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

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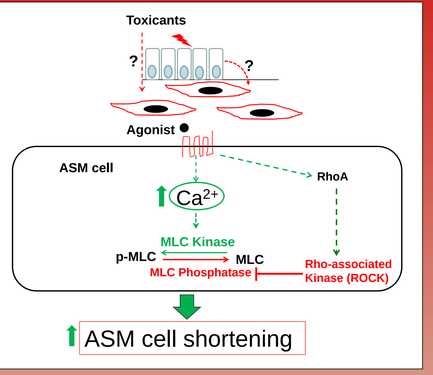
Abstract

Background & Hypothesis: Asthma is an airway disorder characterized by airway inflammation, hyperresponsiveness (AHR) and remodeling. Airway smooth muscle (ASM) cells play a pivotal role in mediating AHR in asthma. Occupational and environmental toxicants induce asthma exacerbations, although the mechanism of action is only partially understood. Salicylic acid (SA) is an irritant and a component in various cosmetic products available in the market. 2, 4-Toluene diisocyanate (TDI), a respiratory sensitizer, is used in polyurethane manufacture. We hypothesized that SA or TDI induces AHR by enhancing carbachol-induced airway narrowing and by altering inflammatory mediator release from airway structural cells. **Methods:** Human precision-cut lung slices (hPCLS) were exposed to vehicle, SA (0.01-10 uM) or TDI (0.01-10 uM) for 24 h. Carbachol (cch) dose-response was conducted and cytokine/chemokine levels were determined in the culture supernatants using Luminex® multi-analyte array for 11 representative mediators (MDC, IFN-g, GM-CSF, MCP-1 & 4, Eotaxin-1,2 &3, TARC,IL-8, IL-6, IL-1b and RANTES). Human airway smooth muscle (HASM) cells were also exposed to SA (0.1 or 1 uM) or TDI (0.01 or 0.1 uM) for 24 h and the basal and cch-induced myosin light chain phosphorylation (p-MLC) was determined by immunoblotting. **Results:** SA-treated hPCLS trended towards enhanced cch-induced bronchoconstriction (Log EC_{50} of cch dose response curve, mean values: 0.11 uM in 10 uM SA compared to 0.29 uM in vehicle, n=3). SA treatment has little effect on any of the 11 inflammatory mediators screened in the hPCLS supernanatants. In HASM cells, SA enhanced both basal and cch-induced p-MLC levels. TDI-treated hPCLS showed enhanced carbachol-induced bronchoconstriction, characterized by increased area under the curve (AUC) (mean values: 228 in 10 nM TDI compared to 157 in vehicle, n=2-3) and decreased Log EC_{25} (mean values: 0.04 uM in 10 nM TDI Vs 0.38 uM in vehicle, n=2-3) with little effect on inflammatory mediator levels in the hPCLS supernatants. In addition, TDI exposure enhanced the basal pMLC level in HASM cells with little effect on cch-induced pMLC levels. **Conclusions:** Our findings suggest that salicylic acid and toluene diisocyanate induce AHR by enhancing pro-contractile signaling in HASM cells, independent of inflammatory mediator release. These observations support a central role for the airway structural cells, especially ASM cells, in mediating SA or TDI-induced AHR.

Toxicant-induced AHR

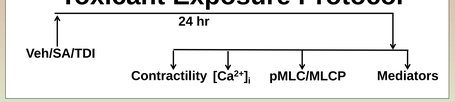
- Toxicants from household environment exacerbate asthma
- Salicylic acid (SA) is an irritant found in cosmetic products and toluene diisocyanate (TDI) is a respiratory sensitizer found in plasticizers.
- We determined the effects of SA and TDI on airway narrowing and pro-contractile signaling in ASM.



