Original Article

Islet Transplantation to the Anterior Chamber of the Eye—A Future Treatment Option for Insulin-Deficient Type-2 Diabetics? A Case Report from a Nonhuman Type-2 Diabetic Primate

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Abstract

Replacement of the insulin-secreting beta cells through transplantation of pancreatic islets to the liver is a promising treatment for type-1 diabetes. However, low oxygen tension, shear stress, and the induction of inflammation lead to significant islet dysfunction and loss. The anterior chamber of the eye (ACE) has gained considerable interest and represents an alternative therapeutic islet transplantation site because of its accessibility, high oxygen tension, and immune-privileged milieu. We have previously demonstrated the feasibility of intraocular islet transplant in mouse and nonhuman primate models of type-1 diabetes and are now assessing its efficacy on glucose homeostasis in a nonhuman primate model of type-2 diabetes. We transplanted allogeneic donor islets (1,500 islet equivalents/kg) into the anterior chamber of one eye in a cynomolgus monkey with high-fat-diet-induced type-2 diabetes. Repeated examinations of the anterior and posterior segments of both eyes were done to monitor the engrafted islets and assess the overall ocular health. Fasting blood glucose level, blood biochemistry, and other metabolic parameters were routinely evaluated to determine the function of the islet graft and diabetes status. The transplanted islets were rapidly engrafted onto the iris and became vascularized 1 month after transplantation. We did not detect changes in intraocular pressure, cataract formation, ophthalmitis, or retinal vessel deformation. A significant lower fasting blood glucose level was observed while the graft was in place, and the transplantation reverts the progression of diabetes. The metabolic markers, hemoglobin A_{1C} and fructosamine, demonstrated improvement following islet transplantation. As a conclusion, intraocular islet transplantation in one eye of a cynomolgus monkey with type-2 diabetes improved its overall plasma glucose homeostasis, as evidenced by short-term measures and long-term metabolic markers. These results further support the future application of the ACE as an alternative site for clinical islet transplants in the context of type-2 diabetes.

Keywords

nonhuman primates, islet transplantation, anterior chamber of the eye, intraocular transplant, diet-induced type-2 diabetes

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Introduction

Diabetes is among the leading causes of death, and its global prevalence of 425 million people in 2017 is expected to rise to 629 million in 2045[1]. Patients with diabetes are at high risk of macro- and micro-vascular pathologies leading to cardiovascular diseases and diabetes-associated retinopathy, neuropathy, and nephropathy[2]. Poorly controlled glycemia rapidly leads to serious health complications such as kidney failure, blindness, and lower limb amputation. The key factor in preventing these complications is mainly dependent on timely and persistent glycemic control through lifestyle changes, antidiabetic medications, and insulin therapy when needed[3,4]. Transplantation of isolated pancreatic islets has emerged as a promising therapy in type-1 diabetes and proven to reduce the exogenous insulin dependency[5–7]. However, limited information is available for the transplantation of islets in type-2 diabetes. The hepatic portal vein is currently the site of choice for clinical islet transplantation. However, liver-specific complications such as low oxygen tension, shear stress, high enzymatic activity, local drug levels, and the induction of immediate blood-mediated inflammatory reaction (IBMIR) lead to significant islets loss immediately after transplantation and islet grafts dysfunction over time. A substantial surplus of transplanted islets is required to compensate for this immediate loss, and the same patient may need to undergo several islet transplantations over time (i.e., involving several donors) to achieve independence from exogenous insulin[6–10]. Therefore, the search for alternative islet transplantation sites remains a priority.

The anterior chamber of the eye (ACE) has gained considerable interest and represents a promising new site for therapeutic islet transplantation because of its high oxygen tension and immune-privileged milieu, among other technical advantages. Major advantages of the ACE as a transplantation site compared to other sites include less invasive surgery for introducing islets[11,12], a smaller number of islets is required per body weight to improve glycemic control[12–15], enhanced accessibility to islet grafts with the optically transparent cornea whereby allowing noninvasive and longitudinal high-resolution monitoring of islet grafts[11,14–18], high potential for local immunosuppression[19], and the possibility for systemic tolerization through anterior chamber-associated immune deviation[20]. We report here a first-of-its-kind case study of a type-2 diabetic nonhuman primate with sustained improvement in its glycemia and overall metabolic control following islet transplantation into the anterior chamber of one eye.

