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Short communication

Cigarette smoke but not electronic cigarette aerosol activates a stress response in human coronary artery endothelial cells in culture

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ABSTRACT

Background: It is generally acknowledged that e-cigarettes are unlikely to be as harmful as conventional cigarettes, but there is little data that quantifies their relative harms. We investigated the biological response to e-cigarette aerosol exposure (versus conventional cigarette smoke exposure) at the cellular level, by exposing human coronary artery endothelial cells (HCAEC) to aqueous filtered extracts of e-cigarette aerosol or cigarette smoke and looking at gene expression changes consistent with a stress response. This included genes controlled by the oxidant-stress sensing transcription factor NFR2 (NFE2L2), and cytochrome P450 family members.

Methods: Cigarette smoke extract (CSE) was created using mainstream smoke from a single cigarette drawn through 10 ml of endothelial cell growth media MV2. Electronic cigarette aerosol extract (eCAE) was created using the same apparatus, using a constant power output of 10.8 w (4.2 V) and 18 mg/ml nicotine solution. eCAE was generated using 5 cycles of 5 s heat with at least 10 s in between each puff to allow the coil to cool, air being drawn through the device at 70 ml/minute.

Results: HCAEC responded to the noxious components in CSE, resulting in activation of NRF2 and upregulation of cytochrome p450. However, eCAE did not induce NRF2 nuclear localisation, upregulation of NRF2-activated genes, or the upregulation of cytochrome p450.

Conclusions: The use of e-cigarettes as a substitute for conventional cigarettes is likely to reduce immediate tobacco-related harm, at least with respect to cardiovascular harms.

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1. Introduction

The rapid growth in the use of e-cigarettes, which deliver nicotine via inhaled aerosol rather than tobacco smoke, has generated debate regarding their potential benefits relative to conventional cigarettes. While e-cigarettes are unlikely to be as harmful as conventional cigarettes, there is little data that quantifies their likely relative harms, and data does not yet exist on the correlates of long-term e-cigarette use. Given this, the development of laboratory models that quantify the biological effects, and therefore likely relative harms, of e-cigarettes and conventional cigarettes is critical.

The levels of chemicals known to be harmful present in e-cigarette aerosol depend on several variables such as the solution used and the battery output voltage (Bahl et al., 2012; Kosmider et al., 2014). Critically, a number of these experiments have been performed with diluted refill solutions (e-liquid), rather than heated aerosol (Bahl et al., 2012). The levels of toxic product will depend on the way the e-cigarette is used (Farsalinos et al., 2015). Moreover, the mere presence of detectable levels of potentially harmful chemicals does not necessarily indicate that e-cigarette aerosol will deliver concentrations required for the chemical to be toxic. In the absence of long-term prospective data on the health correlates of e-cigarette use, there is a need to investigate the potential harmful effects of e-cigarette use. One valuable approach might be to examine the biological response of human primary cells to e-cigarette aerosol.

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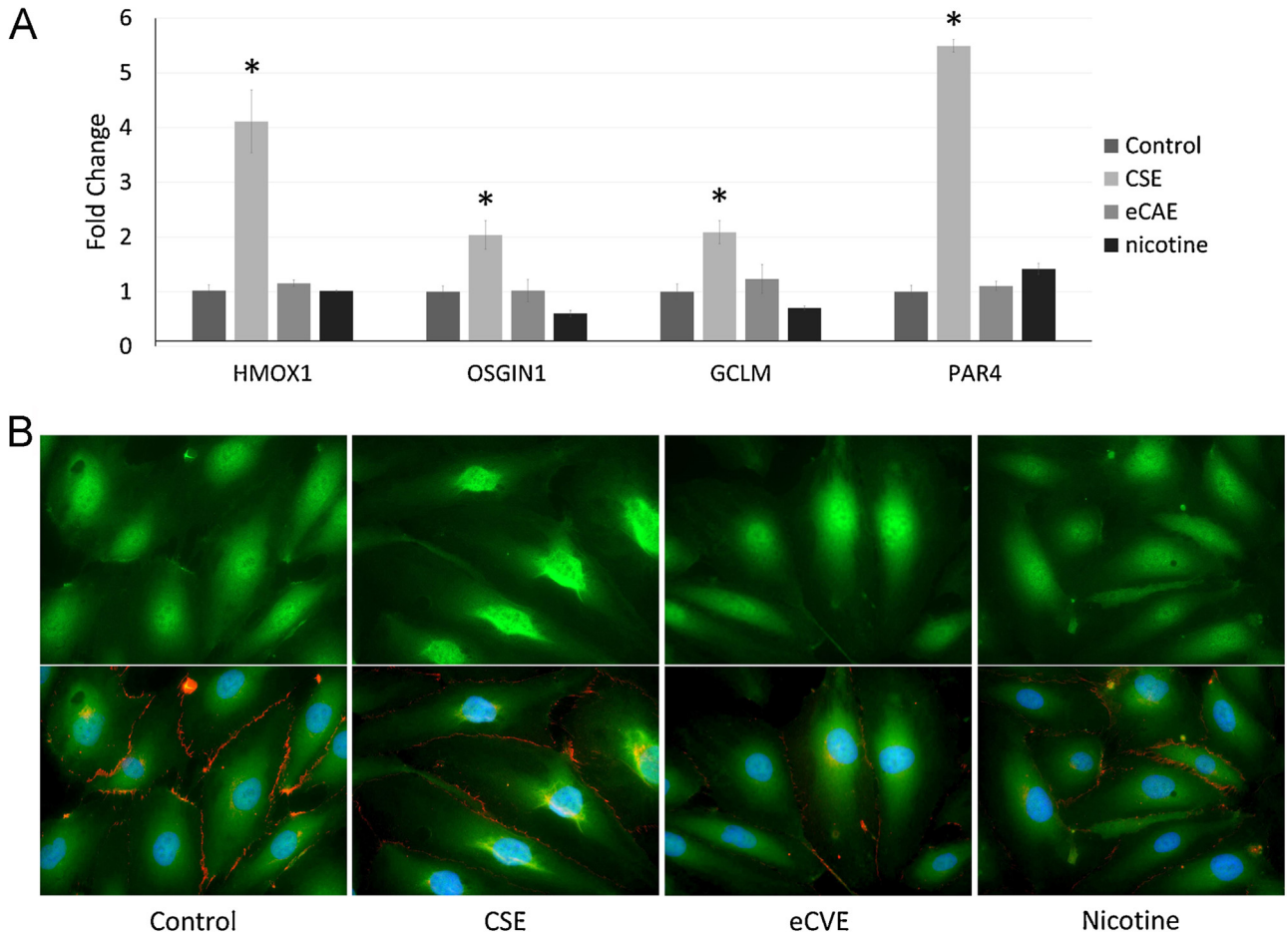

