Synthetic peptides identified from *Palmaria palmata* enhance glucagon-like peptide-1 stability *in vitro* and show acute anti-hyperglycaemic and insulinotropic actions in mice.

C. McLaughlin¹, P.A. Harnedy², P.J. Allsopp¹, E.M. McSorley¹, R.J. FitzGerald², & F.P.M. O'Harte¹

¹School of Biomedical Sciences, Ulster University, Cromore Road, Coleraine, N. Ireland and ²Department of Life Sciences, University of Limerick, Castletroy, Limerick, Ireland.

INTRODUCTION

- Understanding the therapeutic potential of novel protein sources involves liberating peptide fragments followed by bioactivity screening
- Small molecular weight peptides can present highly specific cell receptor binding or pleiotropic physiologic and metabolic activity
- Identified peptide fragments that promote advantageous metabolic activity is a rapidly expanding area of proteomics research
- Many protein fragments and small molecular weight peptides to date have displayed promising DPP4 and ACE inhibitor activity making these molecules highly applicable therapeutics due to high tolerance thresholds versus pharmaceuticals
- Previously published work involving peptides identified and tested within have displayed DPP4 inhibitory activity *in vitro*, however the *in vivo* application was previously unknown
- Three peptides MET-ALA-GLY-VAL-ASP-HIS-ILE (MAGVDHI), ILE-LEU-ALA-PRO (ILAP) and LEU-LEU-ALA-PRO (LLAP) were investigated using *in vitro* and *in vivo* models of glucoregulatory study including a HPLC based approach for DPP4 enzyme activity

AIMS & OBJECTIVES

- Advancing from previously *published in vitro* work, this study aims to investigate the potential pleiotropic activity of *palmaria palmata* protein fragments which may improve markers of obesity and diabetes.
- Cells lines with specific application for diabetes investigation (BRIN-BD11, GLUTag & STC-1) including a HPLC based enzyme activity assay and finally acute IP glucose tolerance of the peptides alone or in combination with GLP-1 were deployed

METHODOLOGY

Panel C/D/E/F - Dose-dependent effects of *Palmaria palmata* derived synthetic peptides on insulin secretion from BRIN-BD11 cells at 5.6 mM glucose. BRIN-BD11 cells were incubated with either MAGVDHI (**Panel D**), ILAP (**Panel E**) or LLAP (**Panel F**) using a range of concentrations (10^{-12} M to 10^{-6} M) for 20 min and insulin release measured by RIA. Values are expressed as mean $\pm$ S.E.M. (n=8). *p<0.05, **p<0.01, ***p<0.001 compared to respective glucose control. (**Panel A**) represents positive insulinotropic controls used in the experiment.

Panel G - secretory effects of peptides on GLP-1 secretion from GLUTag cells at 2 mM glucose. GLUTag cells were incubated with ILAP, LLAP or MAGVDHI using a fixed concentration of peptide (10^{-6} M) for 2 hours and GLP-1 release measured by ELISA. Values are expressed as mean $\pm$ S.E.M. (n=3).

Panel H - Secretory effects of peptides on GIP secretion from STC-1 cells at 2 mM glucose. GLUTag cells were incubated with ILAP, LLAP or MAGVDHI using a fixed concentration of peptide (10^{-6} M) for 2 hours and GIP release measured by ELISA. Values are expressed as mean $\pm$ S.E.M. (n=3).

Panel I - LDH release from BRIN-BD11 cells during co-incubation with ILAP, LLAP or MAGVDHI using a fixed concentration of peptide (10^{-6} M) for 20 min with LDH measured by cytotox assay. Values are expressed as mean $\pm$ S.E.M. (n=3).

Panel J - Effects of marine peptides on cAMP production in BRIN-BD11 cells during acute co-incubation with GLP-1 alone or ILAP, LLAP or MAGVDHI using a fixed concentration of hydrolysate (10^{-6} M) for 20 min. Cells were lysed using cAMP assay lysis buffer with cAMP concentration established using a cAMP specific ELISA. Values are expressed as mean $\pm$ S.E.M. (n=3). *p<0.05, **p<0.01, ***p<0.001 compared to respective glucose only control.

