Electronic Cigarette and Cigarette Smoke Extract Exposure on Abdominal Aorta

A Histochemical Comparison

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Introduction

While cigarette use in adults and adolescence has steadily declined over the last 18 years, electronic cigarettes (e-cigs) are rapidly gaining popularity.¹ E-cig sales in the United States are predicted to reach \$3.5 billion in 2015 and are estimated to exceed cigarette sales within a decade. Unlike a cigarette, which is burned to produce smoke, an e-cig is a device that heats and atomizes nicotine-containing liquid (e-liquid) into a vapor (e-vapor). E-liquid formulations vary by manufacturer, but all typically contain a humectant (eg, polyethylene glycol, propylene glycol, glycerol), nicotine (8.5-22.2 mg/mL), and flavors (eg, tobacco, grape, chocolate).² Many e-cig manufacturers tout their products as being a safe alternative to smoking; however, the health risks of chronic e-cig use have not been rigorously evaluated.

Cigarette smoking is a well-known vascular disease risk factor for a variety of reasons—many stemming from the nicotine, reactive oxygen species (ROS), aldehydes, and polycyclic aryl hydrocarbons present in tobacco smoke.³ In diseases such as abdominal aortic aneurysm and atherosclerosis, it is thought that these compounds in the cigarette smoke modulate or promote inflammatory processes in the vascular wall that lead to vascular smooth muscle cell death, inflammatory cell infiltration, and extracellular matrix (ECM) degradation.³ E-cigs also contain some of the hazardous compounds found in cigarette smoke (eg, nicotine, ROS, aldehydes, polycyclic aryl hydrocarbons), albeit in lesser quantities.²

Although several human studies report only a minimal effect on heart rate and diastolic blood

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pressure following acute e-cig use, there is a paucity of data on the chronic effects of chronic e-cig use on cardiovascular function.^{4,5} Though a model of chronic e-cig exposure has yet to be established, animal and in vitro studies show e-vapor to be proinflammatory and cytotoxic and therefore a potential mediator of vascular disease. For example, acute e-vapor (both nicotine and nicotine-free) exposure increased IL-6 production in human airway epithelial cells^{6,7}; an increase in proinflammatory cytokines IL-1 α and IL-13 was detected in the bronchoalveolar fluid of e-vapor-exposed mice⁸; e-vapor induced a significant increase in the permeability of microvascular endothelial cells in vitro⁹; acute e-vapor exposure significantly increased the number of inflammatory cells in bronchoalveolar fluid⁸⁻¹⁰; and although there are conflicting data, several in vitro studies found e-vapor and e-liquid to be cytotoxic in a variety of cell types.¹¹⁻¹³

Given the similarities between the contents of cigarette smoke and e-vapor, along with data to support e-cigs as proinflammatory and cytotoxic, we hypothesized that arterial tissue incubated in e-liquid-treated media would demonstrate early histological changes consistent with cell death and inflammation. To explore this hypothesis, we chose to compare the effects of e-liquid and cigarette smoke extract (CSE) exposure in an *ex vivo* organ culture model of the abdominal aorta.

Methods

Reagents

Flavorless e-liquid was purchased from Uno Vapor (Davie, Florida). Aqueous CSE was purchased from Fisher Scientific (Pittsburgh, Pennsylvania). Aortic tissue was cultured in serum-free Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, California) containing 1% penicillin, streptomycin, and 4 mM L-glutamine (Invitrogen; Carlsbad, California).

