Effects of an In Vivo Vaping Challenge on In Vitro Interleukin-6 Biosynthesis Pathways in Arterial Endothelial Cells Derived From Human Embryonic Stem Cells.

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RESEARCH LETTER

Effects of an In Vivo Vaping Challenge on In Vitro Interleukin-6 Biosynthesis Pathways in Arterial Endothelial Cells Derived From Human Embryonic Stem Cells

Matthew C. Tattersall, DO, MS*; Stephane Esnault, PhD*; Ron Stewart, PhD; David T. Vereide, PhD; Scott Swanson, MS; Jue Zhang, PhD; John Steill, MS; Nizar Jarjour, MD; Kristin M. Hansen, BS; Claudia E. Korcarz, DVM; Timothy B. Baker, PhD; James H. Stein, MD

Electronic nicotine delivery system (ENDs) use is increasing rapidly and represents a growing public health threat.[1] Newer generation devices heat e-liquids to higher temperatures and can achieve higher serum nicotine concentrations than older devices.[1] The cytokine interleukin-6 (IL-6) mediates atherogenesis and predicts future atherosclerotic cardiovascular disease events. Previous in vitro studies have demonstrated increased IL-6 production by human alveolar macrophages exposed to ENDs condensate.[2] Although there has been considerable focus on the effects of ENDs use on airway inflammation, there are a paucity of data on the effects of ENDs on arterial inflammation. Prior in vitro studies used venous endothelial cells or human umbilical venous endothelial cells as proxies for arterial endothelial cells (AECs), limiting generalizability of their findings. Historically, use of AECs was limited by invasive collection methods. We used a novel in vitro AEC model of human embryonic stem-cell-derived AECs to study differential expression of IL-6 pathway genes after exposure to serum from human participants obtained before and after vaping using third- or fourth-generation ENDs.

This study was approved by the University of Wisconsin Health Sciences institutional review board; participants provided informed consent. Data are available from the corresponding author upon reasonable request. We randomly selected serum from 10 chronic, exclusive ENDs users (exhaled carbon monoxide <5 ppm, positive urine nicotine) and 10 nonvaping, nonsmoking controls from the Cardiac and Lung E-Cigarette Smoking Study (NCT03863509).[3] ENDs users abstained from product use for >8 hours and then completed a 15-minute ad libitum product-use challenge in an externally ventilated room, remotely supervised by a trained observer. ENDs users vaped with their ENDs product and controls rested. Postchallenge serum was collected in ENDs users 15 minutes after the challenge.

We generated purified populations of AECs (H9-CPTC-C13 derivative human embryonic stem cell line).[4] Functional assessments of the AECs such as leukocyte adhesion assays and nitric oxide production were as we reported previously.[4] Consistent with arterial-specific endothelial cell characteristics, the AECs produced greater amounts of nitric oxide compared with

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Correspondence to: James H. Stein, MD, University of Wisconsin School of Medicine and Public Health, Room H4/520 CSC, 600 Highland Avenue, MC 3248, Madison, WI 53792. Email: jhs@medicine.wisc.edu

*M. C. Tattersall and S. Esnault are co-first authors.

