2,4-Dichloro-1-nitrobenzene (DCNB) Modulates Pro-contractile Signaling in Human Airway Smooth Muscle (HASM) Cells

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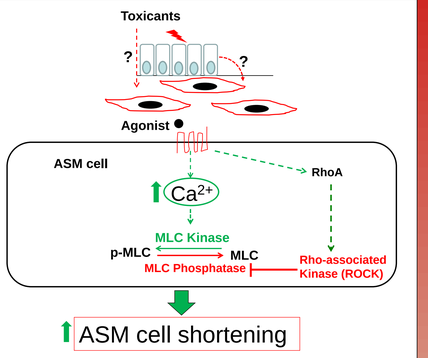
Abstract

Background: 2,4-dinitro-1-chlorobenzene is an organic industrial chemical and a contact sensitizer in occupational setting. Because of its ability to induce localized inflammatory reaction, DCNB is also used as a wart treatment. At occupational settings, inhaled DCNB acts as a respiratory toxicant. Given its sensitizer function in skin, inhaled DCNB may act on airway structural cells to modulate respiratory function. ***Hypothesis:*** To elucidate DCNB effect on human airways, we hypothesized that DCNB acts on human airway smooth muscle (HASM) cells to modulate its contractile and synthetic functions. ***Methods:*** HASM cells were exposed to vehicle, or DCNB (0.01, 0.1,1 uM) for 24 h and basal and carbachol-induced phosphorylation of myosin light chain (MLC) and MLC phosphatase were determined in cell lysates. Vehicle or DCNB-treated HASM cells were loaded with Ca^{2+}-binding dye and carbachol or histamine-induced intracellular Ca^{2+} ($[Ca^{2+}]_i$) was determined. Precision-cut human lung slices (PCLS) were exposed to vehicle or DCNB (0.1, 1 or 10 uM) for 24 h and supernatants were analyzed for a custom array of inflammatory and T_{H2} mediator levels. In PCLS exposed to vehicle or DCNB (10 uM), cilia beat frequency was determined. ***Results:*** DCNB increased MLC phosphorylation in HASM cells, with little effect on MLCP phosphorylation or carbachol/ histamine-induced $[Ca^{2+}]_i$ in HASM cells. In PCLS, upon 24 h exposure, DCNB did not have significant effect on the levels of 11 inflammatory mediators screened in this study. DCNB had little effect on cilia beat frequency. ***Conclusions:*** The industrial chemical DCNB increases myosin light chain phosphorylation in HASM cells, suggesting enhanced ASM cell shortening. DCNB has little effect on inflammatory mediator release or cilia beat frequency, suggesting that irritant injury may not be the primary mechanism of DCNB effect on airways. ***Implications:*** The findings show that the contact sensitizer DCNB modulates signaling mechanisms in an airway structural cell (i.e: ASM cells) independent of inflammatory response.

Toxicants & Lung Health

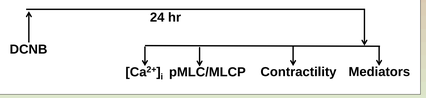
- Toxicants from household environment exacerbate asthma
- Dichloronitrobenezene (DCNB) is an industrial chemical and contact sensitizer
- We determined the effects of DCNB on contractile and synthetic signaling in human airway smooth muscle (HASM) cells.



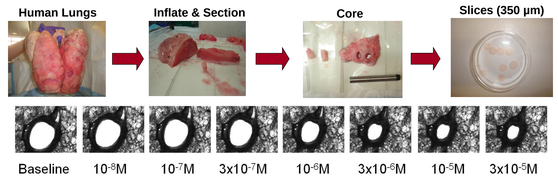
Hypothesis

Toxicant 2,4-dichloro-1-nitrobenzene modulates contractile and synthetic functions in human airway smooth muscle (HASM) cells

Toxicant Exposure Protocol



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



DCNB enhances MLC phosphorylation in HASM cells, with little effect on MYPT1 phosphorylation

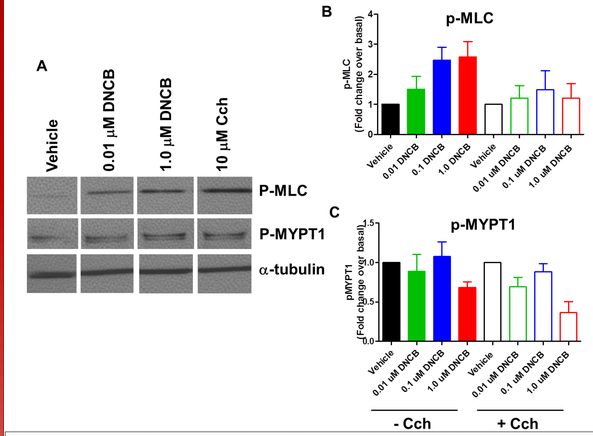


Figure 1. DCNB enhances A & B) Baseline MLC phosphorylation, with little effect on A & C) MYPT1 phosphorylation. (Representative of N=5 donors)

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

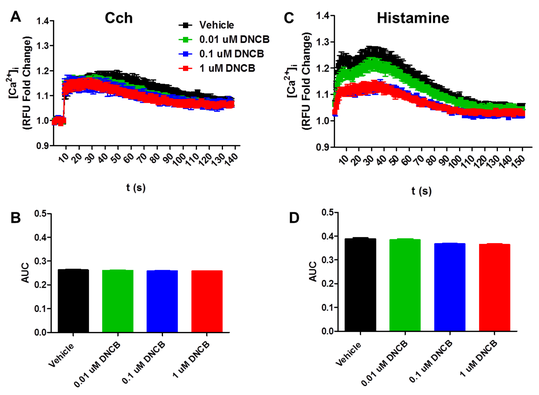


Figure 2. DCNB has little effect on A & B) Cch-induced or C&D) histamine – induced $[Ca^{2+}]_i$ in HASM cells (representative of n=2 donors)