Organ Culture and Histology

Fresh baboon abdominal aorta was harvested and washed in sterile phosphate-buffered saline (PBS) several times by centrifugation at 2000 rpm (375 rcf)

until the washing solution was clear of visible blood. The aorta was cut into 6 pieces of equal size (5 mm $\times$ 3 mm) and flash frozen before being stored at -80°C . Prior to treatment, the tissue was thawed on ice and cultured in serum-free DMEM (control), DMEM containing 40 $\mu\text{L/mL}$ e-liquid, or DMEM containing 100 $\mu\text{L/mL}$ aqueous CSE as previously described.¹⁴ Concentrations of e-liquid and CSE were normalized for nicotine content. Organ cultures were incubated at 37°C in a humidified, 5% carbon dioxide atmosphere for 5 days, and fresh media were exchanged every 24 hours. Tissues were fixed in 10% buffered formalin for 2 hours followed by 70% ethanol prior to embedding and sectioning. Tissue sections were stained with hematoxylin and eosin (H&E, to look for tissue integrity and cell nuclei), Verhoeff–Van Gieson stain (VVG, for elastin fibers), or Masson's trichrome stain (TRI, for collagen and smooth muscle).

Image Analysis

Semiquantification of average elastin fiber density (VVG), vascular smooth muscle density (TRI), and number of nuclei per high-power field (H&E) of the entire tunica media of each sample was measured using the ImageJ analysis software package (National Institutes of Health; Bethesda, Maryland). Data are expressed as means $\pm$ SE. Means were compared using Student *t* test. $P < 0.05$ was considered statistically significant.

Results

Baboon abdominal aortic rings treated with control DMEM showed normal characteristic architecture for smooth muscle cell nuclei and cytoplasm distribution (Figure 1A), while the extracellular elastin fibers were intact and displayed typical waveform patterns (Figure 1B, C). CSE and e-liquid-treated aortic sections stained with VVG (Figure 1E and H, respectively) exhibited elastin fiber fragmentation (arrows) and had significantly decreased elastin fiber density relative to control (Figure 2B). CSE and e-liquid aortic sections stained with TRI (Figure 1F and I, respectively) demonstrated reduced smooth

muscle cell density (Figure 2C), and e-liquid aortic sections had decreased nuclei per high-power field (HPF) relative to control (Figure 2A).

Conclusion

These preliminary results suggest that similar to cigarette smoke, the contents of some e-liquids have the potential to induce elastin fiber degradation and

smooth muscle cell dysfunction in aortic tissues—histologic features also observed in some vascular diseases, such as abdominal aortic aneurysm.¹⁵ While this model has several limitations (ie, small sample size, e-liquid rather than e-vapor extract exposure, relatively short treatment time), our findings provide some insight into the potential effects of e-cigs on the arterial wall and are somewhat

FIGURE 1. Baboon abdominal aorta treated with serum-free DMEM (control; A-C), serum-free DMEM with CSE (CSE; D-F), or serum-free DMEM with e-liquid (G-I) for 5 days and stained with H&E (A, D, G), VVG (B, E, H), and TRI (C, F, I). High-power field focused on tunica media (scale bar is 50 μ m). Control sections (A-C) show regular smooth muscle cell nuclei (A) and intact elastin wave-forms (B). CSE- and e-liquid-treated sections show fragmented elastin fibers (E, H, arrows) and reduced smooth muscle cell cytoplasm (F, I) relative to control (C).

FIGURE 2. Semiquantification of average number of nuclei per high-power field (HPF; A), average elastin fiber density (B), and average smooth muscle cell density (C) of baboon aorta tunica media treated with serum-free DMEM (control), serum-free DMEM with CSE (CSE), or serum-free DMEM with e-liquid for 5 days. Decreased elastin and smooth muscle cell density observed in e-liquid and CSE. Decreased nuclei per HPF also observed in e-liquid. * $P < 0.05$.

consistent with recent data demonstrating the cytotoxic and inflammatory potential of e-cigs.⁶⁻¹³

Given their increasing popularity and the limited data regarding e-cig effects on the cardiovascular system, further research is warranted to address

the deleterious effects of e-cigs compared to conventional tobacco smoke. Future investigation will examine e-cig-vapor-induced inflammatory and apoptotic response of arterial tissue using both in vitro and in vivo models.

