P 08-26

Comparison of epithelial cells inflammatory response from the human respiratory tract after formaldehyde exposure

Gaëlle Bardet[1,2], Charles Persoz[1], Thomas Loret[1], Isabelle Momas[1], Sophie Achard[1], Nathalie Seta[1]

[1]Université Paris Descartes, Faculté de Pharmacie, EA4064 Impact sanitaire des pollutions, 75006 Paris, France

[2]Agence de l'environnement et de la Maîtrise de l'Energie 20, avenue du Grésillé- BP 90406 49004 Angers Cedex 01 France

Correspondence : gaelle.bardet@etu.parisdescartes.fr

BACKGROUND

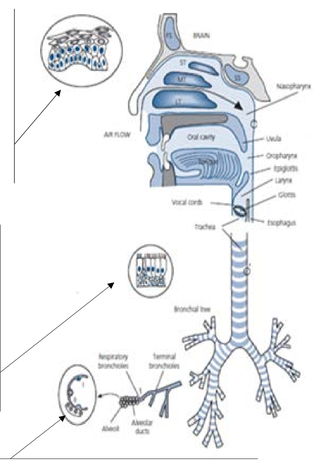
The respiratory tract is directly exposed to inhaled environmental pollutants such as chemicals. Formaldehyde (FA) is a ubiquitous indoor air pollutant known as irritant. Some epidemiological studies revealed an association between FA exposure at low levels and respiratory diseases, especially in young children[1][2][3]. These results do not allow demonstrating causality. Biological proofs are necessary to confirm the impact of this pollutant on the biological response of human airway respiratory cells.

PURPOSE : Our aim was to assess the cellular inflammatory response to FA exposure at environmental levels of epithelial cells from different parts of the human respiratory tract.

MATERIALS AND METHODS

A. AIRWAY EPITHELIAL CELLS

Primary epithelial nasal cells (**hAECN**, Epithelix®) seeded on insert in hAEC medium, 5.10^4 cells/insert



Bronchial cells (**BEAS-2B,** ATCC, USA) seeded on insert in Ham F12 medium, (5% FCS) 4.10^4 cells/insert [5]

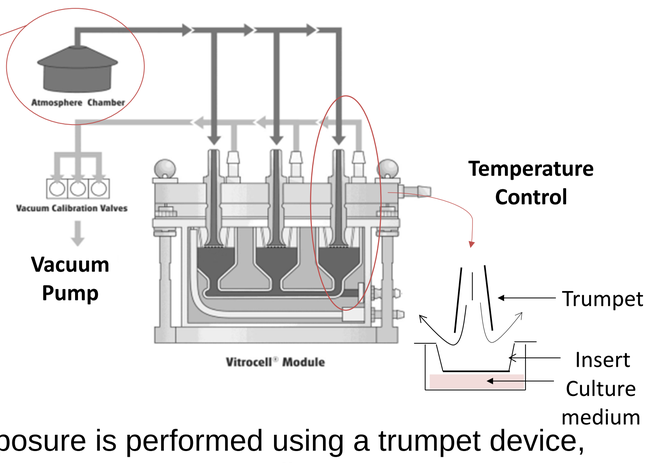
Alveolar cells (**A549,** ATCC, USA) seeded on insert in Ham F12 Medium, (5% FCS) 2.10^4 cells/insert [4][5]

B. AIR-LIQUID EXPOSURE SYSTEM

- Cells were exposed in a **Vitrocell®** module at the air-liquid interface to a dynamic and continu air flow (2 or 5 mL/min)

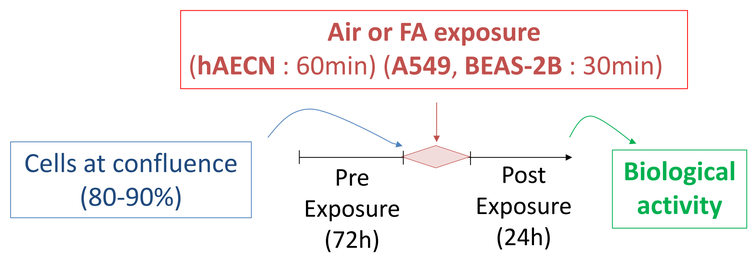
Generation of FA atmosphere

FA atmosphere was generated from liquid FA (Sigma-Aldrich) vaporized in a glass generation chamber. Concentration tested was **50 µg/m³** [4][5]



- Air-liquid exposure is performed using a trumpet device, allowing a direct contact between cells and tested atmosphere

C. PROTOCOL OF EPITHELIAL CELLS EXPOSURE



➢ **Biological activities assessed for *in vitro* models :**

- **Cellular Viability *:*** Metabolic activity using the XTT assay.
- **Inflammatory response** : IL-8 and MCP-1 productions in the cellular supernatant by ELISA method.

