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Original Article

Implanted islet mass influences the effects of dipeptidyl peptidase-IV inhibitor LAF237 on transplantation outcomes in diabetic mice

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ABSTRACT

Background: Previous studies showed inconsistent Results of the effects of dipeptidyl peptidase (DPP)-IV inhibitors on syngeneic mouse islet transplantation. We hypothesized that the implanted islet numbers are critical for the effects of DPP-IV inhibitors on the outcomes of transplantation.

Methods: One hundred and fifty or three hundred islets were syngeneically transplanted under the renal capsule of each streptozocin-diabetic C57BL/6 mouse and recipients were then treated without or with LAF237 (10 mg/kg/day, po) for 6 weeks. After transplantation, recipients' blood glucose, body weight and intraperitoneal glucose tolerance test (IPGTT) were followed-up periodically. The graft was removed for the measurement of β-cell mass at 6 weeks.

Results: In recipients with 150 islets, it was not significantly different between the LAF237-treated group (n = 14) and control group (n = 14) in terms of the blood glucose, body weight, glucose tolerance at 2, 4 and 6 weeks or the graft β-cell mass at 6 weeks. In contrast, in recipients with 300 islets, the LAF237-treated group (n = 24) did have a lower area under the curve of the IPGTT at 4 weeks ($p = 0.0237$) and 6 weeks ($p = 0.0113$) as well as more graft β-cell mass at 6 weeks (0.655 ± 0.008 mg vs. 0.435 ± 0.006 mg, $p = 0.0463$) than controls (n = 24).

Conclusions: Our findings revealed 6-week treatment of LAF237 improves glucose tolerance and increases graft β-cell mass in diabetic mice transplanted with a sufficient number but not a marginal number of islets. These indicate that the effects of DPP-IV inhibitors are influenced by the implanted islet mass.

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At a glance of commentary

Scientific background on the subject

Dipeptidyl peptidase (DPP)-IV inhibitors block the degradation of incretin hormones that promote pancreatic β-cell neogenesis and proliferation, reduce β-cell death as well as preserve β-cell function and mass. However, previous studies showed inconsistent results of the effects of DPP-IV inhibitors on syngeneic mouse islet transplantation.

What this study adds to the field

DPP-IV inhibitor LAF237 treatment improves glucose tolerance and increases graft β-cell mass in diabetic mice syngeneically transplanted with a sufficient number but not a marginal number of islets, indicating the effects of DPP-IV inhibitors are influenced by the implanted islet mass.

Clinical islet transplantation has succeeded in curing patients with type 1 diabetes [1–3]. However, 2 or more transplants are needed in the majority of successful cases, and the insulin independence rate decreases over time [2,3]. Therefore, increasing and maintaining the success rate are critical issues in human islet transplantation. Allograft failure may be caused by immunological factors (such as immune rejection, autoimmune destruction and toxicity of immunosuppressants) and nonimmunological factors (such as insufficient β-cell mass and poor islet engraftment) that have been intensively investigated to improve the result of islet transplantation [4].