Fig. 1. CSE but not eCAE or nicotine increase NRF2-regulated gene expression.
Panel A) Fold change of mRNA expression levels of heme oxygenase 1 (*HMOX1*), oxidative stress growth inhibitor 1 (*OSGIN1*), glutamate-cysteine ligase (*GCLM*) and protease activated receptor 4 (PAR4–from *F2RL3* gene), exposed to 3 sequential treatments of CSE (10%), eCAE (10%) or nicotine (350 ng/ml, 2.16 μM), or vehicle control (*P < 0.05 v all other treatments, n = 4–6). Panel B) Cellular localisation of NRF2 as assessed by immunocytochemistry 2 h after a single treatment. CSE induced a shift in localisation to a predominantly nuclear localisation, co-localising with the nuclear blue dapi staining (bottom row of panel B). Error bars represent the SEM. Numerical results are shown in Supplementary Table 2.

The oxidant-stress sensing transcription factor NFR2 (nuclear factor, erythroid 2-like 2, NFE2L2) is normally sequestered in the cytoplasm through interaction with kelch-like ECH-associated protein 1 (KEAP1), but this interaction is disrupted upon electrophilic attack, allowing NFR2 to translocate to the nucleus and activate gene expression (Müller and Hengstermann, 2012). As such, activation of the NRF2 system is a useful cellular biomarker of biologically relevant levels of free radicals. Combustion products of tobacco activate NFR2-regulated gene expression in the lung and initiate a transcription profile that contributes to protection from the stress induced by tobacco smoke (Rangasamy et al., 2004; Iizuka et al., 2005; Müller and Hengstermann, 2012). The role of NRF2 in the vasculature is less clear; modest activation by physiological laminar flow is thought to protect endothelial cells from oxidative stress (Zakkar et al., 2009). However, when crossed with a hyperlipidaemic mouse, the Nrf2 knockout mouse develops less atherosclerosis, suggesting NRF2 activation contributes to disease progression (Barajas et al., 2011). The upregulation of cytochrome P450 family members by cigarette smoke has also been reported previously, indicating they are another biomarker of toxic compounds found in cigarette smoke (Baker et al., 2001; Zevin and Benowitz, 1999).

We investigated the biological response to e-cigarette aerosol exposure and conventional cigarette smoke exposure at the cellular level, by exposing primary human coronary artery endothelial cells to aqueous filtered extracts of aerosol or smoke and measuring gene expression changes consistent with a stress response.

2. Methods

2.1. Generation of cigarette smoke extract and electronic cigarette aerosol extract

Cigarette smoke extract (CSE) was created using mainstream smoke from a single Marlboro Gold cigarette (7 mg tar, 0.6 mg nicotine) drawn through 10 ml of endothelial cell growth media MV2 (Promocell, C-22120) at a rate of 70 ml/min. Under these conditions, the cigarette was consumed in ~5.5 min resulting in ~385 ml of mainstream smoke being drawn through the solution. Electronic cigarette aerosol extract (eCAE) was created using the same apparatus, using an iStick battery at constant power output (10.8 W, 4.2 V), with an Aerotank Mini atomiser (1.8 Ω) loaded with Haven fluid USA Mix 18 mg/ml nicotine solution (80% vegetable glycerine, 20% propylene glycol). eCAE was generated using 5 cycles of 5 s heat with at least 10 s in between each puff to allow the coil to cool, with air being drawn through the device at 70 ml/min. A visible vapour was generated using these conditions. A fresh pre-soaked heating coil was used for each experiment. A higher power output was selected for generation of eCAE as it has been shown that the levels of potentially harmful chemicals produced by e-cigarettes

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