Use of the *Caenorhabditis elegans* as an alternative model for evaluating the allergen potential of skin sensitizers

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Advisor: Prof Jane Zveiter de Moraes
Animal testing for the production of cosmetic has been banned;

Research about efficient alternative methods;

**skin sensitization**;

ACD (allergic contact dermatitis) – type IV hypersensitivity reaction, induced by repeated contact with sensitizers.

Adverse Outcome Pathway for skin sensitizers

Adverse Outcome Pathway for skin sensitizers

Adverse Outcome Pathway

Key event 2

Induction of cytoprotective genes;

Keap1-Nrf2-ARE pathway

Adverse Outcome Pathway

Key event 2

Induction of cytoprotective genes;

FOXO pathway

• **ORTHOLOGS**: genes in different species that evolved from a common ancestral gene by speciation. Retain the same function in the course of evolution.

- **Mammalian**
  - Keap1-**Nrf2-ARE**
  - JNK-**FOXO**

- **C. elegans**
  - p38 MAPK - **SKN1-ARE**
  - JNK1(MAPK) - **DAF16**

• Both pathways are activated in response to oxidative stress.
Genetic modification in *C. elegans*
<table>
<thead>
<tr>
<th>STRAINS</th>
<th>GENETIC MODIFICATION</th>
<th>ORTHOLOG C.ELEGANS</th>
<th>ORTHOLOG MAMMALIAN</th>
<th>MAMMALIAN SIGNALING PATHWAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF1553-<em>psod3::GFP</em></td>
<td>Addition of GFP molecule in the <em>sod3</em> promoter</td>
<td>DAF-16 – active <em>sod3</em></td>
<td>FOXO (Forkhead box)</td>
<td>JNK-FOXO</td>
</tr>
<tr>
<td>CL2166-<em>pgst4::GFP</em></td>
<td>Addition of GFP molecule in the <em>gst4</em> promoter</td>
<td>SKN-1 – active <em>gst4</em></td>
<td>Nrf2 (nuclear factor erythroid 2-related factor 2)</td>
<td>Keap1-Nrf2-ARE</td>
</tr>
<tr>
<td>N2 BRISTOL</td>
<td>Wildtype</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>
Cell culture x *C. elegans* models

- **Simple Cell Culture model**
  - Very expensive;
  - Cellular Monolayer;
  - Contamination;
  - Loss of phenotypic characteristics;

- **C. elegans model**
  - Low cost;
  - Easy manipulation;
  - Innate immune system;
  - Collagenous cuticle and 4 layers epidermis;
  - Sequenced genome in database;

**Systemic Model**

- **Skin sensitization involves several layers and cells !!!!!**
**WHY C. ELEGANS IS AN ALTERNATIVE MODEL?**

- **Sentience** is the ability of beings to feel sensations and feelings consciously;
- Be sentient means being conscious
- Be sentient is able to be affected positively or negatively

**C. elegans IS NOT SENTIENCE**

**C. elegans model:**

- Nematode – 1mm
- Feeds *E.coli*
- Lives in petri dish
- High reproduction rate (~200 eggs)
- Lives 28-30 days
- Transparent (fluorescent)
- Whole genome was sequenced

Objective:

The present work aims to verify whether the nematode *C. elegans* can help in the evaluation of allergenicity potential.
Growth curve

Sodium Hypochlorite 5%
Sodium Hydroxide 2,5M

Lysing solution

Culture with a large number of gravid hermaphrodites

Only eggs

Counting Worms
- Larvae – Young Adults - Adults
Growth curve

CF1553/DAF-16

CL2166/SKN-1

N2 Bristol

- Adults
- Young Adults
- Larvae

Day 01  Day 02  Day 03  Day 04

Day 01  Day 02  Day 03  Day 04

Day 01  Day 02  Day 03  Day 04

**Chemicals used in the present study**

<table>
<thead>
<tr>
<th>CHEMICAL</th>
<th>Classification LLNA</th>
<th>In vitro classification</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO <em>(Dimethyl sulfoxide)</em></td>
<td>Vehicle</td>
<td>Vehicle</td>
<td>Solvent</td>
</tr>
<tr>
<td>DNCB <em>(2,4-Dinitrochlorobenzene)</em></td>
<td>S (extreme)</td>
<td>S</td>
<td>Solvent</td>
</tr>
<tr>
<td>PFA <em>(Formaldehyde)</em></td>
<td>S (strong)</td>
<td>S</td>
<td>Preservative</td>
</tr>
<tr>
<td>2-MBT <em>(2-Mercaptobenzothiazole)</em></td>
<td>S (moderate)</td>
<td>NS</td>
<td>Preservative</td>
</tr>
<tr>
<td>EU <em>(Eugenol)</em></td>
<td>S (weak)</td>
<td>NS</td>
<td>Preservative</td>
</tr>
<tr>
<td>PROP <em>(Isopropanol)</em></td>
<td>NS</td>
<td>NS</td>
<td>Solvent</td>
</tr>
<tr>
<td>LPS <em>(Lipopolysaccharide)</em></td>
<td>Control +</td>
<td>Control +</td>
<td></td>
</tr>
</tbody>
</table>

