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In vitro model of repeated exposure to different contaminants of human respiratory cells

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Purpose

Humans are permanently exposed to numerous pollutants of different types. The effects of the association between chemical and biological compounds on human respiratory health, and especially asthma, are still unclear. In order to clarify these effects, toxicological evidence is needed in addition to epidemiological observations.

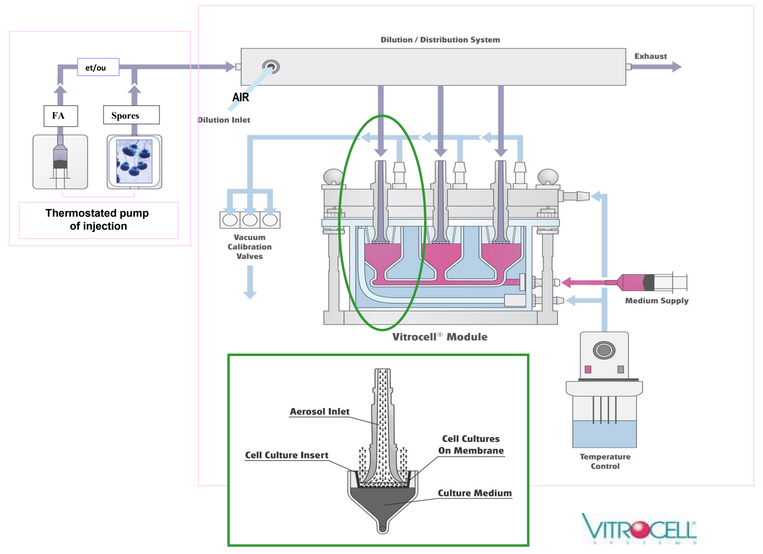
Our aim was to develop an *in vitro* model of exposure using two types of pollutants. The individual or combined *in vitro* effects of a chemical pollutant (formaldehyde-FA) and a biological contaminant (*Aspergillus fumigatus-Asp*), both known to be found in domestic environment and deleterious to respiratory health, were assessed *in vitro* on epithelial respiratory cells using a unique exposure device and stringent conditions of exposure.

Methods

Human alveolar epithelial cells (A549) were sequentially exposed at the air-liquid interface in an Vitrocell exposure module, firstly to environmental level of FA (50µg/m^3) (or Air for Control) during 30 min, and 14h later to viable spores of Asp (7.10^8 spores/m^3, sampled at the inflow level) (or Air for Control) during 30 min.

Controls comprised sequential exposures to each agent alone or to ambient filtered air.

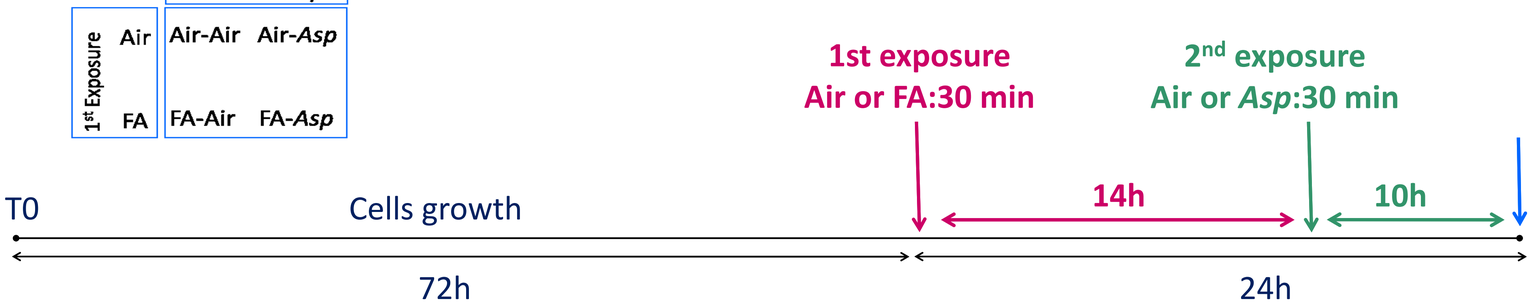
After 10h post-incubation, cellular viability (LDH assay) was assessed. Biomarkers of local inflammation, IL-8 and MCP-1 were assayed by ELISA in the medium removed from apical face of cells, and their mRNA expression was quantified by RT-PCR.