The incretin hormones, including glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide (GLP)-1, improve glucose control in type 2 diabetics by stimulating insulin biosynthesis and secretion [5–7]. Additionally, GLP-1 also suppresses gastric emptying and appetite as well as glucagon secretion [5,6]. Both GLP-1 and GIP are also known to enhance β-cell proliferation and reduce β-cell apoptosis [5–10]. However, they are rapidly catalyzed by dipeptidyl peptidase (DPP)-IV and have a very short plasma half-life [5–7,11]. DPP-IV inhibitors block the degradation of both GIP and GLP-1 [11], thus increasing their circulating levels, which are beneficial for β-cells. Studies done in diabetic rodents have demonstrated DPP-IV inhibitors improve glucose tolerance, β-cell glucose responsiveness, insulin secretion [12] and insulin sensitivity [12,13]; promote pancreatic β-cell survival [14–16], islet neogenesis [14,17] and proliferation [18,19]; reduce pancreatic β-cell death [19–21]; preserve pancreatic β-cell function and mass [19,22–25] and reduces the effect of autoimmunity on islet graft survival [26]. Regarding the effects of DPP-IV inhibitors on syngeneic mouse islet transplantation, a handful of studies have been reported but the results are inconsistent. Kim et al. showed that diabetic mice each receiving 100 islets remained hyperglycemic after sitagliptin treatment. However, the same treatment in 300 islet recipients improved the glucose tolerance and protected the loss of the graft [27]. Samikannu et al. transplanted 200 islets into diabetic mice and found that sitagliptin treatment accelerated the restoration of their blood glucose as well as increased the insulin content, β-cell area and β-cell proliferation in grafts [28]. In contrast, we could not find beneficial effects of posttransplant MK-0431 treatment on transplant outcome or graft insulin content and β-cell mass in diabetic mice each transplanted with 150 islets [29]. Since beneficial effects of DPP-IV inhibitors were shown in recipients with 200 and 300 islets [27,28] but not in recipients with 100 and 150 islets [27,29], we considered the implanted islet number might be important for the effects of DPP-IV inhibitors on transplanted islets. Therefore, we conducted this study to compare the effects of LAF237 treatment on diabetic mice each transplanted with 150 vs. 300 islets.

Material and methods

Animals

We used male inbred C57BL/6 mice (purchased from National Laboratory Animal Center, Taipei, Taiwan), aged 8–12 weeks, as donors and recipients in islet transplantation. Recipients were rendered diabetic by a single intraperitoneal injection of streptozocin (STZ, Sigma Immunochemicals, St. Louis, MO, USA), 200 mg/kg body weight. Before transplantation, their blood glucose levels above 350 mg/dl were confirmed [29]. The protocol of animal experiments was approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital.

Islet isolation

Murine islets were isolated as described previously [29]. Under anesthesia with Zoletil®/Rompun® 2:1 mixture, mouse pancreata were injected with RPMI-1640 (GIBCO BRL, Grand Island, NY, USA) 2.5 ml with collagenase (collagenase from *Clostridium histolyticum*, type XI, Sigma Immunochemicals) 1.5 mg/ml, and then incubated in a 37 °C shaking water bath. Islets were purified by Histopaque-1077 density gradient (Sigma Immunochemicals) and islets with a diameter 75–250 μm were handpicked under a dissecting microscope.

Islet transplantation

We syngeneically transplanted 150 or 300 C57BL/6 mouse islets were under the renal capsule of each STZ-diabetic mouse. The islets were first centrifugated in polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ). With the mouse under anesthesia with Zoletil®/Rompun® 2:1 mixture, the kidney was exposed. After capsulotomy, the tip of the tubing advanced under the capsule and islets were injected [29]. After transplant, recipients' body weight and nonfasting blood glucose were measured periodically. Normoglycemia was defined as nonfasting blood glucose levels <200 mg/dl.

LAF237 treatment

After islet transplantation, recipients were given daily oral gavage with distilled water (control group) or LAF237 (Axon

Medchem, Groningen, Netherlands, 10 mg/kg/day, LAF237 group), for 6 weeks.

Intraperitoneal glucose tolerance test (IPGTT)

After islet transplantation, we performed IPGTT at 2, 4 and 6 weeks. A glucose solution (5%, 1.5 g/kg) was administered intraperitoneally after an overnight fast, and then blood glucose was sampled at 0, 30, 60, 90 and 120 min by tail snipping[27]. The area under the curve (AUC) of IPGTT was the sum of the trapezoidal areas between 0, 30, 60, 90 and 120 min.

