Smoke Inhalation – Decontamination Study of Toxic Substances on an in vitro Model of Human Airway Epithelium

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Background:
Smoke inhalation is nowadays considered as the major cause of morbidity and mortality in victims of fire. The inhalation of mixtures of toxic or corrosive gaseous molecules create cellular damages. Then, an inflammatory cascade is induced, that often leads to acute respiratory distress syndrome (ARDS) and potential death of victims. In order to design efficient solutions for pulmonary decontamination, the toxicity of smokes on lungs has to be qualified and quantified. The following data aim to show our recent advances on the topic, since our communication at the last EAPCCT Annual Congress in 2012.

MATERIAL AND METHODS

A. MucilAir™: Long Shelf Life In Vitro Airway Tissues

Isolation, amplification & seeding

Primary Human Cells

Air-Liquid interface, differentiation

Nose

Trachea

Bronchi

Mucus

Ciliated Cells

Goblet Cells

Basal Cells

Each reconstituted epithelium contains approximately 400,000 cells (basal, goblet, mucus and cilia cells) and can remain at a homeostasis state for more than 1 year. The co-cultured primary cells come from a pool of 14 donors (MucilAir™-Pool) in order that the model is representative of a population heterogeneity.

B. Contamination

Fire smoke may contain several kinds of physical or chemical species, depending on the material that burnt. Essentially there are 3 kinds of components capable of causing damage: particulates, systemic toxins, and respiratory corrosive, irritant or toxic gaseous molecules. Although the toxicity of systemic toxins such as CO or HCN are rather well documented, the effect of pulmonary irritants remains unknown.

For the present study, we chose 3 representative substances that may occur in smoke:

• An acidic corrosive substance: hydrochloric acid (HCl)
• An alkaline corrosive substance: ammonia (NH₄OH)
• An alkylating toxic compound: acrolein (H₂C=CH-CHO)

C. Protocol

20 min apical exposure to 100 µl of contaminant

Among several analytical means to quantify the damage to airway epithelium, the measurement of the trans-epithelial electric resistance (TEER) seems to be the earliest and the most accurate parameter to visualize a detrimental effect on the barrier function. Moreover, this measurement can be performed continuously in order to get kinetics of the damage on airway epithelium.

RESULTS

A. Corrosive Compounds: Hydrochloric Acid (HCl) and Ammonia (NH₄OH)

When exposed to hydrochloric acid or ammonia, the epithelium is able to resist up to an HCl concentration of 25 mM or NH₄OH concentration of 50 mM respectively. Over these critical thresholds, the epithelium completely loses its integrity, within less than 10 minutes, and is unable to recover it.

B. Electrophilic Compound: Acrolein

Acrolein has a much higher toxicity to lung, as the epithelium is able to resist only at a concentration lower than 0.25 mM. Even at extremely low concentration (5 µM), an increase in the cilia beating frequency is observed. To some extent, the damage can be recovered in a few days if the concentration does not exceed 1 mM, but for higher concentration the damage is irreversible.

CONCLUSION

Some attempts of decontamination after a 20-minutes exposure with acrolein (1 mM) were performed, by washing the epithelium with a chemically active solution. These solutions were able to decrease the cytotoxic effect of acrolein, measured with the LDH release from the cells in the culture medium, with a dose-response relationship.

The effect of 3 typical contaminants in fire smoke has been quantified on a 3D reconstituted human airway epithelium. This allows a reliable way to evaluate the toxicity of contaminants on lungs. Among several possibilities to assess their toxicity, the measurement of the trans-epithelial electric resistance (TEER) is the easiest parameter to follow the intensity and the kinetics of the lesions.

With this knowledge, some solutions were designed for pulmonary decontamination in order to avoid irreversible damage to the epithelium. Cytotoxic effect of acrolein could be partially lowered by these solutions, that could be used in case of exposure to this toxic compound.

Finally, not only systemic toxins such as CO or HCN, but also pulmonary corrosive or toxic compounds present in smoke bring a contribution to the airway damage of fire victims.