Published in final edited form as:
Transplant Proc. 2008 March ; 40(2): 382–383. doi:10.1016/j.transproceed.2008.01.009.

β-CELL SPECIFIC CYTOPROTECTION BY PROLACTIN ON HUMAN ISLETS

Toshiyuki Yamamoto, **Camillo Ricordi**, **Atsuyoshi Mita**, **Atsushi Miki**, **Yasunaru Sakuma**, **R. Damaris Molano**, **Alessia Fornoni**, **Antonello Pileggi**, **Luca Inverardi**, and **Hirohito Ichii**
Diabetes Research Institute, University of Miami, Leonard M. Miller School of Medicine, Miami, FL, USA

Abstract

Many cytoprotective agents have been reported to improve islet isolation and transplantation outcomes. However, several cytoprotective agents may improve all cell subsets within an islet preparation, and selected non- β-cells components may have a negative effect on β-cell function and survival (e.g., acinar cells). In this study, we examined the effect of prolactin (PRL) supplementation to the culture medium, to determine whether it could exert β-cell-selective cytoprotection (islet viability and function) towards a possible use of PRL during pre transplant islet culture.

Materials and Methods—Human islets pre-cultured or not with recombinant human PRL (500 μg/L) for 48 hours. Non-β-cells and β-cell-specific fractional viability and cellular composition were assessed by FACS and Laser Scanning Cytometry (LSC). Islet potency was assessed in vivo by transplantation into chemically-induced diabetic immunodeficient mice.

Results—The relative viable β-cell mass and the relative islet β-cell content in PRL group were 28% higher (p=0.018) and 19% higher (p=0.029) than control group, respectively. All transplanted mice achieved normoglycemia in both groups, indicating that PRL treatment did not alter islet function.

Conclusions—PRL treatment improves β-cell-specific viability and survival in human islets in vitro. The development of novel β-cell-specific cytoprotective strategies will be of assistance in improving islet transplantation.

Substantial amounts of non-β-cells are present in the final islet cell products that are used for clinical transplantation. Many cytoprotective agents, such as glucagon-like peptide-1 (GLP-1), lactogens, hepatocyte growth factor, parathyroid hormone-related protein (PTHrP) and insulin-like growth factors (IGF), have been reported to improve islet isolation and transplantation outcomes. These agents may, however, improve all cell subsets within an islet preparation, and selected non- β-cells components, may have a negative effect on β-cell function and survival (e.g., acinar cells) In this study, we examined the effect of prolactin (PRL) supplementation to the culture medium, to determine whether it could exert β-cell-selective cytoprotection (islet viability and function) towards a possible use of PRL during pre-transplant islet culture.

Address correspondence to: Dr. H. Ichii, University of Miami, Leonard M. Miller School and Medicine, Diabetes Research Institute, 1450 NW 10th Miami, FL 33136. E-mail: hichii@med.miami.edu.

11th Congress of the International Pancreas and Islet Transplantation Association (IPITA)
Minneapolis, Minnesota September 15-20, 2007

Materials and Methods

Human islet isolation and culture

Pancreata were procured from brain-death donors and then immediately placed in either pre-oxygenated (30 min) two-layer perfluorocarbon/ University of Wisconsin solution (PFC/UW) (1-2) or with UW alone. All human islets were isolated using the automated method developed by Ricordi et al (3) at the Human Cell Processing Facility of the University of Miami School of Medicine. The islet yield and purity were determined by dithizone staining and then cultured in MM1A culture medium (Mediatech-Cellgro) with or without 500 μg/L of Prolactin (Sigma-Aldrich) for 2 days at 37°C in 5% CO2-humidified atmosphere.