DCNB induces Nrf-2-dependent antioxidant response in HASM cells

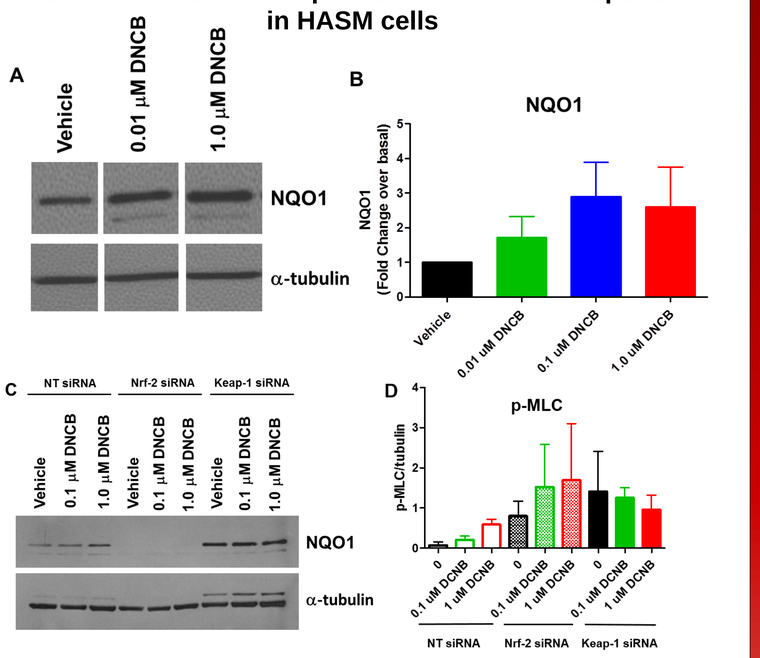


Figure 3. Twenty four h exposure to DCNB A& B) induces NQO1 expression, a marker of Nrf-2 activation (N=5 donors). C) Silencing of Nrf-2 abolishes DCNB-induced NQO1 expression D) with little effect on p-MLC level (n=3 donors).

DCNB has little effect on bronchoconstriction in precision-cut human lung slices (PCLS)

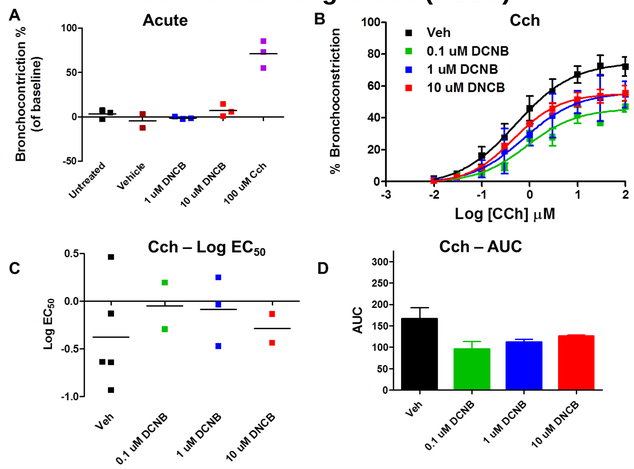


Figure 4. In PCLS A) DCNB has little effect on bronchoconstriction (n=3 donors). B-D) 24 h exposure to DCNB has little effect on carbachol-induced bronchoconstriction (n=2-5 donors).

DCNB has little effect on mediator release and cilia beat in PCLS

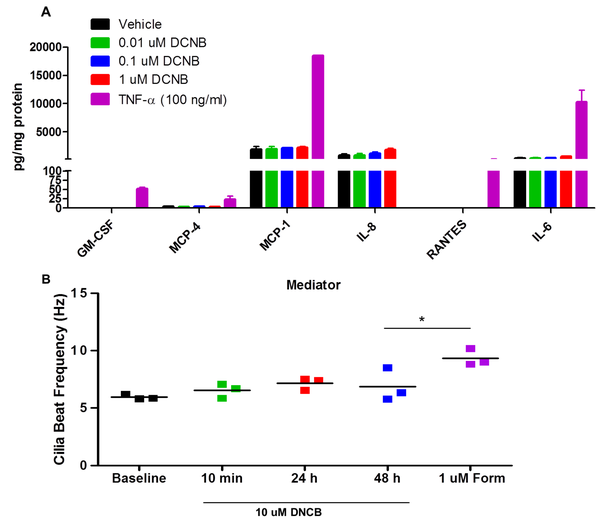


Figure 5. A) 24 h exposure to DCNB has little effect on inflammatory cytokine/chemokine release from PCLS (n=3 donors). B) DCNB (10 min- 48 h) has little effect on cilia beat frequency in PCLS (n=1 donor, with 3 technical replicates)

Summary

- Dichloronitrobenzene (DCNB) enhanced myosin light chain (MLC) phosphorylation in HASM cells, with little effect on agonist-induced $[Ca^{2+}]_i$ or MYPT1 phosphorylation.
- DCNB induced Nrf-2-dependent antioxidant/cytoprotective response in HASM cells.
- In PCLS, DCNB had little effect on inflammatory mediator release, cilia beat or bronchoconstriction.

Significance

The findings indicate that toxicants, such as DCNB, modulate contractile and synthetic signaling in human airway smooth muscle (HASM) cells. Interaction of HASM cells with other airway structural cells may determine the physiological outcome of DCNB exposure.

References

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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

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