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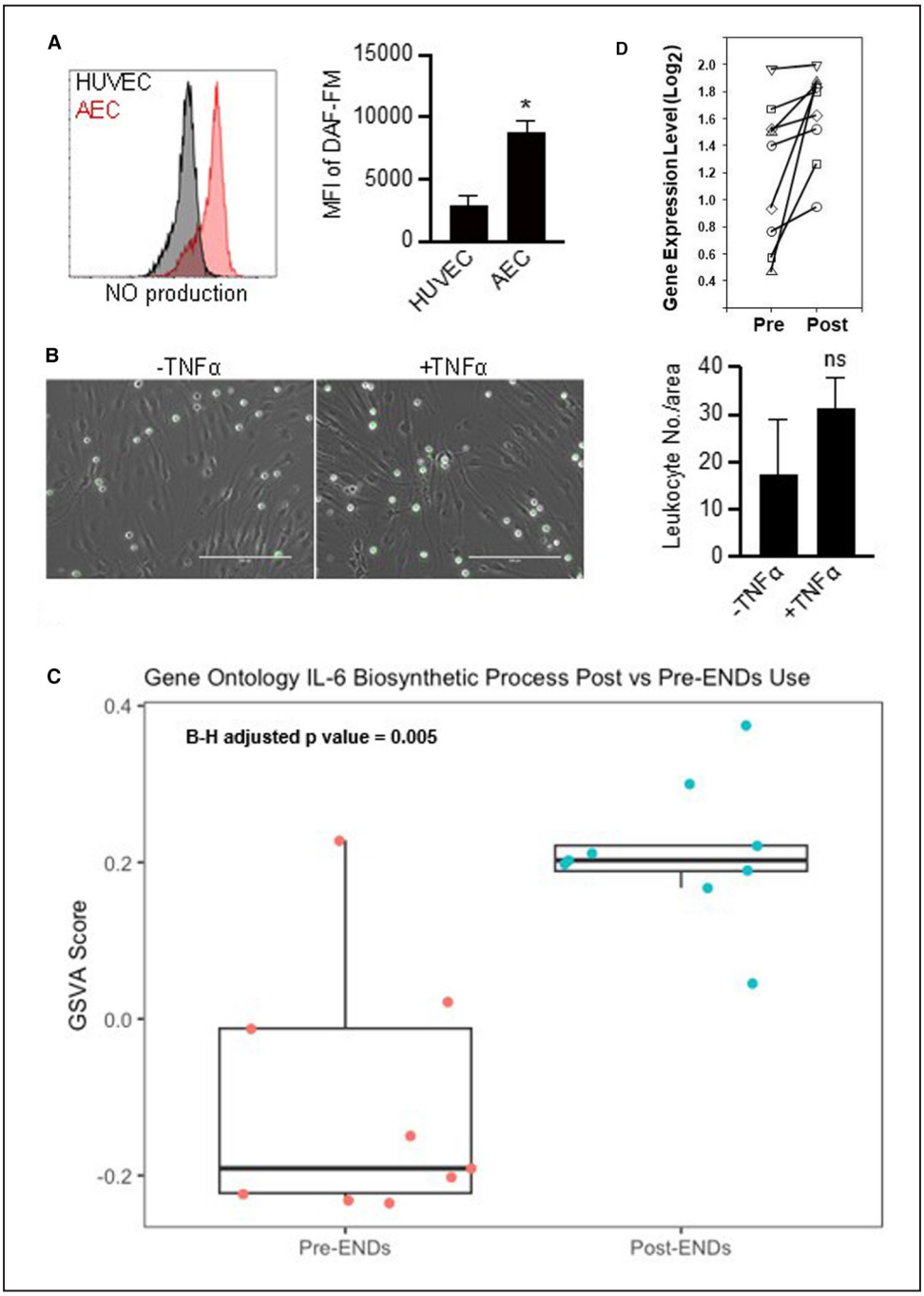

Figure. Arterial-specific endothelial cell characteristics, interleukin-6 biosynthetic gene path analysis, and interleukin IL1β expression.

A, Nitric oxide (NO) production was measured by the intensity of DAF-FM diacetate (4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate). Mean fluorescence intensity (MFI) of DAF-FM was calculated. Data are presented as mean±SD. Student's *t* test; n=3 (*P<0.05). **B**, Leukocyte (round cells) adhesion assay (scale bar, 200 μm) and statistics of leukocyte adhesion assay. Leukocyte numbers were counted for each image. Data are presented as mean±SD. Student's *t* test; n=3 images from 3 independent experiments. **C**, Gene set variational analysis (GSVA) scores of IL-6 gene set. **D**, IL-1β gene expression in arterial endothelial cells (AECs) incubated with serum pre- vs postuse of electronic nicotine delivery systems (ENDs). B-H indicates Benjamini-Hochberg adjusted *P* value for multiple comparisons; HUVEC, human umbilical vascular endothelial cell; IL-6, interleukin-6; ns, not statistically significant; and TNF, tumor necrosis factor.

