Epithelix

in vitro Respiratory Solutions

Ciliopathic and inflammatory effect of Poly (I:C) on *in vitro* 3D human airway epithelium

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Introduction

As first line of defense against airborne pathogens, the airway epithelium reacts through effective mucociliary clearance and secretion of inflammatory mediators. Poly (I:C), a mimetic of respiratory viruses, is capable of activating Toll-like receptor such as TLR3 on the airway epithelial cells. In this *in vitro* study, we would like to better understand the effect of Poly (I:C) on airway epithelial function using a multi-endpoints approach.

1-Testing strategy

An *in vitro* human airway model based on a pool of nasal cells from 14 donors (MucilAir-Pool™) was used to assess acute as well as repeated dose effect of polyinosinic-polycytidylic acid (Poly (I:C)). The epithelial model is fully differentiated and tight, exhibiting efficient mucociliary clearance function (MCC). The tissues* were exposed either to 3; 10 and 30 µg of Poly (I:C) apically in solution (20 µl) during 96h (Single Dose or **SD**), or to 10 µg of poly (I:C) every day during one week (Repeated Dose or **RD**). Trans-epithelial electric resistance (TEER), cytotoxicity (LDH), Mucociliary clearance (MCC) and soluble factor release (Il-8; Il-6; RANTES and INF-λ) were assessed simultaneously.

**The surface of one 24-well format insert is 0.33cm²*

1.1 Single dose exposure (SD)

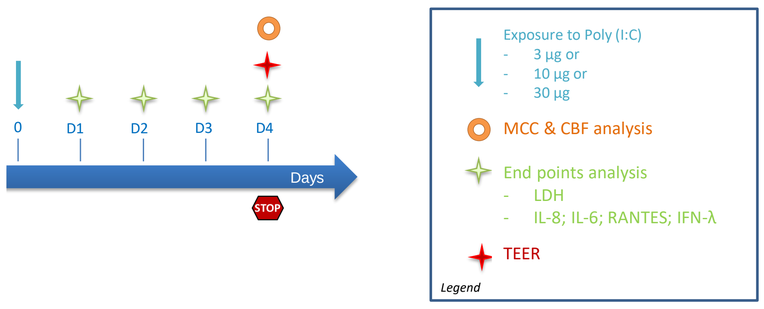


Figure 1: Single dose testing strategy (SD)

1.2 Repeated dose exposure (RD)

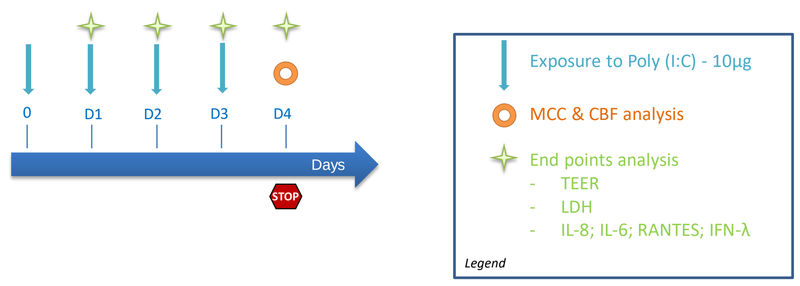


Figure 2: Repeated dose testing strategy (RD)

