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ORIGINAL ARTICLE

Comparison of exendin-4 on beta-cell replication in mouse and human islet grafts

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beta-cell regeneration, beta-cell replication, exendin-4, GLP-1, islet transplantation.

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Conflicts of Interest
All authors declare no conflict of interest.

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Summary

Exendin-4 can stimulate β-cell replication in mice. Whether it can stimulate β-cell replication in human islet grafts remains unknown. Therefore, we compared the effects of exendin-4 on β-cell replication in mouse and human islet grafts. Islets, isolated from mouse and human donors at different ages, were transplanted into diabetic mice and/or diabetic nude mice that were given bromodeoxyuridine (BrdU) with or without exendin-4. At 4 weeks post-transplantation, islet grafts were removed for insulin and BrdU staining and quantification of insulin$^+$/BrdU$^+$ cells. Although diabetes was reversed in all mice transplanting syngeneic mouse islets from young or old donors, normoglycemia was achieved significantly faster in exendin-4 treated mice. Mouse islet grafts in exendin-4 treated mice had significantly more insulin$^+$/BrdU$^+$ β cells than in untreated mice ($P < 0.01$). Human islet grafts from ≤22-year-old donors had more insulin$^+$/BrdU$^+$ β cells in exendin-4 treated mice than that in untreated mice ($P < 0.01$). However, human islet grafts from ≥35-year-old donors contained few insulin$^+$/BrdU$^+$ β cells in exendin-4 treated or untreated mice. Our data demonstrated that the capacity for β-cell replication in mouse and human islet grafts is different with and without exendin-4 treatment and indicated that GLP-1 agonists can stimulate β-cell replication in human islets from young donors.

Introduction

It is generally accepted that β cells form principally by neogenesis until late gestation and that most are formed after birth [1]. Although β-cell replication in adults is very slow [2], the mass of pancreatic β cells is dynamic and can be regulated in an effort to maintain normoglycemia, in response to tissue injury and to increased metabolic demand [1]. Insulin$^+$ β cells have also been found in some patients with longstanding type 1 diabetes, which suggests that new β-cell regeneration occurs despite ongoing β-cell apoptosis [3,4]. This also implies that type 1 diabetes may be reversed after abrogation of autoimmunity.

Numerous studies have investigated β-cell regeneration in diabetic animal models with partial pancreatectomy and with streptozotocin (STZ) treatment, but little emphasis has been placed on β-cell regeneration in islet grafts. It was previously observed that the outcome of islet autotransplantation in pediatric patients was better than the outcome in adolescent patients [5]. One possible mechanism by which this may occur is that β cells in the transplanted islets from young donors have a greater capacity for regeneration. To assess this possibility, we investigated the impact of donor age on β-cell replication in mouse and human islet grafts in diabetic mice by comparing islet grafts from donors at different ages.

Glucagon-like peptide 1 (GLP-1), a potent glucose-dependent insulinotropic peptide hormone secreted by the intestinal L cells, can stimulate β-cell regeneration [6,7]. As the half-life of GLP-1 is very short, exendin-4 which is a potent analog of GLP-1 with a longer half-life was developed. Exendin-4 stimulates β-cell regeneration through neogenesis and/or replication [8,9]. Although exendin-4 was found to improve islet graft function in mice [10–12] and in human transplants [13,14], it remains unknown whether it stimulates β-cell replication in this setting. Therefore, we investigated the effects of exendin-4 on β-cell replication in mouse and human islet grafts.

Materials and methods

Animals

Male C57Bl/6J mice and *Foxn1^{nu}/Foxn1^{nu}* nude mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). These mice were housed in pathogen-free animal facilities at the University of Minnesota. All experiments related to these mice were performed according to the protocol approved by the Institutional Animal Care and Use Committee. Young (8-week-old) and old (retired breeders, over 40-week-old) C57Bl/6 mice were used as donors. Diabetes was induced in C57Bl/6 mice and nude mice (9–12 weeks old) by a single intraperitoneal injection of STZ (Sigma–Aldrich, St. Louis, MO, USA) at 220 mg/kg. Mice with a blood glucose concentration >400 mg/dl for at least two consecutive days were used as recipients. Blood glucose concentrations were determined by glucose meter (Contour Bayer, Tarrytown, NY, USA). All diabetic mice underwent daily insulin treatment for 1–3 weeks before islet transplantation.

Islet isolation and transplantation

Mouse islets were isolated according to a protocol that is similar to a previously published isolation protocol [15,16]. Briefly, we injected 2.5 ml of Hank's balanced salt solution containing 5 mg/ml collagenase from *Clostridium histolyticum* (Serva, Heidelberg, Germany) into the pancreatic duct. The distended pancreas was removed and incubated at 37 °C for 16 min. The islets were purified by centrifugation on gradients comprising three different densities. Islets free of acinar cells, vessels, lymph nodes, and ducts were used for transplantation.