Panel K/L/M - Graphs displays a graphical depiction of a RP-HPLC elution of active GLP-1 (10^{-6} M 7-36 amide) and degraded GLP-1 (9-36) after incubation with DPP4 (5 uM) at 0, 2, 8 and 24 h in a TEA buffer (pH 7.4) (**Panel K**). Using an analytical kinetix C18 column (250 x 10 mm, 3.6 μ m particle size) a 200 μ l injection using a rheodyne 7725i injector attached to a thermo surveyor RP-HPLC machine equipped with a UV/VIS detector set to 214 nm wavelength. **Panel L** represents GLP-1 and DPP4 only. Or co-incubation with the addition of MAGVDHI or ILAP or LLAP. Positive control used Diprotin A. Results are Mean $\pm$ SEM for 2 separate HPLC elution's per time point. ***p<0.001 versus GLP-1 + DPP-4 alone. **Panel M**. Table depicts calculated AUC percentage (%) of final elution's

Panel O/P/Q - Acute actions of MAGVDHI (**Panel O**), ILAP (**Panel P**) or LLAP (**Panel Q**) on IP glucose tolerance in male HsdOla;T0 mice. Glucose was measured prior to (t=0) and 15, 30, 60, 90 and 120 min after administration of glucose alone (18 mmol/kg body weight; IP) or in combination with peptides with and without GLP-1 in combination (25 nmol/kg body weight; IP). Values are expressed as mean $\pm$ S.E.M. for 6 mice. Bottom Panels represent blood glucose AUC for GLP-1 alone or peptide with and without GLP-1 in combination with glucose. Results are mean $\pm$ S.E.M versus IP glucose only group

Prior Investigation of crude hydrolysate

Panel A represents a 60 minute HPLC gradient chromatograph of crude *palmaria palmata* protein hydrolysate generated using food grade Promod proteolytic enzyme. From this, up to 11 different peptide fragments were identified and sequenced using MS/MS. Peptide amino acid sequence was determined and a subsequent synthetic form of the identified peptides were generated for analysis. Of the 11 identified peptides, this poster contains data generated from MET-ALA-GLY-VAL-ASP-HIS-ILE (MAGVDHI), ILE-LEU-ALA-PRO (ILAP) and LEU-LEU-ALA-PRO (LLAP) of which will be referred to as their 1 letter amino acid form hence forth. **Panel B** represents acute insulinotropic activity of the crude hydrolysate of which the peptides were identified from for comparison. Values are expressed as mean $\pm$ S.E.M. (n=8). **p<0.01, ***p<0.001 compared to respective glucose control..

SUMMARY

- Insulin secretion study using BRIN-BD11 cells both ILAP and LLAP stimulated dose-dependent acute insulin secretion with maximum concentrations (10^{-6}) yielding 2-fold insulin secretion above basal glucose control. Peptide MAGVDI had no insulinotropic activity
- GLP-1 secreting GLUTag cells co-incubated with MAGVDHI, ILAP or LLAP (10^{-6} M) failed to increased acute (2 h) GLP-1 secretion above basal glucose control
- GIP secreting STC-1 cells, acute incubation with ILAP or LLAP increased GIP secretion by 1.5- (LLAP) and 2- (ILAP) fold above basal glucose control. Interestingly, MAGVDHI reduced acute GIP secretion under the same experimental conditions
- Using a HPLC approach, DPP4 enzyme activity in the presence of GLP-1 alone or supplemented with MAGVDHI, ILAP or LLAP, seen a significant improvement in active GLP-1 7-36 amide half-life from 1.5 h with DPP4 and GLP-1 only, to 13h (MAGVDHI), 10 h (ILAP) and 8 h (LLAP) with the addition of each of the synthetic marine peptides.
- Acute intraperitoneal (IP) injection of glucose with 25 nmol/kg/BW of ILAP or LLAP, but not MAGVDHI improved glucose excursion over 120 min
- Further IPGTT co-injection of ILAP or LLAP with GLP-1 improved glucose excursion versus GLP-1 only demonstrating that peptides ILAP and LLAP have additive glucoregulatory effects potentially via DPP4 inhibition and insulin secretion within the β -cells. Further investigation is needed to fully elucidate the mechanism of which novel protein fragments could be used as potential diabetes therapeutics