Measurements of plasma DPP-4 activities and GLP-1 concentrations

At 6 weeks after transplantation, recipients' blood was sampled by heart puncture and the plasma was stored at −20 °C. DPP-4 activities were measured with Fluorometric assay by using 7-Amino-4-methylcoumarin (AMC; Cat. Q-1025; Bachem) as standard and H-Gly-Pro-AMC · HBr (Cat. I-1225; Bachem) as substrate. All reagents prepared by assay buffer (25 mM Tris−HCl pH 7.4, 140 mM NaCl, 10 mM KCl and 0.1% BSA). Ten microliters of plasma was incubated with 140 μL substrate (150 μM) for 10 min, and fluorescence intensity was measured at excitation/emission wavelength of 390 nm/460 nm with a spectrophotometer. The DPP-4 activity was expressed as mU/mL where mU = nmol/min. GLP-1 (7−36) concentrations were measured using an ELISA Kit (Linco Research Inc, St. Charles, MO, USA).

Removal of the islet graft

At 6 weeks after transplantation, the recipient intended for graft removal was anesthetized with Zoletil®/Rompun® 2:1 mixture. The kidney capsule with the adherent graft was excised and removed [29]. The graft weight was measured on a Mettler AE200 balance (Mettler Instruments Corp., NJ, USA).

Immunohistochemistry and measurement of β-cell mass of the islet graft

The removed graft was fixed in a formalin solution and then processed for paraffin embedding and sectioning. Immunoperoxidase staining for graft β cells was performed with an insulin antibody (Dako, Denmark) [29]. The graft β-cells and non-β cells were quantified by point counting morphometry on insulin-stained sections. Finally, the β-cell mass was determined by multiplying graft weight by β-cell relative volume [30].

Statistical analysis

Results were expressed as the mean and standard deviation (M ± SD). Paired and unpaired Student's t tests as well as ANOVA were applied to compare mean values in one group, between two groups and multiple comparisons, respectively. The p-value <0.05 was considered significant.

Results

Effects of LAF237 on recipients' nonfasting blood glucose after islet transplantation

In recipients with 150 islets, their nonfasting blood glucose levels decreased gradually in both control group (n = 14) and LAF237-treated group (n = 14) [Fig. 1A]. In the control group, the mean blood glucose was 467 ± 14 and 188 ± 33 at 0 and 6 weeks, respectively ($p < 0.0001$). In the LAF237-treated group, the nonfasting blood glucose was 464 ± 16 and 168 ± 26 mg/dl at 0 and 6 weeks, respectively ($p < 0.0001$). However, there was no significantly difference between 2 groups throughout the study period. By 6 weeks, normoglycemia was achieved in 12/12 (100%) of mice treated with LAF237 versus 8/10 (80%) in the controls ($p = 0.4746$). In recipients with 300 islets, their nonfasting blood glucose levels also decreased progressively in both control group (n = 24) and LAF237-treated group (n = 24) [Fig. 1B]. In the control group, the mean blood glucose was 562 ± 13 and 260 ± 28 at 0 and 6 weeks, respectively ($p < 0.0001$). In the LAF237-treated group, the mean blood glucose was 569 ± 11 and 212 ± 21 mg/dl at 0 and 6 weeks, respectively ($p < 0.0001$). However, there was no difference between the two groups during the study period. By 6 weeks, euglycemia was achieved in 20/24 (83.3%) mice treated with LAF237 versus 17/24 (70.8%) controls ($p = 0.2056$).

Effects of LAF237 on recipients' body weight after islet transplantation

In recipients with 150 islets, both the LAF237-treated group (n = 14) and control group (n = 14) exhibited increased body weight during the 6 weeks after transplantation (LAF237-treated group: from 20.8 ± 0.6 to 24.5 ± 0.4 g, $p < 0.0001$;

