The effects of electronic cigarette vapour on the lung: direct comparison to tobacco smoke

To the Editor:

Electronic cigarette (e-cigarette) usage in the USA has drastically increased in the past 5 years due to age restrictions on conventional cigarettes, aggressive marketing and a perception that e-cigarettes are a healthy alternative. E-cigarettes contain nicotine, water, glycerol, propylene glycol and optional flavouring. On inhalation, the device heats the ingredients into a vapour [1]. While tobacco cigarette smoke is known to cause deleterious effects on the cardiovascular system, angiogenesis and skin capillary perfusion by causing direct injury to blood vessel walls, increased platelet aggregation, microvascular thrombosis [2–4] and inflammation [5], the consequences of e-cigarette vapour exposure on the lung are still largely unexplored [6, 7]. Recently, Lerner *et al.* [8] reported that vapours produced by e-cigarettes and e-cigarette fluids with flavourings induced toxicity, oxidative stress and inflammatory response in human bronchial airway epithelial cells (H292) and fetal lung fibroblasts (HFL1) as well as mouse lung. Garcia-Arcos *et al.* [9] showed that the aerosolised nicotine-containing e-cigarette fluid increased airway hyperreactivity, distal airspace enlargement, mucin production, and cytokine and protease expression in mice, implying potential dangers of nicotine inhalation during e-cigarette use. The inflammatory response to e-cigarette use involved increased neutrophil activation and mucus production [10], and decreased mucociliary clearance [11]. In human embryonic and mouse neural stem cells, human pulmonary fibroblasts [12], and skin and lung cells [13], cytotoxicity of e-cigarette vapour was correlated with the number and concentration of chemicals used to flavour the fluids. We recently showed in the skin flap survival model *in vivo* that nicotine-containing e-cigarette vapour is just as harmful to the microcirculation as tobacco cigarette smoke [4].

In the present study, we examined whether long-term exposure to e-cigarette vapour or nicotine produce the same damaging effect on lung structure and vasculature as tobacco smoke in a rat model *in vivo*.

6-week-old, male Sprague Dawley rats (Envigo Laboratories, Denver, CO, USA) were divided into four groups of eight animals per group and exposed for 5 weeks as follows. 1) Room air. 2) Subcutaneous injections of (−)-nicotine ditartrate (Sigma Aldrich, St Louis, MO, USA) 2 $mg \cdot kg^{-1}$ twice daily; the amount of nicotine for injections was based on that known from previous studies to produce stable plasma nicotine levels of approximately 25 $ng \cdot mL^{-1}$, which is compatible with plasma levels in habitual smokers [14, 15]. 3) Blu E-cigs (Classic Tobacco Flavour (Blu, Charlotte, NC, USA), containing 12 $mg \cdot mL^{-1}$ nicotine) vapour produced in a TE-2E e-cigarette smoking machine (Teague Enterprises, Davis, CA, USA); the coil temperature of the e-cigarettes was within the normal range usually used by vapers (200–250°C). Rats in this group were exposed to 48 mg nicotine per day [4]. Our experimental design, by subjecting rats to e-cigarette vapour, is a major improvement compared to aerosolised e-cigarette liquid, as used by Garcia-Arcos *et al.* [9] in mice. 4) Cigarette smoke exposure in a TE-10z smoking chamber (Teague Enterprises) by burning Kentucky 3R4F reference cigarettes (Tobacco Research Institute, University of Kentucky, Lexington, KY, USA) (4 h per day: 2×2 h with a 1-h rest period in between) as described previously [16]. Total suspended particulate levels in the cigarette smoke chamber were maintained at 60 $mg \cdot m^{-3}$ and nicotine levels at 48–50 $mg \cdot m^{-3}$. Based on plasma nicotine levels (data from our own group [4]), the whole-body exposures of rats to e-cigarettes and tobacco cigarettes were comparable to the use of e-cigarettes and tobacco cigarettes by humans [17]. The work was performed with the approval of the Institutional Animal Care and Use Committee of the University of Colorado Denver Anschutz Medical Campus (Aurora, CO, USA).

