



E-cigarette vapour is not inert and exposure can lead to cell damage

Abstracted from

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 2016; **52**: 58-65. doi: 10.1016/j.oraloncology.2015.10.018. Epub 2015 Nov 4. PubMed PMID: 26547127.

Address for correspondence: WM Ongkeko, Department of Otolaryngology-Head and Neck Surgery, University of California, San Diego, La Jolla, California, United States

Question: What is the cytotoxicity and genotoxicity of short- and long-term e-cigarette vapour exposure on a panel of normal epithelial and head and neck squamous cell carcinoma (HNSCC) cell lines?

Materials and methods *In vitro* experiments were performed on normal epithelial cells as well as head and neck squamous cell carcinoma (HNSCC) cell lines. The widely available cell line HaCat, a spontaneously transformed immortal keratinocyte and the HNSCC cell lines HN30 and UMSCC10B were used. Cells were exposed to nicotine-containing and nicotine-free vapour extract from two popular e-cigarette brands for periods ranging from 48 hours to eight weeks. Cytotoxicity was assessed using Annexin V flow cytometric analysis, trypan blue exclusion and clonogenic assays. Genotoxicity in the form of DNA strand breaks was quantified using the neutral comet assay and γ -H2AX immunostaining.

Results E-cigarette-exposed cells showed significantly reduced cell viability and clonogenic survival, along with increased rates of apoptosis and necrosis, regardless of e-cigarette vapour nicotine content. They also exhibited significantly increased comet tail length and accumulation of γ -H2AX foci, demonstrating increased DNA strand breaks.

Conclusions In conclusion, our study strongly suggests that electronic cigarettes are not as safe as their marketing makes them appear to the public. Our *in vitro* experiments employing two brands of e-cigs show that at biologically relevant doses, vapourised e-cig liquids induce increased DNA strand breaks and cell death, and decreased clonal survival in both normal epithelial and HNSCC cell lines independently of nicotine content. Further research is needed to definitively determine the long-term effects of e-cig usage, as well as whether the DNA damage shown in our study as a result of e-cig exposure will lead to mutations that ultimately result in cancer.

Commentary

Electronic cigarettes (e-cigarettes) are highly popular with over 2.6 million users in the United Kingdom alone.¹ Many issues around e-cigarettes remain controversial and in recent years there has been an almost exponential rise in the number of publications on the topic. Two recent publications of relevance include a Cochrane Collaboration systematic review and meta-analysis that showed e-cigarettes to be a promising smoking cessation aid² and an evidence review by Public Health England that concluded that e-cigarettes were 95% less harmful than smoking.³

The paper by Yu *et al.* is a 'basic science' paper, published by a US pathology research group based in San Diego.⁴ It has caused much controversy beyond the realms of dental research. Press releases quote the senior author saying 'based on the evidence to date, I believe that (e-cigarettes) are no better than smoking regular cigarettes'.⁵ Articles in the UK national press have commented on this publication,⁶ including critical articles dismayed at the misleading nature of the paper and press releases.⁷

This commentary aims to critically review the science of the paper, explore if its conclusions are valid and useful and suggest if there are any clinical implications for oral health care professionals.

Very simply, the paper by Yu *et al.* describes a laboratory based study exposing cell lines to different conditions and investigating for 'cytotoxic and DNA strand break-inducing effects'.⁴ The authors describe using a HaCat cell line to investigate the effects on 'normal epithelium' and head and neck squamous cell carcinoma (HNSCC) cell lines to investigate the effects on those who 'already have HNSCC'. The authors describe the HaCat cell line as 'normal' which is perhaps misleading. HaCat is derived from the epidermis of skin, transformed and known to contain chromosomal abnormalities. It is an immortalised cell line that grows forever. Although more challenging to culture, a primary keratinocyte culture from oral mucosa would have been a more accurate model system.

The cell lines were cultured in media that had been exposed to either e-cigarette vapour (two brands used; either containing nicotine or no nicotine), cigarette smoke or nicotine solution. The vapour or smoke was drawn through the media using negative pressure, filtered and then used to treat the cell cultures.

The main problems with this study are the inadequate reporting of the methods, the way the results and discussions are presented and the comparisons used. The applied methods generally appear to be reasonable although they are lacking critical detail in some areas. One example of this is with regards to how the culture media

were exposed to the smoke/vapour. The paper simply states that it was '*pulled through media using negative pressure*'. No citations to previous methods are provided and it is not clear how consistent media conditions were controlled. Smoke exposure systems for toxicological studies have been in use since the 1930s and several hugely sophisticated systems exist.^{8,9} International standards also exist for these systems (eg ISO), which are recommended by the WHO.^{10,11} The lack of any detail about the technique used in this paper suggests a potentially inadequate and non-standardised procedure. A more suitable alternative for exposing the cells may have been to use a culture chamber into which the smoke/vapour was drawn using controlled flow rates.

Within the results section there is an obvious bias with significant text devoted to the results from the e-cigarette liquids with only one sentence providing results for cells exposed to cigarette smoke extract. Readers are left having to derive the remaining cigarette smoke details themselves from the figures.