Assessment of cellular composition and Beta cell viability

We have recently established the method for the assessment of cellular composition and fractional beta-cell viability in human islets (4). In brief, islets were dissociated using accutase (Innovative Cell Technologies) and dispersed cells were fixed on glass slides with 2.5% paraformaldehyde. The cells were incubated for 2 hours with the following primary antibodies: mouse monoclonal antibody to insulin; rabbit polyclonal antibody to pancreatic polypeptide (PP); mouse monoclonal antibody to glucagons; rabbit polyclonal antibody to somatostain. After washing, samples were incubated for 1h with Alexa Fluor 488 goat anti-mouse IgG, Alexa Fluor 488 goat anti-rabbit IgG antibodies and 4',6-diamidino-2-phenylindole (DAPI). The samples were analyzed using a LSC (CompuCyte). In order to assess the beta cell viability, single cell suspensions were incubated with 1 μM Newport Green PDX acetoxymethylether and 100 ng/mL of tetramethylrhodamine ethyl ester (TMRE) for 30 min at 37°C in PBS. After washing, the cells were stained with 7-aminoactinomycin D (7-AAD) and then analyzed using a FACScan cytometer (Becton Dickinson) with the CellQuest software.

In vivo assessment of islet potency in the diabetic nude mouse model

Animal procedures were approved by the IACUC, and performed in the Preclinical Cell Processing Core. Male athymic *nu/nu* (nude) mice (Harlan Laboratories) were housed in virus-antibody-free rooms in microisolated cages, having free access to autoclaved chow and water. Animals were rendered diabetic via IV administration of 200 mg/kg of Streptozotocin. Nonfasting blood glucose was assessed with a glucometer. Mice with sustained hyperglycemia (>300 mg/dL) were used as islet graft recipients. 1000 human IEQ per recipient were transplanted under the kidney capsule and nonfasting blood glucose values were assessed three times a week. Reversal of diabetes was defined as stable nonfasting blood glucose <200mg/dL. Nephrectomy of the graft-bearing kidney was performed to confirm return to hyperglycemia and exclude residual function of the native pancreas in animals achieving normoglycemia after transplantation.

Statistical analysis

Data were analyzed using Excel for Windows software for descriptive statistics and data plotting. Data are shown as mean standard deviation (SD). Statistical significance was considered for $p<0.05$.

Results

The relative islet β-cell content in the PRL group was 18% higher (p=0.029) than control group. In the PRL group, the relative viable β-cell mass was 26% higher (p=0.018) compared with the control group. Furthermore, the relative viable non β-cell mass in the PRL group was 11% lower (p=0.011) when compared to the control group. All transplanted mice achieved normoglycemia in both groups, indicating that PRL treatment did not alter islet function.

Conclusion

PRL treatment improves β-cell-specific viability and survival in human islets in vitro and in vivo studies. The development of novel β-cell-specific cytoprotective strategies will be of assistance in improving islet transplantation.

Acknowledgements

This work was supported in part by NIH-NCRR, GCRC MO1RR16587, NIDDKRO1-DK55347-IU42RR016603, 5R01 DK25802, ICR 5U42RR016603, JDRFI 4-200-946 and 4-2004-361, and the Diabetes Research Institute Foundation.

References

1. Kuroda Y, Kawamura T, Suzuki, et al. A new, simple method for cold storage of the pancreas using perfluorochemical. Transplantation 1988;46:457–460. [PubMed: 3047936]
2. Ricordi C, Fraker C, Szust J, et al. Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution. Transplantation 2003;75:1524–1527. [PubMed: 12792508]
3. Ricordi C, Lacy PE, Finke EH, et al. Automated method for isolation of human pancreatic islets. Diabetes 1988;37:413–420. [PubMed: 3288530]
4. Ichii H, Inverardi L, Pileggi A, et al. A novel method for the assessment of cellular composition and beta-cell viability in human islet preparations. Am J Transplant 2005;5:1635–1645. [PubMed: 15943621]

Table 1

	Percentage of islet cell	Percentage of whole islet preparation	
	Beta cell	Beta cell	Acinar cell
Control	49.4 ± 7.8%	24.7 ± 6.8%	13.6 ± 6.6%
PRL	56.1 ± 5.6%	34.0 ± 9.5%	13.6 ± 8.4%

Data are shown as means ± standard deviation (SD).