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Amelioration of Diabetes in Mice After Single-Donor Islet Transplantation Using the Controlled Release of Gelatinized FGF-2

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Fibroblast growth factor (FGF)-2 has been recognized to be a key element involved in angiogenesis and a putative factor involved in stem cell-mediated islet regeneration. However, the usefulness of FGF-2 in an islet transplantation setting has not yet been explored. We therefore evaluated the effect of FGF-2 on both islet culture and islet transplantation. Isolated islets were cultured in the presence of 100 ng/ml FGF-2 for a week and then the glucose-responding insulin secretion and insulin contents were measured. Gelatinized FGF-2 (100 ng), which allowed the controlled release of FGF-2, was used for islet transplantation of streptozotocin-induced diabetic mice. Islets (150 IEQ), obtained from a single donor, mixed with gelatinized FGF-2, were transplanted into the subrenal capsule of the mice and the animals were observed for 30 days. Revascularization around the islet grafts was examined. The blood glucose levels were measured and the intraperitoneal glucose tolerance test (IPGTT) was performed. The supplementation of FGF-2 maintained proper insulin secretion and insulin contents in an in vitro culture. The use of gelatinized FGF-2 facilitated revascularization and favorable islet engraftment, thus resulting in an amelioration of the blood glucose levels in diabetic mice. The utilization of FGF-2 showed increased contents of insulin in the islet grafts and revealed a similar pattern as that of normal healthy mice in IPGTT. In contrast, the transplantation of islets without FGF-2 supplementation showed poor revascularization and failed to control the blood glucose levels in the diabetic mice.

Key words: FGF-2; Angiogenesis; Islet transplantation; Diabetes

INTRODUCTION

Because a dramatic success in clinical islet transplantation has been achieved since the introduction of the Edmonton Protocol, islet transplantation has received a great deal of attention as an promising option for the treatment of type 1 diabetes (19). However, such success has usually been achieved in one diabetic patient by the transplantation of islets retrieved from two to four cadaver pancreata (3,19). Considering the imbalance between demand and supply, obtaining success of single-donor islet transplantation would thus be highly advantageous. Toward that goal, anoikis, isolation procedure-associated cell damage, and early events after islet transplantation, such as a lack of oxygen and nutrients and inflammation, should be properly resolved (3). In fact, it has been estimated that only one third of transplanted islets successfully engraft (19). Once revascularization has properly occurred around the transplanted islets, oxygen and nutrients would be delivered to the islets (5,15). Therefore, an early intervention to enhance the engraftment of the transplanted islets and revascularization of the islets could improve the survival and function of the islet graft (5,15).

Previous studies have demonstrated that fibroblast growth factor-2 (FGF-2) plays an important role in in-

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ducing angiogenesis and, moreover, that FGF-2 works as a trophic and chemotactic factor in the development and early regeneration of the pancreatic islets (6–8). FGF-2 has been successfully used as a potent angiogenic inductor in the development of an implantable bioartificial device in mice (17). FGF-2 or FGF-7 has been utilized to expand the islet progenitor populations as a source of putative β-cells (2,6,24). Our previous work has clearly demonstrated that a coculture of isolated islets with pancreatic fibroblasts maintained islet viability, morphology, and glucose-sensitive insulin secretion (12). In the present study, we examined the in vitro effect of FGF-2 in islet culture and then evaluated the benefits of the controlled release of FGF-2 in islet transplantation in diabetic mice.

MATERIALS AND METHODS

Animal Experiments

All procedures performed on animals were approved by the Institutional Animal Care and Use Committee and were thus within the guidelines for the humane care of laboratory animals.

Pancreatic Islet Isolation and Culture

Mouse pancreatic islets were isolated from Balb/c mice of 8–10 weeks, as previously reported (16). Isolated islets were hand selected under a microscope and the resultant islets of 10 islet equivalent (IEQ) were then inoculated into each well of six-well plates in suspension culture condition with RPMI-1640 medium supplemented with 100 ng/ml FGF-2 at 37°C and 5% CO_2 [group 1, G1 islets ($n = 3$)]. As a control, the islets of 10 IEQ were cultured with RPMI-1640 without FGF-2 [group 2, G2 islets ($n = 3$)].

