Using an *in-vitro* human airways model to study the effects of e-cigarettes on bronchial epithelial cells

Vasanthi Bathrinarayanan, P.[1] | **Marshall, L.J.[2]** | **Leslie, L.J.[1]**

[1]School of Engineering and Applied Science, Aston University, Birmingham UK; [2] School of Health and Life Sciences, Aston University, Birmingham UK

Introduction

According to the UK Home Office statistics, in the year 2013, 4.12 million scientific procedures were carried out on animals[1]. A number of reports have shed light on the inadequacy and unreliability of information obtained from using animal models for the study of human diseases [2,3]. For example, many drugs successfully tested on animals fail to translate into human medicine[3]. The metabolic, genetic and cellular differences between animals and human lead to the risk of obtaining erroneous data[4]. Of specific interest to our work, it has been demonstrated that animals and humans do not have the same kind of reaction to cigarette toxins. Moreover, it is difficult to expose laboratory animals to cigarette smoke in the same manner and time frame as human beings[5].

In-vitro models, on the other hand, have proven to be critical in the advancement of human medicine. These techniques have from time-to-time shown to be more accurate, ethical and economical than the more crude animal testing approach[6].

Aim of the study

The prime aim of our study is to use *in-vitro* human airway models as an effective alternative to replace animals in traditional/E-cigarette research.
The objectives of our current work are:

1. To investigate the toxicity of nicotine and its oxidised metabolite, cotinine, on human epithelial cell lines namely BEAS 2B and CALU 3.

2. To examine the toxic effect of the components of E-cigarette solution on the above mentioned two epithelial cell lines.

Materials and Methods

Cell viability assays:

CELL S AND CELL CULTURE

The bronchial epithelial cell line BEAS 2B and adenocarcinoma derived CALU 3 cell line were cultured and maintained under standard conditions (37°C and 5% CO_2) in DMEM:Hams F12 + 10% FCS.

PLATING

Both cell lines were seeded at a density of $5x10^5$ cells/ml in 96 well plates and incubated at 37°C for 24 hours

CHALLENGE ADDITION

The cells were treated with 100µL of varying concentrations of nicotine (1.1µM-75µM), Cotinine (1.5µM-100µM), E-liquid or E-cigarette vehicle and incubated for 24 hours

CELL TITRE BLUE® ADDITION & ANALYSIS

Cell titre blue reagent® was added to each well and incubated for 2 h at 37°C. Fluorescence intensity was measured at 560ex and 590em wavelengths using a Spectramax Gemini plate fluorimeter

Cytokine detection assay:

ELISA was used to determine the amount of IL-6 and IL-8 secreted by the epithelial cells in response to nicotine.
Procedure: Supernatants were collected from each well after being exposed to the different concentrations of nicotine for 24 h. Samples were briefly centrifuged and cell-free supernatants stored at -20°C prior to analysis. IL-6 and IL-8 concentrations in the samples were determined according to the manufacturer's instructions (e–Bioscience).

References

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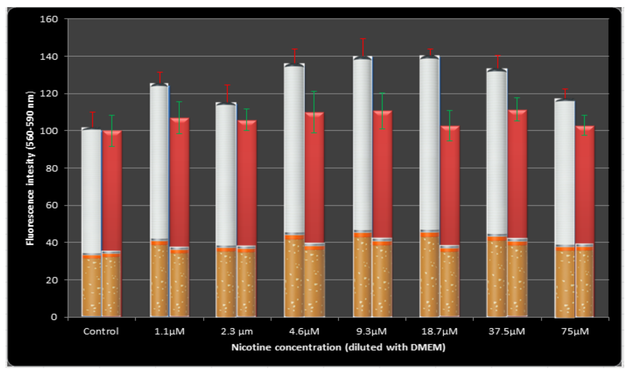
Results

Nicotine/Cotinine do not impact on bronchial epithelial cell viability



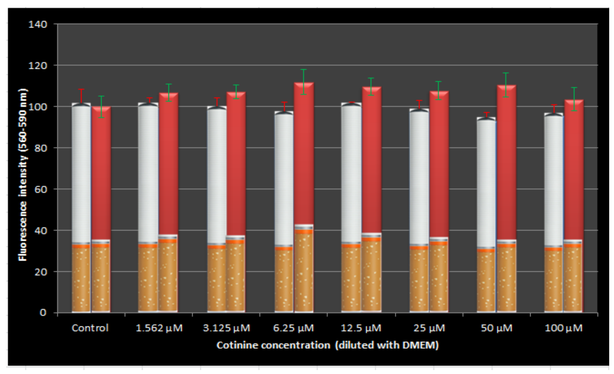


FIGURE 1. Nicotine and cotinine do not affect the metabolic activity of human epithelial cells. The figure clearly shows that there is no decrease in the cell viability of BEAS 2B (white) or CALU 3 (red) post 24 hours exposure to varying concentrations (1.1µM-75µM) of nicotine (left panel) or cotinine (1.5µM-100µM) (right panel). (Mean ±SD, n=3 from triplicate wells).

