Liraglutide, a Long-Acting Human Glucagon-Like Peptide 1 Analog, Improves Glucose Homeostasis in Marginal Mass Islet Transplantation in Mice


The current scarcity of high-quality deceased pancreas donors prevents widespread application of islet transplantation for treatment of labile type 1 diabetes mellitus. Opportunities for the improvement of current techniques include optimization of islet isolation and purification, use of culture with pharmacological insulinotropic agents, strategies to reduce graft rejection and inflammation, and the search for alternative insulin producing tissue. Here, we report our findings on the efficacy of the long-acting human glucagon-like peptide 1 analog, liraglutide, in a mouse model of marginal mass islet transplantation. Liraglutide was administered (200 μg/kg sc twice daily) after a marginal mass syngeneic islet transplant in streptozotocin-induced diabetic BALB/c mice. Time-to-normoglycemia was significantly shorter in liraglutide-treated animals (median 1 vs. 7 d; \( P = 0.0005 \)), even in recipients receiving sirolimus (median 1 vs. 72.5 d; \( P < 0.0001 \)). Liraglutide-treated animals also demonstrated improved glucose tolerance as assessed by an ip glucose tolerance test. Liraglutide discontinuation at postoperative d 90 resulted in diminished glucose tolerance during the ip glucose tolerance test, whereas a late-start liraglutide therapy 90 d after transplant resulted in no improvement. These findings suggest that liraglutide therapy mediates early and late insulinotropic effects. In accord with this hypothesis, insulin/terminal deoxynucleotidyl transferase-mediated deoxyuridine 5-triphosphate nick end labeling fluorescence microscopy showed reduced transplanted β-cell apoptosis in liraglutide-treated recipients 48 h after transplant. In addition, liraglutide resulted in improved glucose-dependent insulin secretion. Overall, our data show that liraglutide has a beneficial impact on the engraftment and function of syngeneic islet secretion. Overall, our data show that liraglutide has a beneficial impact on the engraftment and function of syngeneic islet transplants in mice, when administered continuously starting on the day of transplant. (Endocrinology 149: 4322–4328, 2008)

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Islet transplantation is an emerging therapeutic option for the treatment of select labile type 1 diabetes mellitus patients. The announcement of the first consecutive series of successful islet transplants in type 1 diabetic patients using the Edmonton Protocol (1) sparked renewed interest in the field.

However, several barriers prevent broad application of islet transplantation, even within this select patient group. The relatively limited availability of cadaveric pancreases is especially critical because not every islet isolation attempt currently yields an adequate mass of tissue for transplant. In addition, more than one islet infusion is often necessary to achieve insulin independence. Genetically modified tissue and alternative sources of insulin secreting tissue (porcine or otherwise) could be long-term solutions to this problem. However, many centers are seeking augmentative therapy to islet transplantation. The ideal candidate would both reduce the amount of islet tissue needed to render and maintain the patient insulin independent and be appropriate for concurrent use with immunosuppressive therapy.

Recent reports, including from a multicenter trial of the Edmonton Protocol, show that graft function declines markedly in transplanted patients with time (2, 3). This evanescence of islet graft function may be the combined result of allograft rejection, autoimmune diabetes recurrence, or islet metabolic burnout.

Immunosuppressive agents, although beneficial in preventing islet rejection, may have side effects, including diminishing islet function or inducing islet death. Although initial reports suggested that sirolimus, one such immunosuppressive drug, did not have a detrimental effect on islet function (4, 5), several recent investigations have shown that sirolimus has a negative impact on β-cell function by directly reducing viability, preventing vascular endothelial growth factor-mediated angiogenesis, and preventing neogenesis of β-cells from cotransplanted ductal cells (6–8).