Human islets from nine pancreatic donors were obtained from the JDRF and NIH Islet Cell Resource Centers. They are divided into donors between 14 and 22 years old (18.0 ± 2.9 years old, BMI: 29.6 ± 1.7 $n = 4$) and donors between 35 and 58 years old (47.4 ± 7.8 years old, BMI 27.5 ± 3.3, $n = 5$). The islet purity was 81.3 ± 8.9% and viability was 87.5 ± 2.5% in ≤22-year-old donors and the islet purity was 83.2 ± 10.2% and viability was 93.5 ± 1.5% in ≥35-year-old donors. At least eight islet transplantations were performed in nude mice from each donor.

Islet transplantation was performed as described previously [15,16]. Briefly, PE-50 polyethylene tube containing 100 mouse islets or 2000 IE human islets was inserted beneath the left kidney capsule. Daily nonfasting blood glucose levels of each recipient were measured to monitor islet graft function. Achievement of normoglycemia was defined as blood glucose level in recipient mice <200 mg/dl for two consecutive days and thereafter.

Treatment

Exendin-4 was purchased from Bachem Bioscience (King of Prussia, PA, USA) and dissolved in saline. Bromodeoxyuridine (BrdU) was purchased from Roche Applied Science (Indianapolis, IN, USA). Control recipient mice were given BrdU intraperitoneally at 100 mg/kg daily, staring from the day of transplantation. Exendin-4 treated recipient mice were given exendin-4 intraperitoneally at 10 nM/kg daily and BrdU, staring from the day of transplantation.

Immunofluorescence

At 4 weeks post-transplantation, left nephrectomy was performed to remove islet grafts. Each kidney bearing islet grafts was fixed in 10% formalin solution and embedded in paraffin. Double immunofluorescence staining for insulin and BrdU was done on formalin fixed and paraffin-embedded sections. For insulin labeling, sections were first deparaffinized, rehydrated, and incubated with guinea pig anti-swine insulin (Dako, Carpinteria, CA, USA). Then sections were incubated in the dark with FITC-conjugated, goat anti-guinea pig immunoglobulin. For BrdU labeling, sections were steamed in Retrievagen A and were incubated with biotinylated anti-BrdU and then with Streptavidin-AlexaFluor-594 (Molecular Probes, Eugene, OR, USA). Nuclear staining was done using TOPRO-3 (Molecular Probes). $BrdU^+$ (red nuclei overlapping with TOPRO-3) and $BrdU^-$ (only blue TOPRO-3 nuclei) nuclei in $insulin^+$ (green cytoplasm) β cells were counted by using the confocal microscope [Biorad Radiance 2100 (Biorad, Hercules, CA, USA) laser scanning confocal attached to a Zeiss Axioskop 2 microscope (Carl Zeiss, Oberkochen, Germany)]. All images were taken with a plan apochromat 63× lens. $BrdU^+$ β-cell ratios were calculated as the mean ± SD of $BrdU^+$ β cells over the total of β cells in islets.

Statistics

The significance of differences between the control and treatment groups was determined by the Kaplan–Meier

analysis, one-way analysis of variance and the Student's *t*-test. A *P*-value <0.05 was considered statistically significant.

Results

Exendin-4 improved islet graft function in diabetic C57Bl/6 mice receiving marginal syngeneic islet grafts from either young donors or old donors

With or without exendin-4, normoglycemia was achieved in all diabetic C57Bl/6 mice that received 100 syngeneic islets from young donors (Fig. 1A). However, normoglycemia was achieved significantly earlier in exendin-4 treated mice than that in untreated mice ($n = 10$; $P < 0.05$). Nonfasting blood glucose levels were significantly reduced in exendin-4 treated mice at 4 weeks post-transplantation, compared with untreated mice (Fig. 1B, $P < 0.05$).

With or without exendin-4, normoglycemia was achieved in all mice that received 100 islets from old donors (Fig. 2 A). However, normoglycemia was achieved significantly faster in exendin-4 treated mice ($P < 0.05$). Nonfasting blood glucose levels were not significantly different between untreated mice and exendin-4 treated mice (Fig. 2B).

At 4 weeks post-transplantation, nephrectomy was performed to remove the left kidney bearing islet grafts to confirm islet graft function and to determine β-cell replication. After nephrectomy hyperglycemia was observed in all recipient mice except one exendin-4 treated mouse (Figs 1B and 2B). Thus, exendin-4 improved islet graft function in diabetic mice receiving marginal syngeneic islet grafts from young or old donors.