Protocol of sequential exposure

4 experimental conditi

1st Exposure \ 2nd Exposure	Air	*Asp*
Air	Air-Air	Air-*Asp*
FA	FA-Air	FA-*Asp*



Biological activities

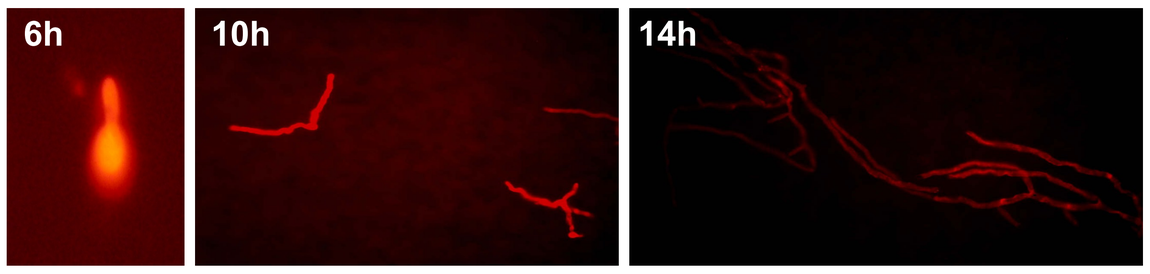
- LDH assay
- IL-8
- MCP-1

Production by ELISA
Expression by RT-qPCR

Preliminary Results

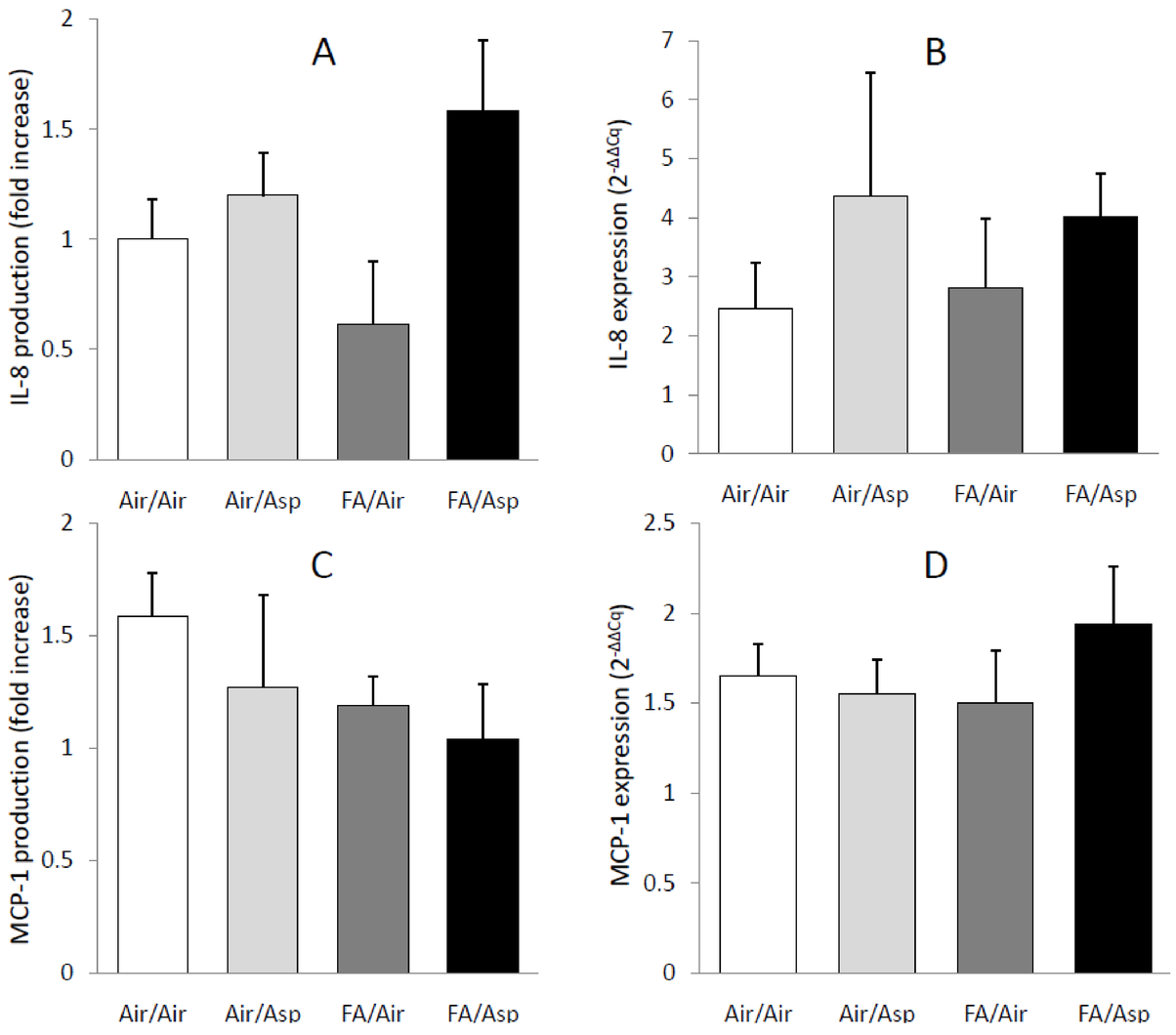
Why did we chose 10h post-incubation after Asp exposure ?

