Author's response to reviews

Title: Inflammatory and cytotoxic effects of acrolein, nicotine, acetylaldehyde and cigarette smoke extract on human nasal epithelial cells

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Author's response to reviews:

Dear Editor,

We would like to thank the Editor and expert reviewers for the detailed review of our manuscript. We have made significant changes to the MS in both its content and wording as outlined below to address the concerns which have been raised. We believe our manuscript is now stronger and we hope that it is now suitable for publication.

Many thanks,
David Comer

Reviewer's report

Title: Inflammatory and cytotoxic effects of acrolein, nicotine, acetylaldehyde and cigarette smoke extract on human nasal epithelial cells
Version: 1 Date: 2 June 2013
Reviewer: Esmaeil Mortaz

Reviewer's report:
The paper is interesting however not well described the points. I have following comments:

1- The Objective of study is obscure.

Our objective was to test the overall hypothesis that as CSE is a toxic substance with complex effects on epithelial cells, we aimed to identify the effects of the more important chemicals within CSE on such cells. The last paragraph of the introduction outlines this.

2- Authors need to describing the some more details of effects CS on inflammatory cells in introduction.

Manuscript edited accordingly
3- The authors must refer to compositions of CS.

Cigarette smoke is a complex substance, containing many thousands of chemicals, many of which have varying immunomodulatory properties. It would be interesting to know the concentrations of acrolein, nicotine and acetylaldehyde contained within CSE. Unfortunately we do not have this information, and we are not aware of any commercial assays available which could be used to address this question.

4- Why the authors selected Nasal EP not the Bronchial?

The responses of bronchial epithelial cells to cigarette smoke have been relatively well characterised,[1-4] but the effects on the nasal epithelium, are also important in respiratory disease, and are not as well understood. Nasal and sinus inflammation is common in COPD and contributes to the decline in lung function.[5]

5- Generally the details of activation such as time points of activation to each experiments not well described.

Manuscript edited accordingly in the results section

6- The amounts of loaded protein to SDS/PAGE not described and densitometry need to data.

Equal amounts of protein (15 µg/lane) were separated in a 10% SDS gel, and transferred to a polyvinylidene difluoride sheet by electroelution with a constant voltage of 100 V for 90 minutes at room temperature. Manuscript edited accordingly to include these details.

Densitometry data has been included for figure 3 for the more relevant bands. The changes within figure 4, which are representative blots, are so striking that densitometry data is not necessary. We feel that there already are a lot of figures and graphs within the manuscript.

7- The activation time for cytokines assays not clear? why some time 4 and some time 24 h??

We chose the 2 exposure times to try to compare our data with other published work. The concentrations of CSE used can range from 100% CSE for 15 minutes [6] to a 1% CSE for 24 hours (in those studies which use a single cigarette to prepare the initial “100%” stock CSE).[7] A study exposing primary human nasal epithelial cells to CSE for 1 h, 2 h, and 4 h reported a time and dose-dependent cytotoxicity of CSE.[8]

8- Whole the Gels for WB not well described.

The manuscript edited accordingly to provide more information on the Western blotting procedure:
Equal amounts of protein (15 µg/lane) were separated in a 10% SDS gel, and transferred to a polyvinylidene difluoride sheet by electroelution with a constant voltage of 100 V for 90 minutes at room temperature.

Level of interest: An article of limited interest
Quality of written English: Not suitable for publication unless extensively edited
Statistical review: Yes, and I have assessed the statistics in my report.

Reviewer’s report
Title: Inflammatory and cytotoxic effects of acrolein, nicotine, acetylaldehyde and cigarette smoke extract on human nasal epithelial cells
Version: 1 Date: 12 August 2013
Reviewer: Martin Liu

Reviewer’s report:
The current study investigated effect of cigarette smoke component (acrolein, acetylaldehyde and nicotine) pretreatment on primary nasal epithelial cell (PNECs) survival and cytokine release, which was studied in comparison to the effect of cigarette smoke extract (CSE). The authors found that pretreatment with 10 and 30µM acrolein augmented IL-8 or IL-6 release by the PNECs in response to LPS stimulation, while same concentrations of nicotine inhibited but acetylaldehyde had no effect. Higher concentrations (50µM) of acrolein and nicotine resulted in apoptotic or necrotic cell death. They also found that 5% CSE not only stimulate IL-8 release, but also induced apoptosis through a mechanism of activating caspase-3 or necrosis. While this is an interesting study, there are comments as following.

