Reducing amyloid-associated beta-cell death during islet culture in diabetic conditions by targeting GLP-1 receptor

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Aim of the study

To study the effect of treatment with exenatide (Byetta), a GLP-1 receptor agonist on amyloid-induced beta-cell death in cultured human islets.

Background

- Diabetes mellitus (DM) belongs to a group of metabolic disorders characterized by chronic hyperglycemia, and is broadly categorized into two categories: Type 1 diabetes (T1D) and Type 2 diabetes (T2D).
- Progressive insulin resistance and islet beta-cell failure are thought to be play major roles in pathogenesis of T2D.
- Islet amyloid polypeptide (IAPP, amylin) is a normal beta-cell hormone that is co-secreted with insulin by pancreatic beta cells.
- IAPP has amyloidogenic sequence and forms amyloid plaques in the islets of Langerhans in T2D patients that are toxic to islet beta cells and contribute to progressive beta-cell dysfunction and death in T2D.
- Exenatide (Byetta) is a glucagon-like peptide-1 (GLP-1) receptor agonist that mimics incretin action to enhance beta-cell function.

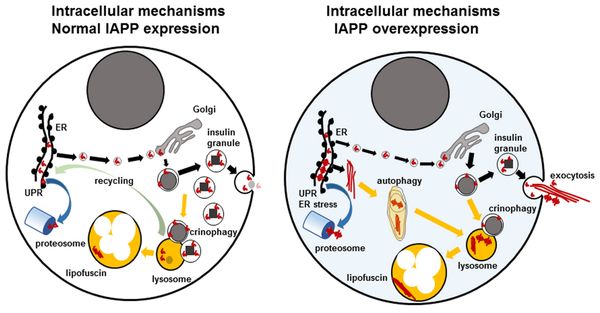

Figure 1. The diagram represents differences between normal and overexpression of IAPP in beta cells.

Source: Raleigh, D et al. (2017). The β-cell assassin: IAPP cytotoxicity, *Journal of Molecular Endocrinology*, *59*(3), R121-40.

Methods and Materials

- Human islets were cultured in CMRL medium (7 days, 37°C) in elevated (11.1 mM) glucose (to form amyloid deposits) in the presence or absence of exenatide (10 mM).
- Paraffin embedded human islet section were double immunolabelled for Insulin (green) and TUNEL (red) to detect apoptotic (death) beta cells.
- The proportion of TUNEL-positive islet beta cells were quantified in islets.
- Amyloid formation in islets was detected by double immunostaining for insulin and thioflavin S.

Results

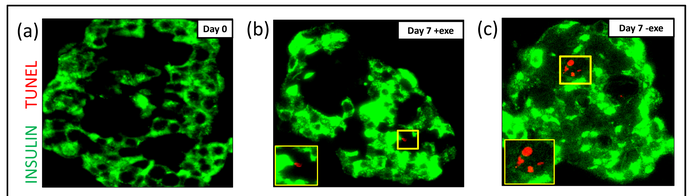

Figure 2. (a)-(c) are representative human pancreatic islets cultured from Day 0, Day 7 +exenatide and Day 7 non-treated (-exe), respectively.