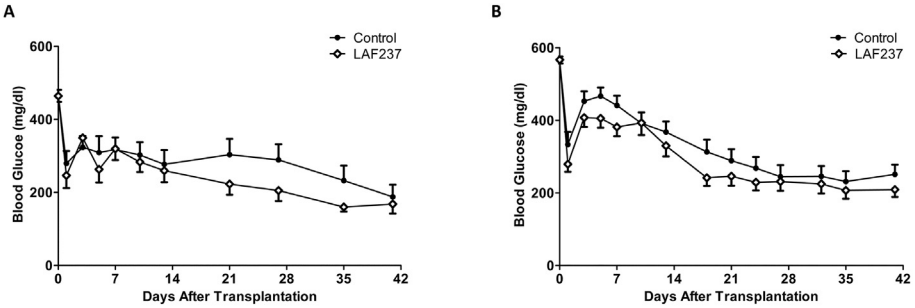

Fig. 1 Evolution of nonfasting blood glucose levels after syngeneic transplantation with 150 (A) or 300 (B) islets in each diabetic mouse with (open diamond) and without (solid circle) LAF237 treatment. Values are mean ± SD.

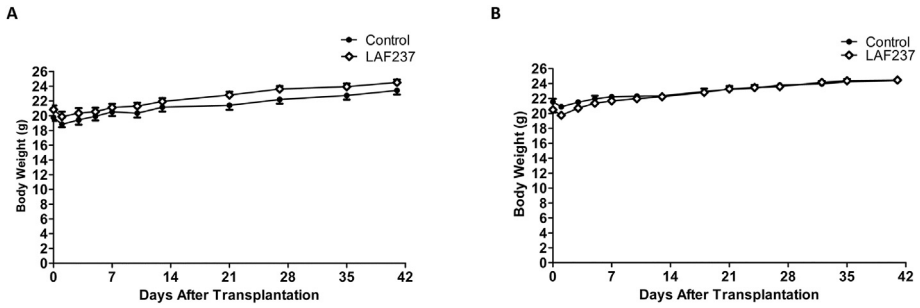

Fig. 2 Evolution of body weight after syngeneic transplantation with 150 (A) or 300 (B) islets in each diabetic mouse with (open diamond) and without (solid circle) LAF237 treatment. Values are mean ± SD.

controls: from 19.8 ± 0.4 to 23.4 ± 0.6 g, $p < 0.0001$) [Fig. 2A]. However, the weight between two groups was not different during the study period. In recipients with 300 islets, both the LAF237-treated group (n = 24) and control group (n = 24) had body weight gain during the 6 weeks after transplantation (LAF237-treated group: from 20.4 ± 0.4 to 24.4 ± 0.4 g, $p < 0.0001$; controls: from 21.6 ± 0.5 to 24.5 ± 0.4 g, $p < 0.0001$) [Fig. 2B]. However, there was no difference between two groups during the study period.

Effects of LAF237 on recipients' intraperitoneal glucose tolerance after islet transplantation

Blood glucose levels at 6 weeks were lower than those at 2 weeks at 60′ and 90′ in controls with 150 and 300 islets and at 60′, 90′ and 120′ in LAF237-treated mice with 300 islets [Supplemental Fig. 1]. In recipients with 150 islets, their blood glucose levels at 30′, 60′, 90′ and 120′ as well as AUC of the IPGTT at 2 weeks (27,410 ± 2347 vs. 32,380 ± 3716 mg· dl, $p = 0.2682$), 4 weeks (28,990 ± 1675 vs. 32,620 ± 3869 mg· dl, $p = 0.3737$) and 6 weeks (22,990 ± 1675 vs. 24,850 ± 2069 mg· dl, $p = 0.4931$) were not significantly different between the LAF237-treated mice and the controls [Figs. 3A and 4A]. In contrast, the IPGTT in recipients with 300 islets revealed the blood glucose levels at 30′, 60′, 90′ and 120' in the LAF237-treated mice were significantly lower than those in the controls at 4 and 6 weeks [Fig. 3B]. Meanwhile, LAF237-treated mice had a lower AUC of the IPGTT at 4 weeks (29,030 ± 1827 vs. 35,730 ± 2209 mg· dl, $p = 0.0237$) and 6 weeks (26,330 ± 1748 vs. 33,340 ± 2125 mg· dl, $p = 0.0113$) [Fig. 4B].