In Vitro Experiments

The G1 and G2 islets were functionally evaluated by insulin secretion index under static incubation. Both groups of islets were incubated at 37°C and 5% CO_2 for 2 h in Krebs-Ringer bicarbonate buffer (KRBB) medium containing 3.3 mM glucose and then the culture medium was replaced with KRBB medium containing 27 mM glucose for 2 h; finally the medium was removed and replaced with KRBB containing 3.3 mM glucose. One hundred milliliter of each culture medium was collected and its insulin level was measured by using Mercodia mouse insulin ELISA kit (Uppsala, Sweden) according to the manufacturer's protocol (20). After a glucose-responding insulin secretion test, both G1 and G2 islets were subjected to insulin content extraction, as previously described (14).

In Vivo Experiments

Diabetes was induced in 8-week-old female Balb/c mice (CLEA Japan, Tokyo, Japan) by streptozotocin (STZ) at 220 mg mg/kg body weight in citrate buffer (pH 4.5, IP), as previously described (14). The animals with a blood glucose level ≥ 350 mg/dl for 2 consecutive days were transplanted with 150 IEQ of freshly isolated mouse islets embedded with gelatinized FGF-2 (100 ng/10 μl) into the left subrenal capsule of the mice (G1 mice, $n = 14$) (21), and the blood glucose levels were measured. As a control, 150 IEQ freshly isolated mouse islets were transplanted into the left subrenal capsule of the mice (G2 mice, $n = 14$). The kidneys bearing islet grafts were removed to measure the insulin contents at 3, 15, and 30 days after transplantation in each group, as previously described (14). The pancreata and livers were removed at the end of the experiments and then were fixed in formalin for histological examinations.

Measurement of the Blood Glucose Levels and Glucose Tolerance Test

The blood glucose levels were measured in both G1 and G2 mice using a portable glucose meter FreeStyle™ (TheraSense, Abbott Park, IL). For the glucose tolerance test, the mice in each group were fasted overnight and blood samples were taken at 0, 10, 20, 30, 60, 90, and 120 min after glucose injection (1 g/kg body weight, IP).

Statistical Analysis

The ANOVA test was used to calculate the significance of the mean values. The significance was determined by the Mann-Whitney U test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

FGF-2 Maintained Islet Function In Vitro

To evaluate the benefit of FGF-2 (100 ng/ml) supplementation in an in vitro culture of mouse islets, we comparatively performed the glucose-responding insulin secretion test in each group. The insulin secretion index (ISI) was significantly better maintained in the G1 islets, which were treated with 100 ng/ml FGF-2, in comparison to the nontreated G2 islets on days 1, 3, and 7 (ISI on day 1, 3.48 ± 0.37 for G1 and 3.16 ± 0.31 for G2; ISI on day 3, 2.62 ± 0.41 for G1 and 1.71 ± 0.37 for G2; ISI on day 7, 1.79 ± 0.33 for G1 and 1.00 ± 0.10 for G2) (Fig. 1A). In parallel, the G1 islets showed a larger insulin content (IC) than G2 islets (IC on day 1, 111.7 ± 3.2 for G1 and 108.6 ± 3.5 for G2; IC on day 3, 83.9 ± 4.3 for G1, and 75.8 ± 3.1 for G2; IC on day 7, 40.4 ± 3.6 for G1 and 9.9 ± 1.6 for G2) (Fig. 1B).

