Journal of Hazardous Materials 344 (2018) 549–557

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Research paper

Assessment of reactive oxygen species generated by electronic cigarettes using acellular and cellular approaches

Jiayuan Zhao[a], Yipei Zhang[b], Jennifer D. Sisler[c], Justine Shaffer[c], Stephen S. Leonard[c], Anna M. Morris[c], Yong Qian[c], Dhimiter Bello[a,b,*], Philip Demokritou[a,*]

[a] Department of Environmental Health, Center for Nanotechnology and Nanotoxicology, Harvard School of Public Health, Boston, MA 02115, USA
[b] Department of Public Health, University of Massachusetts Lowell, MA 01854, USA
[c] Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV 26505, USA

HIGHLIGHTS

- Both acellular and cellular systems found ROS generation in e-cig emissions.
- E-cig features (brand, flavor) highly influence ROS formation.
- Operational parameters (puffing and voltage) highly influence on ROS formation.
- E-cig emission can contain comparable level of ROS compared to tobacco cigarette.
- Influence of parameters should be considered in e-cig toxicological studies.

ARTICLE INFO

Article history:
Received 28 April 2017
Received in revised form 11 October 2017
Accepted 27 October 2017
Available online 29 October 2017

Keywords:
Electronic cigarette
Nicotine
Reactive oxygen species
Oxidative stress

ABSTRACT

Electronic cigarettes (e-cigs) have fast increased in popularity but the physico-chemical properties and toxicity of the generated emission remain unclear. Reactive oxygen species (ROS) are likely present in e-cig emission and can play an important role in e-cig toxicity. However, e-cig ROS generation is poorly documented. Here, we generated e-cig exposures using a recently developed versatile exposure platform and performed systematic ROS characterization on e-cig emissions using complementary acellular and cellular techniques: 1) a novel acellular Trolox-based mass spectrometry method for total ROS and hydrogen peroxide (H_2O_2) detection, 2) electron spin resonance (ESR) for hydroxyl radical detection in an acellular and cellular systems and 3) *in vitro* ROS detection in small airway epithelial cells (SAEC) using the dihydroethidium (DHE) assay. Findings confirm ROS generation in cellular and acellular systems and is highly dependent on the e-cig brand, flavor, puffing pattern and voltage. Trolox method detected a total of 1.2–8.9 nmol $H_2O_{2eq.}$/puff; H_2O_2 accounted for 12–68% of total ROS. SAEC cells exposed to e-cig emissions generated up to eight times more ROS compared to control. The dependency of e-cig emission profile on e-cig features and operational parameters should be taken into consideration in toxicological studies.

1. Introduction

The use of electronic cigarettes (e-cig) have risen considerably in recent years, especially among teenagers [1,2]. E-cig global sales reached $7 billion in 2014, and continue to rise [3]. A great number of manufactures are competing for market shares, and a total of 466 brands and 7700 flavors were documented in 2014 [4]. Battery operated, e-cigs work by heating the e-liquid, which contains humectants (propylene glycol and glycerin), nicotine and flavor additives, to form emissions. Thus, e-cig emission is expected to be chemically complex, and likely strongly dependent on the e-liquid formulation and the design specifications of the e-cig that defines the heating/vaporization process.

There is a general perception that e-cigs are less harmful than tobacco cigarette [5,6]. However, large discrepancy has been reported in the published literature on e-cig emission physico-chemical properties and toxicity [7–19]. This is because e-cig

* Corresponding authors at: Department of Environmental Health, Harvard School of Public Health, Boston, MA, 02115, USA.
E-mail addresses: Dhimiter_Bello@uml.edu (D. Bello), pdemokri@hsph.harvard.edu (P. Demokritou).

https://doi.org/10.1016/j.jhazmat.2017.10.057
0304-3894/

exposure is not a single scenario but highly influenced by e-cig product type, e-liquid composition (flavor), operational parameters and user inhalation pattern [20]. The role of these parameters on both e-cig emission formation and chemical composition has not been studied systematically. Without an adequate understanding of e-cig emission properties and associated toxicological profile, one cannot establish whether e-cig can not cause any harm. For example, because e-liquid contains fewer chemicals and minimizes combustion processes [7], some studies indicate that e-cig emission contain significantly less particulate matter (PM) mass and organic species, therefore e-cig emissions are less cytotoxicity than regular cigarette smoke [11,21]. On the contrary, other studies state that e-cig emissions still poses potential health risks because it contains high numbers of particulate matter (a total of 10^9 particles/cm^3 with peak between 100 nm–200 nm) [12–14] and a complex mixture of chemicals, including propylene glycol, glycerin, nicotine, carbonyls, volatile organic compounds (VOCs) and metals [15–18]. Such discrepancies in the literature are likely due to the differences in the exposure generation by randomly-selected parameters (type, brand, flavor, voltage etc.). Thus, there is real need for a systematic physico-chemical and toxicological characterization of e-cig emission as a function of product features (e.g. brand and flavor) and operational (e.g. voltage) parameters.

