Induction of the Inflammatory Protein Autotaxin – A New Hypothesis for a Mechanism Behind Airways Disease Caused by Aromatic Diisocyanates

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Research Purpose: To find new mechanisms behind health effects caused by aromatic diisocyanates.

Relevance and background: The mechanisms behind health effects induced by isocyanates are largely unknown. This limits the possibilities for diagnostics, treatment and prevention. In a previous cell study we (JH, US) found that toluene diisocyanate (TDI) induced autotaxin (ATX), a protein that produces lysophosphatidic acids (LPAs). LPAs are lipid products that induce inflammation, cellular migration and invasion.

Methods and analyses: We investigated the induction of ENPP2 mRNA and its protein, ATX, in A549 lung epithelial cells, stimulated with low levels of TDI. Purinergic receptor (PR) inhibitors were tested, and receptor interactions were studied using proximity ligation assay (PLA). In plasma samples from 147 workers exposed to TDI, methylene diphenyl diisocyanate (MDI) and naphthalene diisocyanate (NDI) and 87 controls, we analyzed four different LPAs using liquid chromatography-tandem mass spectrometry (LC/MS/MS) and evaluated their associations with biomarkers of exposure. We also assessed possible interactions between the biomarkers of exposure and some symptoms.

The workers included in the study were employed in 11 small isocyanate companies where most of them were engaged in manufacturing processes which included continuous foaming, but also flame lamination and preparations of TDI- and MDI-based polyurethane and isocyanate formulations such as joining and sealing compounds.

Results: In the cell model we have discovered that TDI (10 nM- 50 μM) induce a clear and up to ten times increase in the expression of ATX. A peak was seen at 16 h, affecting both intra- and extracellular levels. Inhibitors of PRs prevented induction of ATX by TDI, and PLA indicated interactions between purinergic receptors. In workers, the levels of total isocyanate metabolites in urine and LPA 18:0 (rS=0.45) as well as total LPAs (rS=0.47) were highly correlated. Furthermore, there was a significant interaction between total isocyanate metabolites in hydrolyzed urine and asthma for LPA 18:0 (pinteraction 0.022; Basthma 0.54, Bnot asthma 0.17). The interaction p-value using the total LPA levels was 0.052. There was also a significant interaction between total isocyanate metabolites in hydrolyzed urine and sneezing for LPA 18:0 (pinteraction 0.037; Bsneezing 0.30, Bnot sneezing 0.14). The interaction p-value for the total LPA levels was 0.021.

Conclusions: Our results show that exposure to aromatic diisocyanates increase the levels of ATX in vitro and the ATX-products LPAs in vivo. Furthermore, at the same exposure level, the concentrations of LPAs are higher in asthmatics and persons with airways irritation than in non-symptomatic persons. This suggests a role of ATX and LPAs in the mechanism of isocyanate induced airways disease. A complex interaction between PRs might trigger ATX. The use of ATX or PR inhibitors may offer a way to reduce ATX and LPA production upon isocyanate exposure which might decrease the airway reactions.

Implications: This study has produced important knowledge about the mechanism behind airway disease of isocyanates (and maybe also in general). This may improve diagnostics, prevention and even treatment of such diseases.

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Monoclonal Antibodies (mAbs) to Methylene Diphenyl diisocyanate (MDI) and Toluene Diisocyanate (TDI) Conjugated Protein: Research and Potential Biomonitoring Applications.

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Diisocyanates are known immunological sensitizers through both dermal and pulmonary routes of exposure. Research into specific pathophysiological mechanisms and immunologically relevant biomarkers of disease have been hampered by the lack of diisocyanate (dNCO)-specific immunochemical reagents.

We have previously reported the production of IgG mAbs that recognize TDI conjugated proteins independent of the specific protein. A hybridoma that produces MDI-protein specific IgM mAbs, which also has good cross-reactivity to hexamethylene diisocyanate conjugated protein, has recently been isolated and cloned. Effort's to create a hybridoma that produces an anti-MDI-protein IgG are ongoing.

The dNCO mAbs have proven useful for several research applications including Western Blotting of diisocyanate conjugated proteins and conjugated proteins from TDI exposed cells; immunochemical staining of dermal tissue and enzyme-linked immunoassays (ELISA). Affinity constants for the TDI mAb are very high (108-1010 M-1) suggesting greater utility for immunoassay and immunohistochemistry, but limited use for immunoprecipitation/protein isolation. TDI conjugated human albumin (TDI-HSA) could only be dissociated from a mAb-TDI-HSA complex under harsh denaturing conditions.

Preliminary data suggest that both the MDI and TDI mAb may have utility in ELISAs for measurement of dNCO conjugated proteins in sera. Immunohistochemical dermal exposure studies where 4% TDI was applied to mouse ears have also been conducted.

These studies demonstrated that TDI-conjugated proteins were located mainly in the stratum cornum and in the epidermis particularly in hair follicles and associated sebaceous glands in the dermis. These preliminary studies suggest potential use of dNCO mAb for assessment systemic and dermal exposure biomarkers and also for research.

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