Nicotine alone is not a strong inflammatory stimulus to epithelial cells

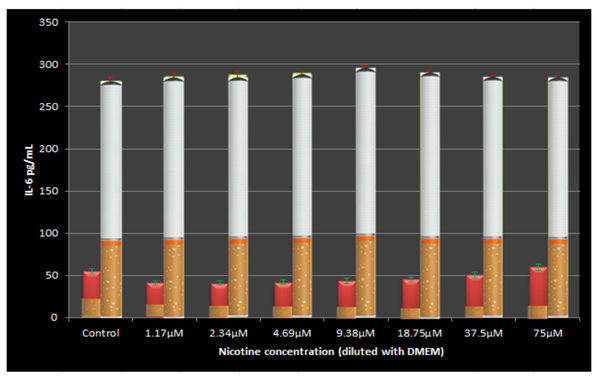


FIGURE 2. Nicotine does not stimulate pro-inflammatory mediator release from BEAS 2B. ELISA was performed to analyse the effect of nicotine on pro-inflammatory cytokine production by BEAS 2B. The figure shows that the amount of IL-6 (red) and IL-8 (white) secreted by the BEAS 2B cells is not stimulated by nicotine at any concentration tested. (Mean ±SD, n=6 from triplicate wells). Predictably, these data are in agreement with human studies[7] and contradictory to mouse studies[8].

E-cigarette vehicle has a detrimental effect on cell viability at high concentrations

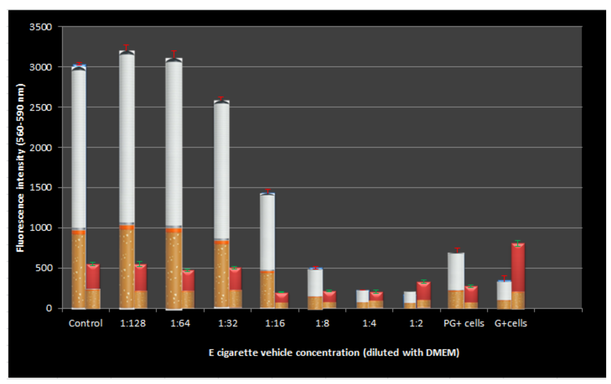


FIGURE 3. E-cigarette components appear toxic to human bronchial epithelial cell lines. The vehicle (carrier) in E-cigarettes often consists of propylene glycol (PG) and glycerol (G). These two components make up ~90% of the total E-cigarette solution. The vehicle was serially diluted with medium and added to the cells. The figure shows the decrease in cell viability of BEAS 2B (white) and CALU (red) as the vehicle concentration increases. (Mean ±SD, n=3 from triplicate wells)

Discussion and conclusions

❖Nicotine and cotinine do not influence the cell viability of BEAS 2B and CALU 3. Moreover, they do not stimulate the production of pro-inflammatory cytokines, namely IL-6 and IL-8.
❖E-cigarette liquid ("medium" nicotine content; extracted from a generic E-cigarette; data not shown) and E-cigarette vehicle cause a dramatic decrease in cell viability at high concentrations. This clearly shows that, for E-cigarette formulations, nicotine does not have an impact on the epithelial cell viability, rather the E-cigarette vehicle consisting of propylene glycol and glycerol mixture has a major influence on the viability of the cells[9].

❖In conclusion we suggest that though E-cigarettes are considered as an aid to stop smoking, this may be counter-productive since the E-cigarette vehicle is toxic to the cells[10].

Future work

❖ Test the effect of E-cigarette liquid containing different concentrations of nicotine on epithelial cell viability.
❖ Perform ELISA to investigate the influence of cotinine and E-cigarette liquid and vehicle on the production of pro-inflammatory cytokines (IL-6 and IL-8).
❖ Expose the human airway cell models to aerosolised nicotine, cotinine and E-cigarette solution using our in-house smoking machine.