Materials and Methods

Husbandry and Handling of the Animal

The experiments were done in accordance with the guidelines and approval from the Institutional Animal Care and Use Committee (IACUC), SingHealth, Singapore. The animal was sourced from PT. Prestasi Fauna Nusantara Ltd, Jakarta, Indonesia, and it was housed in a 2 in 1 tier stainless steel cage with ad libitum access to water through the automatic watering system. Enrichment toys were provided, and fruits were given every day as treats. Parasite check, supplement administration, deworming, random bacterial culture, methicillin-resistant *Staphylococcus aureus* screening, and in-depth physical assessment of the animal were conducted semiannually. In general, National Advisory Committee for Laboratory Animal Research (NACLAR) guidelines were adhered to for the housing condition. The monkey was sedated for surgeries and imaging procedures according to the guidelines using intramuscular ketamine, and isoflurane gas was used at 3%–5% for induction and 1%–2% for maintenance. Heart rate, respiratory rate, and temperature were monitored throughout the procedures.

Induction of Type-2 Diabetes and Experimental Design

There are limited literature and dearth of robust protocols for type-2 diabetes induction in macaque monkeys. Here we report on a macaque with type-2 diabetes following 5 years of exposure to high-fat diet. Type-2 diabetes was induced in a 15-year-old male cynomolgus monkey (*Macaca fascicularis*) by giving 13% high-fat diet (LabDiet, St. Louis, MO, USA) for 5 years (Fig. 1). After the development of diabetes, the plasma blood glucose level of the monkey was monitored twice a day, once in the morning as fasting level before the meal and once in the afternoon before the enrichment fruits were given, by tail prick using an Accu-Chek hand-held glucometer (Roche, Basel, Switzerland). Allogeneic islet transplantation into the ACE was done on post operation day (POD) 0. The animal was given transient systemic therapy with an anti-CD154/CD40 L monoclonal antibody (clone 5C8) in the peri-transplant period at PODs -1, 0, 3, 10, 18, 28, 56, and 84. The efficacy of the islet graft was evaluated by different parameters over the experimental period, such as daily blood glucose level, twice a month fructosamine, monthly hemoglobin A_{1C} (HbA$_{1C}$), bimonthly intra-venous glucose tolerance tests (IVGTTs), and C-peptide analysis in the aqueous humor. IVGTT was performed by giving a 0.5 g/kg body weight bolus dose of glucose intravenously, and the plasma glucose was assessed at predetermined intervals up to 90 min after the glucose injection.

Islet Isolation and Transplantation of the Islets into the ACE

The islets were isolated as previously described[11,21]. In brief, the islets were isolated from the whole pancreas of a healthy donor cynomolgus monkey, using the liberase enzyme in a Ricordi chamber followed by purification with Ficoll gradients. The islets were cultured for 2 days before transplantation. We performed glucose-stimulated insulin secretion (GSIS) assay on the isolated islets as a quality assessment and a stimulation index of 2.98 was measured. The isolated islets were then transplanted into the ACE of the recipient
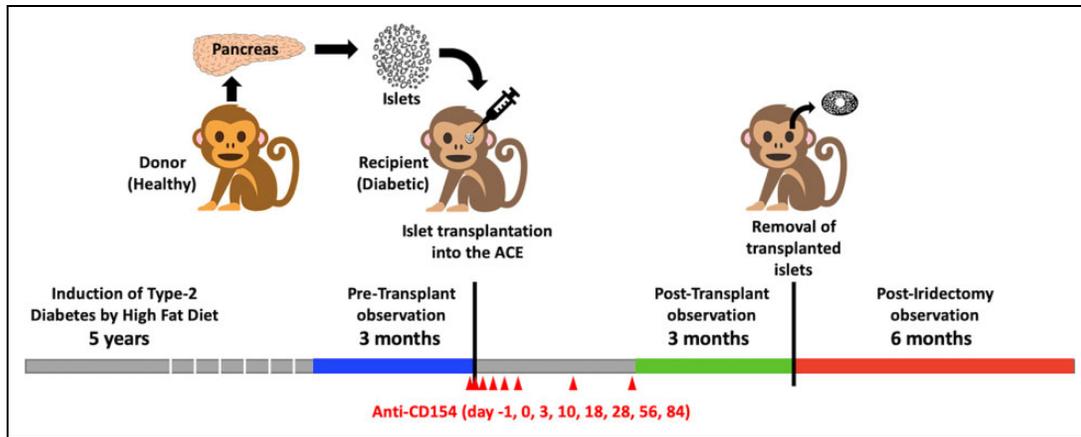
Figure 1. Experimental design. Isolated islets (1,500 islet equivalents/kg) from a healthy donor were transplanted into the anterior chamber of the eye (ACE) of a high-fat-diet-induced type-2 diabetic monkey. Peri-transplantation immunosuppression with anti-CD154 was given at PODs -1, 0, 3, 10, 18, 28, 56, and 84. Fasting blood glucose level, blood biochemistry, and metabolic parameters were evaluated for the graft function comparing the pre-transplant period, post-transplant period, and post-iridectomy period.