The discussion section reflects on several important issues around e-cigarettes and highlights many of the challenges around researching in this area, such as the fast moving nature of product development and variation in use. The authors ultimately conclude that 'electronic cigarettes are not as safe as their marketing makes them appear' and that this *in vitro* experiment has shown e-cigarette vapour to be cytotoxic to epithelial cell lines, with DNA damage occurring.

A critical factor that is omitted from the results, discussions and conclusions (as well as the majority of the resulting press attention) is that the authors were unable to complete the tests on the cells exposed to cigarette smoke due to its high toxicity. Reference is only made to this briefly within the methods section where the authors describe only being able to expose cells for 24 hours due to the level of toxicity. In comparison, the cells exposed to e-cigarette vapour were cultured for up to eight weeks, with the media (containing the relevant e-cigarette vapour extract) refreshed every three days. Somewhat misleadingly, three of the six figures within the results section display the results of the cigarette smoke alongside the results of the e-cigarette vapour. When reading these graphs (eg Fig. 2) the reader would likely draw the conclusion that the e-cigarette vapour caused similar levels of damage to cigarette smoke and indeed the authors have completed (inappropriate) statistical comparisons between the groups. In fact, what these graphs are showing is a comparison between cells exposed to cigarette smoke for 24 hours against cells exposed to e-cigarette vapour for much longer periods (seven days in the case of Fig. 2). The Figure legend partially acknowledges this, stating that cigarette smoke results 'are shown for comparison' but what is meant by this could be made clearer to the reader.

It is surprising that the comparison to cigarette smoke is omitted within the discussion and conclusions of this paper given the statements about smoking made within the paper itself (introduction)

and resulting press releases. Indeed, since 99.5% of e-cigarette users in the UK are smokers or ex-smokers¹² the comparison to cigarette smoke is highly relevant.

In summary, this paper provides useful information that e-cigarette vapour is not inert and exposure can lead to cytotoxic and DNA damaging effects *in vitro*. The authors rightly conclude that further research is needed within this area. However, the authors fail to make the relevant comparisons to cigarette smoke. An alternative conclusion from the results of this study is that epithelial cells can survive in e-cigarette vapour extract for eight weeks but only 24 hours when exposed to cigarette smoke extract.

These results feed into the body of evidence that e-cigarettes are less damaging than cigarette smoke, although not risk free, and continue to support statements such as those from Public Health England that 'e-cigarettes are around 95% less harmful than smoking'.³ Clearly this is an emotive, political and controversial topic. As with any research, oral health care professionals should critically appraise the results of research papers and have a responsibility to provide evidence-based information to their patients.

Richard Holliday^a, Ralf Kist^a, Linda Bauld^b

^aCentre for Oral Health Research, Newcastle University, Newcastle upon Tyne, UK

^bInstitute of Social Marketing, University of Stirling, Sterling, Scotland

1. Action on Smoking and Health (ASH), *Use of electronic cigarettes (vapourisers) among adults in Great Britain. Factsheet*. 2015. http://www.ash.org.uk/files/documents/ASH_891.pdf.
2. McRobbie H, Bullen C, Hartmann-Boyce J, Hajek P. Electronic cigarettes for smoking cessation and reduction. *Cochrane Database Syst Rev* 2014; **12**: CD010216.
3. McNeill A, Brose LS, Calder R, Hitchman SC, Hajek P, McRobbie H. E-cigarettes: an evidence update. A report commissioned by Public Health England. Public Health England, 2015 (PHE publications gateway number: 2015260).
4. 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* 2016; **52**: 58-65.
5. EurekAlert!/AAAs, Press release: Cell harm seen in lab tests of e-cigarettes. Veterans Affairs Research Communications. http://www.eurekalert.org/pub_releases/2015-12/varc-chs122815.php [Accessed 26 February 2016]
6. Knapton S. E-cigarettes are no safer than smoking tobacco, scientists warn. *The Telegraph*, 2015. <http://www.telegraph.co.uk/news/health/news/12073221/E-cigarettes-are-no-safer-than-smoking-tobacco-scientists-warn.html> [Accessed 26 February 2016]
7. Bauld L. No, there's still no evidence e-cigarettes are as harmful as smoking. *The Guardian*, 2015. <http://www.theguardian.com/science/sifting-the-evidence/2015/dec/31/no-theres-still-no-evidence-e-cigarettes-are-as-harmful-as-smoking> [Accessed 26 February 2016]
8. Johnson MD, Schilz J, Djordjevic MV, Rice JR, Shields PG. Evaluation of *in vitro* assays for assessing the toxicity of cigarette smoke and smokeless tobacco. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 3263-3304.
9. Thorne D, Adamson J. A review of *in vitro* cigarette smoke exposure systems. *Exp Toxicol Pathol* 2013; **65**: 1183-1193.
10. Ashley DL, Burns D, Djordjevic M, et al. The scientific basis of tobacco product regulation. *World Health Organ Tech Rep Ser* 2008; (951): p.1-277.
11. Burns DM, Dybing E, Gray N, et al. Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. *Tob Control* 2008; **17**: 132-141.
12. Smoking-Toolkit-Study, Electronic cigarettes in England- latest trends. STS140122. 2015.

Evidence-Based Dentistry (2016) **17**, 2-3. doi:10.1038/sj.ebd.6401143