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GLP-1 AS AN ADJUNCT TO PROLACTIN AND ANTI-CD3 IN TYPE 1 DIABETES TREATMENT

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease where the insulin-producing pancreatic β-cells are destroyed [1]. Affected individuals would have insulin deficiency, leading to the development of hyperglycemia, and unless insulin is provided, the patients will succumb to the disease [1]. The specific triggers of T1DM is still unknown, but many have hypothesized that hygiene and other environmental factors contribute to the pathogenesis of T1DM [1].

Anti-CD3 (aCD3) is a monoclonal antibody that is known to modulate immunity and stop the autoimmune attack on β-cells by T-cells [2]. Prolactin (PRL) is a peptide hormone that plays an important regulatory role in β-cell adaptation to pregnancy [3]. Its known effects include upregulating β-cell mass, proliferation, insulin secretion, and downregulating apoptosis [3,4]. Glucagon-like peptide 1 (GLP-1) is an incretin that is secreted by the L-cells of the distal ileum and exhibits effects such as increasing β-cell mass, proliferation, insulin secretion and satiety while decreasing glucagon secretion and β-cell apoptosis [5,6]. The objectives of this research are to determine if the addition of GLP-1 to PRL+aCD3 treatment would improve β-cell function in diabetic mice and to determine the source of the β-cells in the cured mice.

METHODS

This experiment was conducted using a non-obese diabetic (NOD) mice model. After the T1DM onset in the NOD mice, aCD3 (10 µg/day) was administered to the mice for 5 days, PRL (2.7 µg/day) for 21 days, and GLP-1 (10 µg/day) for 21 days. Upon reaching the 40th week after initial diabetes onset, the mice were sacrificed and the pancreases were taken for immunological studies. β-cell mass, neogenesis, proliferation, and apoptosis were determined by performing immunohistochemistry (IHC). Insulin ELISA was used as an assay for pancreatic insulin content and secretion.

RESULTS

The GLP-1+PRL+aCD3 treatment group had a survival rate of 83.3% (n=6) resulting in a ~1.8-fold increase over the PRL+aCD3 group (n=11) and a ~3.3-fold increase over the aCD3 group (n=16).

	GLP-1 +PRL+aCD3	PRL+aCD3	aCD3
β-cell fraction	0.37 ± 0.10%* (n=4)	0.41 ± 0.11%* (n=7)	0.09 ± 0.03% (n=7)
RFP+ β-cell fraction	91.48 ± 0.36% (n=2)	96.31 ± 1.52% (n=4)	93.51 ± 0.73% (n=2)
BrdU+ β-cell fraction	4.65 ± 1.77%* (n=3)	5.21 ± 3.30%* (n=7)	3.57 ± 2.00% (n=4)
TUNEL+ β-cell fraction	0.17 ± 0.09% (n=3)	0.54 ± 0.16% (n=2)	0.78 ± 0.27% (n=2)

Figure 1. β-cell fraction, RFP+, BrdU+, and TUNEL+ β-cell fractions in various groups. "*" : $p<0.05$ in comparison to the aCD3 group. "n" = number of mice in each group.

DISCUSSION AND CONCLUSIONS

The group treated with GLP-1+PRL+aCD3 had comparable β-cell mass to the PRL+aCD3 group. Evidence for β-cell neogenesis was determined by quantifying RFP+ β-cells. When RFP is expressed on a β-cell, it indicates that the β-cell is pre-existing and is not from neogenesis. Neogenesis did not contribute to the β-cell mass since all three groups shared a similar RFP+ fraction at a value that is near 100%. BrdU was incorporated in the mice's DNA to act as a cell proliferation marker. The BrdU+ β-cell fractions were similar between the GLP-1+PRL+aCD3 and PRL+aCD3 groups, suggesting that GLP-1 does not stimulate β-cells proliferation, above and beyond the effect of PRL. GLP-1, however, may decreases the apoptosis of β-cells, as suggested by the lower TUNEL+ β-cell fraction in the GLP-1+PRL+aCD3 treatment group when compared with the PRL+aCD3 group.

GLP-1 was determined to increase the survival of NOD mice, however, its underlying mechanisms is still unknown. Determining pancreatic insulin content and GLUT2 expression in future experiments would identify such mechanisms.

REFERENCES

1. Daneman D. *Lancet.* **358**:847-858,2006.
2. Herold KC & Taylor L. *Immunol Res.* **28**:141-150, 2003.
3. Huang C. *J Physio.* **591**:1325-1338, 2013.
4. Nielsen JH, et al. *J Mol Med.* **77**:62-66, 1999.
5. Brubaker PL & Drucker DJ. *Endocrinology.* **145**:2653-2659, 2004.
6. Sherry NA, et al. *Endocrinology.* **148**:5136-5144, 2007.