Body weight in mice was measured daily starting from the day of transplantation and exendin-4 treatment. At 1 and 2 days post-transplantation, body weight was reduced. However, it was gradually increased with and without exendin-4. Although the body weight gain in untreated mice was higher than that in exendin-4 treated mice, no significant difference in body weight change was found between untreated mice and exendin-4 treated mice ($P > 0.05$). At 4 weeks post-transplantation, the mean percentage of body weight gain was 9.7 ± 5.1% in untreated C57Bl/6 mice that received 100 islets from young or old donors ($n = 21$) and 6.8 ± 4.3% in exendin-4 treated C57Bl/6 mice that received 100 islets from young or old donors ($n = 21$), compared to the initial body weights before transplantation.

Exendin-4 stimulated β-cell replication in mouse islet grafts from both young and old donors in diabetic C57Bl/6 mice

We gave BrdU to recipient mice daily to label replicated cells and detected insulin$^+$ and BrdU$^+$ cells in each stained islet graft section by using a confocal microscope.

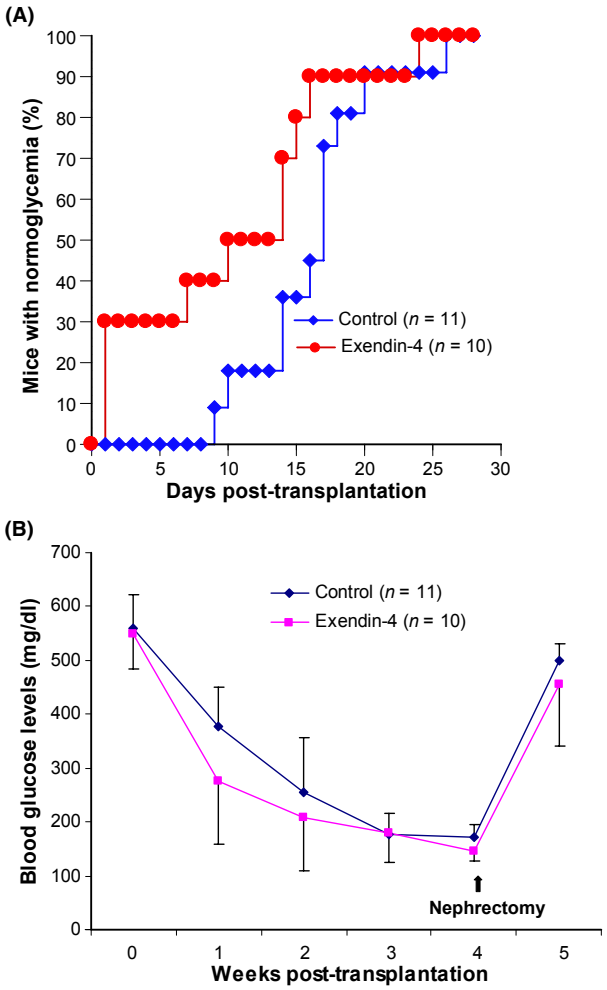

Figure 1 (A) Percentage of normoglycemia in diabetic C57Bl/6 mice received 100 C57Bl/6 islets from young donors and with or without exendin-4 treatment. Untreated mice achieved normoglycemia in 16 ± 4 days ($n = 11$), whereas exendin-4 treated mice required only 10 ± 7 days ($n = 10$, $P < 0.05$). (B) Blood glucose levels in diabetic C57Bl/6 mice received 100 C57Bl/6 islets from young donors and with or without exendin-4 treatment. Data are presented as the mean ± SD.

Without exendin-4 treatment, insulin$^+$/BrdU$^+$ β cells were observed in islet grafts from both young and old donors (Fig. 3A). Islet cells showed both nuclear BrdU label and cytoplasmic insulin staining, thus confirming that replicated cells in the islets are truly β cells. At 4 weeks post-transplantation, the mean percentage of insulin$^+$/BrdU$^+$ β cells were significantly higher in islet grafts from young donors than that in islet grafts from old donors (Fig. 3B). These data therefore indicated that β cells in the islets isolated from both young and old donors can replicate and that, there is greater capacity for β-cell replication in islet grafts from young donors versus older donors in untreated recipient mice.