Gelatinized FGF-2 Facilitated Revascularization Around the Islet Grafts

To investigate whether FGF-2 facilitates revascularization for the islet grafts, we transplanted the mouse islets of 150 IEQ embedded in gelatinized FGF-2 (100

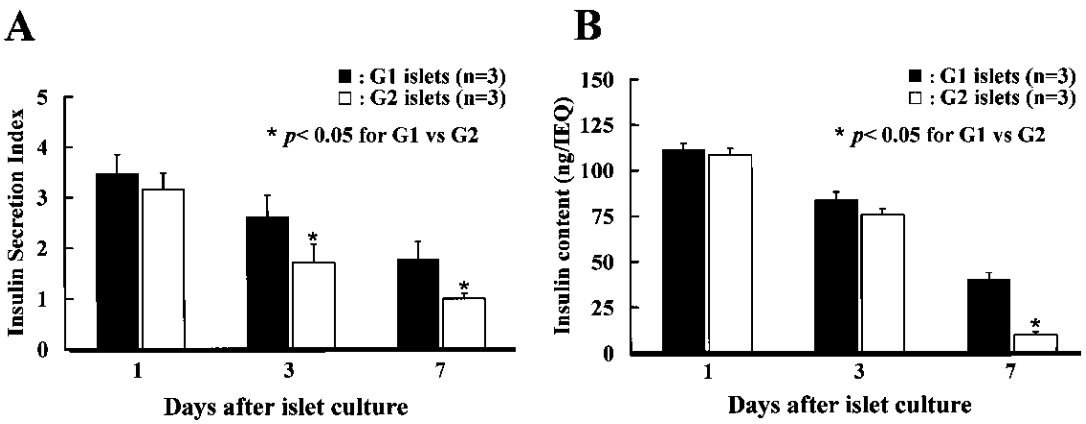

Figure Islet function in vitro. We compared glucose-responding insulin secretion, which was expressed as an insulin secretion index (ISI), and insulin contents between FGF-2 (100 ng/ml)-treated islets (G1, $n = 3$) and untreated islets (G2, $n = 3$). (A) ISI was significantly better maintained in the G1 islets than in the untreated G2 islets. (B). The G1 islets revealed a larger amount of the insulin contents (IC) than the G2 islets.

ng) into the left subrenal capsule of STZ-induced diabetic mice ($n = 9$). The left kidney-bearing islet grafts displayed more developed vessels, as indicated by arrows, around the transplanted islets treated with FGF-2 on days 3, 15, and 30 posttransplant (Fig. 2A). In contrast, no such revascularization was detected at the transplant site of untreated islets even on day 30 (Fig. 2B). Notably, the measurement of the insulin contents of the islet grafts that were treated with FGF-2 (100 ng) revealed a progressive increase in insulin amount in the islet grafts (Fig. 2C). On the other hand, such an increase in insulin content did not occur in the untreated islet transplant.

Transplantation of the Islets Treated With Gelatinized FGF-2 Controlled Blood Glucose Levels

To elucidate the benefits of gelatinized FGF-2 (100 ng)-treated islets of 150 IEQ, we transplanted the islets into the left subrenal capsule in STZ-induced diabetic mice (G1 mice, $n = 5$). As a control, the same number of untreated islets was transplanted into diabetic mice (G2 mice, $n = 5$). The transplantation of islets treated with gelatinized FGF-2 effectively controlled blood glucose levels (280.5 ± 31.4 mg/dl on day 3, 171.8 ± 12.9 mg/dl on day 15, and 163.6 ± 18.8 mg/dl on day 30 in a time-dependent manner, which was significantly better than that of untreated islets, demonstrating blood glucose levels of (303.6 ± 25.8 mg/dl on day 3, 270.1 ± 36.7 mg/dl on day 15, and 255.6 ± 36.6 mg/dl on day 30) (Fig. 3A).

Pancreatic islets in both the G1 and G2 mice showed no insulin-positive staining, thus indicating that ? cells were destroyed by STZ treatment. No insulin-positive cells were detected in the liver of both G1 and G2 mice. The removal of the left kidney bearing islet grafts induced hyperglycemia in G1 mice, thus clearly indicating that islet grafts were responsible for decreasing the blood glucose levels (Fig. 3A). Interestingly, IPGTT showed that diabetic mice with FGF-2-treated islet transplant revealed the blood glucose levels that were similar to those in normal mice (Fig. 3B).

