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Exenatide protects pancreatic islets against brain death-induced inflammation and viability loss

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INTRODUCTION

Pancreatic islet transplantation is an effective therapy for unstable type 1 diabetes mellitus. However, restoration of β-cell function after transplantation and the ensuing improvement in glycemic control depend on a high rate of islet engraftment, which is hindered by the marked destruction of islets during the isolation and transplantation processes. The donor's brain death (BD) is among the main factors contributing for islet loss in this scenario. Exendin-4, a glucagon-like peptide-1 (GLP-1) synthetic analog, exerts anti-inflammatory effects leading to an increase in islet viability *in vitro*. Here, we hypothesized that this drug could alleviate the damage caused by BD on pancreatic islets, improving the quality of such islets for transplantation. Therefore, we proposed a study in a murine model of BD in which the effect of the administration of Exendin-4 (exenatide) was evaluated in respect to islet quality outcomes, such as viability, function, and expression of genes related to inflammation, and endoplasmic reticulum (ER) stress.

METHODS

- Male Wistar rats, weighting 300-350g, were separated into 3 experimental groups: 1) Sham (**control**), 2) **BD**, and 3) BD + Exendin-4 5µg/Kg (**BD+Ex-4**).
- Following pancreas retrieval and islet isolation, viability of dispersed islets was determined by fluorescein diacetate (FDA)/propidium iodide (PI) assay. β-cell function was evaluated by glucose stimulated-insulin release: islets were incubated (1h) in media containing first low (2.8 mM, LG) then high (28 mM, HG) glucose concentrations. The amount of insulin in the supernatant of each sample was measured using a rodent-specific insulin ELISA kit. Stimulation index (SI) was calculated by dividing insulin content of islets stimulated with HG by the insulin content of islets stimulated with LG.
- Gene expression of IL-1β, IL-6, TNF, Bcl-2, CHOP and BIP in pancreatic tissue and isolated islets were assessed by RT-qPCR, while apoptosis was investigate by western blot and immunohistochemistry for active-caspase 3.

RESULTS

Islet isolated from the BD group showed a striking reduction in viability as compared to control and BD+Ex-4 groups (**Figure 1A**). Accordingly, islets from BD rats had an increased number of dead cells (PI positive) as compared to the other groups (**Figure 1B**).

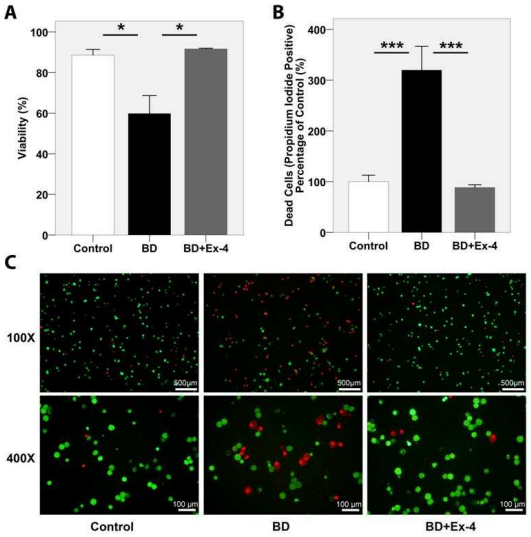

Fig. 1: Viability of isolated islets. A) Percentage of living cells. **B)** Percentage of dead cells. **C)** Representative photomicrographs of dispersed islet cells after staining with FDA/PI fluorescent dyes. Sample number = 3 per group. * P< 0.05; *** P< 0.001.

Islet isolated from the BD group showed a significant decrease in insulin secretion after stimulation with high glucose as compared with control and BD+Ex-4 groups (**Figure 2A**).

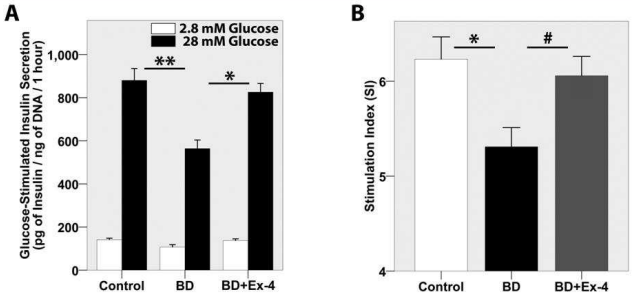

Fig. 2: Insulin release after glucose stimulation. A) Glucose-stimulated insulin secretion after incubation with first low glucose (1h) and then high glucose (1h) concentrations. **B)** Insulin stimulation index (insulin concentration in samples stimulated with high glucose / insulin concentration in samples stimulated with low glucose). Sample number = 3 by group. Fifteen islets were used by experiment (in triplicates). * P< 0.05; **P< 0.01; #P< 0.10.

A robust and significant increase in *IL-1β* gene expression was seen in the pancreatic tissue of animals from BD group *vs.* control and BD+Ex-4 (**Figure 3A**). *TNF* expression was increased in BD group *vs.* control animals; however, it did not differ significantly *vs.* BD+Ex-4 animals (**Figure 3B**). IL-6 was similar among groups. Moreover, none of these cytokines were differently expressed among islets isolated from the 3 experimental groups (data not shown).

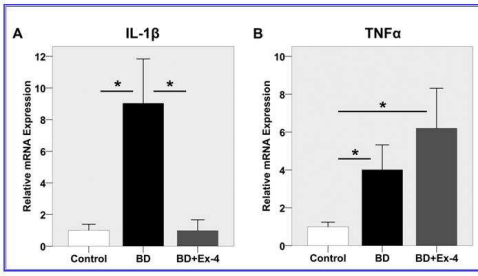

Fig. 3: Gene expression of pro-inflammatory cytokines in pancreatic tissue. A) Relative quantification of IL-1β. **B)** Relative quantification of TNF. Data are expressed in n-fold change in relation to a sample calibrator. *GADPH* gene as used as the reference gene. Sample number = 6 per group. * P< 0.05.

Interestingly, endoplasmic-reticulum (ER) stress genes, Chop and Bip, were increased in islets isolated from BD animals as compared to the other groups (**Figure 4A and 4B**). Our data also suggest a greater expression of *Bcl-2* in islets originated from the BD group (1.36 ± 0.12 *vs.* 1.0 ± 0.25 (control) *vs.* 0.93 ± 0.28 (BD+Exe-4) arbitrary units; P= 0.11).

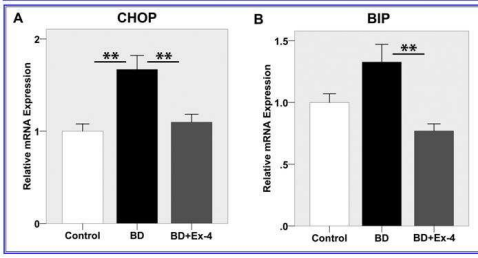

Fig. 4: Gene expression of ER stress genes in isolated islets. A) Relative quantification of *Chop*. **B)** Relative quantification of *Bip*. Data are expressed in n-fold change in relation to a sample calibrator. *GADPH* gene as used as the reference gene. Sample number = 6 per group. ** P< 0.01.

Active-caspase 3 protein concentrations, measured by both western blot and IHC analyses, were similar among islets from the 3 experimental groups (data not shown), indicating that apoptosis is not the main mechanism of death in islets from BD animals.

CONCLUSIONS

- Our data indicate an increase in the inflammatory state and ER stress as well as a reduction in the cell viability and function of islets isolated from the BD group. Exendin-4 administration following the establishment of the BD seems to protect the islets against such deleterious effects.

Competing interests/financial disclosure: Nothing to declare.
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