Identification of polyphenol regulators of the insulin-sensitive transcription factor FOXO1a

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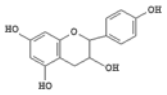
Introduction

The aim of this project is to identify food compounds which induce insulin-like effects on cells. Compounds with these properties may help to alleviate some of the problems caused by the insulin insensitivity of type 2 diabetes mellitus (T2DM). Normally, when blood glucose levels are elevated, insulin is released from the pancreas, stimulating liver, muscle, fat and brain cells to take up glucose from the blood and either use it or store it as glycogen via the insulin signalling pathway, a phosphorylation cascade within the cell, ultimately regulating various effectors involved in glucose regulation, including phosphorylation and inactivation of the transcription factor, FOXO1a.

Oligoproanthocyanidins

Proanthocyanidins are part of a group of compounds called polyphenols found in a wide variety of plants. Proanthocyanidins are polymerised flavonoids and are major constituents of amongst others, grape seed, pine park and berries.
Recently we found in cell culture studies that black tea polyphenols called theaflavins stimulate the insulin signalling pathway at an as yet undetermined point (Cameron *et al*, 2008) and currently we are studying the mechanism of the effect and working out whether it is shared by other dietary compounds. The basic flavonoid structure is shown below :



Results

Chromatography/ cell culture/ Western blotting

Human Embryonic Kidney 293 (HEK293) cells were stimulated with increasing concentrations of grape seed extract (GSE) and Western blotting was employed to detect phosphorylated FOXO1a. This confirmed that a concentration of 100µg/ml induced FOXO1a phosphorylation (data not shown). Similar effects were found with lingonberry (LB) and fractions from pine bark (PBE), and cranberry (CB) (Fig 1). After column chromatography on a Supelco Discovery polyamide column to which proanthocyanidins adsorb, a 100µg/ml concentration of each fraction generated was then applied to HEK293 cells and subsequent western blotting for phosphorylated FOXO1a confirmed that the bound proanthocyanidin rich fractions of each sample phosphorylated FOXO1a and the unbound fractions did not.

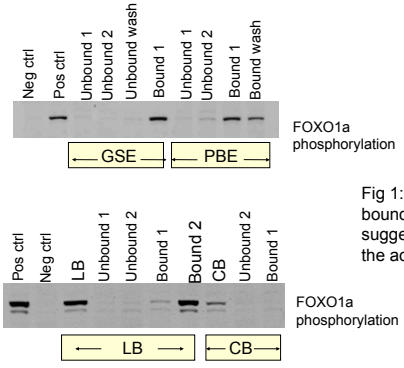


Fig 1: Western blot analysis of bound and unbound fractions suggests bound fractions contain the active compound(s).

Liquid chromatography /mass spectrometry

To determine the composition of each sample, further analysis was then carried out using normal phase HPLC on a LCQ Deca system. Oligoproanthocyanidins (OPCs) were confirmed to be highly represented in each fraction which phosphorylated FOXO1a (Fig 2, 3).

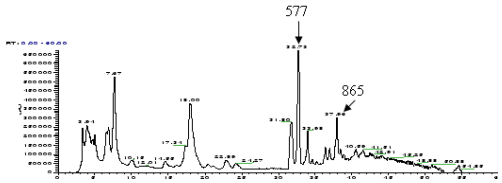


Fig 2: Photodiode array (PDA) data of active pranthocyanidin rich fraction of PBE. Accompanying MS data confirmed enrichment of OPCs.

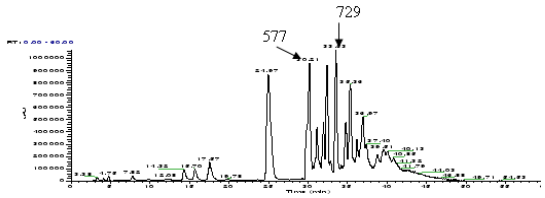


Fig 3: PDA data of active hydrophobic fraction of GSE. Accompanying MS data confirmed enrichment of OPCs.

Current work

Samples have been collected from each peak and MS data suggested most of these peaks only represented one or two compounds. These purified fractions are currently undergoing Western blot analysis to identify the active components.

Future work

•To determine the mechanism of action of the compounds, including investigation of potentiating effects of trace minerals on the compounds that we identify

•To investigate structure/activity relationships for OPCs on this pathway

References

Cameron, AR *et al*. Black tea polyphenols mimic insulin/ insulin-like Growth factor-1 signalling to the longevity factor FOXO1a. *Aging Cell.* (2008). **7**. 69- 77.

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