In vitro reconstituted human airway epithelium model to assess the impact of indoor air pollutants on the inflammatory response

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Purpose

Epidemiological studies have suggested an association between indoor air pollution and human respiratory health, especially airway inflammation. Formaldehyde (HCOH) is a major indoor air pollutant and is known as an airway irritant and is suspected to favor respiratory disease as asthma.

The respiratory tract is permanently exposed to numerous pollutants of atmospheric. Airway epithelium acts as a physical barrier ; it is the first line of defense against the inhaled toxicants including chemicals as CHOH.

Reconstituted 3D human nasal epithelium can be considered as the nearest model to nasal epithelium tissue, in view of the pseudo-layer structure combining three types of cells: basal cells, ciliated cells and goblet cells secreting mucus.

A novel method, using Dextran as carrier miming the mucus, can be used for applying chemicals to the apical surface of the airway epithelia

To assess the inflammatory response of the reconstituted 3D human nasal epithelium after formaldehyde exposure

Materials and Methods

- 3D-reconstituted human airway epithelia, MucilAir™ (Epithelix, Suisse) were cultivated at the air-liquid interface in a defined medium (MucilAir medium, Epithelix) and incubated in a CO_2 incubator (37°C, 5% CO_2, constant humidity).
- 3D-MucilAir™ (2 patients, triplicates) were exposed to 50, 100 and 200 µM HCOH as following : 10µL HCOH of the concentration tested were applied on the apical surface of the epithelium on a Dextran tablet (figures 1 and 2) .
- Saline buffer (NaCl 0,9%, $CaCl_2$ 1,25mM, HEPES 10mM) and Cytomix (LPS 200 µg/mL, TNFα 500 ng/mL, SVF 1%) were used as negative and positive controls, respectively.
- Morphology and cilia beating were observed under contrast microscope every day.
- Tissue Integrity was performed 48h before exposure (d-2) and at day 4 (d4) and day 11 (d11) by Trans-Epithelial Electrical Resistance (TEER) measurement (EVOMIX, World Precision Instruments, UK).
- Culture medium was collected every day on the basal side and quantification of IL-8 and MCP-1 productions were assessed by ELISA assay (Invitrogen, France).

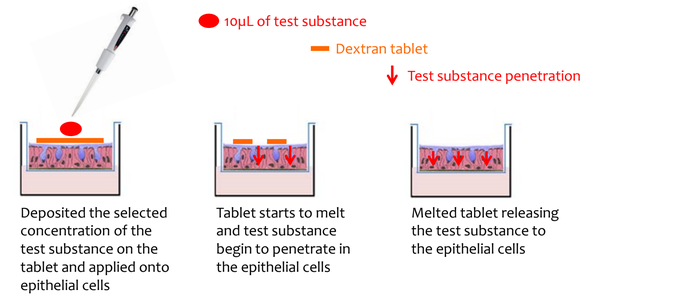

Figure 1 : Principle of exposure with the Dextran tablet *(Huang et al., 2012)*

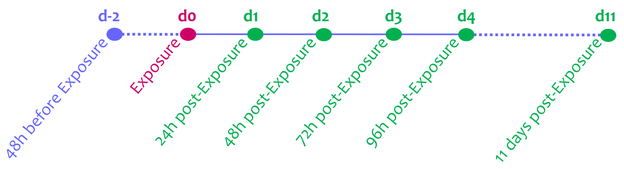

Figure 2 : Experimental design for the exposure at test substance

Results

Each reconstituted human airway epithelium (3D-MucilAir) was followed during 1 month before exposure :

- TEER measurement to verify that all the selected 3D-MucilAir are tights, and
- IL-8 and MCP-1 quantification in the culture medium

The values presented on the Table I are the mean ± standard error of the different data obtained during 1 month without any treatment for both the patient 1 and the patient 2.

A variability from patient to patient was observed but at the same time a good reproducibility for each patient was obtained.

	Patient 1	Patient 2
TEER (Ω/cm²)	2522 ± 45	1787 ± 68
IL-8 (pg/mL)	2439 ± 712	1512 ± 150
MCP-1 (pg/mL)	993 ± 97	608 ± 71

Table I : 3D-MuciAir followed during 1 month (n=24 per patient)

Results obtained with patient 1 and patient 2 after various exposures are similar in term of profile of inflammatory response. Just data of patient 1 were presented.

Cytomix exposure

No tissue alteration (TEER) and absence of inflammatory response after 11 days for Controls. No impact on the tissue integrity after Cytomix exposure (TEER : from 2106 to 1987 Ω/cm²).

IL-8 release increased three-fold compared to the Controls, at d1 and return to baseline between d2 and d11 progressively (figure 1).

Profile reversed for MCP-1. Important decrease at d1 then slow increase to control's levels between d2 and d11 (figure 2).