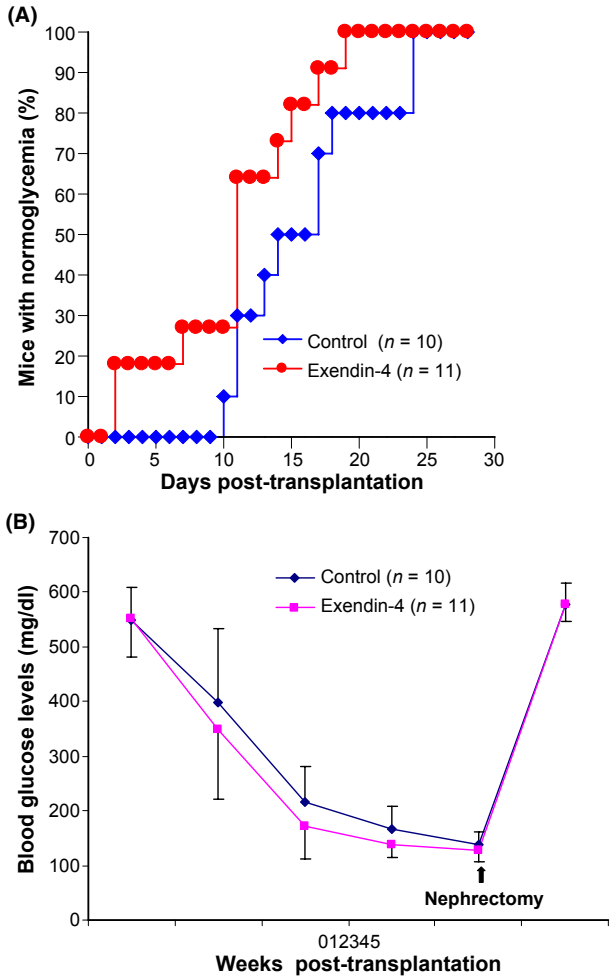

Figure 2 (A) Percentage of normoglycemia in diabetic C57Bl/6 mice received 100 C57Bl/6 islets from old donors and with or without exendin-4 treatment. Untreated mice achieved normoglycemia in 16 ± 5 days (n = 10), whereas exendin-4 treated mice required 11 ± 6 days (n = 11, $P < 0.05$). (B) Blood glucose levels in diabetic C57Bl/6 mice received 100 C57Bl/6 islets from old donors and with or without exendin-4 treatment. Data are presented as the mean ± SD.

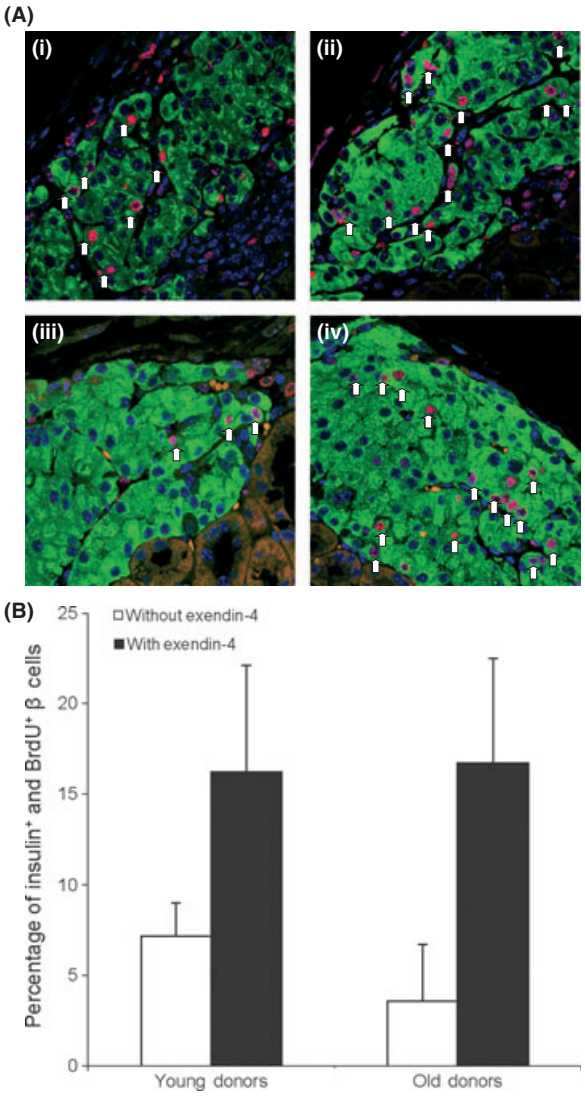

Figure 3 (A) Double immunofluorescent staining for insulin (green color) and bromodeoxyuridine (BrdU) (red color) in islet grafts in diabetic C57Bl/6 mice received C57Bl/6 islets from young donors (i); in diabetic C57Bl mice received C57Bl/6 islets from young donors and exendin-4 treatment (ii); in diabetic C57Bl mice received C57Bl/6 islets from old donors (iii); and in diabetic C57Bl/6 mice received C57Bl/6 islets from old donors and exendin-4 treatment (iv). Insulin$^+$/BrdU$^+$ β cells (arrowheads) in the islet grafts under the kidney capsule can be seen. (B) Percentage of insulin$^+$/BrdU$^+$ β cells in total insulin$^+$ β cells in C57Bl/6 islet grafts at 4 weeks post-transplantation. Islet grafts from younger donors or old donors were harvested by removing left kidney bearing islet grafts in C57Bl/6 mice with and without exendin-4 treatment. Data are presented as the mean ± SD. At 4 weeks post-transplantation, the mean percentage of insulin$^+$/BrdU$^+$ β cells was 7.2 ± 1.8% in untreated C57Bl/6 mice with islet grafts from younger donors (n = 6) and 16.2 ± 5.2% in exendin-4 treated mice with islet grafts from younger donors (n = 11, $P < 0.01$); 3.6 ± 3.1% in untreated C57Bl/6 mice with islet grafts from old donors (n = 7) and 16.7 ± 5.8% in exendin-4 treated C57Bl/6 mice with islet grafts from old donors (n = 8, $P < 0.01$).