References

1. CDC Office on Smoking and Health. Smoking and tobacco use; data and statistics; tables, charts, and graphs; trends in current cigarette smoking. 2013. CDC Web site. http://www.cdc.gov/tobacco/data_statistics/tables/trends/cig_smoking/index.htm. Accessed July 7, 2015.
2. Cheng T. Chemical evaluation of electronic cigarettes. *Tob Control*. 2014;23(suppl 2):ii11-ii17. doi:10.1136/tobaccocontrol-2013-051482.
3. Morris PB, Ference BA, Jahangir E, et al. Cardiovascular effects of exposure to cigarette smoke and electronic cigarettes. *J Am Coll Cardiol*. 2015;66(12):1378-1391. doi:10.1016/j.jacc.2015.07.037.
4. Yan XS, Ruiz CD. Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. *Regul Toxicol Pharmacol*. 2015;71(1):24-34. doi:10.1016/j.yrtph.2014.11.004.
5. Vansickel AR, Cobb CO, Weaver MF, Eissenberg TE. A clinical laboratory model for evaluating the acute effects of electronic "cigarettes": nicotine delivery profile and cardiovascular and subjective effects. *Cancer Epidemiol Biomarkers Prev*. 2010;19(8):1945-1953. doi:10.1158/1055-9965.EPI-10-0288.
6. Wu Q, Jiang D, Minor M, Chu HW. Electronic cigarette liquid increases inflammation and virus infection in primary human airway epithelial cells. *PLoS One*. 2014;9(9):e108342. doi:10.1371/journal.pone.0108342.
7. Husari A, Scd AS, Talih S, Ms YH, El-Sabban M, Zaa-tari G. Original investigation acute exposure to electronic and combustible cigarette aerosols: effects in an animal model and in human alveolar cells. *Nicotine Tob Res*. 2015;1-7. doi:10.1093/ntr/ntv169.
8. Lerner CA, Sundar IK, Yao H, et al. Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. *PLoS One*. 2015;10(2). doi:10.1371/journal.pone.0116732.
9. Schweitzer KS, Chen SX, Law S, et al. Endothelial disruptive pro-inflammatory effects of nicotine and e-cigarette vapor exposures. *Am J Physiol Lung Cell Mol Physiol*. 2015;309(2):L175-L187. doi:10.1152/ajplung.00411.2014.
10. Lim H Bin, Kim SH. Inhalation of e-cigarette cartridge solution aggravates allergen-induced airway inflammation and hyper-responsiveness in mice. *Toxicol Res*. 2014;30(1):13-18. doi:10.5487/TR.2014.30.1.013.
11. Cervellati F, Muresan XM, Sticozzi C, et al. Comparative effects between electronic and cigarette smoke in human keratinocytes and epithelial lung cells. *Toxicol In Vitro*. 2014;28:999-1005. doi:10.1016/j.tiv.2014.04.012.
12. Bahl V, Lin S, Xu N, et al. Comparison of electronic cigarette refill fluid cytotoxicity using embryonic and adult models. *Reprod Toxicol*. 2012;34(4):529-537. doi:10.1016/j.reprotox.2012.08.001.
13. Yu V, Rahimy M, Korrapati A, et al. Electronic cigarettes induce DNA strand breaks and cell death independently of nicotine in cell lines. *Oral Oncol*. 2015;52:58-65. doi:10.1016/j.oraloncology.2015.10.018.
14. Ghosh A, Pechota LVTA, Upchurch GR, Eliason JL. Cross-talk between macrophages, smooth muscle cells, and endothelial cells in response to cigarette smoke: the effects on MMP2 and 9. *Mol Cell Biochem*. 2015;410(1-2):75-84. doi:10.1007/s11010-015-2539-3.
15. Ailawadi G, Eliason JL, Upchurch GR. Current concepts in the pathogenesis of abdominal aortic aneurysm. *J Vasc Surg*. 2003;38(3):584-588. doi:10.1016/S0741-5214(03)00324-0.