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Delivery of nicotine aerosol to mice via a modified electronic cigarette device

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ABSTRACT

Background: Although both men and women use e-cigarettes, most preclinical nicotine research has focused on its effects in male rodents following injection. The goals of the present study were to develop an effective e-cigarette nicotine delivery system, to compare results to those obtained after subcutaneous (s.c.) injection, and to examine sex differences in the model.
Methods: Hypothermia and locomotor suppression were assessed following aerosol exposure or s.c. injection with nicotine in female and male mice. Subsequently, plasma and brain concentrations of nicotine and cotinine were measured.
Results: Passive exposure to nicotine aerosol produced concentration-dependent and mecamylamine reversible hypothermic and locomotor suppressant effects in female and male mice, as did s.c. nicotine injection. In plasma and brain, nicotine and cotinine concentrations showed dose/concentration-dependent increases in both sexes following each route of administration. Sex differences in nicotine-induced hypothermia were dependent upon route of administration, with females showing greater hypothermia following aerosol exposure and males showing greater hypothermia following injection. In contrast, when they occurred, sex differences in nicotine and cotinine levels in brain and plasma consistently showed greater concentrations in females than males, regardless of route of administration.
Discussion: In summary, the e-cigarette exposure device described herein was used successfully to deliver pharmacologically active doses of nicotine to female and male mice. Further, plasma nicotine concentrations following exposure were similar to those after s.c. injection with nicotine and within the range observed in human smokers. Future research on vaped products can be strengthened by inclusion of translationally relevant routes of administration.

1. Introduction

Since their introduction to the U.S. market, use of electronic cigarettes (e-cigarettes) has risen dramatically, particularly in youth in grades 6–12 (Bunnell et al., 2015; McMillen et al., 2015). For example, recent data from national surveys conducted by the Centers for Disease Control show that over 10% of male youth reported e-cigarette use in the last 30 days compared to less than 5% of adult males (Table 1). While significantly fewer female youth reported past 30-day use (~8%) compared to male youth, their recent use still remains twice that of adult females. Yet, examination of prevalence figures for more frequent use (i.e., some days or every day) reveals that the percentage of users of both sexes is higher for adults than for youth. Hence, the overall percentages across frequency suggest that youth are more likely to try e-cigarettes whereas adults are more consistent in their use, with similar percentages of men and women reporting regular use. This interpretation is consistent with previous literature reporting that adults use e-cigarettes primarily for smoking cessation (Dawkins et al., 2013) whereas adolescents use primarily for experimentation (Hughes et al., 2015), although longitudinal analysis suggests increasing adolescent use over time (Lippert, 2016).

The use of e-cigarettes by women and men argues for inclusion of both sexes in research on biological mechanisms and consequences associated with their use. To date, however, most preclinical research on tobacco and the nascent research on e-cigarettes have focused on examination of nicotine effects in male rodents following injection. Recently, several laboratories have reported on the development of methods to expose rodents to nicotine and/or tobacco via inhalation (George et al., 2010; Ponzoni

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Table 1
Self-reported e-cigarette use among male and female youth and adults.[a]

Current E-cigarette Use	CDC National Youth Tobacco Survey 2014		CDC National Adult Tobacco Survey 2013	
	Male N (weighted%)	Female N (weighted%)	Male N (weighted%)	Female N (weighted%)
Past 30-day use	1156 (10.3%)	833* (8.1%)	809 (4.7%)	854* (3.6%)
Some days	1021 (9.2%)	782* (7.6%)	691 (23.2%)	640 (25.7%)
Every day	135 (1.2%)	51* (0.6%)	163 (5.5%)	169 (5.1%)

[a] N = number of individuals who responded positively to the indicated question (weighted% of total number of males or females surveyed). For the youth survey, the use of e-cigarette use options were presented as "0 days", "1 or 2 days", "3 to 5", "6 to 9", "10 to 19", "20 to 29", and "all 30 days". 1–29 days were recoded as "Some days", 30 was recoded as "Everyday".

* $P \leq 0.001$ (males vs females) based upon Wald test.

et al., 2015; Smith et al., 2015). While many of these studies concentrated primarily on examination of the effects of inhaled nicotine on the pulmonary system or on developmental or toxicological effects (McGrath-Morrow et al., 2015; Misra et al., 2014; Smith et al., 2015; Sussan et al., 2015), a few studies have investigated behavioral effects of inhaled tobacco smoke (Bruijnzeel et al., 2011; de la Pena et al., 2014, 2015; Harris et al., 2010; Yamada et al., 2010) or nicotine vapor generated by bubbling air through a nicotine solution (George et al., 2010; Gilpin et al., 2014) and one lab compared the effects of chronic exposure to cigarette smoke or e-cigarette vapor (Ponzoni et al., 2015). However, none of these studies examined sex differences and only the latter study focused on a model of e-cigarette exposure. Further, most of these studies were conducted in rats. The primary metabolic enzyme for nicotine in rats is in the CYP2B family (Nakayama et al., 1993), whereas the primary enzyme in mice is CYP2A5 (Murphy et al., 2005; Siu et al., 2006), which is more closely related (84% sequence homology) to CYP2A6 (Murphy et al., 2005), the predominant liver enzyme in humans that metabolizes nicotine to cotinine (Messina et al., 1997). Hence, mice may represent a better animal model for studies with a pharmacokinetics component (Matta et al., 2007; Siu et al., 2006).

