UNDERSTANDING THE ROLE OF SKIN METABOLISM IN ALLERGIC CONTACT DERMATITIS: GLUTATHIONE AS A CLEARANCE SYSTEM

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SKIN ALLERGY AOP

Source to Outcome Pathway

Need to understand clearance processes in skin

The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins, OECD, 2012
GLUTATHIONE IN BIOLOGICAL SYSTEMS

GSH important in detoxification of xenobiotics in liver, kidneys

Glutathione metabolism in human skin has not been fully characterised yet. Study focused on presence of enzymes or conjugation of specific species.


Average skin area: 2m² in adults
GST activity in human skin:
30 nmol/min/µg cytosolic protein (epidermis) (Harris et al, 2002)
90 nmol/min/mg of whole skin (van Eijl et al, 2012)

GST activity in human liver:
119 nmol/min/mg (Baars et al, 1981)

<table>
<thead>
<tr>
<th>Total GSH (nmol/g)</th>
<th>Technique used</th>
<th>Number of donors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>480 (epidermis) 85 (dermis)</td>
<td>Ellman’s reagent- GR recycling assay</td>
<td>6 (healthy)</td>
<td>Shindo et al (1994)</td>
</tr>
<tr>
<td>250-450 (epidermis) 280-530 (dermis)</td>
<td>Ellman’s reagent- GR recycling assay</td>
<td>7 (mean 21y.old) 9 (mean 71y.old)</td>
<td>Rhie et al (2001)</td>
</tr>
<tr>
<td>195 (healthy whole skin) 442 (lesional skin)</td>
<td>Ellman’s reagent- GR recycling assay</td>
<td>5 (healthy skin) 6 (ICD or ACD on hands)</td>
<td>Kaur et al (2001)</td>
</tr>
</tbody>
</table>
GLUTATHIONE CONJUGATION

Reactivity of electrophiles with GSH has been studied *in chemico* (Gerberick et al 2004, Schultz et al 2007, Enoch et al 2012).

Objectives:
- Demonstrate depletion of GSH in biologically relevant system
- Adaptive response after exposure to model sensitisers
GLUTATHIONE CONJUGATION

HaCaT cell line evaluation as a skin model
Model sensitisers: 1-X-2,4-dinitrobenzene with X= F, Cl, Br

GSH is depleted quickly in cells (metabolism by conjugation).

Nrf2 activation after 2h is observed.

Repletion of GSH after 24hrs is observed with variations between compounds at “tipping point” (10uM)
GLUTATHIONE OXIDATION

**Analysis by LC-MS:**
- GSSG m/z 613.10 > 355.10
- GSH (derivatised with iodoacetic acid) m/z 366.02 > 173.80

- No GSSG above Limit of Quantification
- Require inhibitor of Glutathione Reductase
- Recycling of GSSG and provision of fresh GSH happen within 1h

**Phenylacetaldehyde dose response at 10min**
- GSH in µM
- Toxicity observed after 2h

**Benzaldehyde dose response at 10min**
- GSH in µM
- Toxicity observed after 2h
3D SKIN MODELS

Single exposure:
Proof of principle that metabolism takes place in model

Daily exposure: -> Build a scenario that is closer to consumer exposure.
Model sensitiser at non toxic dose for 2h followed by 22h of recovery time.
Aim: Evaluate GSH level in human skin.

Practical considerations:

1) Use frozen skin from various sources.
   - ✓ Higher number of samples to be expected
   - X Loss of metabolic activity
   - X No information about potential skin conditions in donors

2) Use of ex vivo skin obtained post surgery.
   - ✓ Information about potential skin conditions in donors
   - ✓ Potential preservation of metabolic activity
   - X Limited number of samples

3) Use of commercially available ex vivo skin.
   - ✓ Higher number of samples and easy format
   - X No information about skin conditions in donors
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THANK YOU