Glucagon-like peptide 1 (GLP-1) receptor agonists are emerging therapeutic options in the field of type 2 diabetes (9). GLP-1, an incretin hormone, exerts effects through the specific GLP-1 receptor (GLP-1R), including stimulation of insulin secretion, suppression of glucagon secretion, slower gastric emptying, and increased satiety. Native GLP-1 administration has limited therapeutic benefit due to its short biological half-life (several minutes). However, GLP-1 analogs, including exenatide (half-life 60–90 min) and liraglutide (half-life 13 h when administered sc) (10), are more

First Published Online May 29, 2008

Abbreviations: AUC, Area under the curve; BrdU, 5-bromo-2-deoxyuridine; GLP-1, glucagon-like peptide 1; GLP-1R, glucagon-like peptide 1 receptor; IPGTT, ip glucose tolerance test; TTN, time to normoglycemia; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine 5-triphosphate nick end labeling.

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.
promising candidates. GLP-1 analogs have recently been discovered to have beneficial effects as adjunct therapy in type 1 diabetes mellitus models (11, 12), and in pancreatic islet transplant preclinical models (13–16). Furthermore, some groups have studied these analogs in human islet transplantation, which has provided evidence that isolated islets remain responsive to GLP-1R agonists (17, 18). Liraglutide is an especially attractive candidate for islet transplantation; in addition to its long half-life, it has been reported to maintaining glycemic control in type 2 diabetes with a low risk of hypoglycemia (10, 19–22).

However, current literature on the therapeutic role for GLP-1 analogs is limited to observational rather than mechanistic studies in islet transplantation. Furthermore, there is a need to understand whether documented functional effects of GLP-1 analogs in islet transplant can circumvent immunosuppressive toxicities on islets, especially those of sirolimus, which are of particular concern and have been well described elsewhere (6–8, 23).

Here, we report our investigation of liraglutide in islet transplantation. We investigated both the effect of liraglutide on islet engraftment in the short and long term. Furthermore, we investigate the mechanism of liraglutide action in the setting of islet transplantation. Finally, we sought to investigate the ability of liraglutide to improve islet engraftment in the presence of the immunosuppressive agent sirolimus at clinically relevant blood levels. Our studies make use of a syngeneic mouse marginal mass islet transplant model described previously (24), and to the best of our knowledge, provide the first report of liraglutide use in islet transplantation.

Materials and Methods

Animals and reagents

BALB/c mice (age 8–10 wk) were obtained from the Health Sciences Laboratory Animal Services at the University of Alberta (Edmonton, Canada). All animals were housed using conventional methods following Canadian Council on Animal Care guidelines under approval of the University of Alberta Health Sciences Animal Care and Use Committee. BALB/c mice were used as either islet transplant recipients or syngeneic donors. All chemicals were purchased from Sigma-Aldrich (Oakville, Ontario, Canada) unless otherwise noted. Liraglutide was provided by Novo Nordisk ( Bagsvaerd, Denmark).

Diabetes induction

Streptozotocin (200 mg/kg ip) was used to induce diabetes in BALB/c mice at least 5 d before islet transplantation. Diabetes was defined as nonfasting blood glucose levels more than or equal to 18.0 mmol/l for two or more consecutive days before transplantation.

Islet isolation

Islets were isolated from BALB/c mice as described previously with minor modifications (25, 26). Briefly, mouse pancreata were digested with collagenase (1.0 mg/ml in Hanks' buffered saline solution) and purified by Ficol-density centrifugation. Handpicked islets were washed twice with Hanks' buffered saline solution before short-term culture in Connaught Medical Research Laboratories (CMRL-1066) medium supplemented with 10% fetal bovine serum; islets were cultured for a maximum of 2 h before transplantation.

Islet transplantation

A marginal mass of islets (250 islets) was handpicked and aspirated into PE50 tubing using a microsyringe, and attached to a custom-built rotor for low-velocity (100 × g) centrifugation into a pellet suitable for transplantation. Islets were transplanted under the left renal capsule of diabetic mice under isoflurane anesthesia.

Blood glucose measurements

Tail capillary blood glucose levels were measured on alternate days using OneTouch Ultra (LifeScan, Inc., Milpitas, CA) glucose meter.

