Effects of Gypenosides on glucagon-like peptide-1 (GLP-1) release from intestinal L-cells

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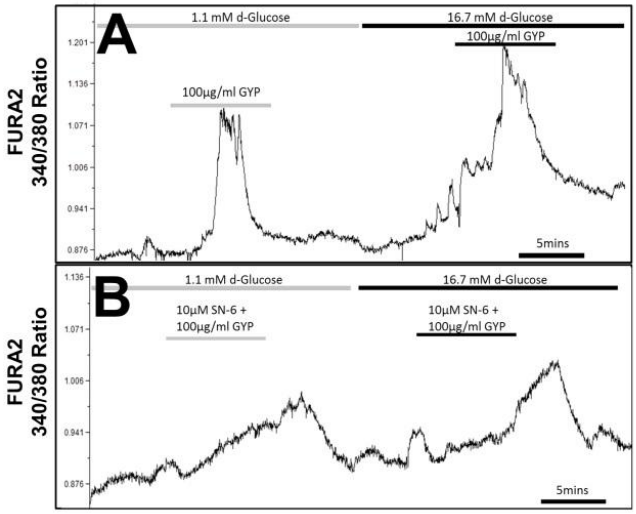
Introduction: Gypenosides are glycosides extracted from the plant *Gynostemma Pentaphyllum*. Gypenosides are known for their glucose lowering effects both *in vitro* and *in vivo* [1], although their mechanism of action is unclear. GLP-1 is an incretin hormone produced in gut L-cells which enhances β-cell insulin release. Methods to enhance endogenous GLP-1 may improve glucose control in diabetes. This study aimed to elucidate gypenosides effects on the function of GLP-1 secreting intestinal L-cells using the well characterised GLUTag L-cell model [2].

Methods: GLUTag cells were maintained in DMEM (1g/L glucose) supplemented with 10% FBS, 50U/ml penicillin/streptomycin and 2mM L-Glutamine. Gypenoside extract was prepared by dissolving gypenosides in absolute ethanol. Metabolic activity was determined by MTT assay following treatment of GLUTag cells with gypenosides (0.39 to 200µg/ml) for 24 h. RNA was extracted from GLUTag cells following 24h culture with 50 µg/ml gypenosides and L-cell specific (glucagon (GCG), proprotein convertase1/3 (PC1/3), sodium-glucose transporter (SGLT-1), glucokinase (GCK) and NFκB1) gene expression changes quantified by qPCR. Gypenoside effects on intracellular calcium were determined in FURA-2 loaded GLUTag cells at both low and high glucose concentrations, both in the absence and presence of specific ion channel blockers. GLP-1 release from GLUTag cell monolayers was assessed during 1h treatment with gypenosides and quantified using a total GLP-1 ELISA Kit (Millipore).

Results: Cell metabolic activity increased following 24h culture in media containing 50 & 100µg/ml gypenosides (n=8; p<0.01). Gypenoside culture down-regulated GCG, PC1/3 and NFκB1 gene expression (P<0.01). Gypenosides (50 & 100 µg/ml) stimulated acute GLP-1 secretion (n=4) from GLUTag cells at both low (1.1 mM) (2.7-7.5 fold P<0.01) and high (16.7 mM) glucose (3.6-13.5 fold P<0.01) concentrations. An increase in intracellular calcium was observed after gypenoside treatment at both low and high glucose concentrations, which was unaffected by L-type (nifedipine and verapamil) and T-type (mibefradil) calcium channel blockers. However, sodium-calcium exchanger (NCX) reverse mode inhibitor, SN-6, blocked gypenoside-induced increase in intracellular calcium.

Acute effects of gypenosides on cytosolic calcium in GLUTag cells alone and in the presence of NCX reverse mode inhibitor SN-6



Responses shown are representative for 44 cells (A) and 78 cells (B) from 3 independent experiments.

Conclusion: The increased cell viability and altered gene expression by gypenoside suggests that gypenosides may modulate L-cell function. Interestingly, acute gypenosides exposure increases intracellular calcium and stimulates GLP-1 secretion from GLUTag cells possibly via activation of NCX reverse mode. Further studies are required to determine the interaction between gypenoside and NCX in gut endocrine cells.

References:

1. Lokman EF et al. (2015) Evid Based Complement Alternat Med:120572.
2. Drucker DJ et al. (1994) Mol Endocrinol 8:1646-55.