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At the end of the exposure, rats were sacrificed and lungs were inflated with 1% low melting point agarose at 25 cmH_2O pressure. The mean alveolar airspace enlargement was measured using an automated image analyser (ImageJ; National Institutes of Health, Bethesda, MD, USA) and calculated as a percentage of total airspace *versus* tissue density [16]. While less sensitive than the stereological method, the alveolar airspace area measurements (based on our extensive experience) accurately reflect lung morphological changes.

All data are presented as mean±SEM. Statistical analysis was performed using two-way ANOVA followed by Tukey's honest significant difference post-test and two-tailed, unpaired Student's t-tests. The alveolar airspace enlargement measurements within each exposure group were analysed using GraphPad Prism (GraphPad, La Jolla, CA, USA) with one-way ANOVA and Tukey's multiple comparisons test.

As shown in figure 1a, exposure of rats to subcutaneous injections of nicotine, e-cigarette vapour or cigarette smoke in the smoking chamber for 5 weeks led to significant ($p<0.01$) emphysematous lung destruction when compared to RA controls. The mean±SEM alveolar airspace area (figure 1b, black bars) for the room air control, nicotine, e-cigarette vapour and cigarette smoke groups were 71±5.1%, 83±2.7%, 86±2.0% and 84±3.3%, respectively. There were statistically significant differences in alveolar airspace enlargements between room air controls and the experimental e-cigarette vapour, nicotine and cigarette smoke groups ($p<0.01$).

In emphysema, along with the airway space enlargement, there is also visible loss of peripheral vasculature [18]. To assess capillary vessel (<100 µm) density, lung sections were stained for von Willebrand factor. Three fields per slide (total 24 fields per eight-animal group) were counted by two independent investigators in a blind manner. As shown in figure 1b (white bars) the capillary count was significantly ($p<0.01$) decreased in all three treatment groups. The differences were also significant between the nicotine alone and e-cigarette vapour and cigarette smoke groups ($p<0.02$).

Our results clearly demonstrate that e-cigarettes are as damaging to pulmonary structures as traditional tobacco cigarettes. The emphysematous changes seen in cigarette smoke-exposed rat lungs are also abundantly apparent in e-cigarette- and nicotine-treated rat lungs (figure 1). Other than a common ingredient, nicotine, e-cigarettes and tobacco cigarettes are fundamentally different. The e-cigarette fluid contains three main ingredients (nicotine, propylene glycol and glycerine) and is vaporised, whereas tobacco cigarettes, besides nicotine, contain over 7000 chemical compounds and involve the combustion of tobacco. Surprisingly, in a rat model, both produce very similar, devastating effects on the lungs.

While in our study, serum levels of nicotine and cotinine were higher in the cigarette smoke group than in the e-cigarette vapour group [4], the amount of lung tissue destruction was similar across both exposure groups. Nicotine and cotinine plasma levels in the cigarette smoke-exposed rats were comparable with those found in smokers, and our values corroborated prior published nicotine and cotinine levels in rats exposed to tobacco smoke [19].