DISCUSSION

The FGF-2 receptor has been identified in the adult mouse β-cell and its in vivo signaling attenuation leads to the onset of diabetes (7). This finding encouraged us to use FGF-2 for the maintenance of functional culture of islets. The addition of FGF-2 into the islet culture significantly maintained glucose-responsive insulin-secreting activity of the islets, but the effect decreased in a time-dependent manner, which was considered to be due to the decreased viability of the cultures islets. Cell-to-cell and/or cell-to-matrix interaction is essential for maintaining the homeostasis of various tissues and organs. The isolation of the islets from the pancreas and subsequent tissue culture disrupt such interactions. The utilization of an adequate cellular matrix should be considered to achieve a successful long-term islet culture.

Oxygen tension plays a major role in the regulation of the expression of vascular epithelial growth factor (VEGF) in the endothelial cells that exist in the islets (18) and VEGF facilitates revascularization of islet grafts (23). The islet isolation procedure itself induces hypoxia in islets, which results in the activation of hypoxia-responsive elements binding to the promoter region of VEGF. In fact, after the islet isolation the expression of VEGF in endothelial cells inside the islets is dramatically increased. However, such a hypoxic stimu-

lation was insufficient to induce revascularization around the islet grafts in our experiments, as shown in Figure 2B. In vivo, the blood flow in the islets is considerably higher than that of other pancreatic tissues, thus suggesting that metabolism of the islets is very active and an absence of oxygen and nutrients would seriously diminish the functions of the islet grafts. Thus, islet graft revascularization is an important step towards successful islet transplant. This process might be significantly impaired and delayed for up 10–14 days after islet transplantation (5,8,10,11,15). In addition, VEGF-induced angiogenesis has been reported to be dependent on FGF-2 (9). Thus, to facilitate revascularization of the islet grafts, we utilized a gelatinized form of FGF-2 to gradually release the molecule around the site of transplantation.

The use of gelatinized FGF-2 successfully induced the vascular network around the islet grafts, allowing the amount of insulin content of the islet grafts to be maintained, while decreasing the blood glucose levels in diabetic mice. Unfortunately, complete normoglycemia was not achieved by the transplantation of single-donor islets (150 IEQ) using gelatinized FGF-2. This may be due to the loss of islet mass to some extent immediately after transplantation, which was induced by graft instability and hyperglycemia- and cytokine-mediated apoptosis (1–5,10,11,13,19). However, interestingly, in IPGTT, FGF-2-utlized single-donor islet transplant was found to be able to rapidly secrete insulin with an effective response to the blood glucose levels, in which the pattern was similar to that of normal mice (Fig. 3C). These results suggest that the use of FGF-2 enhanced the glucose sensitivity of the transplanted islets, thereby resulting in a quick insulin secretion after a glucose challenge. In-

