Evaluation of in vitro embryo-toxicity tests for Chinese herbal medicines

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Evaluation of *in vitro* embryotoxicity tests for Chinese herbal medicines

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The validated embryonic stem cell test to predict embryotoxicity *in vitro*

Andrea E M Seiler\(^1\) & Horst Spielmann\(^2\)

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Background
Traditional Chinese Medicine

• Widely applied with long history
• Promote both mothers’ & fetuses’ health
• Effective in many pregnancy disorders
• Few embryotoxic effects
• Lacking scientific evidence of its claimed applications

Therapeutic Approaches

• Chinese medicines
• Acupuncture
• Food therapy
• Tai Chi exercise
• Qi Gong
• Cupping
• Tui Na (Physical therapy)
• Die Da
• Gua Sha
Chinese medicines

Origins
• Plant original (85%) = Chinese herbal medicines (CHMs)
• Animal original (10%)
• Mineral original (5%)

Chinese Pharmacopeia
• 6,000 CHMs
• 250 CHMs commonly for pregnancy
• 31 CHMs forbidden during pregnancy
Prevalence during pregnancy

Prevalence of Application %

Western Countries
- Canada: 61%
- United Kingdom: 58%
- United States: 45%
- Australia: 36%
- Norway: 36%
- Finland: 14%
- Sweden: 12%
- Japan: 9.1%
- China: 1%

Asian Countries
- Hong Kong: 56%
- Taiwan: 50.9%
- Rhode Island: 46%
- Norway: 33%

Canada
United Kingdom
United States
Australia
Norway
Finland
Nigeria
Sweden
Hong Kong
Japan
China
Taiwan
2015 Nobel Prize in Physiology or Medicine

William C. Campbell
Satoshi Ōmura
Youyou Tu
Aim

- CHMs have been widely used during pregnancy, but feto-embryo safety tests are lacking.
- Here we evaluated *in vitro embryotoxicity tests (IVTs)* as *alternative methods in assessing developmental toxicity* of CHMs,
- to report the importance of validated in vitro toxicity tests for the safety testing of CHMs.
Previous Work - Report

- Ten CHMs were selected and classified as strongly, weakly and non-embryotoxic.
- All test CHMs were authentication qualified.
- Three well validated IVTs and prediction models (PMs) were compared.
  - embryonic stem cell test (EST),
  - micromass (MM)
  - whole embryo culture (WEC)
All strongly embryotoxic CHMs were predicted by MM and WEC PM2. While all weakly embryotoxic CHMs were predicted by MM and WEC PM1. All non-embryotoxic CHMs were classified by EST, MM, but over-classified as weakly embryotoxic by WEC PM1.
Our findings

• Overall predictivity, precision and accuracy of WEC determined by PM2 were better than EST and MM tests.

• Compared with validated chemicals, performance of IVTs for CHMs was comparable.

• So IVTs are adequate to identify and exclude embryotoxic potential of CHMs in this training set.
Pentaherbs Formulation (PHF): 2:2:1:2:2, and clinical effects have been proved.

<table>
<thead>
<tr>
<th>Pharmaceutical Name</th>
<th>English Name</th>
<th>Chinese Name</th>
<th>CHM images</th>
<th>Yield (%)</th>
<th>Clinical Dose</th>
<th>LD$_{50}$ (g/kg) (mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flos ionicae</td>
<td>Honeysuckle Flower</td>
<td>Jin Yin Hua</td>
<td></td>
<td>34.2%</td>
<td>6-15g (100-250mg/kg)</td>
<td>67.6-81.7</td>
</tr>
<tr>
<td>Cortex moutan</td>
<td>Tree Peony Bark</td>
<td>Mu Dan Pi</td>
<td></td>
<td>23.4%</td>
<td>6-9g (100-150mg/kg)</td>
<td>3.4</td>
</tr>
<tr>
<td>Cortex phellodendri</td>
<td>Amur Corktree Bark</td>
<td>Huang Bai</td>
<td></td>
<td>13.7%</td>
<td>3-12g (50-200mg/kg)</td>
<td>2.7</td>
</tr>
<tr>
<td>Herba menthae</td>
<td>Common Mint</td>
<td>Bo He</td>
<td></td>
<td>28.6%</td>
<td>3-6g (50-100mg/kg)</td>
<td>3.3</td>
</tr>
<tr>
<td>Rhizoma atratyloides</td>
<td>Swordlike Atractylodes Rhizome</td>
<td>Cang Zhu (CZ)</td>
<td></td>
<td>34.0%</td>
<td>3-9g (50-150mg/kg)</td>
<td>≥ 5.0</td>
</tr>
</tbody>
</table>
Objectives

• To evaluate and predict the embryotoxicity potentials of a CHM formula used for the treatment of atopic dermatitis by Embryonic Stem Cell Test (EST)

Methods

• EST - Differentiation assay (embryonic stem cells)
• EST - Cytotoxicity assay (embryonic stem cells & fibroblast)
• Skin irritation test – cytotoxicity assay (EPI-200 skin tissues)
Overview of EST Protocol

A.