➢ **Results :** Arithmetic mean ± standard deviation (N=3). Cytokine concentrations compared to basal level with Student's *t*-test. Difference was significant if $p < 0.05$. **Logiciel R (R version 2.11.1, www.r-project.org)**

RESULTS

Cellular Viability

Alveolar A549, bronchial BEAS-2B cell lines and human nasal primary cell cultures hAECN were exposed in an air-liquid interface with AIR or FA (50 µg/m³) during 30 min for A549 and BEAS-2B, 60 min for hAECN.

➢ Whatever the experimental conditions and cell type, cellular viability was **unchanged** after 24 h of exposure.

Inflammatory response

24 h after exposure, cytokines production in the cellular supernatant was measured. Basal level corresponds to 24 h cytokine production of cells without exposure.

Fig. 1. IL-8 production 24 h after AIR or FA exposure.
* p< 0.05, ** p< 0.01, *** p< 0.001 *versus* basal level.

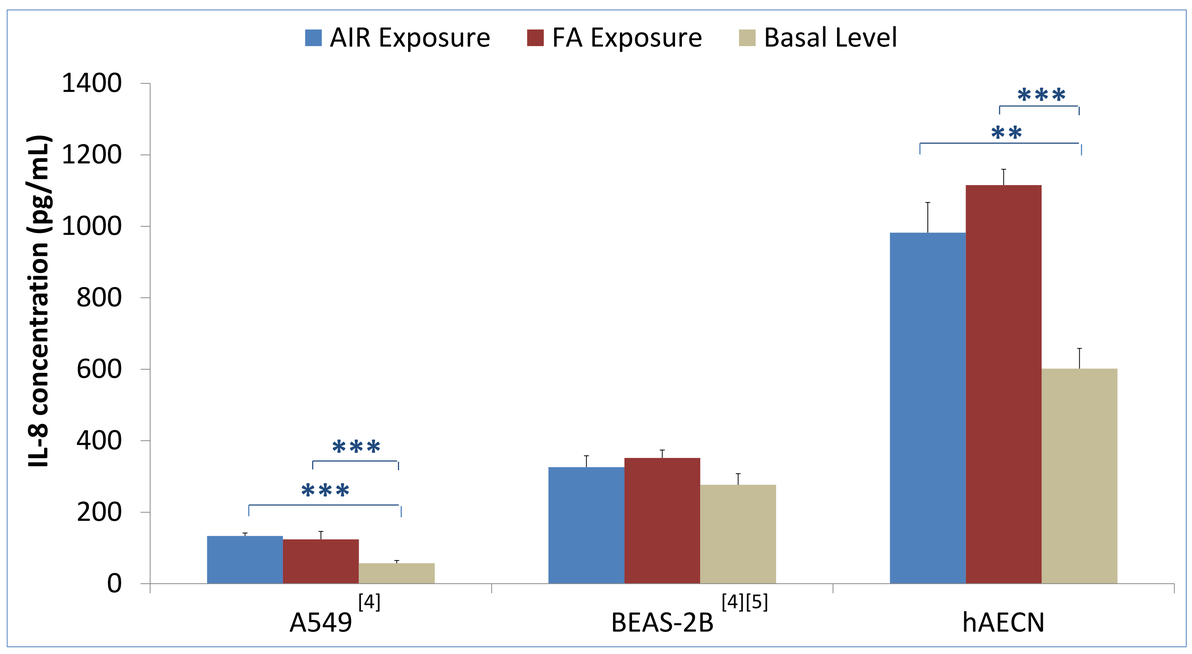
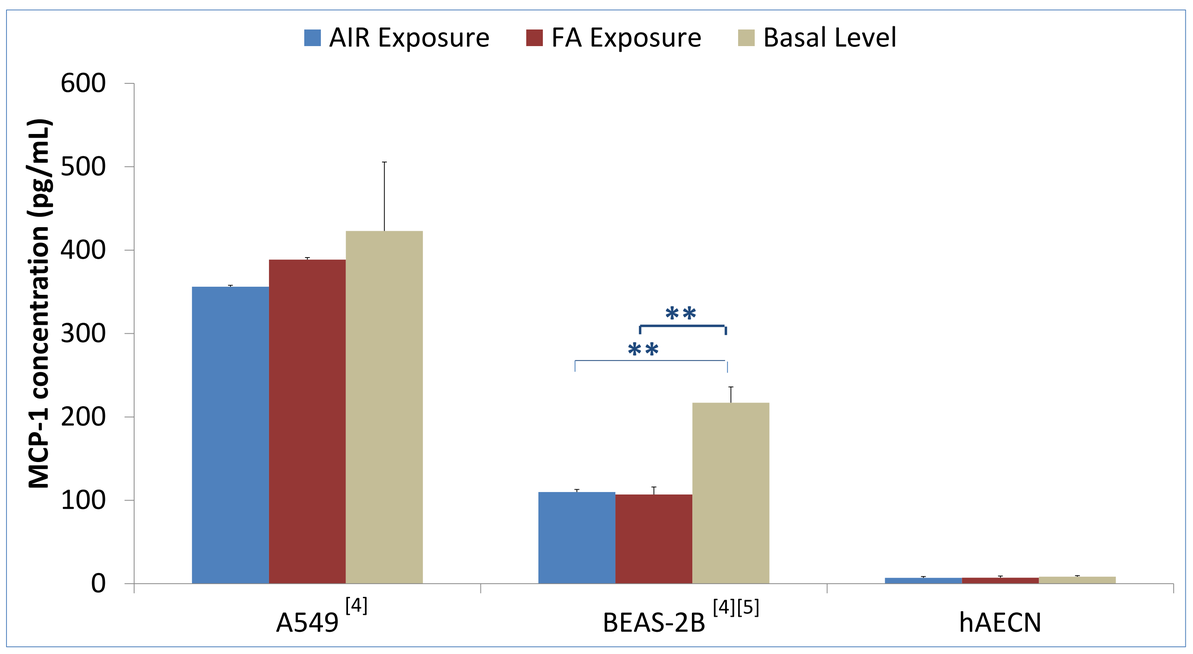


