

Effect of Formaldehyde On Corneal Epithelial Cells In An Air-Liquid Culture Model

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Purpose

Gaseous formaldehyde (FA) is well known for its irritative effects on eyes, nose and throat. It is the main indoor air pollutant with concentrations ranging from 8 to 95 microg.m-3 and up to 224 microg.m-3 that are higher than those found in outdoor air (4.7-15.7 microg.m-3; up to 23 microg.m-3). Characterized by a pungent and suffocating smell, it is released from many sources such as construction products, adhesives, detergents, insecticides, cosmetics, tobacco..., and its role on the ocular surface is still poorly understood. Dry eye disease and ocular allergy are growing ocular surface diseases in occidental countries. Our aim was to study the inflammatory effect of gaseous formaldehyde on the expression of the chemokines CXCL8/IL-8, CCL2/MCP-1 and adhesion molecule-1 CD54/ICAM-1 by human corneal epithelial cells in an air-liquid culture.

Methods

Human corneal cells (HCE, RCB 13, Riken Cell Bank, Tsukuba, Japan) at 4.10⁴ cell/insert were grown until subconfluence on transwell inserts before being placed in exposure chambers (Vitrocell®, Germany; Fig. 1).

Four conditions were tested for 30 min:

standard medium without (negative control) or with TNFalpha (2ng/ml, positive control) or, after removal of the supernatant, AIR or FA (50 microg/m3) generated from a known amount of FA, nebulized into a glass chamber.

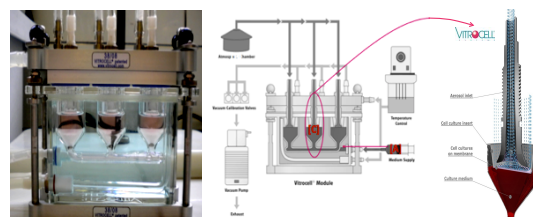
Then, the cells were returned to standard medium for 24 hours.

ASSAYS:

- Bicinchnonic Acid Protein assay and LDH activity assessment (Siemens, Germany),
- CXCL8/IL-8 and CCL2/MCP-1 ELISA assays in the supernatant (R&D Systems),
- Flow cytometry analysis of ICAM-1 (Dako) and MCP-1 (R&D Systems) expressions
- Immunofluorescence stainings of MCP-1 on the inserts after a TNFalpha prestimulation at 1ng/mL. One-way ANOVA with post-hoc Bonferroni test was used to compare the conditions between them.

Figure 1: Left: exposition system (VITROCELL® Systems GmbH, Waldkirch, Germany) is composed of 3 exposition chambers containing 3 inserts in which cells were seeded.

Right: detail of the air flux



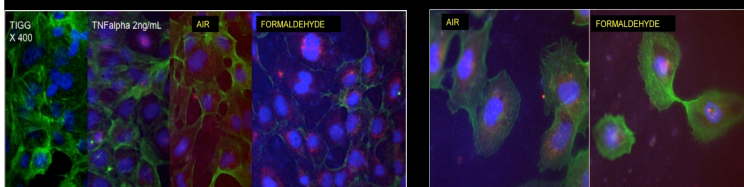
Results

Protein assessment showed a decrease for FA and TNF without any statistically significant difference when compared to DMEM and Air (Fig. 3A). The LDH (Fig. 3B) activity significantly increased when cells were exposed to AIR or FA with FA values higher than Air values. Air did not induce chemokine secretion in contrast to FA and TNF with FA < TNF (Fig. C, D). CD54 showed an increasing trend but without statistical significance (Fig. 3E). The immunostainings (Fig. 2) showed an increase of IL-8 and MCP-1 expressions in HCE following Air and FA with higher levels for FA. These expressions were confirmed for MCP-1 using flow cytometry (Fig. 3F, 3G). Moreover, we found an increase of these expressions under TNF/LPS inflammatory stimulations.

Figure 2:

MCP-1 : no stimulation

IL-8 : LPS stimulation



MCP-1 : LPS stimulation

MCP-1 : TNF stimulation

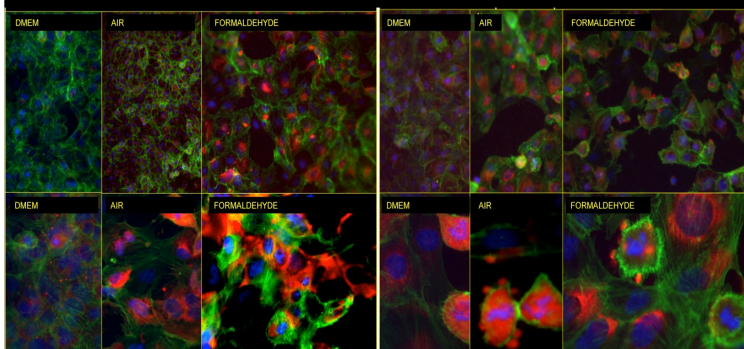
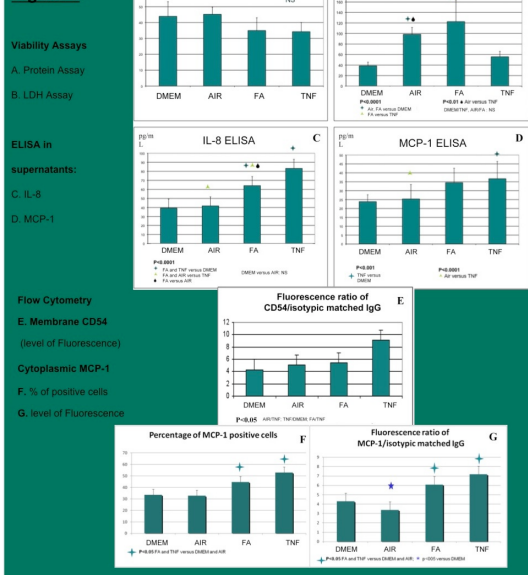


Figure 3:



Conclusion

This new air-liquid culture model mimicks the *in vivo* exposure of cornea. We have shown that exposure to air alters cell viability compared with incubation in standard medium, without inducing secretion of IL-8 or MCP-1. However, formaldehyde also alters cell viability and appears to induce the production of proinflammatory chemokines. These results confirm that the corneal cells are able to initiate pro-inflammatory mechanisms and apoptosis following exposure to formaldehyde.

These findings may explain the clinical manifestations of ocular surface irritation and discomfort observed in indoor environments and reported in the literature.

References

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