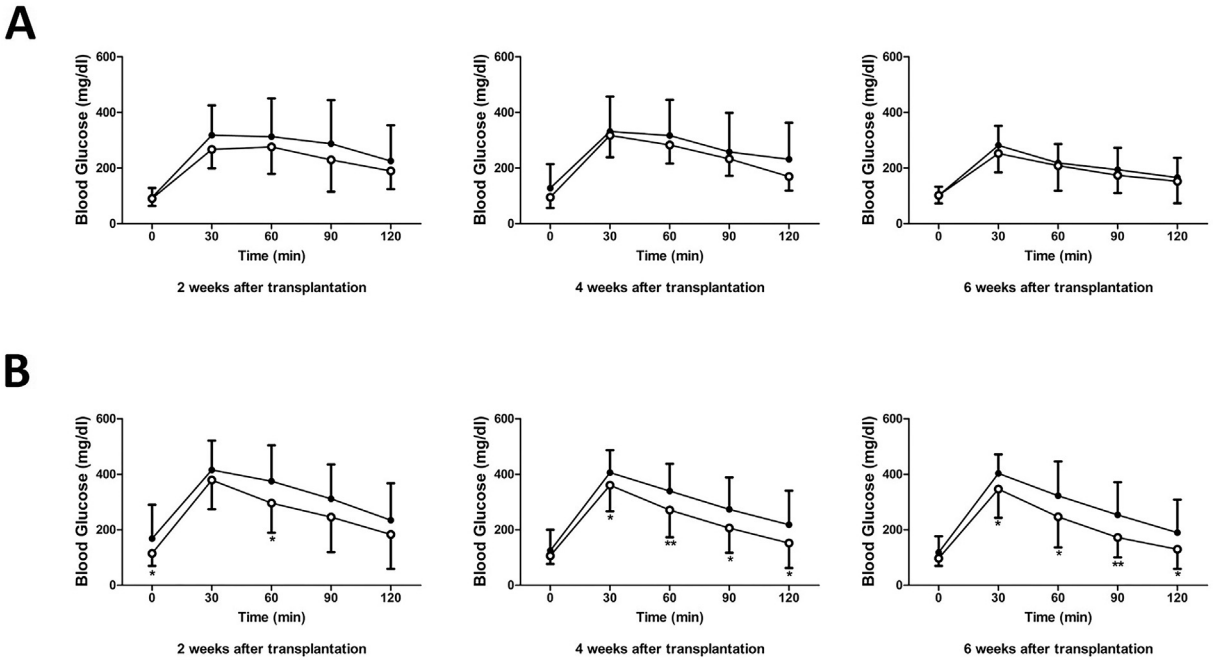

Fig. 3 The intraperitoneal glucose tolerance test at 2, 4 and 6 weeks after syngeneic transplantation with 150 (A) or 300 (B) islets in each diabetic mouse with (open circle) and without (solid circle) LAF237 treatment. Values are mean ± SD. *$p < 0.05$, **$p < 0.01$ vs. control.

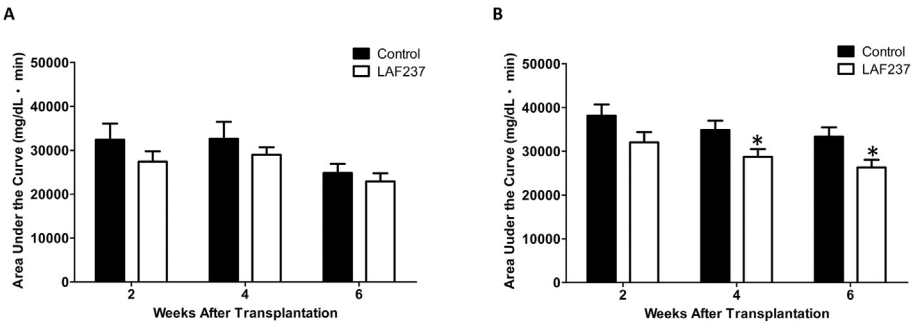

Fig. 4 The area under the curve of intraperitoneal glucose tolerance test at 2, 4 and 6 weeks after syngeneic transplantation of 150 (A) or 300 (B) islets in each diabetic mouse with (open column) and without (filled column) LAF237 treatment. Values are mean ± SD. *$p < 0.05$ vs. control.