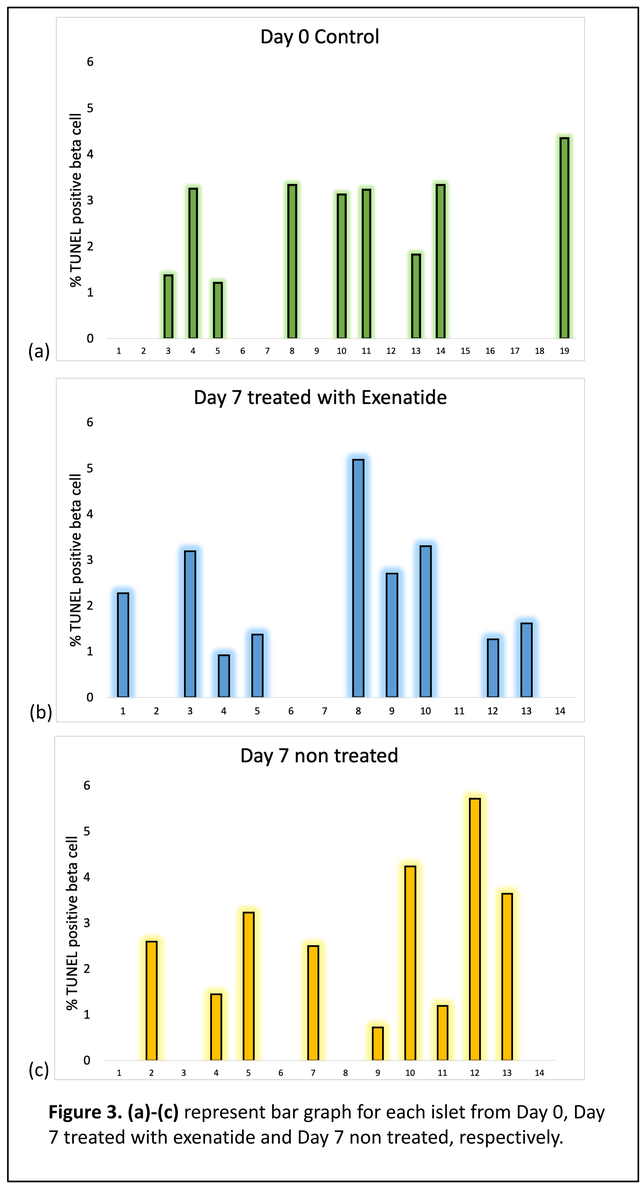

Figure 3. (a)-(c) represent bar graph for each islet from Day 0, Day 7 treated with exenatide and Day 7 non treated, respectively.

- The majority of islets formed amyloid during 7 days culture in elevated glucose.
- Treatment with exenatide reduced amyloid formation in cultured islets.
- The proportion of TUNEL-positive beta cells in isolated islets (day 0) was low suggesting good quality of isolated islets. More than half of the islets in Day 0 had no TUNEL-positive beta-cell (Figure 2).
- Islets treated with exenatide for 7 days had improved beta-cell survival than non-treated 7-day cultured islets. The maximum proportion of TUNEL-positive beta-cells in non-treated and treated 7-day cultured islets was 5.19% and 5.71%, respectively (Figure 3).
- The mean percentage of TUNEL-positive beta cells for day 0, day 7 with exenatide and day 7 non-treated were 1.32% ± 0.37%, 1.56% ± 0.42% and 1.81% ± 0.50%, respectively (Figure 4).
- The mean percentage of the TUNEL positive to beta cell ratio for Day 7 treated with exenatide was lower than Day 7 non treated. However, this difference was not statistically significant due to small sample size in this pilot study.
- ANOVA single factor statistical test was performed. (p-value = 0.72, $p > 0.05$). A set of student t-tests were done to confirm statistical significance. (p-values = 0.73, 0.43 and 0.70, $p > 0.05$).

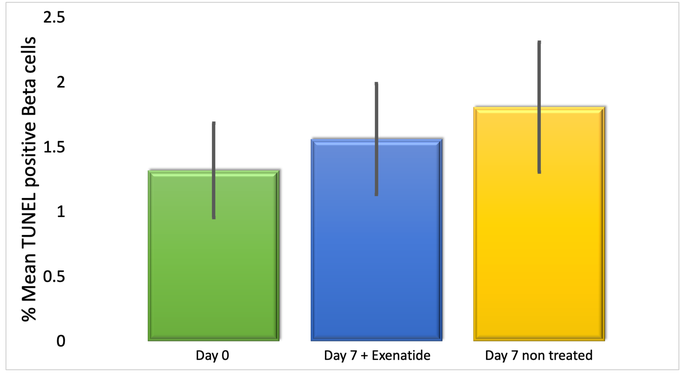

Figure 4. The histogram represents mean percent of TUNEL positive beta cells on the y-axis for the three groups on x-axis: Day 0, Day 7 treated with exenatide (10nmol/L), and Day 7 non treated. The error bars represent standard error of mean (SEM).

Conclusion

- Treatment with exenatide reduced amyloid formation in cultured islets.
- The number of TUNEL-positive beta cells were lower in 7-day cultured islet treated with exenatide as compared to non-treated cultured islets. Further studies are in-progress to validate findings of this pilot study with larger sample size.
- Treatment with GLP-1 receptor analogues may provide a new strategy to reduce amyloid-induced beta cell death.