report from the FDA's Tobacco Product Scientific Advisory Committee (Samet et al., 2011) which concluded that menthol cigarette smoking was a risk factor for development of nicotine dependence, was challenged by tobacco industry representatives (Heck et al., 2011) who argued that there was no scientific evidence to support such causal relationships. The clinical evidence on the role of menthol in nicotine addiction is limited, and at times inconclusive. For example, while some studies suggest that menthol cigarette smokers may be more dependent and find it harder to quit smoking (Ahijevych and Garrett, 2010; Fagan et al., 2010; Foulds et al., 2010) this is not consistently observed (Hyland et al., 2002; Muscat et al., 2009).

We aimed to conduct a clinical experiment to examine if menthol has independent rewarding effects, and whether it interactively alters the reward from nicotine. We used e-cigarettes which provide an ideal system for examining the inhaled effects of menthol. To achieve adequate increases in nicotine levels we adapted an e-cigarette exposure paradigm (Vansickel et al., 2010). Since menthol has been shown to disproportionately influence use by younger smokers, we conducted this study in older adolescents. Because even tobacco products that are not labeled as being mentholated may contain small concentrations of menthol (Giovino et al., 2004; Reid, 1994), we examined concentrations of menthol that were either barely perceptible or similar to levels in commercial menthol e-liquids. We hypothesized that menthol would have independent, concentration-dependent effects on liking/wanting e-cigarettes, and would also interact with nicotine to alter liking/wanting e-cigarettes. Secondarily, we examined whether menthol independently or interactively (with nicotine) altered stimulant effects, nicotine withdrawal, and taste and coolness. Finally, we explored whether menthol altered e-cigarette value as well as e-cigarette use during an ad-libitum period.

2. Methods

2.1. General procedures

Experimental procedures were approved by the Yale School of Medicine Human Investigations Committee and followed National Advisory Council on Drug Abuse guidelines for substance use research in children and adolescents, and administration of drugs for research purposes (NACDAa, 2006; NACDAb, 2012).

Participants were recruited from local high schools, colleges, and through online advertisements and flyers. Participants had to be between 16 and 20 years of age, have used e-cigarettes with nicotine for the past year (at least 10 days in the past month), with baseline urinary cotinine levels of > 500 ng/ml, not currently trying to quit smoking or e-cigarette use, not have any physical or psychological conditions which would increase risk of participation, and report having tried menthol-flavored tobacco products (58 of the 981 participants screened had never tried menthol products). Participants who were > 18 years old provided informed consent. Parental permission and minor assent was obtained for those < 18 years old. Participants received a physical examination (by an APRN) and a clinical evaluation (by a licensed clinical psychologist) to rule out concerning physical or psychological conditions, and substance use disorders (other than nicotine use disorder). Eligible youth participated in laboratory sessions at the John B. Pierce Laboratory in a temperature controlled and ventilated room (with air exchange 11 times/hour). Participants were asked to abstain from cigarette (confirmed by breath CO levels < 10 ppm, Micro Direct, Inc., Lewiston, ME) and e-cigarette use for at least 10 h before each session. At the end of the study participants met with a licensed clinical psychologist who encouraged them to rethink their tobacco use and explore quitting options.

Table 1
Demographic information participants in Pilot and Main Study.

Variable	Pilot Study (N = 10)	Main Study (N = 60)		
		0 mg/ml Nicotine (N = 21)	6 mg/ml Nicotine (N = 22)	12 mg/ml Nicotine (N = 17)
Age, M (SD)	18.1 (0.8)	18.8 (0.68)	18.8 (0.81)	18.9 (0.83)
Sex, N (%)				
Male	7 (70%)	10 (47.6%)	11 (50.0%)	8 (47.1%)
Race/Ethnicity, N (%)				
White	9 (90.0%)	16 (76.2%)	19 (86.4%)	14 (82.4%)
Asian	0 (0.0%)	0 (0.0%)	0 (0.00%)	0 (0.00%)
Hispanic	1 (10.0%)	0 (0.0%)	2 (9.09%)	2 (11.8%)
Black	0 (0.0%)	1 (4.76%)	0 (0.00%)	0 (0.00%)
Biracial/Other	0 (0.0%)	4 (19.0%)	1 (4.55%)	1 (5.88%)
E-cigarette Use				
Days in past month, M (SD)	17.4 (3.3)	24.7 (7.4)	22.8 (9.0)	25.6 (5.1)
Menthol in e-cigarette, N (%)	4 (40%)	13 (61.9%)	11 (50.0%)	9 (52.9%)
Cigarette Use				
Smokers, N, (%)	10 (100%)	15 (71.4%)	18 (81.8%)	15 (88.2%)
Menthol cigarette smokers N (%)	5 (50%)	11 (52.4%)	10 (45.5%)	9 (52.9%)
Intake cotinine levels (ng/ml); M (SE)	960.2 (76.2)	1202 (87)	769 (61.8)	847 (65.1)

2.2. Pilot chemosensory experiment

Fourteen participants participated in one session (see Table 1 for demographics). We used V2 Cigs™ (VMR Products LLC) with refillable cartridges which were filled with AmericaneLiquidStore® Tobacco Flavor e-liquids containing 70/30 propylene glycol (PG)/vegetable glycerin (VG) and varying concentrations of L-menthol (obtained from Sigma-Aldrich, USP). Two commercial American Liquid Store® menthol e-liquid flavors were also tested and concentrations of menthol in these e-liquids were determined using liquid chromatography/mass spectrometry (LC/MS/MS).

Cartridges were filled with 500 μL of e-liquid and were used only in a single session. During each session, presentation of a non-menthol control trial was followed by the presentation of 5 increasing concentrations of menthol (0.5%, 1.5%, 2.5%, 3.5%, 5%, a commercial menthol flavor, and a commercial mint flavor, with 5 min breaks between trials to reduce sensitization/desensitization of menthol's sensory effects (Cliff and Green, 1994). At the end of each trial, participants rated coolness and overall flavor intensity.

2.3. Main study procedures

Sixty participants who reported e-cigarette use, with or without concurrent cigarette use, (31 females, 29 males) participated in 3 laboratory sessions separated by at least 48 h (see Table 1 for demographics).

Using randomization stratified by gender, we first assigned each participant to a single nicotine concentration [0 mg/ml, low (6 mg/ml), high (12 mg/ml)]. Combined with their assigned nicotine concentration), participants received, all three menthol concentrations [no menthol (NM) = 0%, low menthol (LM) = 0.5%, high menthol (HM) = 3.5%], each during a separate session, in counterbalanced order. Nicotine concentrations were chosen to represent those commonly used by youth (Morean et al., 2016). The research assistants and the