human umbilical venous endothelial cells (Figure [A]).[4] Due to higher nitric oxide production, there were no significant differences in leukocyte adhesion between the AECs that were treated with or -without tumor necrosis factor-alpha (Figure [B]).[4] On day 8 of the differentiation protocol, AECs were exposed for 6 hours to 50% (1 mL) human sera from the pre- and postproduct challenge conditions. The AECs were lysed and harvested using the buffer RLT from a Qiagen RNeasy Kit (Qiagen, Valencia, CA) with β-mercaptoethanol. Total RNA was sequenced on a NextSeq 2000. The DESeq2 bioinformatic toolkit was used to identify differential expression genes. Because we focused on cytokine-mediated inflammation, we searched for gene ontology pathways that included "INTERLEUK." *Z* scores of normalized natural $\log_2$ transcripts per million gene expression values were determined. Gene set variational analysis was used to interrogate for enriched gene sets. *P* values were adjusted for multiple comparisons using the Benjamini-Hochberg method.

ENDs users were a mean (SD) 28.9 (8.1) years old with a body mass index of 25.8 (3.1) kg/m^2 and controls were 33.6 (17.7) years old with a body mass index of 23.4 (3.1) kg/m^2. Baseline serum measures of nicotine, C-reactive protein, and hemoglobin A1c were similar between ENDs users and controls. Compared with controls, ENDs users had nominally increased expression of the IL-6 pathway at baseline (gene set variational analysis$_{\text{nominal}}$ P=0.03); differences were not significant after Benjamini-Hochberg adjustment (P=0.98). Our primary finding was that ENDS users, post challenge, had higher activation in IL-6 biosynthetic pathway genes using gene set variational analysis analysis (P_{nominal}=0.00005, Benjamini-Hochberg adjusted P=0.005) (Figure [C]). Because IL-1β enhances the production of IL-6 by nonimmune cells such as fibroblasts and endothelial cells, we extracted gene expression values for IL1β. In all 10 subjects, IL1β expression levels increased in AECs after activation with sera obtained post-ENDs challenge, compared with sera obtained before the challenge (Figure [D]). The "real-world" product-challenge approach of our study is limited by an inability to distinguish between toxic component contributions such as carbonyl compounds, nicotine, and particulate matter, as well sample size, which limited power for statistical adjustments and detection of small effect sizes.

This is the first study, to our knowledge, deploying a novel model using human embryonic-derived AECs with arterial-specific functional capabilities as an in vitro means to investigate cytokine transcriptomic changes following an acute, in vivo ENDs product ("vaping") challenge. The AECs we used had characteristic in vivo arterial-specific functions including nitric oxide production and leukocyte adhesion rates that are pivotal in the pathophysiologic processes of endothelial activation and atherogenesis.[4] Our findings are consistent with recent work highlighting the deleterious effects of ENDs use on vascular endothelium.[5] Our study extends those findings by (1) establishing a disease-modeling approach that uses in vitro AECs with functional characteristics of in vivo AECs, and (2) demonstrating increased IL-6 pathway upregulation by AECs exposed to serum of ENDs users compared with controls and among ENDs users following an acute ENDs product challenge.

ARTICLE INFORMATION

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Affiliations

Department of Medicine, Division of Cardiovascular Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI (M.C.T., K.M.H., C.E.K., J.H.S.); Department of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine, The University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI (S.E., N.J.); Morgridge Institute for Research, Madison, WI (R.S., D.T.V., S.S., J.Z., J.S.); and University of Wisconsin Center for Tobacco Research and Intervention (UW-CTRI), University of Wisconsin School of Medicine and Public Health, Madison, WI (T.B.B.).

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Disclosures

None.

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