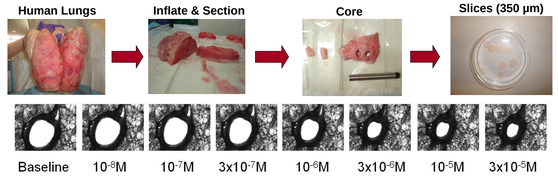
Hypothesis

Toxicants salicylic acid (SA) and toluene diisocyanate (TDI) modulate ASM cell shortening to elicit AHR

Toxicant Exposure Protocol



Human precision-cut lung slices (PCLS)[1]



SA enhances carbachol-induced airway narrowing in PCLS

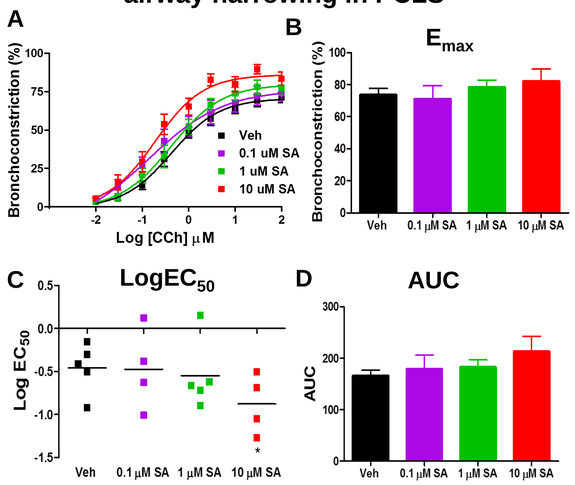


Figure 1. SA enhanced cch-induced airway narrowing in PCLS. (n= 4-5 donors, *p=0.043 Veh Vs 10 µM SA)

TDI has little effect on carbachol-induced airway narrowing in PCLS

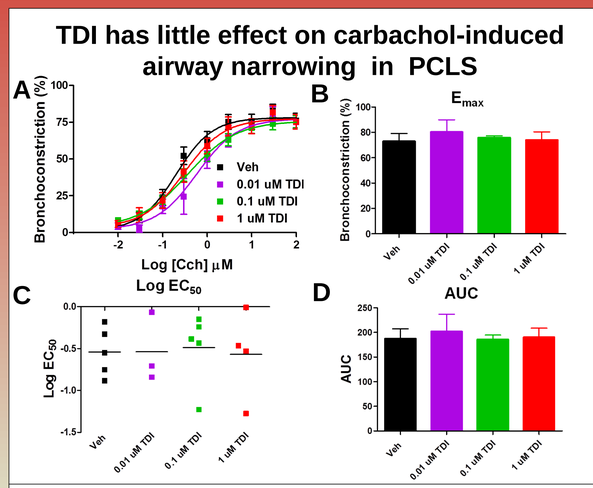


Figure 2. TDI has little effect on Cch-induced airway narrowing (n=3-5 donors)

SA or TDI has little effect on agonist-induced $[Ca^{2+}]_i$ in HASM cells

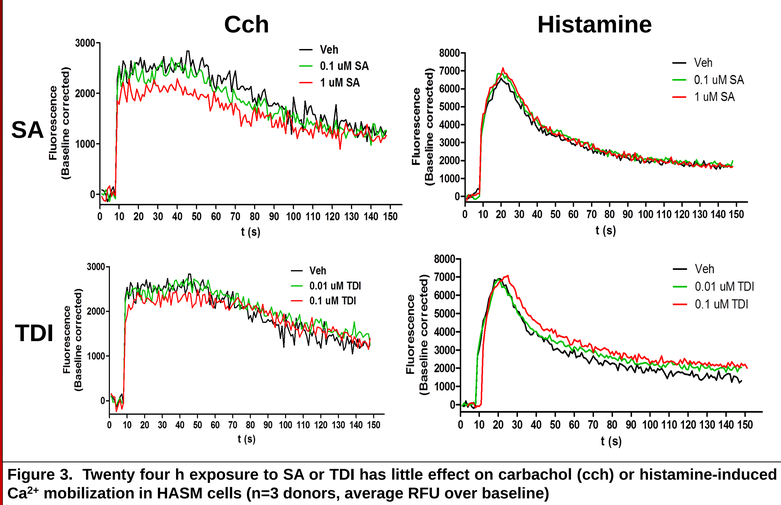


Figure 3. Twenty four h exposure to SA or TDI has little effect on carbachol (cch) or histamine-induced Ca^{2+} mobilization in HASM cells (n=3 donors, average RFU over baseline)