Intraperitoneal glucose tolerance test (IPGTT)

At time points indicated, animals were challenged with a 3 g/kg body weight ip dextrose bolus after an 18-h fast. Blood glucose was monitored before injection, and at 5, 15, 30, 60, and 120 min.

Graft explant by nephrectomy

To confirm the graft-dependent normoglycemia, animals with functional grafts had their islet transplants explanted by left nephrectomy. Transplant recipients were placed under isoflurane anesthesia and had their left kidney exposed. A surgical clip was used to occlude the renal vessels and ureter. The left kidney was dissected from the animal. The explanted graft was either prepared for histology or snap frozen for total graft insulin content. Animals were monitored after nephrectomy for 5 d; a return to hyperglycemia confirmed graft function over naive pancreas β-cell regeneration.

Sirolimus and blood drug levels

Sirolimus (Rapamune; Wyeth-Ayerst Laboratories, Madison, NJ) was purchased as solution from the University of Alberta Pharmacy and administered at 0.2 mg/kg as an ip injection. We selected this dose of sirolimus based on a pilot study in our laboratory that showed that a dose of 0.2 mg/kg sirolimus resulted in trough levels in the range of 5.93 ± 3.1 ng/ml (mean ± sem, measured by the University of Alberta Hospital, Special Investigations Unit), which corresponds to a clinically relevant exposure.

Serum insulin levels

To determine serum insulin, animals were terminally bled under isoflurane anesthesia by cardiac puncture. Serum was prepared by allowing blood to clot at room temperature for 15 min, and separated by centrifugation at 2000 rpm for 10 min. Serum was collected and stored at −20 C. Rodent insulin concentration in serum was measured using a commercial ELISA kit (LINCO Research, Inc., St. Charles, MO).

5-Bromo-2-deoxyuridine (BrdU) assay for β-cell proliferation

For studies of proliferation, BrdU was supplemented to the drinking water of experimental animals at a concentration of 1 mg/ml. BrdU supplemented water was administered starting on the day of transplantation for a period of 2 wk. At 2 wk after transplant, the islet graft was explanted.

Histology

Animals designated for histological analysis had their islet-bearing kidney harvested, fixed in formalin, and prepared in paraffin-embedded blocks for sectioning. Guinea pig anti-insulin antibody (Dako, Mississauga, Ontario, Canada) at a dilution of 1:500 was used for insulin staining. Secondary biotinylated goat antimouse antibody (visualized with avidin-biotin complex with diaminobenzidine as a chromagen) or tetramethylrhodamine isocyanate conjugated goat antimouse antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was used for detection of the primary antibody. Using the method described by Emanuelli et al. (27), islets were stained for insulin and apoptotic cells concurrently to quantify the ratio of apoptotic to nonapoptotic β-cell. The DeadEnd fluorometric terminal deoxynucleotidyl transferase-mediated deoxyuridine 5-triphosphate nick end labeling (TUNEL) kit (Promega Corp., Madison WI) was used as per manufacturer’s instructions to detect apoptotic cells.
FIG. 1. Return to normoglycemia and daily blood glucose level after marginal mass islet transplantation. Diabetic BALB/c animals were transplanted with a marginal mass of syngeneic islets. Blood glucose was measured on alternate days after transplantation. Survival analysis of TTN, defined as the first post-transplant day of sustained nonfasting blood glucose less than 12.0 mmol, is shown here using the log-rank method for the following treatment groups. A, Vehicle-treated (solid black, n = 20) vs. liraglutide-treated recipients (interrupted black, n = 20) (P = 0.0003). B, Sirolimus-treated (solid gray, n = 10) vs. combination sirolimus plus liraglutide-treated recipients (interrupted gray, n = 10) (P = 0.0001). Mean nonfasting blood glucose levels of these recipients are shown in C and D. N, Day of nephrectomy.

4',6-Diamidino-2-phenylindole or hematoxylin/eosin was used as counterstains for immunofluorescence and light microscopy, respectively.