Major Comment:
1. The authors cited a literature and provided concentrations of nicotine and acrolein in the alveolar lining fluid in smokers are 30µM and 80µM, respectively, and thus, concentrations of 30µM nicotine or acrolein are reasonable in the current study. However, could the authors provide the concentration of acetylaldehyde in the smokers airway fluid? It had been reported that acrolein exerts its effect at micromolar range, but acetylaldehyde has biological effect at mili-molar range on the bronchial epithelial cells (Wang et al. Am J Respir Cell Mol Biol. 2001; 25: 772-9). If the in vivo acetylaldehyde concentration is higher than acrolein or nicotine, it would be worthy to examine higher concentration of acetylaldehyde effect on the cells.
The paper which the reviewer refers to established the effects of acetylaldehyde on matrix remodelling, along with proliferation and contraction of bronchial epithelial cells in 3 dimension gels, using up to 5 mM acetylaldehyde concentration.[9] However, other groups, also studying airway epithelial cells and lung fibroblasts in cell culture models, used acetylaldehyde in the µM range in experiments which had end points more similar and relevant to our work.[10]

We acknowledge that it would be of interest to use a broader range of concentrations, along with further chemicals, but we had to be selective in the experiments which we performed due to the relative limited availability of the primary cells themselves from the brushing procedure.

2. The authors concluded that nicotine is immunosuppressive in PNEC cultures. To this reviewer, nicotine has anti-inflammatory effect rather than "immunosuppressive" effect.

Manuscript edited accordingly

Minor comment:

1. Figure 3, 4, 5 and 6, are not matching with figure legends.

Manuscript edited accordingly

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests

Reviewer's report
Title: Inflammatory and cytotoxic effects of acrolein, nicotine, acetylaldehyde and cigarette smoke extract on human nasal epithelial cells
Version: 1 Date: 13 August 2013
Reviewer: T-C Lee

Reviewer's report:
In this manuscript, the authors tried to reveal whether acrolein, nicotine and acetylaldehyde, chemicals presented in volatile cigarette smoke, play as immunomodulatory agents in cultured primary nasal epithelial cells (PNECs). They simply treated PNECs obtained from healthy subjects with these agents before stimulating by Pseudomonas aeruginosa lipopolysaccharide (PA LPS) and then determined the levels of IL-8, NF-κB, and caspase-3 by different
techniques. Their results showed that acrolein is pro-inflammatory whereas nicotine immunosuppressive in PNEC cultures. Acetaldehyde was no immunomodulatory activity. However, this study using a simple cell system and toxic doses makes difficult to draw conclusion regarding to inflammatory activity.

The major comments as following:

1. It is unclear why the authors used these agents at 30 or 50 #M. Are these concentrations relevant to CS? Apparently, the doses used are very toxic epithelial cells. It may not suitable for studying their immunomodulatory activity. The authors should determine the IC50 of these agents against PNECs.

Acrolein has been estimated to reach concentrations of up to 80 µM in the respiratory tract lining fluids as a result of smoking.[11] However, maximum stimulation of IL-8 was achieved in primary small airway epithelial cells after stimulation with 30 µM acrolein for 18 h, decreasing thereafter with higher concentrations attributable to increased cytotoxicity.[10] Similar results in terms of the cytotoxic effects of acrolein have been reported using the human bronchial cell line HBE1, with no detectable apoptosis using a flow cytometry method after exposure to 25 µM acrolein for 12 h, but clearly present with a more prolonged exposure time.[12]

In our experiments we have also demonstrated that the optimal acrolein concentration was in the 30 µM range, with a marked reduction in cell viability at 60 µM using the MTT assay, making any release of IL-8 difficult to interpret at this concentration (MTT data not included in manuscript).

Smoke-borne acrolein contains 118 µg of acrolein per cigarette.[13] Acolein is highly water soluble (http://www.inchem.org/documents/ehc/ehc/ehc127.htm). The molecular weight is acrolein is 56 g/mol. Therefore, considering the fact that we dilute our “100%” CSE by a factor of 20 before being used in experiments, an estimate of the concentration of acrolein would be in the order of 2 µM in the CSE itself.

Determining the IC50 would not alter the concentration of acrolein or nicotine which would be considered optimal for these particular experiments.

2. NFkB activity should be determined using reporter assay system.

We acknowledge that measuring NFkB activity using a reporter assay system is the gold standard. However, the use of Western blotting to measure phospho-NFkB is broadly accepted as a valid and robust measure of its activity.[14-15]

3. The interaction between PNECs and immune cells should be studied.

We agree this would be an interesting area for future studies.

Minor Essential Revisions
4. Several figure numbers on figure pages are incorrect.

Manuscript edited accordingly

5. The statistical comparison shown in Figure 1 is weird.

Figure 1 does not have any stats - it is the ICC image. In Figure 2, we have shown the differences that were elicited using the Kruskal-Wallis test method. This is the non-parametric equivalent of a one way analysis of variance. We regard it as appropriate to use non-parametric statistics given our sample size.

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests

Reference List