Reactive oxygen species (ROS) are a group of highly reactive and often short-lived radicals and include hydroxyl radicals, superoxide anions, singlet oxygen, alkoxyl, and alkylperoxy radicals [22]. Hydrogen peroxide (H_2O_2), in comparison to other ROS mentioned above, is relatively stable and in acellular systems it is formed from terminating reaction of various radicals, including hydroxyl radicals. ROS represent one important mechanism of emission-induced health effects because their presence can initiate pathological processes and greatly contribute to oxidative stress, damage of important biomolecules, including DNA, proteins, and lipids, and sustained pro-inflammatory responses. ROS and oxidative stress are involved in numerous diseases of the airways, cardiovascular system, neurological disorders and cancers [23,24]. The ability to generate ROS and induce oxidative stress by tobacco smoke has been categorized as a driving factor in smoking-related diseases [25–27]. Limited research already indicates that e-liquid and e-cig emissions induced oxidative stress *in vitro* [28–32] and *in vivo* [33], and this is indicative of the important role of ROS in e-cig induced cytotoxicity [34]. However, e-cig ROS characterization at present is limited and results are often contradictory in the literature. This is likely because research on e-cig is still an emerging area and, more importantly, e-cig emissions can be influenced by parameters such as e-liquid flavor and heating wire status [35–37] and user puffing patterns. For example, two studies found similar ROS generation from e-cig and tobacco cigarette, using both acellular [38] and cellular assays [39]. On the contrary, several other studies found e-cig induced less oxidative stress *in vitro* in bronchial epithelial cells [40–42] and endothelial cells [34], as well as from human blood biomarker analysis following controlled human exposures [43]. Thus, there is a need to investigate the influence of e-cig brand, e-liquid composition, operational parameters and puffing pattern on ROS generation in a systematic manner, using both acellular and cellular methodologies.

In this manuscript, we report for the first-time the results of a comprehensive e-cig ROS characterization as a function of the aforementioned influencing parameters, using complementary assays in acellular and cellular systems. The recently developed in our group versatile e-cig exposure generation system (Ecig-EGS) was used to precisely control the e-cig operational parameters for emission generation [20]. ROS was measured by collecting the emission in trapping reagents followed by liquid chromatography-electrospray ionization-tandem-mass spectrometry (LC-ESI–MS/MS), electron spin resonance (ESR) for ROS speciation, and cellular ROS utilizing the dihydrorthidum (DHE) fluorescent probe in human small airway epithelial cells (SAEC).

2. Methods

2.1. Generation and sampling of e-cig exposure

2.1.1. E-cig exposure generation system (Ecig-EGS)

The recently developed by the authors Ecig-EGS platform was used to generate real world e-cig exposure for ROS characterization as indicated in Fig. 1 [20]. In brief, a single port e-cig generator (ECAG, e∼Aerosols, LLC, Central Valley, NY), which is fully programmable and enables precise control of the puffing pattern and e-cig operational voltage, was connected to an e-cig (Fig. 1). The cylindrical mixing chamber connected to ECAG had a volume of 7 L. Generated e-cig emission and the dilution air were introduced into the mixing chamber through two separate ports and thoroughly mixed in there. The Ecig-EGS was connected with real time instrumentation and time integrated sampling for physico-chemical characterization of emission [44,45].

In this study, a commonly used advanced e-cig with refillable tank was used [46,47]. The residence time of the mixing chamber was set at 60 s to mimic the "washout time" of a smoker's lungs in active smoking [48].

2.1.2. Influence of e-cig brand, e-liquid flavor, puffing protocol and operational voltage

For baseline experiments, tobacco flavor (10 mg/mL nicotine) e-liquid from a popular e-cig brand A was used. The e-cig was operated at 3.7 V, a standard voltage according to the manufacturer. A modified puffing protocol (MPP), which reflected real world e-cig smoking behavior [10,49] was applied. MPP defines puffing regime as: puff volume, 55 mL; puff duration, 4 s; and puffing interval, 30 s.

To systematically investigate the influence of the aforementioned parameters, one out of these four parameters was modified each time and its effect on ROS content was then compared with the baseline experiment using an array of acellular and cellular techniques as described below. In total, two popular e-cig brands (brand A and B), two flavors (tobacco flavor, fruit flavor–10 mg/mL nicotine), two puffing protocols (MPP and the standardized Federal Trade Commission protocol (FTC): puff volume, 35 mL; puff duration, 2 s; and puffing interval, 60 s [50] and three voltage scenarios (3.7, 4.8 and 5.7 V) were used.

2.1.3. E-cig emission sampling

For ROS characterization: Generated e-cig emissions were bubbled through fritted head impingers (porosity A (145–174 μm) tip; Ace glass Inc., NJ) containing trapping regents corresponding to the analytical methods (Fig. 1). For Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) method (LC-ESI–MS/MS), 10 mL of 100 μM trolox in 1 mM phosphate buffer (pH = 7.4) was used. For ESR analysis, a 5 mL vial was placed inside the impinger containing 1.7 mL of 500 mM 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO; Sigma Aldrich, MO). The samples were immediately frozen post-sampling until analysis. For the two cellular assays (DHE and MTS), 20 mL of small airway basal medium (SABM, Lonza Inc., Allendale, NJ) was used. The samples were stored at 4 °C until cell exposure experiments. The sampling duration was 30 min.

Blanks were collected in a similar fashion by bubbling only pretreated room air (cleaned through charcoal and high-efficiency particulate filters) through the impinger for 30 min containing the same trapping reagents as above.

For nicotine characterization: Nicotine was measured in the SABM sample as a way to normalize the dose. The medium was refrigerated to −80 °C prior to analysis.