TREATMENT WITH GLP-1 AGONISTS IMPROVES B-CELL SURVIVAL IN AMYLOID FORMING ISLETS DURING EXVIVO CULTURE

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INTRODUCTION

- Type 1 diabetes (T1D) is an autoimmune disease caused by selective auto-immune destruction of pancreatic islet β-cells by autoreactive T-lymphocytes leading to insulin deficiency and life-time insulin therapy.[1]
- Islet transplantation by the Edmonton protocol has provided a promising approach for treatment of T1D,[2] but it is currently limited by the poor long-term survival of islet grafts due to both immune (graft rejection) and non-immune factors (islet amyloid, inadequate oxygen and nutrient supply).[2]

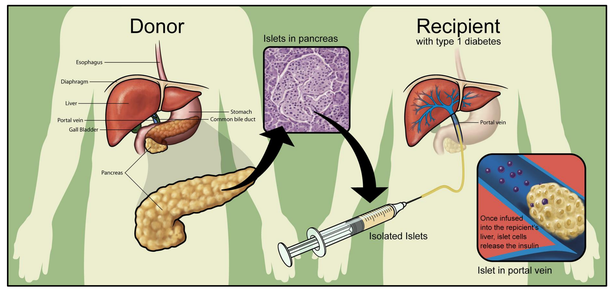

Figure 1. A diagram showing the major steps of islet transplantation (Edmonton protocol). Islets from cadaveric donors are isolated from pancreas, re-suspended in a physiological solution and infused into liver portal vein.

- Islet amyloid forms by aggregation of human islet amyloid polypeptide (hIAPP), a β-cell hormone co-secreted with insulin. Amyloid formation is a characteristic of T2D but also occurs rapidly in islets during pre-transplant culture and post-transplantation which contributes to graft dysfunction.[3]

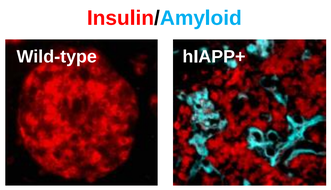

Figure 2. hIAPP-expressing mouse islets form amyloid similar to that observed in human islets during T2D and islets grafts in T1D.

- Glucagon-like peptide-1 (GLP-1) agonists such as exenatide (short-acting), and liraglutide (long-acting) mimic the action of GLP-1 to trigger the release of insulin and are currently are used in T2D to improve glucose control.[4]

HYPOTHESIS

Treatment with GLP-1 agonists can improve survival of islets during *ex vivo* culture by reducing amyloid-induced β-cell apoptosis.

METHODS

- Isolated islets from hIAPP-expressing transgenic mice, an animal model of amyloid-associated T2D, were cultured in Ham's-F10 medium supplemented with 16.7 mmol/l glucose, 0.5% (wt./vol.) BSA, 50 U/ml penicillin, 50 µg/ml streptomycin and 50 µg/ml gentamycin.
- Islets were treated with exenatide (10 nmol/L), liraglutide (10 nmol/L) or a vehicle (as control) for 7 days (medium was changed twice per week).
- Paraffin-embedded islet sections were immunolabelled for insulin by incubation with a guinea pig anti-insulin antibody (4°C, overnight, Sigma) followed by incubation with Alexa 488 conjugated anti-guinea pig (1 hour, room temperature, Invitrogen).
- TUNEL staining was performed by incubating islet sections in TUNEL reagent mixture (30 minutes, 37°C, Roche) following insulin immunostaining.
- Micrographs were taken using an inverted Zeiss fluorescence microscope.
- Statistical analysis was performed using one-way ANOVA or Student's *t*-test ($p<0.05$ was taken as level of significance).

RESULTS

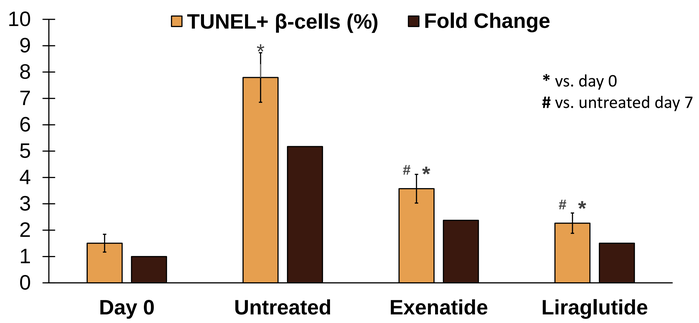

Figure 3. Percentage of TUNEL+ β-cells (mean ± S.E.M) and fold change in TUNEL+ β-cells between conditions.

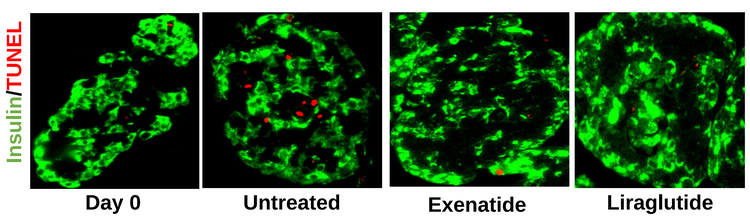

Figure 4. Representative micrographs from untreated and treated paraffin-embedded mouse islets immunolabelled for insulin and TUNEL.

CONCLUSION

- Freshly isolated islets from hIAPP-expressing mice which lack amyloid had a low rate of apoptosis, but β-cell apoptosis was increased in amyloid-forming islets during 7-day culture.
- Treatment with GLP-1 agonists (exenatide or liraglutide) significantly reduced β-cell apoptosis in the 7-day cultured amyloid-forming islets as compared to non-treated islets.
- The number of TUNEL+ β-cells in was lower in liraglutide treated as compared to exenatide treated amyloid-forming islets, suggesting that long-acting GLP-1 agonists may provide a better protection against amyloid-induced β-cell death during *ex vivo* culture.

SUMMARY

Treatment with GLP-1 agonists may provide an effective approach to enhance islet survival during pre-transplant culture period thereby enhancing long-term survival of islet grafts in patients with T1D.

Long-acting GLP-1 agonists may have higher potency in protecting islet β-cells from amyloid toxicity than short-acting GLP-1 agonists.

Future Research: Treatment of cultured human islets with a GLP-1 agonist.

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