Fig. 2. MCP-1 production 24 h after AIR or FA exposure.
* p< 0.05, ** p< 0.01, *** p< 0.001 *versus* basal level.



➢ Level of IL-8 production depends on the cell type : A549 < BEAS-2B < hAECN

➢ IL-8 production increases after FA or AIR exposure of A549 and hAECN compared to basal level. (Figure 1)

➢ MCP-1 production depends on the cell type : A549 > BEAS-2B > hAECN

➢ MCP-1 production decreases after FA or AIR exposure of BEAS-2B compared to basal level. (Figure 2)

➢ **Cytokines production levels after AIR or FA exposure differ depending on the cell type and measured cytokine**

➢ IL-8 production of hAECN appears to be modulated after AIR and FA exposure in an air-liquid interface compared to basal level.

➢ hAECN are primary cultures from human nasal epithelium whereas A549 and BEAS-2B are immortalized cell lines.

➢ Cellular viability of hAECN was unchanged 24 h after 60 min of AIR or FA exposure which wasn't observed to A549 and BEAS-2B cell lines.

We can consider to repeat hAECN exposure once or twice at regular time intervals.

hAECN seems to be a more relevant cellular model

CONCLUSION

Nasal epithelium is first in contact with inhaled pollutants and the first defensive barrier. The use of human nasal primary culture cells to investigate the cellular inflammatory response to FA exposure (50 µg/m³) is more appropriate than immortalized cell lines to mimic the effect of inhaled pollution. This cellular model is relevant to assess the impact of repeated exposures at regular time intervals in order to get close to the real conditions.

REFERENCES :

[1] Eder et al., 2006 [2] Franklin, 2007 [3] Rumchev KB 2002 [4] Persoz et al., 2010 [5] Persoz et al., 2012