The Effects of Hypoxia and Cytotoxic Stress on β-cells during Transplantation

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Introduction

Type I diabetes is an autoimmune disorder in which the islets of Langerhans are destroyed, creating a deficiency in insulin and a lack of blood sugar regulation. The Schulze Diabetes Institute is researching islet cell transplantation as an appropriate therapy to replace destroyed islets. A major obstacle in the transplant process is protecting islet cells from fatal hypoxic and cytotoxic stresses that lower graft viability.

Current models of syngeneic islet transplantation estimate that 60% of β-cell mass undergo apoptosis within the first 3 days post-transplantation (1).

In this study, we used rat insulin-producing cells to mimic the process of islet cell transplantation by exposing them to low oxygen environments and different concentrations of three inflammatory cytokines. The purpose was to reveal the overall combined effects of hypoxia and cytotoxic stress on graft viability and to observe which cytokine had the most potent effect.

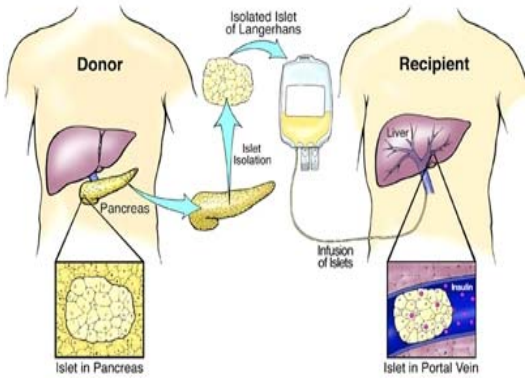


Figure 1. Islets are transplanted through the portal vein

Methods

•The rat insulinoma cell line, INS-1, was cultured in appropriate RPMI 1640 medium supplemented with appropriate growth factors and grown in 37°C in 5% CO_2

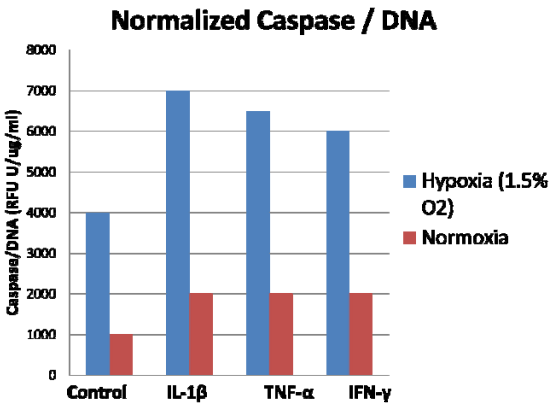
•INS-1 cells were passaged via trypsinization and placed in 6-well plates where they were treated with the following concentrations of cytokines

Treatment	Concentrations administered		
IL-1β	10 ng/mL	50 ng/mL	100 ng/mL
TNF-α	1000 U/mL	5,000 U/mL	10,000 U/mL
IFN-γ	100 U/mL	500 U/mL	1,000 U/mL
Control	No cytokine treatment		

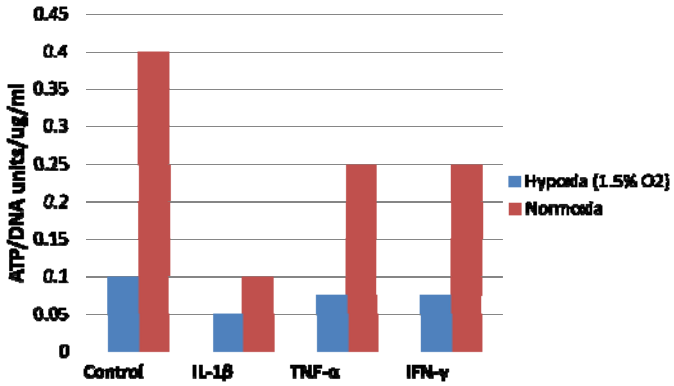
•INS-1 cells with treatment were placed in either a normoxic 37°c, 5% CO_2 incubator or a hypoxic 1% O_2 incubator for 24 hours

•Cell viability was assessed using ATP, Caspase and Oxygen Consumption Rate assays

•This data was normalized to the amount of DNA that was present



Normalized ATP/DNA



Predicted Results

•Caspase levels will be higher in hypoxic conditions and overall higher in cytokine- treated cells compared to untreated cells

•ATP levels will be lower in hypoxic conditions and overall lower in cytokine-treated cells compared to untreated cells

•Oxygen consumption rate will be higher for cells in normoxic conditions and not treated with cytokines

•Future directions include testing which combinations of cytokines provide the most synergism and potency

•Testing antioxidants, JNK inhibitors, and tocopherols to prevent INS-1 cell dysfunction and death in the presence of cytokines and hypoxia

References

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3. Papas, KK, Colton, CK, Nelson, RA, Rozak, PR, Avgoustiniatos, ES, Scott III, WE, Wildey, GM, Pisania, A, Weir GC and Hering, BJ. (2007). Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice. *American Journal of Transplantation*. 7:707-713.