Insulin/BrdU immunofluorescence histology

Formalin-fixed and paraffin-embedded sections were hydrated and subsequently processed for antigen retrieval by microwave in sodium citrate buffer. After a blocking step using 20% normal goat serum, slides were stained with 1:500 guinea pig anti-insulin and 1:40 mouse anti-BrdU, (Calbiochem, VWR Canlab, Mississauga, Ontario, Canada). Secondary antibodies (goat anti-guinea pig Alexa 488, 1:200 and goat anti-mouse Alexa 594, 1:200; both from Invitrogen, Burlington, Ontario, Canada) were used for fluorescent detection. Slides were prepared with ProLong Gold antifade plus 4',6-diamidino-2-phenylindole (Invitrogen). Appropriate controls without either primary antibody were prepared with each experiment.

Islet graft insulin content

Islet grafts dissected from the underlying kidney were snap frozen in liquid nitrogen for subsequent insulin extraction in acid-ethanol by homogenization and ultrasonic cell membrane disruption. Rodent insulin concentration of the neutralized extract was measured using a commercial ELISA kit (LINCO Research).

Statistical analysis

Data were analyzed using GraphPad Prism (version 4.0a; GraphPad Software Inc., San Diego, CA). Statistical tests used in specific analyses are cited in Results; P values less than 0.05 were considered statistically significant. Graphical representation of data is represented as mean ± SEM, unless otherwise indicated in the figure legends.

Results

Liraglutide improves engraftment in marginal mass islet transplant recipients

Diabetic BALB/c animals were transplanted with a marginal mass of 250 syngeneic islets, and randomized to four treatment groups: vehicle (n = 20); liraglutide (200 mg/kg sc twice daily, n = 20); sirolimus (0.2 mg/kg ip, n = 10); or sirolimus plus liraglutide (n = 10). The extent of hyperglycemia among transplant recipients was not found to be statistically different among treatment groups (P = 0.3631 using one-way ANOVA); blood glucose levels on the day of transplant were 25.6 ± 1.1, 23.5 ± 1.0, 24.3 ± 1.4, and 23.50 ± 1.3 mM, respectively, in treatment groups (Fig. 1, C and D). Blood glucose was monitored every second day until 25 d after transplant, and every 5 d thereafter. Engraftment was monitored as the time to normoglycemia (TTN) defined as the first post-transplant day of sustained blood glucose less than 12.0 mM in each animal. Log-rank survival analysis was conducted among groups (Fig. 1, A and B). TTN was found to be shorter in liraglutide-treated animals when compared with vehicle (median 1 vs. 7 d; P = 0.0003). Furthermore, liraglutide shortened TTN in sirolimus-treated marginal mass islet transplant recipient animals (median 1 vs. 7.25 d; P < 0.0001). Interestingly, TTN was not different between liraglutide and liraglutide plus sirolimus-treated recipients (P = 0.8176), suggesting that liraglutide protected against the delayed engraftment of marginal mass islets from sirolimus. Upon nephrectomy, all animals returned to their pre-transplant hyperglycemic status, indicating graft-dependent normoglycemia and not regeneration of naive pancreatic β-cell function.

Liraglutide has a beneficial effect on glucose tolerance in islet transplant recipients independent of satiety

Because liraglutide and other GLP-1 analogs are known to induce satiety (28, 29), we sought to investigate further whether the reduced TTN in liraglutide-treated animals was simply due to reduced food intake and/or reduced body weight in liraglutide-treated animals.

