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Effects of Insulin-Like Growth Factor-1 and Donor Age on Transplantation of Porcine Neonatal Pancreatic Cell Clusters

J.-H. Juang, C.-H. Kuo, and N.-K. Yao

ABSTRACT

Porcine neonatal pancreatic cell clusters (NPCCs) isolated from 1- to 3-day-old pigs cured diabetic nude mice more than 14 weeks after transplantation. To shorten the latent period between transplantation and reversal of hyperglycemia, we investigated the effects of insulin-like growth factor-1 (IGF-1) and NPCCs isolated from 1-month-old pigs after transplantation. Pig pancreata were cut into fragments, collagenase digested, and then cultured. Three hundred and 2000 NPCCs were transplanted under the kidney capsule of nondiabetic and diabetic nude mice, respectively. After transplantation, the graft-bearing kidneys were removed to measure insulin content. NPCCs isolated from 1- to 3-day-old pigs were cultured with or without IGF-1 for 6 days. The stimulation index was not significantly different between the 2 groups at 1, 2, or 4 weeks. Moreover, at 4 weeks after transplantation of 300 NPCCs to nondiabetic nude mice yielded comparable graft insulin content as the recipients of NPCCs precultured with or without IGF-1. Two thousand cultured NPCCs isolated from 1-to 3-day-old pigs or 1-month-old pigs were transplanted into diabetic nude mice. The blood glucose levels of diabetic recipients in both groups decreased at the same rate after transplantation, achieving normoglycemia at 8 weeks. The graft insulin content at 12 weeks was not different between the 2 groups. Our data indicated that isolated NPCCs cultured with IGF-1 showed no beneficial effects on insulin secretion and transplantation; NPCCs isolated from 1-to 3-day-old and 1-month-old pigs displayed similar effects on transplantation.

SINCE 1990, islet transplantation has led to insulin independence in humans with type 1 diabetes mellitus.[1] However, a large number of islets—usually >6000 islet equivalents/kg body weight—is needed to achieve normoglycemia.[2] Unfortunately, the number of available organs has leveled despite the ever-growing number of patients on the transplant waiting list. To overcome this supply problem, islet tissues from animal sources have been considered for xenotransplantation. One potential source of tissue for the treatment of diabetes is porcine neonatal pancreatic cell clusters (NPCCs) isolated from 1- to 3-day-old pigs, which are easily isolated, capable of secreting significant quantities of insulin in response to an in vitro glucose challenge, and show growth potential.[3–5] Moreover, recent studies have shown that NPCCs can cure diabetes in pancreatecomized pigs[6] and nonhuman primates.[7] However, it requires more than 14 weeks to reach adequate beta-cell mass and maturity to cure diabetic nude mice after transplantation of NPCCs.[4] Several beta-cell growth factors have been shown to increase beta-cell proliferation and expand beta-cell mass in vitro and/or in vivo.[8] Among them, insulin-like growth factors (IGFs) play an essential role in DNA synthesis and proliferation of beta-cells, acting as islet survival factors.[9–11] In addition, NPCCs isolated from 1-month-old pigs were larger and contained more insulin

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than those from 1- to 3-day-old pigs.[12] Thus, in the present study, we tested whether NPCCs isolated from 1-month-old pigs and NPCCs precultured with IGF-1 were beneficial for transplantation.

MATERIALS AND METHODS

Animals[5,12]

Donor pancreata were obtained from 1- to 3-day-old and 1-month-old neonatal pigs of either gender. Male athymic nude Balb/c mice, age 8–12 weeks, were used as recipients of the NPCCs. Mice were rendered diabetic by intravenous injection of 90 mg/kg alloxan (Sigma Chemical Co, St Louis, Mo, United States) which was freshly dissolved in 1 mmol/L hydrochloric acid 14 days before transplantation.

Preparation and Culture of NPCCs[5,12]

Each pancreas was cut into ~1–2 mm^3 fragments before digestion with collagenase (type V, Sigma Immunochemicals) in a water bath at 37°C.[5,12] Filtered and washed digest was placed in RPMI maintained at 37°C (5% CO_2, 95% air) in humidified air.