With exendin-4 treatment, more insulin$^+$/BrdU$^+$ β cells were observed in islet grafts from both young donors and old donors (Fig. 3A). At 4 weeks post-transplantation, the mean percentage of insulin$^+$/BrdU$^+$ β cells was significantly greater in exendin-4 treated mice than that in untreated mice that received islet grafts from younger donors ($P < 0.01$, Fig. 3B). The mean percentage of insulin$^+$/BrdU$^+$ β cells was also significantly greater in exendin-4 treated mice than that in untreated mice that received islet grafts from old donors ($P < 0.01$). Therefore, our data indicate that exendin-4 can stimulate β-cell replication in mouse islet grafts from both young and old donors.

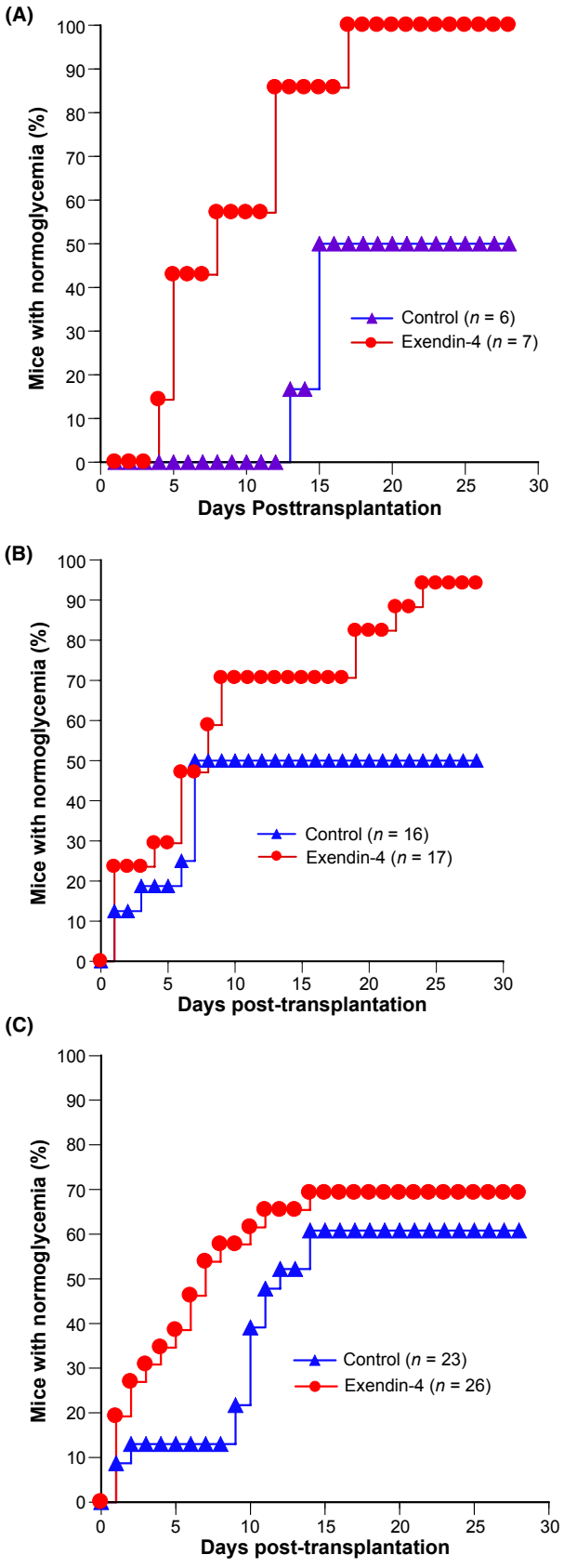

Figure 4 (A) Percentage of normoglycemia in diabetic nude mice received 100 C57Bl/6 islets from old donors and with or without exendin-4 treatment. At 4 weeks post-transplantation, normoglycemia was achieved in 50% untreated nude mice ($n = 6$) and 100% treated nude mice ($n = 7$, $P < 0.05$). (B) Percentage of normoglycemia in diabetic nude mice received 2000 IE human islets from ≤22-year-old donors and with or without exendin-4 treatment. At 4 weeks post-transplantation, normoglycemia was achieved in 50% untreated diabetic mice ($n = 16$) and in 94% exendin-4 treated diabetic nude mice ($n = 17$, $P < 0.01$) that received human islets from younger donors. (C) Percentage of normoglycemia in diabetic nude mice received 2000 IE human islets from ≥35-year-old donors and with or without exendin-4 treatment. At 4 weeks post-transplantation, normoglycemia was achieved in 52% untreated ($n = 21$) and 74% exendin-4 treated diabetic nude mice ($n = 23$, $P < 0.05$) that received islet from old donors.