Body weight of all recipients was monitored weekly (Fig. 2A). Transplant recipients exhibited a recovery in body weight with time after transplantation, regardless of treatment group (Fig. 2A; P < 0.0001 analyzed by two-way repeated measured ANOVA). Although liraglutide-treated animals experienced lower body weight when compared with control cohorts, this treatment factor did not reach statistical significance in the two-way ANOVA analysis (P = 0.0528).
To assess glucose tolerance in the absence of food intake as a confounding factor, we additionally conducted an IPGTT on all transplant recipients 2, 4, 8, and 12 wk after transplant to quantify glucose tolerance in these animals. Repeated measures two-way ANOVA on area under the curve (AUC) (Fig. 2C) of the glucose curve (Fig. 2B) revealed a treatment and time effect on glucose tolerance (P < 0.001). The Bonferroni post hoc test showed statistically significant AUCs between liraglutide and vehicle-treated animals (P < 0.05) at all time points. Furthermore, a statistically significant difference was found in the glucose tolerance of liraglutide plus sirolimus-treated animals when compared with sirolimus-alone treated animals (P < 0.05 at 4 and 8 wk after transplant). These findings suggest that reduced food intake alone does not explain improved nonfasting glycemia in liraglutide-treated animals.

Continuous liraglutide administration starting on the day of transplant has a beneficial impact on islet graft-dependent glucose homeostasis

In experiments described previously, liraglutide was administered twice daily starting on the day of transplant and sustained until postoperative d 90. To examine if such early and sustained liraglutide administration was absolutely required to retain a beneficial impact on glucose homeostasis, a cross-over style experiment was conducted. Randomly selected animals in the liraglutide-only group (n = 5 of 20) were discontinued of liraglutide on post-transplant d 91. Furthermore, five randomly selected animals from the vehicle group were initiated on liraglutide therapy at post-transplant d 91. At post-transplant d 120, all animals were challenged with an IPGTT (Fig. 2D). Although not statistically significant, the d-90 mean IPGTT AUCs of vehicle-treated recipients started on liraglutide appear lower than that of vehicle controls. This trend was not due to selection bias because animals were assigned by random assignment to either group. Statistical analyses were conducted using paired analyses to afford the most stringent statistical test.

We found that vehicle-treated recipients and vehicle-treated animals that receive delayed onset liraglutide maintained stable IPGTT AUC values from post-transplant d 90 to 120 (Mann-Whitney U test, P = 0.8857 and 0.8413). However, if liraglutide was discontinued after d 90 after transplant, the AUC increased from a mean of 954.6 to 1922.3 mm × min (P < 0.05), reaching a similar poor glucose tolerance as that of vehicle-treated recipients. Furthermore, analysis of animals on continuous liraglutide shows no statistical difference in IPGTT AUC between post-transplant d 90 and 120 (P = 0.4206).

Administration of liraglutide immediately after islet transplant prevents β-cell apoptosis

To investigate why liraglutide must be administered early after transplant, we investigated whether liraglutide has any impact on β-cell proliferation or apoptosis in the immediate post-transplant period.

To investigate β-cell proliferation, we assayed cumulative BrdU incorporation during the first 2 wk of transplant. Because β-cell proliferation is less than 1% when assayed at a single time point (30–32), we chose to continuously administer BrdU in the drinking water for 2 wk to increase the sensitivity of our assay using the protocol of Teta et al. (33). A separate cohort of transplant animals from the ones described previously was transplanted with a marginal mass islet transplant and administered BrdU in its drinking water starting at the day of transplant. The four treatment groups were as in the preceding experiments (n = 5 animals per group). Animals had their islet graft-bearing kidney harvested 2 wk after transplantation. Kidney histological sections were costained for BrdU and insulin. The percentage of BrdU positive insulin staining cells (proliferated β-cells) was quantified in each tissue sample using a sufficient number of sections to achieve 100 β-cells per transplanted animal (Fig. 3, A and C). Statistical analysis showed no
statistically significant difference in percentage of BrdU-positive among insulin-positive cells among the four groups (one-way ANOVA, $P = 0.3447$).

