



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)

Plant extraction



MCF7 exposure



Figure 1. Experimental procedure.

Results

- 28 cardiac glycosides (CG) or aglycones were detected in the methanolic extract of *D. lanata* using LCMS. The five major CGs are shown in Fig. 2.



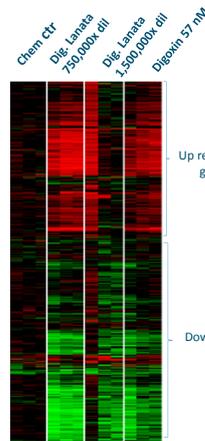
Digitalis lanata



Ranking	Name	Concentration (µg/g DW)	Stdev	Glycoside Type
1	Lanatoside C	7420	262	Tetraglycoside
2	Lanatoside B	2482	143	Tetraglycoside
3	n.i.	2432	59	Diglycoside
4	α-AcDigoxin	1696	25	Triglycoside
5	Digoxin	931	25	Triglycoside

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

- 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.



Metacore pathway analysis up regulated genes (n=74)

- GO Processes:** Regulation of whole metabolism including biosynthesis and transcription
- GO Molecular Function:** DNA/nucleic acid binding
- GO Localisation:** Nucleus

Metacore pathway analysis down regulated genes (n=73)

- No pathways significantly affected!

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.

- 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).

a						b							
barview	rank	batch AT	cmap name AT	dose	cell	score AT	barview	rank	batch AT	cmap name AT	dose	cell	score AT
	1		676 proscillaridin	8 µM	MCF7	1		1		676 proscillaridin	8 µM	MCF7	1
	2		705 proscillaridin	8 µM	MCF7	937		2		752 strophanthidin	10 µM	MCF7	923
	3		711 lanatoside C	4 µM	MCF7	916		3		726 digoxigenin	10 µM	MCF7	921
	4		684 digoxigenin	11 µM	MCF7	905		4		684 digoxigenin	11 µM	MCF7	918
	5		684 digoxigenin	11 µM	MCF7	904		5		686 helveticoside	7 µM	MCF7	900
	6		758 digoxigenin	10 µM	MCF7	904		6		758 digoxigenin	10 µM	MCF7	893
	7		728 digoxigenin	10 µM	MCF7	881		7		686 lanatoside C	4 µM	PCI	887
	8		686 lanatoside C	4 µM	MCF7	873		8		682 helveticoside	7 µM	PCI	885
	9		751 helveticoside	7 µM	MCF7	872		9		685 digoxin	5 µM	MCF7	882
	10		686 helveticoside	7 µM	MCF7	865		10		711 helveticoside	7 µM	MCF7	850
	11		752 strophanthidin	10 µM	MCF7	848		11		711 lanatoside C	4 µM	MCF7	850
	12		685 digoxin	5 µM	MCF7	848		12		751 helveticoside	7 µM	MCF7	849
	13		707 ouabain	5 µM	MCF7	800		13		705 proscillaridin	8 µM	MCF7	804
	14		720 digoxin	5 µM	MCF7	797		14		637 thiosolanin A	100 nM	PCI	774
	15		711 helveticoside	7 µM	MCF7	786		15		684 digoxigenin	11 µM	MCF7	773

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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