NPCCs Culture With IGF-1 Treatment

Isolated NPCCs were cultured in RPMI medium containing IGF-1 (100 ng/mL) for measurement of insulin content at 1, 2, and 4 weeks.[5,12,13] NPCCs secretory response to glucose was determined at 2 and 4 weeks using static incubation.[5,12] NPCCs were incubated in RPMI medium supplemented with 100 mg/dL glucose and then 500 mg/dL glucose for 120 minutes, respectively. The media were collected at 120 and 240 minutes for insulin measurement. Stimulation indices were calculated by dividing the amount of insulin release at 500 mg/dL glucose by that released at 100 mg/dL glucose.

Transplantation of NPCCs

After 6 days of culture, 300 and 2000 NPCCs were transplanted into nondiabetic and diabetic nude mice, respectively.[5,12] NPCCs were centrifuged in PE-50 tubing connected to a 200-μL pipette tip. A capsulotomy was performed in the lower pole of the mouse kidney. The tip of the tubing was advanced under the capsule of the upper pole, the site of final injection.

Insulin Content of NPCCs and the Graft

One hundred fifty NPCCs were sonicated. The removed graft-bearing kidneys were homogenized in acid ethanol.[5,12,13] After sonication or homogenization, the samples were extracted overnight at 4°C. On the following day, they were centrifuged at 2400 rpm for 10 minutes (NPCCs) or 30 minutes (kidney), and the supernatant stored at −20°C. The pellet was again sonicated or homogenized in acid *ethanol,* and insulin extracted overnight. After centrifugation, this second was added to the first extraction sample. Insulin was measured using radioimmunoassay with an INSI-PR kit (CIS US Inc, Bedford, Mass, United States).

Statistical Analysis

Results were expressed as mean values and standard errors of the mean (M ± SEM). We used unpaired Student t test for comparisons between the 2 groups. A value of $P < .05$ was significant.

RESULTS

Effects of IGF-1 on Beta-Cell Function and Transplantation

The insulin content of 150 NPCCs isolated from 1- to 3-day-old pigs cultured with versus without IGF-1 was comparable at 1 (6.6 ± 1.9 vs 7.5 ± 1.2 μg; $P = .66$), 2 (6.6 ± 0.7 vs 8.2 ± 0.5 μg; $P = .10$), and 4 (3.7 ± 1.0 vs 4.3 ± 1.0 μg; $P = .21$) weeks. In addition, the stimulation index of those cultured with versus without IGF-1 was not significantly different at 1 (2.6 ± 0.2 vs 2.1 ± 0.4; $P = .51$), 2 (5.0 ± 0.7 vs 6.3 ± 0.8; $P = .41$), and 4 (4.3 ± 0.7 vs 3.7 ± 0.5; $P = .21$) weeks. Furthermore, at 4 weeks after transplantation of 300 NPCCs to nondiabetic nude mice, the mean insulin content of the grafts was comparable with that of recipients of NPCCs precultured with versus without IGF-1 (18.7 ± 5.6 vs 17.2 ± 0.9 μg; $P = .75$).

Effects of NPCCs Isolated From 1- to 3-Day-Old Versus 1-Month-Old Pigs on Islet Transplantation

In diabetic recipients transplanted with 2000 NPCCs isolated from 1- to 3-day-old and 1-month-old pigs, the blood glucose in both groups decreased at the same rate after transplantation and normoglycemia was achieved at 8 weeks (Fig 1). The body weight did not significantly differ between the 2 groups. Additionally, their insulin content of the graft was comparable at 12 weeks after transplantation (7.9 ± 1.5 and 5.4 ± 1.1 μg in 1- to 3-day-old and 1-month-old NPCCs recipients, respectively; $P = .055$).

DISCUSSION

Previously, Korbutt et al showed that it required 8 and more than 14 weeks to reach an adequate beta-cell mass and maturity to cure diabetic nude mice after transplantation with 2000 and 1000 NPCCs, respectively.[4] To shorten the latent period between transplantation and hyperglycemia reversal, we investigated the effects of IGF-1 and donor age. It had been demonstrated that IGFs stimulate DNA synthesis and proliferation as well as inhibit beta-cell apoptosis,[9–11] which may increase beta-cell mass, thereby benefiting transplantation outcomes. However, in the present study, we observed the insulin content and stimulation index of NPCCs cultured with versus without IGF-1 to be not significantly different at 1, 2, and 4 weeks. Furthermore, at 4 weeks after transplantation of 300 NPCCs to nondiabetic nude mice, the mean insulin content of the grafts was comparable with that of recipients of NPCCs precultured with versus without IGF-1. These findings were consistent with those of Lopez-Avalos et al who showed no significant increase in DNA and insulin content in NPCCs treated with growth factors, including IGF-1. In addition, although increased insulin content of NPCCs was achieved in vitro by addition of a combination of fetal calf serum, IGF-I, nicotinamide, and sodium butyrate, the increase did not shorten the time to achieve normoglycemia after transplantation.[14] The dose, timing, and duration of IGF-1 treatment may partly explain why there was no response of NPCCs to growth factors that had previously been reported to induce proliferation of