<table>
<thead>
<tr>
<th>Day 0</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction of EB formation</td>
<td>EB differentiation</td>
<td>EB outgrowth and differentiation into contracting cardiomyocytes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Day 0**
  - Cell culture (hanging drops)
  - Cultivation of EBs (suspension culture)
  - Cultivation of EBs (24-well plates)

- **Day 3**
  - Cultivation of EBs (suspension culture)

- **Day 5**
  - Cultivation of EBs (24-well plates)

- **Day 7**
  - Endpoint determination (microscope)

- **Day 10**
  - Endpoint determination (microscope)

B.

<table>
<thead>
<tr>
<th>Day 0</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeding cells (96-well plates)</td>
<td>Change of medium</td>
<td>Change of medium</td>
<td>Endpoint determination (MTT assay)</td>
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</tr>
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</table>
## Summary I - EST results

**Classification:**
(A Seiler, nature protocols, 2011)

<table>
<thead>
<tr>
<th>Functions Results</th>
<th>JYH</th>
<th>MDP</th>
<th>HB</th>
<th>BH (Mint)</th>
<th>CZ</th>
<th>PHF formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>I&gt;II, I&gt;III</td>
<td></td>
<td></td>
<td><strong>I&gt;II, I&gt;III</strong></td>
<td>I&gt;II, I&gt;III</td>
<td>I&gt;II, I&gt;III</td>
<td></td>
</tr>
<tr>
<td>II&gt;I, II&gt;III</td>
<td>I&gt;II, I&gt;III</td>
<td></td>
<td></td>
<td>I&gt;II, I&gt;III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I&gt;II, I&gt;III</td>
<td></td>
<td></td>
<td>I&gt;II, I&gt;III</td>
<td>I&gt;II, I&gt;III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-embryotoxic</td>
<td></td>
<td></td>
<td></td>
<td>Non-embryotoxic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weakly embryotoxic</td>
<td></td>
<td></td>
<td></td>
<td>Weakly embryotoxic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strongly embryotoxic</td>
<td></td>
<td></td>
<td></td>
<td>Strongly embryotoxic</td>
<td></td>
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</tr>
</tbody>
</table>

**Classification (EST):**

- Non-embryotoxic
- Non-embryotoxic
- Weakly-embryotoxic
- Non-embryotoxic
- Non-embryotoxic
- Non-embryotoxic

**N number:**

- 3~4

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<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>English</th>
<th>Chinese</th>
<th>Chinese PinYin</th>
<th>IC50 3T3</th>
<th>IC50 D3</th>
<th>ID50 D3</th>
<th>Functions</th>
<th>EST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flos ionicrae</td>
<td>Honeysuckle Flower</td>
<td>金银花</td>
<td>Jin Ying Hua</td>
<td>375.4</td>
<td>1151</td>
<td>360.9</td>
<td>10.0496685</td>
<td>9.78446441</td>
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<tr>
<td>Herba menthae</td>
<td>Common Mint</td>
<td>薄荷</td>
<td>Bo He</td>
<td>122.6</td>
<td>444.8</td>
<td>252.7</td>
<td>11.5672772</td>
<td>9.25628838</td>
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<tr>
<td>Cortex moutan</td>
<td>Tree Peony Bark</td>
<td>牡丹皮</td>
<td>Mu Dan Pi</td>
<td>113.8</td>
<td>316.7</td>
<td>342.8</td>
<td>15.9099555</td>
<td>10.7164418</td>
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<tr>
<td>Cortex phellodendri</td>
<td>Amur Corktree Bark</td>
<td>黄柏</td>
<td>Huang Bai</td>
<td>399.4</td>
<td>3.119</td>
<td>1.37</td>
<td>-3.8623964</td>
<td>1.80279936</td>
</tr>
<tr>
<td>Rhizoma atratyloides</td>
<td>Swordlike Atractylodes Rhizome</td>
<td>苍术</td>
<td>Cang Zhu</td>
<td>400.5</td>
<td>1198</td>
<td>467.5</td>
<td>11.3703204</td>
<td>10.3466115</td>
</tr>
<tr>
<td>Formula (PHF)</td>
<td></td>
<td></td>
<td></td>
<td>231.2</td>
<td>258.5</td>
<td>347.1</td>
<td>9.40031001</td>
<td>8.56197037</td>
</tr>
</tbody>
</table>
Conclusion

mEST in vitro embryotoxicity test

• Penta Herbs Formulation (PHF) should not be used during pregnancy

• The potentially embryotoxic *Amur Corktree Bark* to be eliminated from HPF and replaced by another CHM
In Asia countries, many pregnant women avoid Western medicines but opt for Chinese medicines to prevent its adverse effects on their fetuses. However, it is not very clear how safe the Chinese medicines are being used during pregnancy and if there are any toxicity or side-effects to both the mothers and babies. Here, we provide general and in-depth information regarding the safety concerns of Chinese herbal medicines during pregnancy.
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German Federal Institute for Risk Assessment (BfR)
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