Published in final edited form as:
Transplant Proc. 2009 ; 41(1): 343–345. doi:10.1016/j.transproceed.2008.10.064.

The Effect of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) in Islet Transplantation

Yasunaru Sakuma[a, f], **Camillo Ricordi**[a,b,c], **Atsushi Miki**[a], **Toshiyuki Yamamoto**[a], **Atsuyoshi Mita**[a], **Scott Barker**[a], **R Molano Damaris**[a], **Antonello Pileggi**[a,b], **Yoshikazu Yasuda**[d], **Toshihiko Yada**[e], and **Hirohito Ichii**[a,b,c,*]

[a]Cell Transplant Center, Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

[b]Department of Surgery, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

[c]Transplant Institute, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA

[d]Department of Surgery, Jichi medical University

[e]Department of Physiology, Jichi medical University

Abstract

Pituitary adenylate cyclase-activating polypeptide (PACAP) is an islet substance serving as an intra-islet amplifier of glucose induced insulin secretion similar to exendin-4. It has been reported that systemic administration of PACAP maintained beta-cell mass, delayed the onset of hyperglycemia and protected beta cells from glucose toxicity. Moreover, PACAP increases glucose-stimulated insulin release in vitro and in vivo. In this study, we investigated the possibility of PACAP use in human islet transplantation.

Methods—Human islets were cultured in the presence or absence of PACAP (10^{-12}M) for 48 hours. Beta-cell viability by FACS, cellular composition analysis by iCys/LSC and glucose stimulated insulin secretion were assessed. In vivo, islets were transplanted beneath the kidney capsule of Streptozotocin induced diabetic immunodeficient mice. Intravenous glucose tolerance test (IVGTT) was also performed in presence or absence with PACAP (Peptide International; 1.3 nmol/kg).

Results—There were significant improvements in terms of beta cell viability and cellular composition between islets cultured with or without PACAP (respectively; $P<0.05$). Moreover, glucose stimulated insulin secretion significantly improved in islets cultured with PACAP, compared to controls, respectively; $P<0.05$). Treatment of recipient mice with PACAP resulted in beneficial effects on insulin secretion (PACAP vs. control, l3.2 vs. 1.9 mU/l), in IVGTT. However, no significant difference in glucose level between the two groups was observed.

Conclusions—Our study indicated that PACAP significantly improved beta cell viability and survival during culture, and increased insulin secretion in vitro and in vivo. However, blood glucose levels in vivo after an IVGTT did not significantly improve probably due to increased glucagon secretion from alpha cells. PACAP supplementation to culture medium could be of assistance in improving clinical islet transplantation outcomes.

[*]**corresponding author contact information**: Hirohito Ichii, M.D, Ph.D., Cell Transplant Center, Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, 1450 NW 10th Avenue (R-134), Miami, Florida 33136, Phone: (305) 243-3700, Fax: (305) 243-4404, hichii@med.miami.edu.

Islet transplantation has become a favorable therapy for patients with Type 1 diabetes (1, 2). However, further improvements are still required to maintain insulin independence for a long time. Previous studies have identified several modifications to maximize both islet yield and quality, thereby increasing the successful islet transplantation. The neuropeptide pituitary adenylate cyclase–activating polypeptide (PACAP) is ubiquitously distributed not only in the central and peripheral nervous systems but also in the pancreatic islets and exerts a variety of effects (3). Recent studies revealed the potential effect of PACAP on insulin secretion in a glucose-dependent manner (4). Accumulating evidence in animal studies points to a physiological importance of PACAP in the regulation of the insulin response to feeding (5). The aim of the present study is to investigate the function of PACAP in human islet cells and the possibility of PACAP use in clinical islet transplantation.

MATERIAL AND METHODS

Human islet isolation and culture

Pancreata were obtained from multiorgan cadaveric donors after cerebral death. Islets were isolated using a modified automated method and purified on density gradients (1). After isolation, human islets were cultured in Miami defined culture medium (Mediatech-Cellgro, Herindon, Va) with the presence or absence of PACAP (10^{-12}M, Peptide International, Louisville, KE) for 48 hours 37°C in a 5% CO2–humidified atmosphere.

Fractional beta cell viability assay and cellular composition assay

Dissociated human islets were assessed for cellular composition and fractional beta cell viability as previously reported (6). The cells were incubated for 2 hours with the following primary antibodies: mouse monoclonal antibody to insulin (Neo Markers, Fremont, CA); rabbit polyclonal antibody to pancreatic polypeptide (DAKO, Carpenteria, CA); mouse monoclonal antibody to glucagons (R&D systems, Minneapolis, MN); and rabbit polyclonal antibody to somatostatin (DAKO). The samples were analyzed using a laser scanning cytometer (iCys/LSC, CompuCyte, Cambrige, MA). To assess the beta cell viability, single cell suspensions were incubated with 1 μmol/L Newport Green PDX acetoxymethylether (NG; Molecular Probes, Eugene, OR) and 100 ng/mL of tetramethylrhodamine ethyl ester (TMRE, Molecular Probes). After washing, the cells were stained with 7-aminoactinomycin D (7-AAD, Molecular Probes) and then analyzed using a FACScan cytometer (Becton Dickinson, Mountain View, Calif) with the CellQuest software.