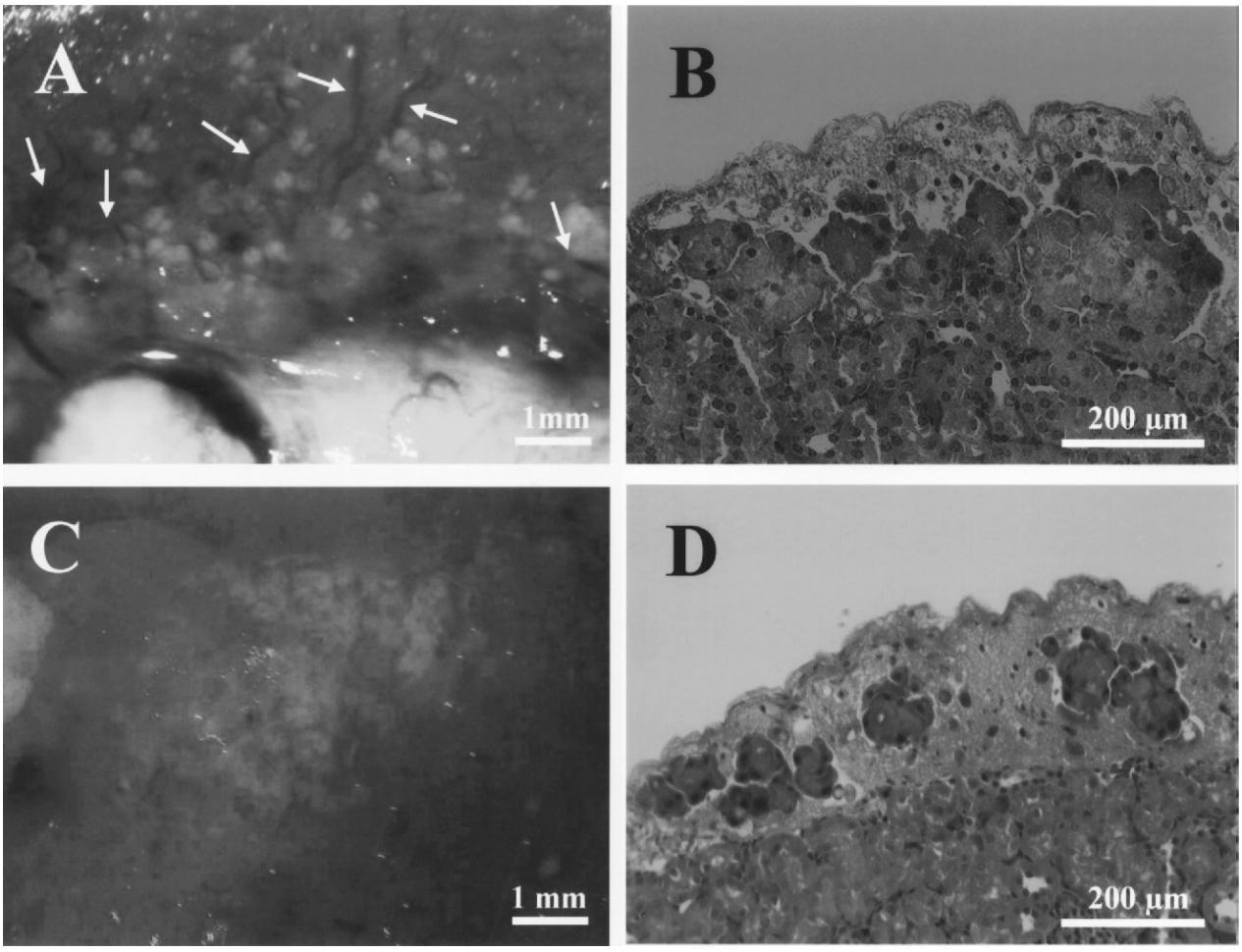

Figure 2. Revascularization around islet grafts. (A) The left kidney bearing the islets treated with gelatinized FGF-2 (100 ng) (G1 islets) displayed more developed vessels, as indicated by arrows, around the transplanted islets on day 3 posttransplant. This vascular network was properly maintained for up to day 30. (B) On the other hand, revascularization was not detected in the transplant site of untreated islets (G2 islets) on days 3, 15, or 30 posttransplant. Microscopically, the G1 islets showed bigger clusters of islets than the G2 islets on day 30.