TDI enhances basal MYPT1 phosphorylation in HASM cells

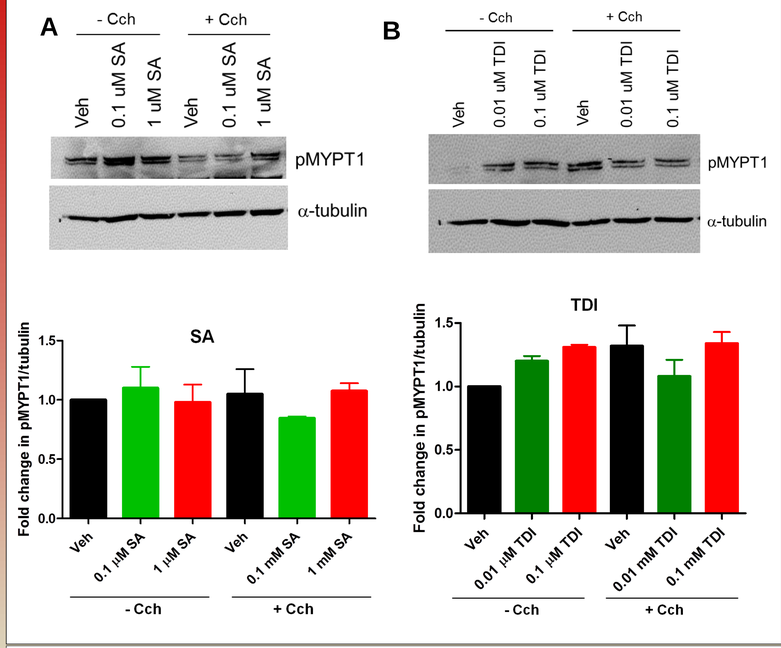


Figure 4. A) SA has little effect on basal MYPT1 phosphorylation whereas B) TDI marginally enhanced basal MYPT1 phosphrylation in HASM cells. (n=3 donors).

SA & TDI enhance MLC phosphorylation in HASM cells

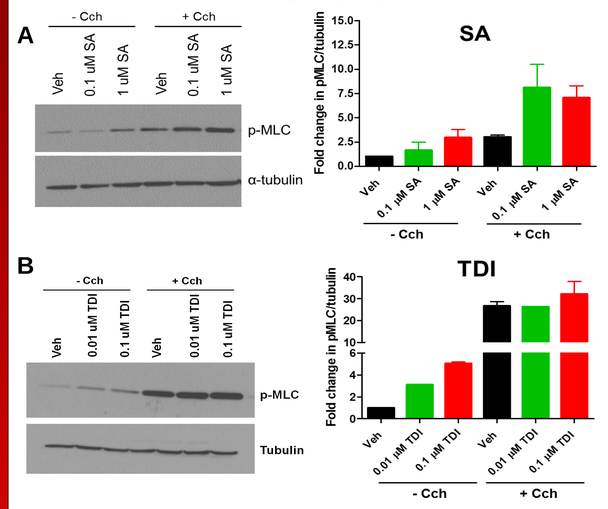


Figure 5. A) SA and B) TDI enhance basal MLC phosphorylation in HASM cells. (n=3 donors)

Summary

1. Salicylic acid (SA) enhances carbachol-induced airway narrowing in PCLS, while TDI has little effect on cch-induced airway responsiveness
2. Salicylic acid or toluene diisocyanate (TDI) has little effect on agonist-induced Ca^{2+} mobilization in HASM cells.
3. Salicylic acid and toluene diisocyanate enhanced MLC phosphorylation in HASM cells, while toluene diisocyanate enhanced MYPT1 phosphorylation.

Significance

Salicylic acid-induced AHR is mediated through altered contractile signaling in HASM cells. The smooth muscle-centric signaling can be a novel therapeutic target for toxicant-induced AHR

References

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2. Lauenstein L, Switalla S, Prenzler F, Seehase S, Pfennig O, Förster C, Fieguth H, Braun A, Sewald K. Assessment of immunotoxicity induced by chemicals in human precision-cut lung slices (PCLS). *Toxicol In Vitro.* 2014 Jun;28(4):588-99.
3. Joseph Jude, Cynthia Koziol-White, Jacqueline Scala, Edwin Yoo, William Jester, Christopher Maute, Pamela Dalton, Reynold Panettieri, Jr. Formaldehyde Induces Rho-associated Kinase Activity to Evoke Airway Hyperresponsiveness. *Am J Respir Cell Mol Biol.* First published online 5 May 2016 as DOI: 10.1165/rcmb.2015-0254OC.

Acknowledgments

This work is supported by Research Institute for Fragrance Materials (RIFM), NIH Training Grant T32-ES019851 & P30-ES013508 (CEET, University of Pennsylvania).