To investigate β-cell apoptosis, we used a histological TUNEL assay. A third series of marginal mass islet transplants was conducted using similar procedures described previously using the same four treatment groups ($n = 5$ animals per group). Because the rate of apoptosis among transplanted murine β-cells is highest (previously documented to be as high as 40%) in the immediate post-transplant period and tapers to very low levels after 2 wk (34), we focused our investigation of apoptosis during the early post-transplant time period. Animals had their islet graft-bearing kidney harvested 48 h after transplantation. Graft histological sections were costained for insulin and apoptotic nuclei (TUNEL method). The percentage of TUNEL-positive (apoptotic) among insulin-positive (β-) cells was quantified in each tissue sample using a sufficient number of sections to achieve 100 insulin positive cells per graft (Fig. 3, B and D). Statistical analysis revealed a significantly lower rate of β-cell apoptosis in liraglutide-treated animals compared with their control cohorts (one-way ANOVA, $P < 0.05$), regardless of sirolimus cotreatment.

**Liraglutide does not change stored insulin within islet grafts**

Because apoptosis of transplanted β-cells is prevented by liraglutide, we expected that liraglutide-treated animals would have a higher graft insulin content compared with their respective control cohort. However, graft insulin content was not significantly higher in liraglutide-treated animals when compared with vehicle-treated animals, or in liraglutide plus sirolimus cotreated animals when compared with sirolimus-treated animals ($n = 10$ per group, Fig. 4). We did find that grafts harvested from sirolimus-treated animals contained a statistically lower amount of insulin compared with those from vehicle-treated animals (Dunn’s multiple comparison test, $P < 0.01$).

**Liraglutide improves glucose-dependent insulin secretion**

To investigate further the mechanism by which liraglutide improves glucose homeostasis in marginal mass islet transplant recipients, we measured insulin-dependent glucose release in animals treated with a single dose of liraglutide. Our pilot investigation using streptozotocin-induced diabetic BALB/c mice revealed that liraglutide does not change stored insulin within islet grafts.
mice showed that the majority of animals, regardless of glucose stimulation or liraglutide therapy, had serum insulin levels below the threshold of detection. In normoglycemic naive mice, we measured the serum insulin concentration and blood glucose below the threshold of detection. In normoglycemic naive mice, we discovered that neither baseline (pre-glucose administration) serum insulin nor blood glucose levels were statistically different between liraglutide and vehicle-treated animals. After stimulation with glucose, liraglutide-treated animals showed much higher serum insulin when compared with vehicle-treated. **Single asterisk** (***) indicates statistically significant (P < 0.05) difference when compared with baseline, **double asterisk** (**) when compared with vehicle-treated.

**Discussion**

Here, we have shown a potentially clinically translatable application of liraglutide therapy to augment the function of an experimental marginal mass islet transplant. Liraglutide administration reduced the time required to achieve normoglycemia (time to engraftment) in transplanted animals and provided better long-term glycemic control. Furthermore, we have demonstrated that these characteristics of liraglutide are present even when sirolimus therapy is administered concurrently.

We found that the body weight of recipients increases after transplantation. Although not statistically significant, there is a notably reduced rate of body weight increase in liraglutide-treated animals. This is an expected observation because liraglutide promotes satiety and thereby reduces food consumption. The difference between liraglutide and vehicle groups in our transplant model is less pronounced than expected, likely resulting from higher rates of persistent diabetes in recipients of marginal mass islet grafts in the absence of liraglutide. Because animals in the liraglutide-treated group remained normoglycemic despite body weight gain, we hypothesize that improved islet graft function in this group is not an artifact of reduced insulin requirement associated with lower body weight. Furthermore, liraglutide-treated recipients perform better than their nonliraglutide-treated control group on the IPGTT, which eliminates reduced satiety as the sole reason for better glycemic control in liraglutide-treated animals.

We have clarified that liraglutide must be given early and on continuous exposure to have beneficial effects on islet transplant function. Immunohistology experiments clarify that β-cell apoptosis, which is known to occur at a high rate in the immediate post-transplant period, is significantly reduced by liraglutide treatment. In addition, mice treated with a single dose of liraglutide show increased glucose-dependent insulin secretion. These observations led us to conclude that liraglutide acts to improve islet engraftment through these two mechanisms at least.

