Metabolomics and its application to novel food testing

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The possible introduction of genetically modified (GM) foods in the European market has engendered concern amongst consumers and environmentalists regarding their safety. As part of the EU project NOFORISK (www.noforisk.org) we have developed MS-based metabolomics as a foundation for novel food safety and risk assessment. Within the project in vitro and in vivo experiments were performed with wild type (control) and GM potatoes which had been modified with respect to their glycoalkaloid (GA) biosynthetic pathway; specifically down regulation of α-Solanine glucosyltransferase-1 (McCue et al, 2006). Besides specific affects attributable to glycoalkaloid levels unintended effects accompanying GM were also sought out. The experimental systems analysed were Caco2 (intestinal) and Hep G2 (hepatic) cell lines for human absorption and metabolism studies, respectively. In addition this was extended to feeding trials wherein the comparative effects of wild type and GM potato consumption by hamsters were followed at the a variety of levels: plasma, urine and organ (liver, intestine etc).

Materials & Methods

Potato Material

Thirty one potatoes lines were used for project comprising genetically modified Desiree (down regulated with regard to α-solanine content), Desiree control (tissue culture), Desiree empty vector, Desiree Wild type (parent material) and fifteen cultivars with varying glycoalkaloid content. The plants were grown in a Tygan Tunnel in a duplicated random plot design. Post-harvest the tubers were freeze-dried and milled.

Cell line Experiments

To assess the intestinal transport and absorption across human intestinal epithelial cell barrier. Mono-layers of Caco2 cells were exposed to solutions of extracted GAs from GM and non-GM potato, extracts of potato with GAs removed and synthetic potato GAs, α-solanine and α-chaconine. Metabolomics was carried out on exposed cells and media. To model metabolism of the bioavailable potato metabolites, media from Caco2 experiment was removed for exposure to HepG2 cells. Exposure was carried out in Transwell® plates for twenty-four hours (Fig. 1). After exposure the media from the bottom of the plate was removed for metabolomic analysis by GC/MS and LCMS

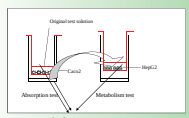

Figure 1 – Invitro absorption and metabolism model

Feeding Experiments

A ninety day feeding experiment was carried out on Golden Syrian hamsters. Nine groups n=10 were fed a diet containing control feed and feed containing 20, 40, 60% of freeze dried potato. Groups contained Wild Type only, GM only, solanine spiked GM to give the same glycoalkaloid ratio as wild type and chaconine spiked Wild Type to give same glycoalkaloid ratio as GM. Bio-fluids and tissues were analysed by LC and GC-MS

Results

Potato glycoalkaloid content

The total glycoalkaloid content of the GM potato line (SGT 9-2) used in the cell line studied and animal feeding trials fall within the natural variation of cultivars studied for comparison. (Fig.2).
The marked difference is in the α-chaconine to α-solanine ratio figure 3

Potato metabolomics

LC/MS analysis of the potato lines show that the machine controls (Desiree and Solanum Phureja, an Andea potato) have segregated from each other and away from the rest of the cultivars; as expected. The segregation of Desiree (control) and Desiree WT (derivation of the GM material), both the same germplasm but grown a different times, suggests an environmental factor is evident. In addition the segregation of Desiree away from the rest of the cultivars suggest that this cultivar may be unique.. However the GM (and controls) derived from Desiree are close dispersed the parent. (Fig 4).

The analogous GC-MS analyses showed that the analytical controls group well again with a tentative segregation of GM v cultivars (Fig 5).

With the addition of other GM lines not associated with GA biosynthesis production the GM lines cluster even more and are not separated by specific modification on any PCA score. This suggests that perhaps the GM process that caused separation Figure 6.

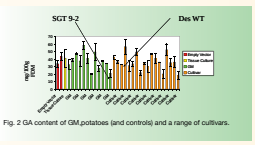

Fig. 2 GA content of GM,potatoes (and controls) and a range of cultivars.