Exendin-4 improved human islet graft function in diabetic nude mice

As a control, we transplanted 100 C57Bl/6J islets from old donors into diabetic nude mice. Normoglycemia was achieved in 50% untreated nude mice and 100% treated nude mice (Fig. 4A). Normoglycemia was also achieved in 50% untreated diabetic nude mice and in 94% exendin-4 treated diabetic nude mice that received human islets from ≤22-year-old donors (Fig. 4B, $P < 0.01$); in 61% untreated and 69% exendin-4 treated diabetic nude mice that received islets from ≥35-year-old donors (Fig. 4 C, $P > 0.05$). To confirm human islet graft function, we removed the left kidney bearing islet grafts in recipient mice which had islet graft function at 4 weeks post-transplantation. The return of hyperglycemia in these mice confirmed islet graft function. Thus, exendin-4 improved human islet graft function from young donors. At 4 weeks post-transplantation, body weight gain was found in recipient mice that restored normoglycemia. However, there was no significant difference in body weight change between untreated mice and exendin-4 treated mice (data not shown).

Exendin-4 stimulated β-cell replication in human islet grafts from young donors in nude mice

As the control, insulin$^+$/BrdU$^+$ β cells were detected in C57Bl/6 islet grafts in nude mice received C57Bl/6 islets and with or without exendin-4 treatment. However, more insulin$^+$/BrdU$^+$ β cells were found in islet grafts from exendin-4 treated mice (Fig. 5A). In untreated nude mice that did not return to normoglycemia, β-cell replication was also seen in islet grafts. At 4 weeks post-transplantation, the mean percentage of insulin$^+$/BrdU$^+$ β cells was significantly higher in islet grafts from exendin-4 treated mice than that in islet grafts from untreated mice (Fig. 5

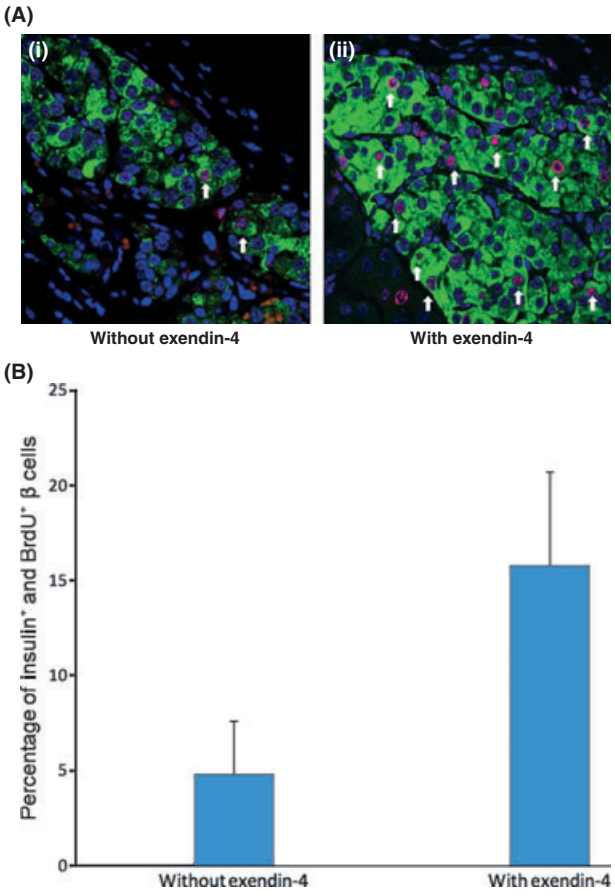

Figure 5 (A) Double immunofluorescent staining for insulin (green) and bromodeoxyuridine (BrdU) (red) in retired breeder C57Bl/6 islet grafts in untreated diabetic nude mice (i) and in exendin-4 treated diabetic nude mice (ii). (B) At 4 weeks post-transplantation, the mean percentage of insulin$^+$/BrdU$^+$ β cells was 4.8 ± 2.8% in C57Bl/6 islet grafts from untreated nude mice (n = 5) and 15.8 ± 4.9% in C57Bl/6 islet grafts from exendin-4 treated nude mice (n = 7, P < 0.01).

