In vitro safety assessment of herbal preparations: a toxicogenomics approach

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Background
Using animals to test the safety of food or feed ingredients is under debate since both the ethics and the predictive capacity for human toxicity are questioned. As a result there is a strong demand for alternatives for animal testing. Here we report an in vitro approach to assess the toxicity of complex plant metabolite mixtures.

Objective
The aim of the present work is to explore the usefulness of transcriptomics on in vitro cell systems for the safety assessment of complex food and feed products using herbal preparations as models.

Method
The human breast carcinoma cell line MCF-7 was exposed for 6 h to a methanolic extract of Digitalis lanata, and to digoxin, one of the major cardiac glycosides of D. lanata. RNAs were subjected to whole genome gene expression analysis using microarrays. In order to identify potential hazardous activities in the extracts, the expression profiles were subjected to 1) Metacore pathway analysis and 2) a comparison with profiles of 1309 biologically active compounds in the Connectivity Map, a publicly available transcriptome database. (Connectivity Map, www.broadinstitute.org/cmap)

Results
- The extract of Digitalis lanata and pure digoxin induced similar gene expression profiles in MCF7 cells (Fig. 3).
- Metacore pathway analysis indicated activation of the whole metabolism, DNA binding and transcription (Fig. 3). Cardiac glycosides are known to inhibit topoisomerases which might explain the activation of DNA binding.
- Comparison of MCF7 expression profiles of D. lanata and digoxin to that of profiles induced by 1309 biologically active compounds in CMAP, demonstrated a very strong positive correlation with effects of cardiac glycosides or their aglycones including digoxin (Fig. 4, orange frame).

<table>
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<th>Ranking</th>
<th>Name</th>
<th>Concentration (µg/g DW)</th>
<th>Stddev</th>
<th>Glycoside</th>
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<tr>
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<td>Lanatoside C</td>
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<td>262</td>
<td>Tetraglycoside</td>
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<td>2482</td>
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<tr>
<td>3</td>
<td>n.i.</td>
<td>2432</td>
<td>59</td>
<td>Triglycoside</td>
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<td>4</td>
<td>α-Digoxin</td>
<td>1696</td>
<td>25</td>
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<tr>
<td>5</td>
<td>Digoxin</td>
<td>931</td>
<td>25</td>
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</table>

Figure 2. Major cardiac glycosides in the methanolic extract of Digitalis lanata detected by LCMS. (n.i.: not identified)

Figure 3. Hierarchical cluster analysis and pathway analysis of gene expression profiles of MCF7 cells treated with a methanolic extract of D. lanata or pure digoxin. Exposures were performed in triplicate.

Figure 4. Connectivity Map results (top 15) for digoxin (a) and D. lanata (b) treated MCF7 cells.

Conclusion
Toxicogenomics tools like Metacore pathway analysis and particularly expression databases like the Connectivity Map can be very useful for detecting hazardous activities in a complex plant matrix.

Acknowledgements
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