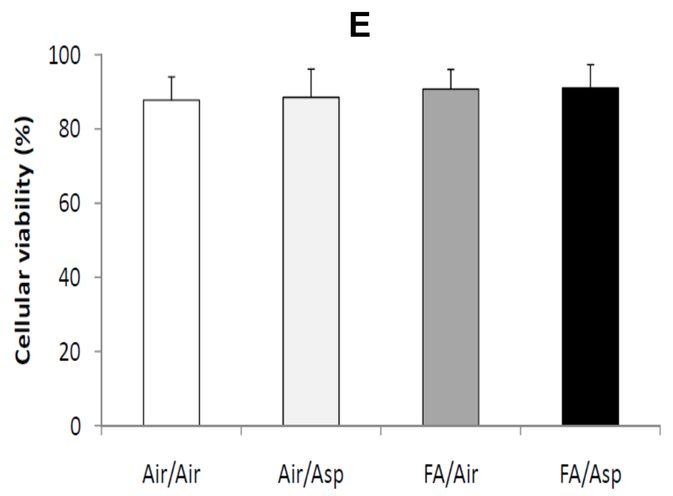
Because 10h is the necessary time to have an early hyphae stage for the growth of *Asp*. At 6h spores are just germinated and at 14h hyphae are too developed.



Results

Sequential exposure to air, FA or Asp did not impact cellular viability (E). Single exposure to FA or *Asp* and sequential exposure to FA then air did not induce significant changes of production or expression of inflammatory cytokines. However, sequential exposure to air then *Asp* tended to induce IL-8 production (A) and expression (B). When combined, FA followed by *Asp* exposure increased IL-8 production (A) and MCP-1 expression (D).





After exposure of A549 cells, cytokines production, cytokines expression and cellular viability was assessed: **A** IL-8 production ; **B** IL-8 expression ; **C** MCP-1 production ; **D** MCP-1 expression ; **E** cellular viability

Conclusion

These results show that a combined exposure to different types of environmental pollutants can be modelled *in vitro* with concomitant assessment of effect on pulmonary cell viability and measurement of inflammatory cytokine response. Extending this experimental approach to other pollutant combinations is warranted to reach a first estimate of the complexity of interactions between pollutants in the pulmonary cell sensitization processes .

References : Persoz *et al.*, *Toxicol Lett*, 2010 and 2011