Perifusion

Selected islet preparations were analyzed for their response to a dynamic stimulation assay *in vitro* (6). Briefly, islets were perifused in the same buffer for 10 minutes and then sequentially exposed to 11-mM and 3-mM glucose, and 25-mM KCl. Fractions were collected every 2 minutes with 3-mM glucose, and every minute during stimulation. The collected fractions were then assessed for human insulin concentrations by ELISA (Mercodia Inc., Winston Salem, NC).

In Vivo assessment of islet potency in chemically induced diabetic immuno deficient mouse

Animals were rendered diabetic via single intravenous administration of 200 mg/kg of Streptozotocin (Sigma, St Louis, MO). Mice with sustained hyperglycemia (>300 mg/dL) were used as islet graft recipients. 2000 IEQ of human islets preparations (n=4) were transplanted under the kidney capsule of diabetic immunodeficient mice (7).

Intravenous glucose tolerance test

At least 1 month after transplantation, mice were fasted overnight and then blood glucose and insulin levels were assessed by IVGTT. Mice received an intravenous glucose bolus (2g/kg bw) with or without PACAP (1.3 nmol/kg). A blood sample was taken from the retrobulbar, intraorbital capillary plexus at selected time points after injection.

Statistical Analysis

Data were analyzed using Excel for Windows software for descriptive statistics and data plotting. Data are shown as mean values ± standard deviations (SD). Statistical significance was considered for $P < .05$.

RESULTS

Significant improvements regarding beta cell viability were observed (P<0.05). In cellular composition assay, surviving beta cell mass during culture also significantly increased (control vs. PACAP, 15.0% vs. 19.2%, respectively; P<0.05). Moreover, insulin secretion was statistically augmented after loading of 11-mM glucose, but showed no difference after KCL immersion glucose stimulated insulin secretion. *In vivo* insulin secretion by glucose tolerance test using islet transplantation model showed significant improvement in PACAP group, compared to controls (34 vs. 3.0 IU/ml, respectively; P<0.05). However, no significant difference in glucose level was observed.

DISCUSSION

Our study indicated that PACAP significantly improved beta cell viability and survival during culture, and increased insulin secretion in vitro and in vivo. However, in spite of the marked increase in insulin secretion in vivo, the glucose level was not affected. The exact mechanism behind this finding is not known but may be the consequence of increased secretion of glucagon or epinephrine, contributing to elevated plasma glucose levels, which suggests that PACAP administration resulted in reduced insulin sensitivity (8).

Considering from the *in vitro* studies, PACAP supplementation to culture medium could be of assistance in improving clinical islet transplantation outcomes.

Acknowledgments

This work was supported in part by NIH-NCRR, GCRC MO1RR16587, NIDDK RO1-DK55347-IU42RR016603, 5R01 DK25802, ICR 5U42RR016603, JDRFI 4-200-946 and 4-2004-361, and the Diabetes Research Institute Foundation.

References

1. Ricordi C. Islet transplantation: a brave new world. Diabetes. 2003; 52:1595. [PubMed: 12829621]
2. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000; 343:230. [PubMed: 10911004]
3. Arimura A. Perspectives on pituitary adenylate cyclase activating polypeptide (PACAP) in the neuroendocrine, endocrine, and nervous systems. Jpn J Physiol. 1998; 48:301. [PubMed: 9852340]
4. Filipsson K, Kvist Reimer M, Ahren B. The neuropeptide pituitary adenylate cyclase-activating polypeptide and islet function. Diabetes. 2001; 50:1959. [PubMed: 11522660]
5. Yada T, Sakurada M, Filipsson K, et al. Intraperitoneal PACAP administration decreases blood glucose in GK rats, and in normal and high fat diet mice. Ann N Y Acad Sci. 2000; 921:259. [PubMed: 11193831]

6. 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. [PubMed: 15943621]
7. Ranuncoli A, Cautero N, Ricordi C, et al. Islet cell transplantation: in vivo and in vitro functional assessment of nonhuman primate pancreatic islets. Cell Transplant. 2000; 9:409. [PubMed: 10972339]
8. Filipsson K, Pacini G, Scheurink AJ, et al. Am J Physiol. 1998; 274:E834. [PubMed: 9612241]