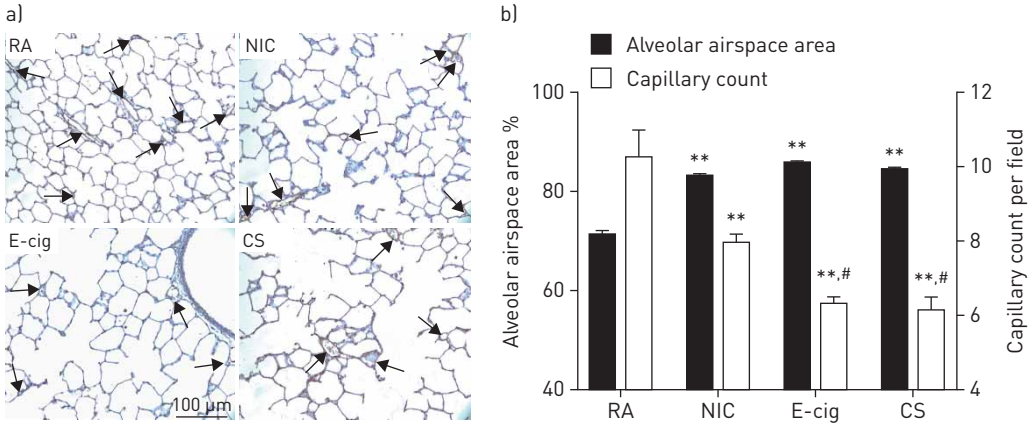

FIGURE 1 The effects of electronic cigarette vapour (E-cig), nicotine (NIC) and cigarette smoke (CS) exposure on the lung structure and blood vessel count in comparison to room air (RA)-exposed controls. a) Lung morphology and lung vasculature (visualised by staining for von Willebrand factor) after 5 weeks of exposure. Arrows indicate capillary vessels. b) Black bars represent alveolar air space enlargements (percentage of total airspace *versus* tissue density per visual field; n=8 rats, three fields per slide). Significant differences in alveolar airspace enlargements were found between treatment groups and RA controls. White bars show capillary vessel count per field (n=8 rats, three fields per slide). Data are presented as mean±SEM. **: $p\leqslant0.01$ for differences between RA controls and all three treatment groups; #: $p\leqslant0.02$ indicates differences in NIC alone *versus* E-cig and CS groups.

It is possible that the particles within the vapour with a hydrodynamic diameter of 2.5 µm or less (known as fine particulate matter), rather than the nicotine itself or in conjunction with nicotine, have a drastic negative effect on lung morphology. Fine particles are concerning because they can penetrate lung tissue and the blood stream, causing serious health effects. A recent study [20] demonstrated that the fine particulate matter within e-cigarette vapour alters platelet function to the same extent as the particulate matter within tobacco smoke. Just as seen with conventional cigarettes [16], the exposure to e-cigarette vapour causes decreased density of the lung vasculature (figure 1), meaning that as seen in emphysema patients [18], both airway and vascular cells are affected, resulting in alveolar airspace enlargement and disappearance of peripheral vasculature.

There are some limitations to our study. First, there is no standard e-cigarette or "vaping" machine. To best match the nicotine content of the e-cigarette vapour to cigarette smoke [17], we used an AirChek 52 pump (SKC Inc., Eighty Four, PA, USA) to determine the levels of nicotine at multiple settings within the TE-2E and TE-10z chambers. A second limitation was that we only evaluated Blu electronic cigarettes. Third, common to all translational research, equating results from experimentation in rats may not necessarily translate into similar results in humans. While our study was designed to imitate the exposure levels of rats to the use of e-cigarettes and tobacco cigarettes by people, they may not replicate the exact smoking experience for human users. However, until a randomised controlled trial can be performed in humans, this rat model will likely be one of the most appropriate to reference when counselling patients on e-cigarette smoking cessation.

In summary, our findings in an experimental model clearly indicate that e-cigarettes are just as toxic as tobacco cigarettes and that long-term exposure to nicotine vapour can cause significant lung damage; it is not a safe alternative to tobacco smoke.

The US Food and Drug Administration (FDA) now has regulatory authority over e-cigarettes and can regulate product and e-cigarette fluid design features, such as nicotine content and delivery, voltage, fluid formulations, and flavours. The FDA recently announced its strategy, which includes forcing e-cigarette manufacturers to lower the amount of nicotine in their products to "non-addictive levels". However, it is not clear what non-addictive levels are and if they indeed impact pulmonary toxicity.

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