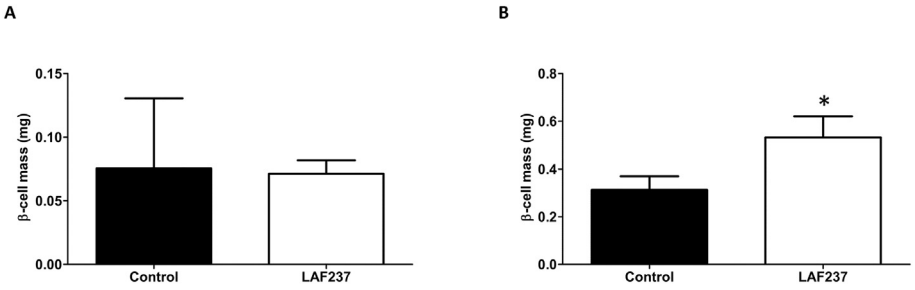

Fig. 5 The graft β-cell mass at 6 weeks after syngeneic transplantation of 150 (A) or 300 (B) islets in each diabetic mouse with (open column) and without (filled column) LAF237 treatment. Values are mean ± SD. *$p < 0.05$ vs. control.

Effects of LAF237 on recipients' plasma DPP-4 activities and GLP-1 concentrations

At 6 weeks after transplantation, LAF237-treated mice had a lower plasma DPP-4 activities [3.057 ± 0.467 mU/mL (n = 7) vs. 3.393 ± 0.877 mU/mL (n = 10), $p = 0.7696$] and higher plasma GLP-1 concentrations [5.456 ± 1.242 pmol/l (n = 9) vs. 4.660 ± 1.455 pmol/l (n = 10), $p = 0.6861$]. However, the differences between two groups did not reach statistical significance.

Effects of LAF237 on the graft β-cell mass

At 6 weeks after transplantation, in recipients with 150 islets, their graft β-cell mass (LAF237: 0.071 ± 0.001 vs. controls: 0.075 ± 0.006 mg, $p = 0.9346$) [Fig. 5A] were comparable in both groups. However, in recipients with 300 islets, the LAF237-treated group [Fig. 6B] had more prominent grafts than controls [Fig. 6A]. The LAF237 group had 1.5-fold more graft β-cell mass when compared to the control group (0.655 ± 0.008 mg vs. 0.435 ± 0.006 mg, $p = 0.0463$) [Fig. 5B].

Discussion

In the present study, we used a syngeneic subrenal capsule transplantation model to test whether the implanted islet numbers influence the effects of DPP-IV inhibitors on transplantation outcomes. Previously, we demonstrated that, after syngeneic transplantation of 150 and 300 mouse islets to each diabetic mouse, there were 18% and 100% achieved normoglycemia at 4 weeks, respectively [31]. For recipients with 150 islets, we found 6-week exendin-4 [32] but not MK-0431 [29] treatment was beneficial for transplantation outcome and increased graft β-cell mass, indicating GLP-1 receptor agonists are more effective than DPP-IV inhibitors in enhancing transplanted islets. This can be explained by the differential effects of the pharmacological actions of GLP-1 agonists versus the physiological potentiation of endogenous GIP and GLP-1 via DPP-IV inhibition [15,33]. In this study, we further investigated the effects of LAF237 on diabetic recipients transplanted with 150 and 300 islets and demonstrated that LAF237 improved glucose tolerance and increased graft β-cell mass only in those with 300 but not 150 islets. Since substantial β-cell death occurred in islet grafts in the diabetic recipient soon after transplantation [34], it is not surprised that the beneficial effects of LAF237 were not observed in recipients transplanted with a small number of islets, same as our previous study with MK-0431 [29]. The reason why the islet mass can influence the effect of DPP-IV inhibitors is that additional transplanted islets can enhance the growth and function of the islet graft [30]. Because both LAF237 and more transplanted islets can enhance the growth and function of the islet graft, the beneficial effects of LAF237 was demonstrated only in diabetic mice transplanted with a sufficient number but not a marginal number of islets.