In the present study, a commercially available tank-based e-cigarette (Brown and Cheng, 2014) was modified to permit rodent exposure to aerosolized e-liquids (i.e., solutions containing a vehicle of propylene glycol and/or vegetable glycerin with nicotine and added flavors). Hypothermia and locomotor suppression, characteristic effects of nicotine in mice (Damaj, 2001), were assessed following inhalational exposure to nicotine aerosol or after subcutaneous (s.c.) injection with nicotine in female and male mice. As a preliminary step towards verifying similar mechanisms, reversal of these effects following injection of the noncompetitive nicotine receptor antagonist mecamylamine was also assessed. Subsequently, plasma and brain concentrations of nicotine and its major metabolite cotinine (Benowitz et al., 1983; Petersen et al., 1984) were measured. Results reported here serve as proof-of-principle for a novel device capable of translationally relevant delivery of nicotine aerosol for use in mechanistic studies of behavioral and biological effects of e-cigarettes. This apparatus has also been used to deliver aerosolized stimulants to rodents (Marusich et al., 2016).

2. Materials and methods

2.1. Subjects

Adult male and female ICR mice (25–35 g) [Harlan/Envigo Laboratories, Frederick, MD] were singly housed in polycarbonate cages with hardwood bedding in a temperature-controlled environment (20–24 °C) with a 12 h light-dark cycle (lights on at 0600). All mice had *ad libitum* access to food and water while in their home cages. The studies were carried out in accordance with federal and state regulatory guidelines and were IACUC-approved.

2.2. Drugs and chemicals

Mecamylamine HCl and (−)-nicotine hydrogen tartrate salt (Sigma-Aldrich, St. Louis, MO) were dissolved in physiological saline (Patterson Veterinary, Devens, MA), and the pH was adjusted to approximately neutral (pH ~ 7), as necessary. (−)-Nicotine free base (Sigma-Aldrich) was mixed with a 50:50 propylene glycol and glycerin solution (Sigma-Aldrich). Doses of nicotine for injection are expressed as mg/kg of the base. Nicotine and mecamylamine were injected subcutaneously (s.c.) at a volume of 10 ml/kg. Concentrations for aerosol administration are expressed as mg/ml in the e-cigarette tank, and may not be representative of the actual amount of nicotine inhaled.

Chemicals and reagents for the analysis of biological samples were purchased commercially and included nicotine (Sigma-Aldrich), cotinine (Toronto Research Chemicals, Toronto, ON), nicotine-d3 (Cambridge Isotope Laboratories, Tewksbury, MA), cotinine-d3 (Santa Cruz Biotechnology, Dallas, TX), ammonium acetate (Sigma-Aldrich), and formic acid and acetonitrile (Fisher Scientific, Fair Lawn, NJ). An internal standard solution was prepared in methanol (Fisher Scientific) containing 48 μg/ml nicotine-d3 and 38 μg/ml cotinine-d3. Working solutions containing both nicotine and cotinine were prepared in methanol at concentrations of 10,000 and 100 ng/ml.

2.3. Apparatus

Aerosol was generated using a modified commercially available electronic cigarette (Fig. S1). An iStick 30W variable wattage (eLeaf, Irvine, CA) supplied power (7W) to a CE5-S tank/clearomizer with bottom dual coil atomizer (1.8 Ω) (Aspire, Kent, WA). Air/aerosol was pumped (1 L/min) through the bottom of the tank and into an EZ-177 Sure-Seal 1L mouse induction anesthesia chamber (10 cm × 10 cm × 10 cm) [EZ-Anesthesia, Palmer, PA] via Tygon tubing (Fisher Scientific, Pittsburgh, PA) and controlled by 3-way stopcocks (Grainger, Raleigh, NC). The aerosol generation system was placed in a hood to avoid exposure of laboratory technicians to aerosol. Mouse locomotor activity was assessed in separate clear Plexiglas activity chambers (47 cm × 25.5 cm × 22 cm). Each chamber was surrounded by two arrays of 4 × 8 infrared photocell beams, interfaced with software for automated data collection (San Diego Instruments, San Diego, CA). Temperature readings were taken using a BAT-12 Microprobe Thermometer with RET-3 Rectal Probe (PhysiTemp Instruments Inc., Clifton, NJ). Analgesia was measured by a Tail Flick Analgesia Meter (IITC Inc. Life Science, Woodland Hills, CA).