*Chemicals used in the present study*
Determination of Letal Concentration 50% - LC50

- N=10-12 worms

50 µl - M9 medium
50 µl – Chemicals solution

DEAD WORMS AFTER 24H WERE COUNTED
<table>
<thead>
<tr>
<th>CHEMICAL</th>
<th>N2 Value</th>
<th>CL2166 Value</th>
<th>CF1553 Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO ( (\text{Dimethyl sulfoxide}) )</td>
<td>1% ( R^2 = 0.9645 )</td>
<td>1% ( R^2 = 0.9765 )</td>
<td>1% ( R^2 = 0.9432 )</td>
</tr>
<tr>
<td>DNBC ( (2,4-\text{Dinitrochlorobenzene}) )</td>
<td>1.2 mM ( R^2 = 0.9984 )</td>
<td>2.5 mM ( R^2 = 0.9652 )</td>
<td>2.5 mM ( R^2 = 0.9652 )</td>
</tr>
<tr>
<td>PFA ( (\text{Formaldehyde}) )</td>
<td>20 mM ( R^2 = 0.9801 )</td>
<td>40 mM ( R^2 = 0.9797 )</td>
<td>20 mM ( R^2 = 0.9801 )</td>
</tr>
<tr>
<td>2-MBT ( (2-\text{Mercaptobenzothiazole}) )</td>
<td>5.0 mM ( R^2 = 0.9604 )</td>
<td>2.5 mM ( R^2 = 0.9524 )</td>
<td>2.5 mM ( R^2 = 0.9524 )</td>
</tr>
<tr>
<td>EU ( (\text{Eugenol}) )</td>
<td>0.5 mM ( R^2 = 0.9829 )</td>
<td>0.5 mM ( R^2 = 0.9829 )</td>
<td>1.25 mM ( R^2 = 0.9829 )</td>
</tr>
<tr>
<td>PROP ( (\text{Isopropanol}) )</td>
<td>170 mM ( R^2 = 0.9765 )</td>
<td>170 mM ( R^2 = 0.8738 )</td>
<td>170 mM ( R^2 = 0.8738 )</td>
</tr>
<tr>
<td>LPS ( (\text{Lipopolysaccharide}) )</td>
<td>1.0 µg/ml ( R^2 = 0.9383 )</td>
<td>0.5 µg/ml ( R^2 = 0.9289 )</td>
<td>1.0 µg/ml ( R^2 = 0.9383 )</td>
</tr>
</tbody>
</table>
**RACIONALE**

1. **Most allergenic potential**
2. Higher activation of signaling pathways
3. Increased worms fluorescence intensity

_C. elegans strains used:_
- CF1553 – PoD3::GFP – DAF16
- CL2166 – PoGST4::GFP – SKN1
Selection of the exposure time

- 4h - short
- 10h - moderate
- 24h - long

50 µl - M9 medium
50 µl – Chemical solution
Selection of the exposure time

4h - SHORT EXPOSURE

10h - MODERATE EXPOSURE

N=10-15 worms
Selection of the exposure time

24h - LONG EXPOSURE

N=10-15 worms
Analysis of the expression of the JNK-DAF16 signaling pathway - CF1553
Analysis of the expression of the p38MAPK-SKN-1 signaling pathway - CL2166
Fluorescence intensity analysis by the *ImageJ* software

- CF1553
  - JNK-DAF16
  - JNK-FOXO

- CL2166
  - p38MAPK-SKN1
  - Keap1-Nrf2-ARE

* N=15-20 worms
CONCLUSIONS and PROSPECTIVES

- The CL2166 strain, which emit fluorescence when the Keap1-Nrf2-ARE signaling pathway is activated, showed promising potential to predict the allergenicity.

- The CF1553 strain, which emit fluorescence when the JNK-FOXO signaling pathway is activated, was not able to predict the allergen potential of chemicals using the fluorescence emission test;

- These results must be checked by other tests, such as Real Time-PCR, as well as a greater number of chemicals need to be tested to confirm the potential of the approach.
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