The apparent disparity between our histological and insulin content studies may be due to the complex interdependence of graft insulin content on β-cell death, β-cell proliferation/neogenesis, β-cell burnout, glycemic environment, and insulin synthesis, storage, and exocytosis. The nonlinear relationship among these factors on physiological outcome is complex to unravel and is outside the scope of the present study. However, the aforementioned investigations do show that islet graft insulin storage is not increased with liraglutide treatment.

Sirolimus-treated transplant recipients showed reduced graft insulin content compared with vehicle-treated controls. This may be accounted for by the reduced, although not statistically significant, rates of β-cell proliferation and newly increased levels of β-cell apoptosis in islet grafts harvested from sirolimus-treated animals. Our data confirm earlier reports (6–8) that sirolimus can have a detrimental impact on islet graft health and function.

Sirolimus' effects are observed even in the presence of liraglutide. We found that the body weight of recipients increases after transplantation. Although not statistically significant, there is a notably reduced rate of body weight increase in liraglutide-treated animals. This is an expected observation because liraglutide promotes satiety and thereby reduces food consumption. The difference between liraglutide and vehicle groups in our transplant model is less pronounced than expected, likely resulting from higher rates of persistent diabetes in recipients of marginal mass islet grafts in the absence of liraglutide. Because animals in the liraglutide-treated group remained normoglycemic despite body weight gain, we hypothesize that improved islet graft function in this group is not an artifact of reduced insulin requirement associated with lower body weight. Furthermore, liraglutide-treated recipients perform better than their nonliraglutide-treated control group on the IPGTT, which eliminates reduced satiety as the sole reason for better glycemic control in liraglutide-treated animals.

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Future investigation of liraglutide in the field of islet transplantation is warranted. In the preclinical setting, data on liraglutide use in allogeneic islet transplant would be highly valuable. Because liraglutide can reduce apoptosis in transplanted β-cells, we hypothesized that liraglutide would help delay islet allorejection by reducing the immunogenic danger signal associated with peri-transplant graft tissue damage. Evaluation of liraglutide for any species-specific differences (mouse vs. human) in the setting of islet transplantation, although beyond the scope of the current work, would be of interest; some of these features have been described in vitro, but not in situ (30). In addition, exploring the influence of the anatomical location of islet transplantation on liraglutide-improved engraftment would also add value to this field.

This report provides novel support for the use of liraglutide as adjunct therapy for improving glycemic control islet transplantation. We have shown that liraglutide improves glucose homeostasis in the marginal mass islet transplant model without the need for exogenous insulin. Second, liraglutide's effects are observed even in the presence of
concurrent sirolimus therapy at a clinically relevant exposure. Furthermore, we have clarified that liraglutide must be administered both immediately after islet transplantation and continuously thereafter to maximize the beneficial effects offered by this therapy. Finally, we have provided some insights into the mechanism of liraglutide action, and have identified both reduced β-cell apoptosis and improved glucose-dependent insulin secretion from liraglutide administration.

Acknowledgments

We thank Deb Dixon for her assistance in mouse islet isolation, Rena Pavlick and Ryan Edgar for their assistance with the care of investigational animals, and Dr. Colin C. Anderson for reviewing this manuscript.

Received April 9, 2008. Accepted May 21, 2008.

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A.M.J.S. is a scholar supported through the Alberta Heritage Foundation for Medical Research. S.M. is a recipient of the Alberta Heritage Foundation for Medical Research M./Ph.D. Studentship, the Canadian Institutes for Health Research Walter & Jessie Boyd and Charles Roberts Centre, 2000 College Plaza, Edmonton, Alberta, Canada T6G 2C9. E-mail: shapiro@ualberta.ca.

Disclosure Statement: S.M., W.T., J.A.E., C.T., and A.M.J.S. have nothing to declare. L.B.K. is an employee and stockholder of NovoNordisk.

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Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

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