Human Bronchial Epithelial Cells Exposed at the Air – Liquid Interface

Department of Medical and Surgical Sciences, Institute of Neurology, Catanzaro, Italy

Keywords: Nicotine; E-liquid; E-cigarette; Myocardial infarction; Cell viability; Augmented cellular levels

Introduction

Battery-operated electronic nicotine delivery systems devices use heat to produce an inhalable aerosol from a liquid mixture of nicotine, avouring chemicals and humectants. e e-cig aerosol is a complex mixture of ne and ultra ne particles and gases that contains in addition to nicotine, at least 30 di erent chemicals, including metals. Although ENDS devices use similar scienti c principles to generate an aerosol from an e-liquid, there are signi cant di erences in device con gurations between the various generations of electronic nicotine delivery systems.

is creates major challenges for electronic nicotine delivery systems -related research, as standardized assessments are absent; there are more than 2800 di erent models of electronic nicotine delivery systems from 466 identi ed brands; plus over 7700 unique e-liquid avours. is is a signi cant public health concern, since, as demonstrated by the 2019–2020 outbreak in the United States, exposures to electronic nicotine delivery systems aerosols can induce potentially fatal e-cigarette or vaping-associated lung injury (EVALI) [1]. is clearly demonstrates that little is known regarding the long-term pulmonary e ects of inhaling ENDS heated and aerosolized humectants, nicotine as well as avours. In dual-users of both conventional cigarettes and e-cigs, use of e-cigs leads to declines in lung function, increased air ow resistance and signi cantly increased risk of having a myocardial infarction. Inhaling a 2-s e-cig pu can result in airway deposition of 6.25 × 10 10 particles that can interact with epithelial cells along the entire respiratory tract. Studies of human bronchial epithelial cells exposealmost exclusively in all electronic nicotine delivery systems. While PG and VG are “generally recognized as safe” (GRAS) food additives, their safety for the lungs following aerosolization has not been established. Furthermore, thermal degradation of VG and the chemical interactions of the e-liquid components produce emissions of carbonyls, including formaldehyde and acetaldehyde, known to be potent threats to human health. Unlike rst-generation e-cigs, where aerosol levels of toxic chemicals, including formaldehyde (0.02 to 0.37 µg/pu), were up to 600 times lower than those found in cigarette smoke (0.9 to 11.9 µg/pu), second and third-generation e-cig aerosols contain formaldehyde at similar or higher levels (1.8 µg/pu) than those found in cigarette smoke. Design features of third-generation e-cigs allow for user adjustment of: (1) atomizer resistance, responsible for heating the e-liquid, and (2) battery voltage, that provides power to the device.

Discussion

e combination of a given resistance and voltage a ects e-cig aerosol physicochemical composition. Low resistance combined with high voltage increases the amount of aerosol produced, the intensity of the taste and the throat hit. ose user-altered settings are used to create di erent vaping styles, including sub-ohm vaping or cloud chasing, popular among younger e-cig users [3]. Sub-ohm atomizers (resistance < 0.5 Ω) produce large exhaled clouds, potentially leading to exposure to elevated levels of carbonyls. Besides heating conditions, the composition and constituents’ ratios in the e-liquid also in uence chemical levels found in the aerosol, as do e ects related to the chemistry of the e-cig avoring agent. Cinnamaldehyde, the major avouring chemical found in cinnamon- avoured e-iquids, and diacetyl, associated with butter avours, are two of the Flavour and Extract Manufacturers Association high-priority avouring chemicals for respiratory hazard, when inhaled by workers. ese avouring chemicals impair lung function and cause irreversible lung damage (bronchiolitis obliterans, i.e., popcorn lung).

ese user-modi able factors can signi cantly impact toxicity of the inhaled e-cig aerosol [4]. us, studies examining how e-cig devices’ adjustable components a ect aerosol composition and comparing the toxicity of these generated aerosols are urgently required. e present study was designed to determine the in uence of atomizer resistance and battery voltage on e-cig aerosol composition and cellular toxicity. A physiologically-relevant ALI in vitro model was employed to investigate the e ects on lung cells of cinnamon- and butter- avored e-cig aerosols pr19(t Alun)8(gC-4.9(d t)r0[n- [*4;i-2ni)4(2h0io)1C8lo-2nim- [*tion a ce-2ni

evidence supporting regulations on e-cig device design features and e-liquid manufacturing. e ALI exposures allow cells to be exposed to all the aerosol components, including the particulate and gas phases. We used a customized ALI exposure system from Vitrocell Systems GMBH (Waldkirch, Germany) that enables direct exposure of cells to various aerosols. Our customized ALI system is composed of a Vitrocell 6/4 stainless steel exposure module for 4 × 6 well/24 mm diameter inserts, which is connected to a distribution system for the Vitrocell 6 modules. is exposure system is also equipped with a quartz crystal microbalance (QCM) sensor for Vitrocell 6, which has a performance resolution 10 ng/cm2 per second. is is in addition to a Vitrocell 6/3 stainless steel exposure module for 3 × 6 well/24 mm diameter inserts, which is connected to a clean air distribution system for air control-exposed cells. We used medical grade compressed air to supply our clean air distribution system, and thus this air was used for aerosol dilution and for our control group exposures [5]. Overall, the exposure modules are composed of seven chambers: four for e-cig aerosol exposures, including one chamber with the QCM, and three for medical grade compressed air exposures. To study the e ect of butter- and cinnamon- avoured e-cig aerosols on cellular toxicity of H292 cells, we connected the third-generation e-cig device (Scireq®), operating with the device settings and topography pro le described above, to the Vitrocell ALI exposure system. e cells were seeded on distinct transwell inserts, which were independently grown for 21 days at the ALI. During each experiment, 3 cell inserts were randomly assigned to a di erent treatment, either e-cig aerosols (n = 3) or medical grade compressed-air (n = 3), and then exposed simultaneously to the respective test atmosphere via our in vitro inhalation exposure system [6]. For scienti c rigor, the same experiment was performed independently on three separate occasions (which were done on 3 di erent days). Also, we used 2 di erent medical grade compressed air control groups, one for each avored e-cig aerosols. Diluted with 1 L/min of medical grade compressed air, the e-cig aerosol concentrations were measured with the QCM placed inside the cell chamber. While the cells were directly exposed in the ALI exposure system, warm water (36–37 °C) was circulated around the chambers via a water bath. e exposure chambers were cleaned a er each exposure. H292 cells were exposed to either e-cig aerosols or medical grade compressed air for 2 h per day for 1 or 3 consecutive days [7]. A er the last exposure, cells were incubated at 37 °C for 24 h and biological endpoints were