Our results are consistent with those of Kim et al. who mentioned in discussion that diabetic mice each receiving 100 islets remained hyperglycemic and were not improved by sitagliptin treatment in their pilot studies [27]. In contrast,

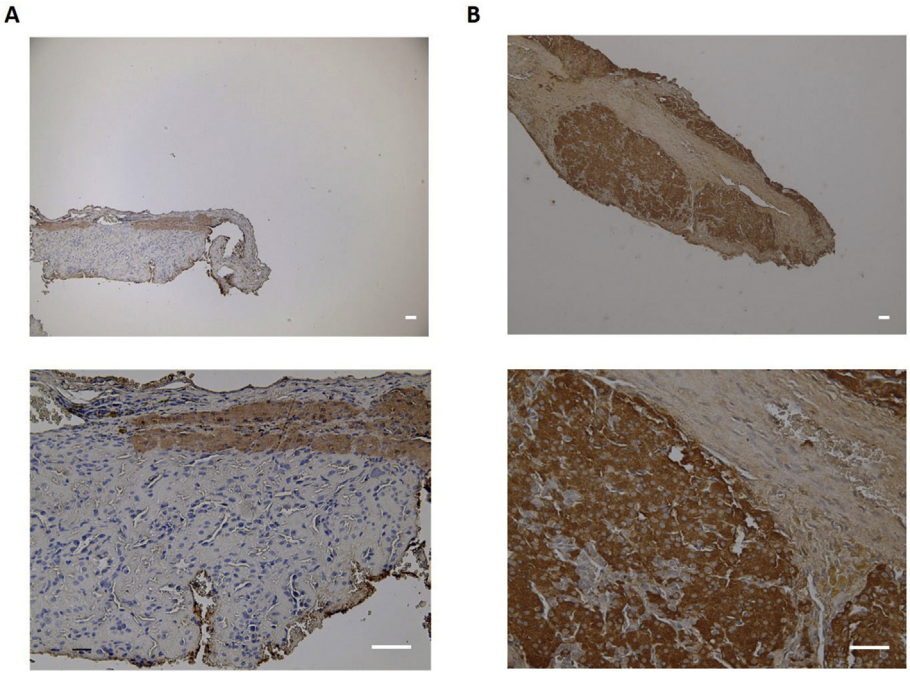

Fig. 6 Immunohistochemistry with insulin staining (brown color) of the control (A) and LAF237-treated (B) grafts at 6 weeks after syngeneic transplantation of 300 islets. Upper panel: 100X, Lower panel: 400X, Scale bar: 100 μm.

they showed sitagliptin treatment improved the recipient's glucose tolerance and protected the loss of the graft in recipients with 300 islets [27]. However, our study designs are more sophisticated and convincing than theirs. First, we used freshly isolated islets instead of rAD-TK-infected islets. Therefore, the blood glucose levels in our control mice decreased gradually after transplanted but Kim et al. showed they progressively increased from 1.5 weeks on and ~40% of the mice died during the course of the study. Second, we daily administered to mice with constant amount of LAF237 by oral gavage instead of sitagliptin in the diet chow by which daily drug dose was inconsistent. Third, we quantified graft β-cell mass directly by point counting morphometry instead of quantification of PET imaging which was dependent on the amount of β-cells infected with rAD-TK. Finally, using above mentioned methods, we direct compared the effects of LAF237 treatment on diabetic mice each transplanted with 150 vs. 300 islets.

