Prohaptten-activation by human cutaneous cytochrome P450 isoenzymes – identified with a modified KeratinoSens assay

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BACKGROUND/AIMS

Skin is a uniquely susceptible target organ for allergic contact dermatitis to environmentally encountered haptens that can form antigens. The prevailing mechanistic explanation for these types of allergic responses requires some type of chemical activation that drives binding of the hapten or prohaptten to macromolecules to form an antigen. Some haptens are sufficiently reactive to bind directly but other less reactive prohaptens require metabolic activation in the skin to haptens. As an approach to reducing the need for experimental animals one has attempted to develop non-animal alternatives an example of which is the KeratinoSens assay. However, the identification of prohaptens in these assays has proven to be challenging because of inconsistent prohaptten bioactivation. Preincubation or coincubation of the prohaptten with single cytochrome P450 isoenzyme (CYP) cocktails or CYP-containing microsomes are gene-rally very cytotoxic. Accordingly the use of the cytosolic S9 fraction prepared from rat liver has been tried. One limitation of this approach is that rat liver S9 does not contain human skin CYPs that might be capable of activating these prohaptens. Therefore our aim was to redesign the protocol by spiking rat liver S9 with human skin CYPs and measure the activation of NRF2.

METHODS

KeratinoSens cells were seeded in 96-well plates in medium without G418. Each 96-well plate was seeded in duplicate to examine the luciferase activity and the viability at the same time. Carvoxime and creosol were dissolved in DMSO and diluted to obtain six concentrations. Then, the solutions were diluted 25-fold without or with S9 fraction and CYP cocktail or single CYP 1B1. CYP cocktail and single CYP 1B1 were prepared as described previously (Bergström et al., 2007). Each solution was added in triplicate so that the final S9 concentration was 1%. After 48h incubation the luciferase activity and the viability were examined. Quantification of luciferase was used as a measure of activation of NRF2 and has been validated as a useful *in vitro* system for assessing the skin sensitizing potential. MTT assay was used to assess the cellular cytotoxicity. Cinnamic aldehyde was included in each 96-well plate as a positive control since it does not require metabolic activation as well as control wells with cells and solvent for treatment without or with S9 fraction and CYP cocktail. The cutaneous metabolom of the fragrance creosol was analyzed by HR-MAS NMR (High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance) following its topical application to a 3D human skin equivalent.

RESULTS

CONCLUSIONS

- The fragrance creosol could be characterized as a prohaptten by HR-MAS NMR after its application to a 3D human skin equivalent.
- Using a modified KeratinoSens assay, addition of the prohaptens carvoxime and creosol to the spiked preparation strongly activated NRF2 whereas heat inactivated preparations showed no reactivity.
- These results confirm that carvoxime and creosol are specifically activated by CYPs.
- Spiking of rat liver S9 with cutaneous CYPs increases the sensitivity of the KeratinoSens assay in identifying prohaptens specifically activated by cutaneous CYP isoenzymes.

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Literature: Bergström et al. (2007) A skin-like cytochrome P450 cocktail activates prohaptens to contact allergenic metabolites. J Invest Dermatol, 127, 1145-53; Natsch, A. & T. Haupt (2013) Utility of rat liver S9 fractions to study skin-sensitizing prohaptens in a modified KeratinoSens assay. Toxicol Sci, 135, 356-68.