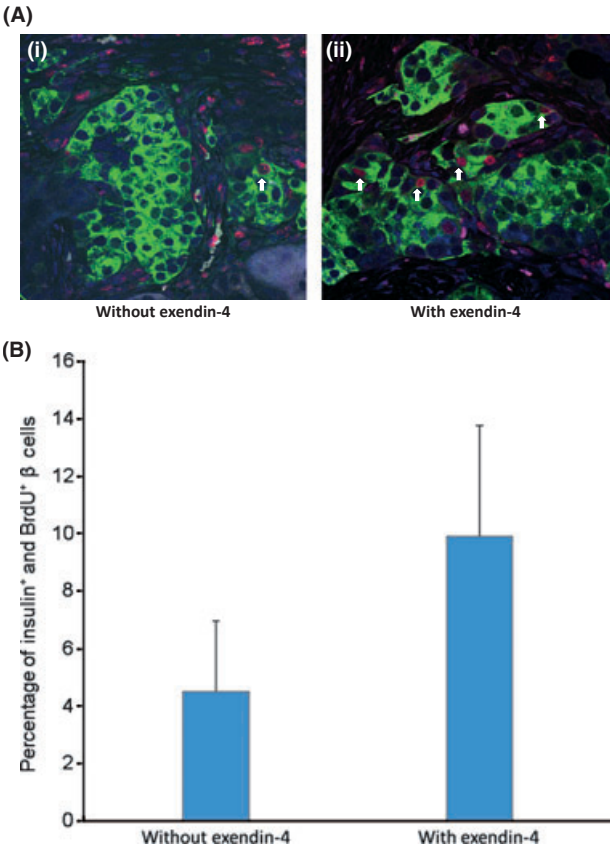

Figure 6 (A) Double immunofluorescent staining for insulin (green) and bromodeoxyuridine (BrdU) (red) in human islet grafts from 19-year-old donor in untreated diabetic nude mice (i) and in exendin-4 treated diabetic nude mice (ii). (B) Percentage of insulin$^+$ and BrdU$^+$ double labeled β cells in total insulin$^+$ β cells in islet grafts from ≤22-year-old young donors at 4 weeks post-transplantation. The mean percentage of insulin$^+$/BrdU$^+$ β cells was 4.5 ± 2.4% in islet grafts from untreated mice (n = 16) and 9.9 ± 3.9% in islet grafts from exendin-4 treated mice (n = 17, P < 0.01).

B, P < 0.01). Thus, β cells in islet grafts from mice over 40 weeks of age were able to replicate in nude mice and exendin-4 stimulated additional β-cell replication in these mouse islet grafts from older mice.

In nude mice with and without exendin-4, insulin$^+$/BrdU$^+$ β cells were observed in islet grafts from ≤22-year-old young donors. However, more insulin$^+$/BrdU$^+$ β cells were found in islet grafts from exendin-4 treated mice (Fig. 6A). At 4 weeks post-transplantation, the mean percentage of insulin$^+$/BrdU$^+$ β cells was significantly higher in human islet grafts from exendin-4 treated mice than that in human islet grafts from untreated mice (Fig. 6B, P < 0.01). In those human islet grafts from ≥35-year-old donor, few insulin$^+$/BrdU$^+$ β cells were detected with or without exendin-4 treatment. Thus, our data indicated that β cells in human islet grafts from ≤22-year-old young donors, but not from ≥35-year-old donors, able to replicate in nude mice. Exendin-4 stimulated β-cell replication in islet grafts from ≤22-year-old young donors but not from ≥35-year-old donors.

Discussion

A decreased capacity for β-cell replication has been shown to correlate with age in mice [17–20] and in humans [21–23]. Similarly, islet autotransplantation was found to be more successful in preadolescents than in adolescents suggesting that age may be a factor in replication capacity of human islet grafts [5]. In this study, although β-cell replication was detected in mouse islet grafts from young and old donors, β-cell replication was found only in human islet grafts from ≤22-year-old donors and not

from ≥35-year-old donors. Our study therefore confirms and extends the prior findings indicating a loss of β-cell replicative ability beyond early adulthood in humans.

Although all untreated recipient mice achieved normoglycemia at 4 weeks post-transplantation, we observed better metabolic control in mice that received mouse islets from old donors than that in mice that received islets from young donors, despite the relatively low levels of β-replication in islet grafts from old donors at 4 weeks after transplantation. The better metabolic control in mice receiving islets from old donors was most likely because of them receiving a greater β-cell mass at the time of transplantation. In human clinical islet transplantation, transplanted islets are counted as islet equivalents but in rodent islet transplantation, transplanted islets are counted as islet absolute numbers. Mouse islets from old donors tend to be larger and higher in insulin content than islets from young donors [24]. Therefore, more β cells and insulin in islet grafts from old donors may contribute to better metabolic control in the recipient mice.

