Salicylic acid and toluene diisocyanate enhance carbachol-induced bronchoconstriction in human precision-cut lung slices (hPCLS)

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Abstract
Asthma is characterized by airway inflammation, hyperresponsiveness (AHR), and remodeling. Human airway smooth muscle (HASM) cells are pivotal in mediating AHR in asthma. TDI induces airway exacerbations, but the mechanism is only partially understood. Salicylic acid (SA) is a known respiratory irritant and a, toluene diisocyanate (TDI) is a respiratory sensitizer. We hypothesized that SA or TDI alone induces AHR by enhancing carbachol (cch) induced airway narrowing. Human precision-cut lung slices (hPCLS) were exposed to vehicle, SA or TDI (0.01-10μM) for 24h. Cch dose-response was conducted. Cytokine/chemokine levels were determined in supernatants using Luminex multi-analyte array for mediators (IL-1α, IL-1β, IL-6, IL-8, IL-10, and TNF-α). HASM cells were exposed to SA or TDI (0.01 or 0.1μM) for 24h, basal and cch-induced myosin light chain phosphorylation (p-MLC) was determined by immunoblotting. 4A. TDI enhanced cch-induced bronchoconstriction (EC50) cch dose response curve, mean (SEM): 0.1μM-μM SA v. 0.25μM (vehicle). In HASM cells, SA enhanced both basal and cch-induced p-MLC levels. TDI-treated hPCLS enhanced cch-induced bronchoconstriction, characterized by increased area under the curve (mean values: 228±10M TDI v. 137±vehicle) and decreased LogEC50 (mean values: 0.05μM-10μM TDI v. 0.38±vehicle). TDI exposure enhanced basal p-MLC levels in HASM cells, but not cch-induced p-MLC levels. SA and TDI had little effect on cytokine/chemokine levels. Therefore, SA and TDI induce AHR by enhancing pro-contractile signaling in HASM cells, independent of inflammatory mediator release, supporting a central role for airway structural cells in mediating SA or TDI-induced AHR.

Hypothesis
Salicylic acid (SA) and toluene diisocyanate (TDI) modulate ASM cell shortening to eliciting AHR.

Conclusions
- Study establishes that PCLS can be utilized as an in vitro platform to study the physiological impact of materials on human lungs.
- Salicylic acid enhances MLC phosphorylation in HASM cells and agonist-induced airway narrowing in PCLS.
- TDI enhanced basal MYPT1 phosphorylation, with little effect on agonist-induced airway narrowing in PCLS.
- Overall, data suggests these materials modulate bronchial response through genomic or epigenetic mechanisms in structural cells of the airway, and target ASM cells to elicit airway hyperresponsiveness.

References
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Acknowledgments

Figure 1. SA and TDI enhance basal MLC phosphorylation in HASM cells (n=3 donors).

Figure 2. Twenty four h exposure to SA or TDI has little effect on carbachol (cch) or histamine-induced Ca2+ mobilization in HASM cells (n=5 donors, average RFU over baseline).

Figure 3. A) SA has little effect on basal MYPT1 phosphorylation whereas B) TDI enhanced basal MYPT1 phosphorylation in HASM cells (n=3 donors).

Figure 4. SA enhanced cch-induced airway narrowing in PCLS (n=4-5 donors, *p=0.043 Veh vs 1μM SA).

Figure 5. TDI has little effect on cch-induced airway narrowing (n=3-5 donors).