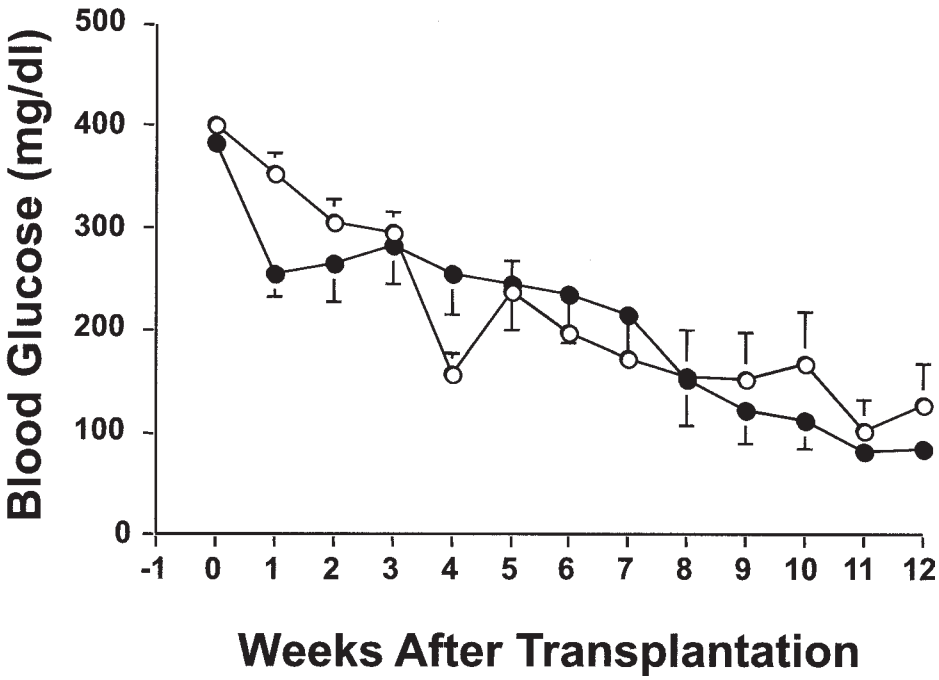

Fig 1. Evolution of blood glucose in diabetic recipients transplanted with 2000 NPCCs isolated from 1- to 3-day-old (open circle) and 1-month-old (closed circle) pigs. Data are expressed as mean ± SE.

beta cells and/or differentiation duct cells. However, further studies are needed to explore the underlying mechanism(s).

Regarding donor age, we have investigated the characteristics of NPCCs isolated from 1-month-old pigs and their effects on transplantation.[12] The mean yield of NPCCs isolated from 1- to 3-day-old and 1-month-old pigs was comparable. However, soon after isolation the latter were larger than the former, containing more insulin after a 6-day culture. The stimulation indices of NPCCs isolated from 1- to 3-day-old and 1-month-old pigs were not significantly different. Nondiabetic recipients of NPCCs isolated from 1- to 3-day-old versus 1-month-old pigs showed similar graft beta-cell mass and function in both groups. In the present study of diabetic recipients transplanted with 2000 NPCCs isolated from 1- to 3-day-old and 1-month-old pigs, the blood glucose in both groups decreased at the same rate after transplantation; normoglycemia was achieved at 8 weeks, which was consistent with a previous study.[4] Additionally, we observed their graft insulin content was comparable at 12 weeks after transplantation. In terms of growth and function, NPCCs isolated from 1-month-old pigs are not better than those from 1- to 3-day-old pigs. Presumably, significant NPCCs maturation takes more than 1 month after birth. Further studies on the characteristics of young pig islets are needed to know their maturation process. In conclusion, our data indicated that isolated NPCCs cultured with IGF-1 showed no beneficial effects on in vitro function and transplantation, and NPCCs isolated from 1- to 3-day-old and 1-month-old pigs had similar effects on transplantation.

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