In the present study, the metabolic improvement in recipients with 150 and 300 islets is contributed by transplanted β cells because LAF237 was demonstrated no glucose lowering effect in streptozotocin-diabetic rats [35] and we excluded recipients with β-cell regeneration in the endogenous pancreas. In diabetic mice transplanted with 300 islets, LAF237 further improved glucose tolerance and increased graft β-cell mass. Regarding the protective effects of LAF237 on syngeneic islet transplants, they may include inhibition of β-cell apoptosis [36,37], stimulation of β-cell replication [38], suppression of oxidative stress [39], enhancement of angiogenesis [40], anti-inflammatory properties [41], as well as increase of endothelial cell proliferation, blood flow in islet grafts, and microvessel density [28] shown by previous studies with GLP-1 receptor agonists and DPP-IV inhibitors.

In this study, the blood glucose levels in recipients with 300 islets were higher than those with 150 islets. This can be explained by their initial severity of diabetes because recipients with 300 islets had higher baseline blood glucose level (562 ± 13 and 569 ± 11 mg/dl in control and LAF237 group, respectively) than that with 150 islets (467 ± 14 and 464 ± 16 mg/dl in control and LAF237 group, respectively). Actually, the reduction of blood glucose in recipients with 300 islets (302 and 357 mg/dl in control and LAF237 group, respectively) was more than that with 150 islets (279 and 296 mg/dl control and LAF237 group, respectively) at 6 week. We found the LAF237 group in mice transplanted with 300 islets had 1.5-fold more graft β-cell mass when compared to the control group. This may result from improved engraftment and enhanced β-cell proliferation of the islet graft. In contrast, we previously demonstrated 6-week exendin-4 treatment in mice transplanted with 150 islets increased their graft β-cell mass by 2.3-fold. Again, GLP-1 receptor agonists showed more powerful than DPP-IV inhibitors in enhancing transplanted islets. In our study, even though LAF237-treated recipients with 300 islets had better glucose tolerance at 4 and 6 weeks and more graft β-cell mass than the controls at 6 weeks after transplantation, their non-fasting blood glucose levels were comparable. The results of glucose tolerance should be more reliable than blood glucose levels because the blood glucose was randomly measured, either before or after eating, but IPGTT was performed after fasting. To adjust different number of mice transplanted with 150 islets (n = 14) and 300 islets (n = 24), we analyzed IPGTT data of 14 mice randomly selected from controls and LAF237-treated recipients in 300-islet group, respectively. The results revealed LAF237-treated mice had a lower AUC of the IPGTT at 4 weeks (25,700 ± 1683 vs.

37,230 ± 3587 mg· dl, $p = 0.0073$) and 6 weeks (24,010 ± 1604 vs. 34,760 ± 3214 mg· dl, $p = 0.006$) which were similar to those from 24 mice.

In clinical islet transplantation, sitagliptin has been investigated in allo- and auto-transplant recipients. A 6-month treatment with the combination of pantoprazole and sitagliptin was associated with positive effects on alloislet recipients transplanted with 1–4 infusions (mean 12,046 IEQ/kg), indicated by decreased HbA_{1C} at 1 month and the reduction of insulin doses at 6 months, with three participants became insulin independent [42]. Since the effect did not persist when treatment had been withdrawn, it was postulated that the benefit was more likely due to the glucose-lowering action of sitagliptin rather than its effect on β–cell regeneration. By contrast, a 12-month randomized controlled trial of sitagliptin treatment did not affect the rate of insulin independence, insulin dose and metabolic outcomes in recipients with islet autotransplantation after total pancreatectomy for severe chronic pancreatitis [43]. In that series, mean transplant islets were 4400 IEQ/kg by which about one-third of patients could achieve insulin independence [44]. The different Results in above-mentioned studies can be partly explained by our finding that the benefits of sitagliptin are dependent on the transplanted islet numbers.

Conclusions

We showed 6-week LAF237 treatment improved glucose tolerance and increased graft β-cell mass in diabetic mice syngeneically transplanted with a sufficient number but not a marginal number of islets. It indicates that the effects of DPP-IV inhibitors are influenced by the implanted islet numbers. Further studies are warranted to examine the potential use of DPP-IV inhibitors in human islet transplantation.

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Conflicts of interest

The authors declare no conflicts of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2020.10.002.

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