Our data indicated that β cells have the ability to replicate in mouse islet grafts from either young or old donors in untreated recipient mice. However, more replicated β cells were detected in mouse islet grafts from younger donors than that in islet grafts from old donors. Hyperglycemia *per se* did not appear to promote increased β-cell replication in mouse islet grafts as there were high percentages of β-cell replication in recipient mice in which normoglycemia was restored either early or late in the clinical course (data not shown). In a unique double-islet transplanted mouse model, significant difference of β-cell replication in mouse islet grafts from both young and old donors was not found, in response to hyperglycemia which was induced 3 weeks after transplantation [25]. The different observation is may be because of the transplanted models.

In contrast to our findings in mice, we found significant replication of β cells in human islet grafts from ≤22-year-old donors but not from ≥35-year-old donors. As BMIs in the two groups were similar (29.6 ± 1.7 vs. 27.5 ± 3.3), the β-cell replicative difference was not attributable to the body weight of donors. Our data are consistent with a previous study which found no evidence of β-cell replication in the islets of >30-year-old humans [23]. Our findings therefore show that the capacity for β-cell replication in mouse and human islet grafts is significantly different in this respect and should be taken into account when interpreting experiments employing mouse islets.

Exendin-4 or GLP-1 has been previously shown to have beneficial effects on islet transplantation by improving metabolic control and reversing hyperglycemia in mice [10–12,26]. Furthermore, it has been shown that exenatide (synthetic exendin-4) improves islet graft function in patients with type 1 diabetes and facilitates achievement of insulin independence with fewer islets [14]. However, none of these previous studies examined for evidence of GLP-1 or exendin-4 stimulation of β-cell replication in islet grafts. Our results clearly showed that exendin-4 treatment enhanced the reversal of diabetes in mice that received a marginal number of mouse islets from young and old donors and human islet grafts. Although exendin-4 can stimulate insulin synthesis and prevent β-cell apoptosis, we looked more closely at the β-cell population in islet grafts for evidence of cell replication. To demonstrate cell replication among β cells in islet grafts, recipient mice were given BrdU daily for 4 weeks. In contrast to a recent study that failed to show exendin-4 treatment stimulates β-cell replication in islet grafts in mice [27], we detected many insulin$^+$/BrdU$^+$ β cells in islet grafts indicating that β cells were undergoing cell replication. A possible reason for the negative findings in the previous article was that they administered BrdU only 6 h or 24 h before harvesting islet grafts. As the replication rate of β cells in adults is very slow, long-term BrdU treatment is necessary for successful labeling of replicating β cells. Similarly, it has also been previously reported that there was no increase in replication of β cells when BrdU was given only 24 h before harvesting pancreases in NOD mice treated with anti-CD3 monoclonal antibody that reversed diabetes [28]. However, when BrdU was given for 2 weeks or 4 weeks, we could detect insulin$^+$/BrdU$^+$ β cells in NOD mice [9,29]. Here, we found that exendin-4 could stimulate β-cell replication in mouse islet grafts from both young and old donors. Interestingly, there was no significant difference in β-cell replication between exendin-4 treated mice that received islet grafts from younger donors and exendin-4 treated mice that received islet grafts from old donors. In contrast, we found that exendin-4 could only stimulate β-cell replication in islet grafts from ≤22-year-old donors but not from ≥35-year-old donors. Again it indicated that the capacity for β-cell replication in mouse and human islet grafts is different under exendin-4 treatment.

Although exendin-4 associated early post-transplant hypoglycemia has been reported [27], we did not find it in our study. In contrast to the marginal dose of islets used in our study, a high dose (500) of islets was transplanted in that study. Therefore, it is likely that the post-transplant hypoglycemia in that study was not a consequence of exendin-4 therapy but was rather because of unregulated insulin release from dying β cells in the islet grafts, a complication which is less problematic in the marginal dose experiments that we conducted.

It has been reported that pre-existing β-cells are the major source of new β cells during adult life and after pancreatectomy in mice [30] and that all β cells contribute

equally to islet growth and maintenance [31]. We observed that only some, but not all β cells in islets respond to exendin-4 treatment by undergoing mitotic division. Further studies are needed to explore whether these replicated β cells are from progenitor cells and to identify specific pathways that govern β-cell replication in islet grafts under exendin-4 treatment. Further studies are also needed to determine whether immunosuppressive drugs interrupt the effect of exendin-4 on β-cell replication.

In conclusion, our study demonstrated that the capacity for β-cell replication in mouse and human islet grafts is different with and without exendin-4 treatment. Our studies also indicated that targeting GLP-1 receptors can be used to stimulate human β-cell replication in islets from young donors or in young patients with diabetes.

Authorship

LT and JG: contributed equally to this study and share the first authorship. ZG and BT: participated in research design. ZG and TDO'B: participated in the writing of the paper. LT, JG, GW, and HY: participated in the performance of the research. LT, JG, ZG, and TDO'B: participated in data analysis.

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