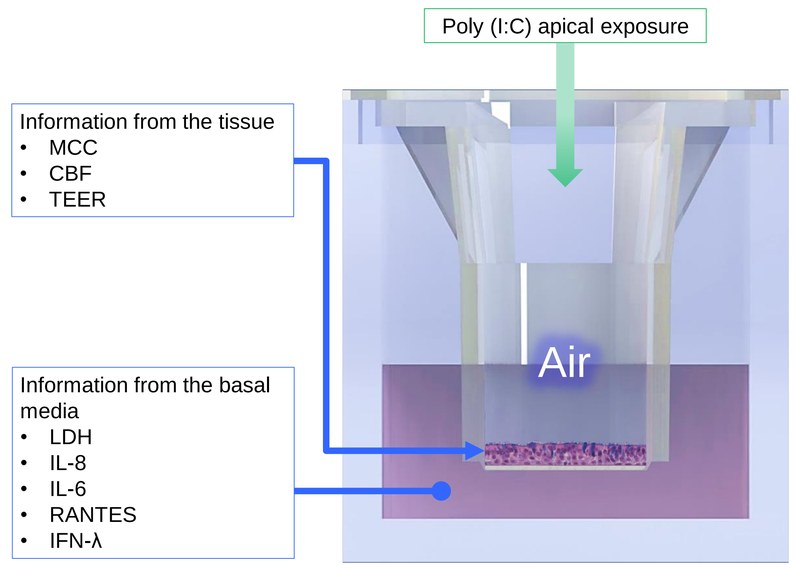


Figure 3: Exposure and endpoints on a 24-well format MucilAir™ Pool insert

2- Experimental results

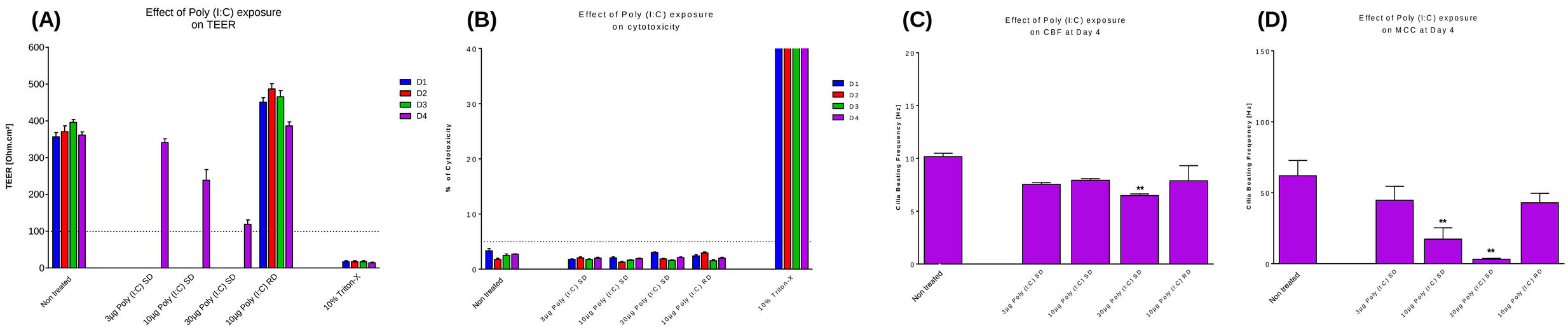
2.1 Markers of toxicity

Triton X-100 (10%) was used as a positive control to lyse the cells inducing loss of tissue integrity and 100% cytotoxicity,

- **Fig A**: Single doses of Poly (I:C) induced dose dependent decrease of TEER. Repeated 10 µg exposure of Poly(I:C) didn't affect tissue integrity.
- **Fig B**: Poly (I:C) didn't induce cytotoxicity (≤ 5 %) for all tested conditions.

2.2 Effect on cilia activity

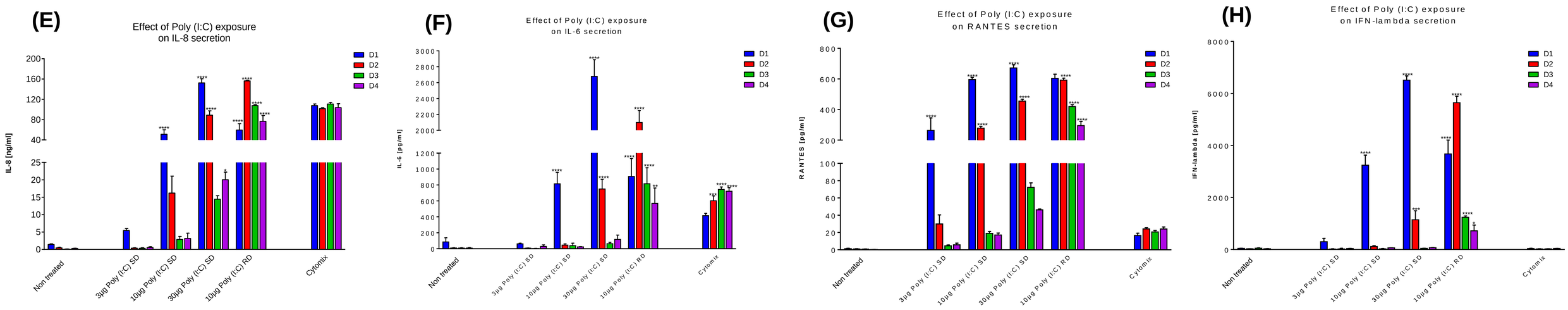
- **Fig C**: Exposure to Poly (I:C) induced a decrease of cilia beating frequency at Day 4. The decrease was only significant for single dose of 30 µg SD.
- **Fig D**: Single exposure to Poly (I:C) induced a dose dependent decrease of mucociliary clearance at Day 4, while repeated dose exposure didn't induce significant change of MCC.



2.3 Soluble factors release

Cytomix was used as positive control to induce inflammation of the tissues.

- **Fig E**: Exposure to Poly (I:C) induced a dose dependent increase of IL-8 release. Single dose exposure showed transient increases, while repeated dose exposure induced a stable increase of IL-8 concentration, comparable to Cytomix.
- **Fig F**: Single dose exposure to Poly (I:C) induced dose dependent, transient increase of IL-6 release, while repeated dose induced sustainable stimulation of IL-6 release.
- **Fig G**: Single dose exposure to Poly (I:C) induced a high, transient and dose dependant increase of RANTES release, while repeated dose induced longer stimulation of RANTES release.
- **Fig H**: Exposure to Poly (I:C) induced a high, transient and dose dependant increase of Interferon lambda release with a peak at Day 1 (for Single dose) or 2 (for repeated dose).



*All data shown for are the means +/- SEM errors from 3 biological replicates of MucilAir-Pool™ . Statistical comparison was performed using one-way ANOVA with Dunnett's post-tests comparing Poly (I:C)s to Non treated (Prism 6.0 GraphPad, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).*

Conclusion

Altogether these data suggest that Poly (I:C) induce a potent and typical inflammatory response with progressive loss of tissue integrity and mucociliary clearance function. Therefore, Poly (I:C) could be used as positive control to benchmark the effect of airborne substances on airway epithelium.