Author's response to reviews

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

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Author's response to reviews: see over
Dear Editor,

We would like to thank the Editor and expert reviewers for the detailed review of our manuscript. Reviewers 1 and reviewer 2 have regarded the revision to be satisfactory. Please see below our response to reviewer 3.

Many thanks,

David Comer

Reviewer 3

In the revised manuscript, the authors answered some questions raised by the reviewers. However, one major issue whether the experimental doses of acrolein, nicotine, and acetylaldehyde are relevant to those in CSE is not clearly answered. According to their estimation, the concentration of acrolein in CSE is 2 \( \mu \text{M} \) that is 15 to 25 folds lower than those used in this manuscript. The authors should discuss this point in their manuscript.

We estimate that the concentration of acrolein in CSE is approximately 2 \( \mu \text{M} \). However, for the purpose of these experiments, we feel it is of much greater relevance to use concentrations of acrolein which would be similar to the concentrations present in the respiratory tract lining fluid of smokers. This has been estimated to be 80 \( \mu \text{M} \).[1] With this in mind, it is plausible that the concentration of acrolein with the respiratory tract lining fluid accumulates throughout the day in a smoking subject, that is, assuming that more than a single cigarette is smoked. In fact, previous studies using CSE have been guided by the fact that the average smoker smokes in excess of 13 cigarettes per day, and used intermittent CSE exposures at 2 h intervals in an effort to replicate this very effect in cell culture models.[2]

Furthermore, we question the relevance of expanding the discussion in order to compare these various chemicals in isolation to predicted concentrations within CSE, which may distract from the main message of our paper. CSE is a complex substance, containing several thousand chemicals, some of which may interact with each other. So, to compare a concentration of a chemical within CSE with the isolated form of the chemical would be an oversimplification. We therefore would prefer not to redraft the discussion to include a narrative comparing isolated chemicals with estimated concentrations within CSE.
In the last paragraph of Discussion, the authors mentioned that acrolein heightened IL-8 release but still caused loss of cell viability. Therefore, the values of IC50 are important references for the readers to understand the toxic ranges of chemicals studied.

IC50 establishes the effectiveness of a given substance to inhibit a biological function. In these experiments we have measured cell activation by measuring IL-8 release and NF κB activation. Loss of cell viability was established by a number of assays in response to various chemicals. It is not clear to us what the reviewer suggests should be measured to determine IC50. We have not used any inhibitors in these experiments.

Despite acrolein causing loss of cell viability, there still remained a heightened release of IL-8. It is likely levels of IL-8 will accumulate during the period acrolein is exposed to the cells, and after a sufficient exposure time and concentration, loss of cell viability emerges and release of IL-8 plateaus. This supports the notion that acrolein stimulates the cells themselves, and after a certain time point, a toxic level is reached, and the loss of cell viability becomes more pronounced.

**Minor Essential Revisions**

1. The legend of Figure 1 is unclear. There are 4 panels without clear description.

The manuscript has been adapted in order to make the legend more clear.

2. Since ELISA was used to determine the amount of IL-8 in the cultural medium, the data of Figure 2 should be presented by the concentrations of IL-8 instead of % Control. Then, the reader can know whether the treatment of tested chemicals to PNEC cells affects the basal levels of IL-8.

The data, as it is currently presented, demonstrates the effects the various chemicals have on IL-8 release when compared to basal release, without any form of chemical exposure. The control IL-8 concentration was 1310 ± 223 pg/ml, which has now been included in the legend for figure 2.