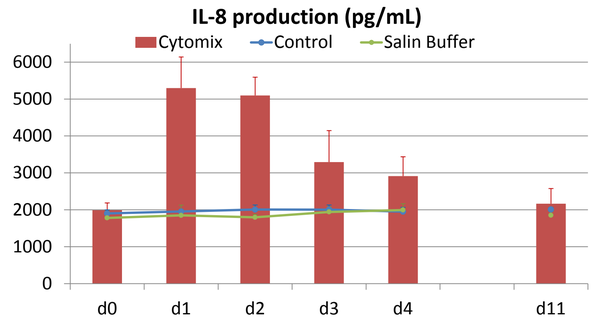

Figure 1 : IL-8 production after Cytomix exposure

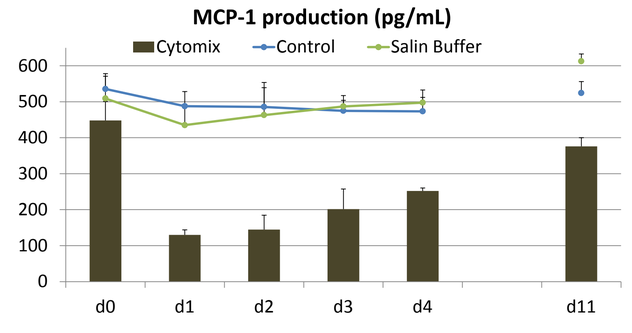

Figure 2: MCP-1 production after Cytomix exposure

Formaldehyde exposure

IL-8 production (figure 3) :

No modulation of inflammatory response was observed after HCOH exposure at 50µM.

After HCOH exposure at 100µM , dose and time responses were shown : IL-8 increase from d1 to d3 and return to baseline progressively .

24h after exposure at 200µM (d1) IL-8 increased with a 1.5 factor compared to the Control then decreased from d2 to d11 in association with the loss of tissue integrity (table II).

MCP-1 production (figure 4) :

MCP-1 release had an inverted curve compared to IL-8, whatever the experiment .

No alteration was observed after HCOH exposure at 50 and 100µM while a low loss of ***tissue integrity*** appeared at 200µM showing epithelium suffering (Table II).

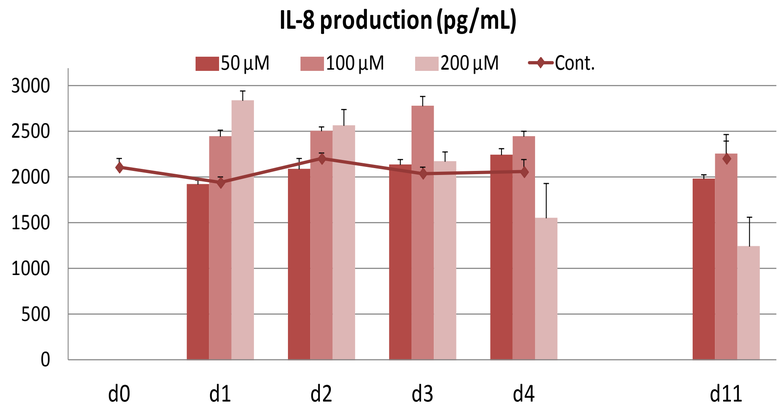

Figure 3 : IL-8 production after Formaldehyde exposure

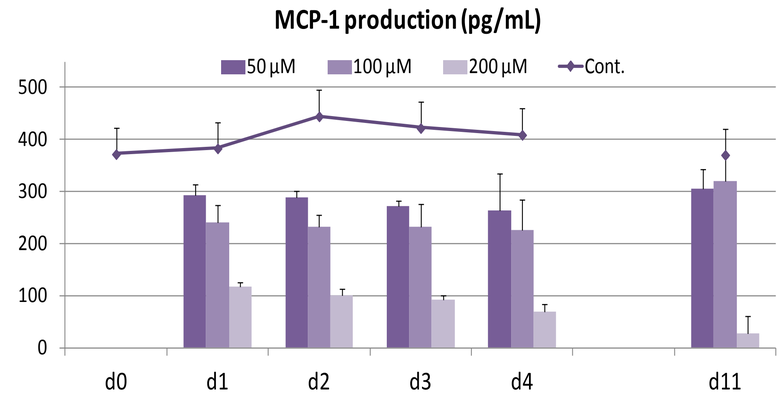

Figure 4 : MCP-1 production after Formaldehyde exposure

TEER measurement (Ω/cm²) after Formaldehyde exposure			
[CHOH] / Day	50 µM	100 µM	200 µM
d-2		2069 ± 125	
d4	2073 ± 146	2020 ± 56	1464 ± 79
d11	2074 ± 105	1998 ± 82	1541 ± 120

Table II : Tissue integrity after CHOH exposure

Discussion - Conclusion

The airway epithelial cells occupy a central position in the genesis of various respiratory pathologies. The development of appropriate *in vitro* model to study the relation between inhalable pollutant and respiratory diseases is needed.

Reconstituted 3D human nasal epithelium can be considered as the nearest model to nasal epithelium tissue, in view of the pseudo-layer structure combining three types of cells: basal cells, ciliated cells and goblet cells secreting mucus. Given the presence of the mucus at the apical side, prolonged exposure with pollutants like formaldehyde can be achieve by passive diffusion of the pollutant into the mucus.

Our results show that a such model is adapted to mimic environmental exposure at low levels of inhalable pollutants and to assess their impact on the inflammatory response.