

Isothiocyanates as Skin Sensitizers

*Bioactivation and Distribution in Skin and
Draining Lymph Nodes*

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2010

Akademisk avhandling för avläggande av filosofie doktorsexamen i kemi som, med medgivande av Institutionen för Kemi, Göteborgs Universitet, kommer att försvaras offentligt fredagen den 26:e november kl 13.15 i föreläsningssal KB, Kemigården 4, Göteborgs Universitet och Chalmers tekniska högskola. Avhandlingen kommer att försvaras på svenska.

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Abstract

Development of contact dermatitis can be caused by electrophilic compounds (haptens) or prohaptens that come into contact with the skin. A prohaptent is a compound seemingly harmless at first sight that can be transformed into reactive metabolites by metabolizing enzymes in the skin to become a hapten. The prerequisites for a compound to induce contact allergy are, absorption into the skin, bioactivation of prohaptens, and reaction with endogenous proteins. The hapten-protein complex will be further processed into antigens inducing an immune response. The major aims of this thesis were: to investigate the distribution of topically applied sensitizing isothiocyanates in human- and mouse skin, to localize hapten-bound proteins in lymph nodes of exposed mice, and to explore the possibility of isothiocyanates to be formed in the skin via bioactivation.

In this thesis the isothiocyanate moiety was used as a reactive handle on three fluorescent compounds; fluorescein isothiocyanate (FITC), rhodamine isothiocyanate (TRITC), and rhodamine B isothiocyanate (RBITC). The non-sensitizers fluorescein and rhodamine were used as controls. The distribution of the fluorescent compounds, of varying reactivity, in skin was visualized and FITC and TRITC were mainly located in the *stratum corneum* (SC) whereas fluorescein was evenly distributed and rhodamine did not penetrate into the skin to any significant degree. Even though TRITC was an extremely strong sensitizer and mainly stayed in SC, the majority of the lymph node cells of TRITC-exposed mice were fluorescent. The fluorescence was found to be incorporated in cytosolic proteins and we identified high mobility group box 1 (HMGB1) as a haptenated protein. HMGB1 is a nuclear binding protein that can also be secreted. It is described as an endogenous adjuvant and is e.g. important for DC migration. Thus, this protein might play several roles in the development of allergic contact dermatitis. To rule out which role HMGB1 plays is beyond the scope of this thesis but of major interest for future investigations.

Isothiocyanates can be identified as degradation products from substituted thioureas. Diphenylthiourea (DPTU) is a known skin sensitizer used as a vulcanization accelerator in the production of e.g. neoprene. The versatile usage of neoprene is due to the multifaceted properties of the material, it is e.g. stretchable, waterproof, and chemical- and abrasion-resistant. It has resulted in numerous case reports of dermatitis patients allergic to DPTU. Also, simultaneous reactions to DPTU and phenyl isothiocyanate (PITC) in patients have been reported. Hence, the ability of DPTU to form reactive metabolites by skin metabolism was investigated. Using a skin-like cytochrome P450 (CYP) cocktail, four metabolites of DPTU were identified and adducts from trapping experiments revealed that PITC was formed upon bioactivation and not only by thermal degradation of DPTU. PITC was further metabolized into phenyl isocyanate (PIC), an even stronger sensitizer than PITC. The major adducts formed are thought to be derived from sulfenic, sulfinic and sulfonic acids of DPTU. Thus, the acids of DPTU and/or PITC, and PIC might be responsible for the allergenic activity of DPTU.

In conclusion, isothiocyanates are, due to the isothiocyanate moiety, potent contact allergens. After topical exposure, isothiocyanates bind to SC, but a sufficient amount enter the skin and are further transported to the draining lymph nodes able to cause sensitization. In the draining lymph nodes, different cell types are target cells, and HMGB1 was identified as a target protein. It is of future interest to trace hapten target proteins *in vivo* to be able to reveal the mechanisms behind the development of contact allergy more in detail. Furthermore, we have shown that isothiocyanates can be formed by metabolic activation of prohaptens by skin CYP enzymes. Knowledge regarding biotransformation of compounds is important to be able to predict allergenic activities of compounds not allergenic themselves. Using adequate metabolites for patch testing could increase the possibility of a correct diagnosis which otherwise would have been missed due to a too low concentration of the metabolite formed in the test situation.

Keywords: Allergic contact dermatitis, Covalent binding, Biotransformation, Diphenylthiourea, Cytochrome P450, Gel electrophoresis, Isothiocyanate, Laser scanning confocal microscopy, Local lymph node assay, Metabolism, Mouse ear swelling test, Penetration, Prohaptent, Sensitization, Skin, Two-photon microscopy. ISBN: 978-91-628-8201-3