ABSTRACT

The work presented in this thesis emphasises the importance of considering skin metabolism in toxicity assessment of chemicals used in contact with the skin. Conversion of seemingly harmless chemicals to reactive metabolites by xenobiotic-metabolising enzymes can lead to the development of contact allergy. These compounds are referred to as prohaptens and the activation process is known as bioactivation or metabolic activation. Once a person is sensitised, the allergy remains throughout life since no curative therapy is known. Thus, it is important to identify prohaptens and evaluate their sensitising potency in order to make reliable risk assessments. The overall aims of this thesis were: to investigate mechanisms of cutaneous bioactivation, to explore structure-allergenic activity relationships for prohaptens, and to develop a skin-like cytochrome P450 (CYP) cocktail for use in studies of CYP-mediated skin metabolism.

In this study, the bioactivation of conjugated alkenes and α,β-unsaturated oximes, two previously unknown classes of prohaptens, was investigated. *In vivo* and *in vitro* studies of metabolic activation and allergenic activity showed that conjugated dienes and α,β-unsaturated oximes are activated by CYP to form highly reactive and sensitising epoxy and nitroso species respectively. The structure-activity relationship that was formulated showed that conjugated dienes in or in conjunction with a six-membered ring are prohaptens, and that an α,β-unsaturation is necessary for an oxime to be a sensitiser. In addition, increased steric hindrance around the α,β-unsaturation of oximes leads to a reduction in sensitising capacity. The isolated alkenes and saturated oximes studied were found to be non-sensitising. An acyclic alkene and a similar acyclic oxime were found to be considerably less sensitising compared to their cyclic analogues. This knowledge could be used in the design and selection of non-allergenic alternatives to currently-used allergenic chemicals.

Knowledge regarding the biotransformations which take place in the skin is of importance in the design of robust assays for the prediction of contact allergenic activity. For this reason, a skin-like CYP cocktail was developed, based on studies of CYP expression in human skin. The cocktail consists of the five most common CYP isoforms found in the skin, but with a higher total CYP content in order to increase the metabolic activity in *in vitro* experiments. This CYP cocktail was found to convert a conjugated diene, an α,β-unsaturated oxime and an allylic alcohol into skin sensitising metabolites. Combined with an *in vitro* predictive assay, this skin-like CYP cocktail would provide a metabolic step which would make such an assay prohapten detecting.

Keywords: Allergic contact dermatitis, Bionucleophile, Cytochrome P450, Dendritic cell, Epoxide, FCAT, Glutathione, LLNA, Metabolism, Metabolic activation, Nitroso, Prohapten, Sensitisation.

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