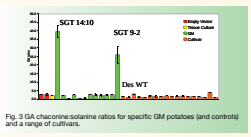

Fig. 3 GA chaconine:solanine ratios for specific GM potatoes (and controls) and a range of cultivars.

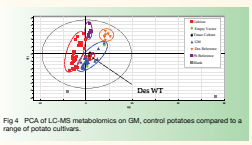

Fig 4 PCA of LC-MS metabolomics on GM, control potatoes compared to a range of potato cultivars.

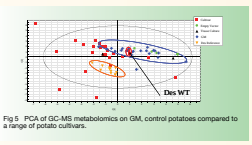

Fig 5 PCA of GC-MS metabolomics on GM, control potatoes compared to a range of potato cultivars.

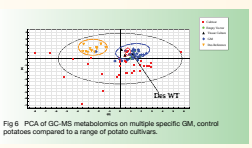

Fig 6 PCA of GC-MS metabolomics on multiple specific GM, control potatoes compared to a range of potato cultivars.

Cell Line Experiments

PCA plots of the GC/MS analysis of non-polar extracts of Caco-2 cells show that the cells exposed to extracts of freeze dried potato with glycoalkaloids removed segregate from the cells that have been exposed to glycoalkaloids. The compounds that are driving this segregation are fatty acids; C14-C19. (Fig 7)

There was also a slight separation in the PCA of the cells exposed to potato extract and an equivalent amount and ratio of synthetic GAs (Fig 8)

GC/MS analysis of polar extracts of Caco-2 cells shows a dose dependency irrespective of treatment Figure 9.

PCA of the GC/MS metabolomics data of the media (+ potato extract) before and after absorption to Caco2 cells and following exposure of the latter to the Hep G2 cells showed clear separations and therefore significant changes in the chemistries.

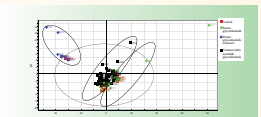
Fig 7 PCA of GC-MS analysis of non-polar extracts of Caco-2 cells following exposure to potato extracts with and without their Gas.

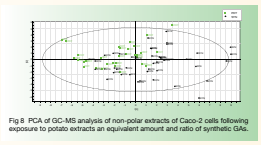
Fig 8 PCA of GC-MS analysis of non-polar extracts of Caco-2 cells following exposure to potato extracts an equivalent amount and ratio of synthetic GAs.

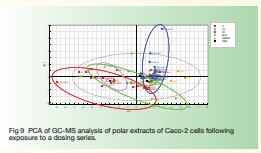
Fig 9 PCA of GC-MS analysis of polar extracts of Caco-2 cells following exposure to a dosing series.

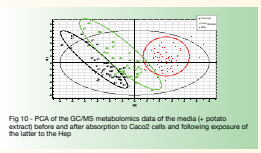
Fig 10 - PCA of the GC/MS metabolomics data of the media (+ potato extract) before and after absorption to Caco2 cells and following exposure of the latter to the Hep

Animal Feeding Trials

The data for this trial is currently under analysis but the PCA plots of Direct Infusion Mass spectrometry (DIMS) obtained from urine, faeces and plasma shows some grouping for treatments but results are inconclusive (data not shown). This is not surprising since these biological matrices are not normally sampled for long duration exposures. However the lack of changes suggests that the systems associated with plasma, urine and faeces have not been adversely affected by the GA exposure or any changes associated with the GM event in the potato.

Conclusions

Clearly the metabolomics approaches generate a rich source of data for any toxicological studies and have been shown here to be a significant step forward with regard to the level and degree of detail that can be obtained within a framework of food safety and the risk assessment of novel foods.

As the data from the feeding trial with animals becomes available the relevance of these platform technologies to “real life” should be confirmed and help establish these approaches as the way forward for food safety and risk assessment

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