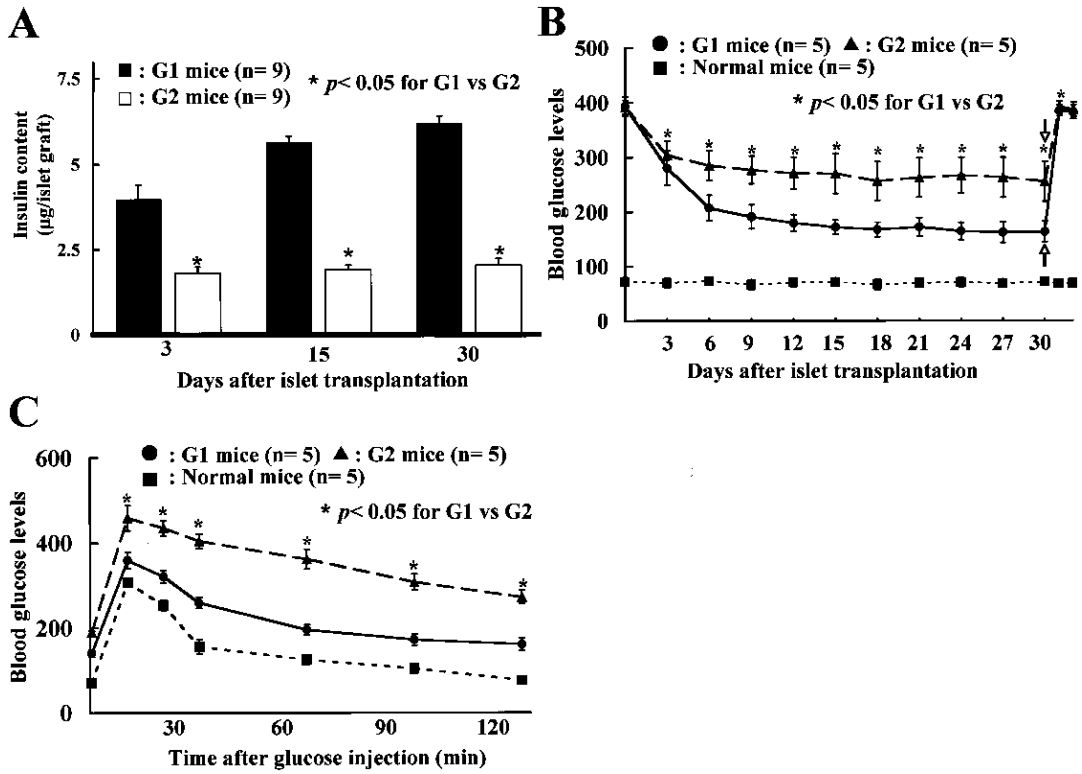

Figure 3. Blood glucose levels and glucose tolerance test in diabetic mice and insulin contents of islet grafts. (A) The transplantation of islets treated with gelatinized FGF-2 controlled blood glucose levels in the G1 mice better than that of the untreated islets in the G2 mice. The removal of the left kidney bearing islet grafts induced hyperglycemia in the G1 mice, clearly indicating that islet grafts were responsible for decreasing blood glucose levels. (B) IPGTT showed that the G1 mice showed a similar pattern in the blood glucose levels as normal healthy mice. (C) The G1 mice showed significantly higher amount of insulin contents in the islet grafts than the G2 mice. Notably, the insulin contents increased from day 3 to day 15 in the G1 mice; however, no such increase was observed in the G2 mice.

vestigators previously reported that possible spontaneous β-cell replication after syngeneic islet transplantation under a minimum stress (1,2,13,24). However, the effectiveness was limited by several factors, including donor age, endotoxin-free isolation procedures, transplantable islet mass, and a rapid correction of hyperglycemia in patients with islet transplant (1,13). Some of these factors, except for the donor age, should be addressed by improving the isolation and transplant procedures. The increase in insulin content in the islet grafts suggests that FGF-2 may have a capacity to restore β-cell function after islet transplant and, possibly, to regenerate the islet grafts. We are now investigating the direct role of FGF-2 in islet grafts based on long-term follow-up experiments.

The islet isolation and transplantation procedures induce apoptosis in the islets and reduce the number of islet grafts. Further investigations, such as combination of FGF-2 and antiapoptotic molecules, are thus required in order to eventually achieve the goal of an effective single-donor islet transplantation. We have currently found that antiapoptotic pentapeptide V5 significantly prevents apoptosis in hepatocytes that were transplanted in the spleen of mice suffering from acute liver failure (22).

In conclusion, the supplementation of FGF-2 (100 ng/ml) allowed for the favorable maintenance of insulin content and glucose responsiveness of insulin secretion of the islets in vitro. The transplantation of the islets treated with gelatinized FGF-2 ameliorated blood glucose levels and increased the amount of insulin content of the islet grafts, thus resulting in a normal response to the glucose tolerance test.

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