type-2 diabetic monkey (Fig. 2A). The transplant was done under sterile conditions in an operating room. One drop of a miotic agent, 3% pilocarpine (Alcon, Geneva, Switzerland), was instilled into the left eye of the primate half an hour before transplantation, letting the pupil constrict to have a maximum space of the iris bed. The peri-ocular regions were sterilized with povidone-iodine, followed by washing with physiological saline. A self-sealing incision was made on the clear cornea using a disposable 23G needle, 3 mm away from the cornea–sclera junction at the temporal quadrant. We transplanted a total of 12,000 islets (1,500 islet equivalents/kg) into the left eye only as a metabolic transplant. The isolated islets were withdrawn into a customized 25G thin-walled blunt cannula attached to a polyethylene tubing and held upward to let the islets settle down by gravity for 3 min. The cannula was then inserted into the pre-made self-sealing incision, and the islets were injected slowly into the ACE over 5 min while letting out the excess fluids from the eye via the entry incision. Total fluid injected together with the islets was expected to be around 150 µl. An air bubble was created at the center to prevent the islets from settling on the pupil. The cells were equally distributed over the whole iris by gently moving the eye. The animal was put in a supine position with the head facing upward for 2 hr, giving the transplanted islets time to attach to the iris. One drop of 0.5% levofloxacin (Cravit, Santen Pharmaceutical, Osaka, Japan) was instilled immediately after the procedure.

Iridectomy

To show the effect of the transplanted islets on blood glucose homeostasis, we removed the engrafted islets together with the iris on POD 186. The surgery was done under sterile condition in the operation room. Peri-ocular region and conjunctival sac of the surgery eye were cleaned with a standard ocular sterilization procedure. Peripheral corneal

paracentesis 2–3 mm was made at 12 o'clock position using a sterile disposable 2.8 mm slit blade (NANOedge Ophthalmic blades, Madhu Instruments Pvt. Ltd, New Delhi, India) without injuring the iris. The disposable 25G end-grasping forceps (Alcon) was inserted into the ACE, and the iris was then grabbed and pulled slowly to detach from the trabecular meshwork before extracting the whole iris out from the paracentesis (Fig. 2B, C). The incision was closed using a 10° suture, and topical antibiotic/steroids, 0.3% tobramycin, and 0.1% dexamethasone (Tobradex ointment, Alcon) were given for 3 days to prevent infection.

Ophthalmological Examinations

The intraocular pressure (IOP) was assessed using a Tono-Pen AVIA Vet Veterinary Tonometer (Reichert Technologies, Depew, NY, USA) after the instillation of a drop of 1% lignocaine. The graft and the anterior portion of the eye were examined by a standard slit-lamp examination. The posterior portion of the eye, retinal fundus images, retinal nerve fiber layer (RNFL), and retinal thickness were evaluated using Spectralis (Heidelberg Retinal Tomography, Heidelberg, Germany).

Analysis

All data were plotted and analyzed using GraphPad Prism version 6.03 (GraphPad Software, CA, USA). The data shown are mean $\pm$ SD, and a nonparametric Kruskal–Wallis test was used to compare the groups. P value ≤ 0.05 was considered significant. The area under the curve (AUC) and frequency distribution curve were developed using the same software. All data points from the pre-transplant period (POD –101 to POD 0), post-transplantation period (POD 97 to POD 185), and data from the post-iridectomy period (POD 186 to POD 348) were used for analysis. We excluded data from POD 0 to 96 to rule out the full engraftment and
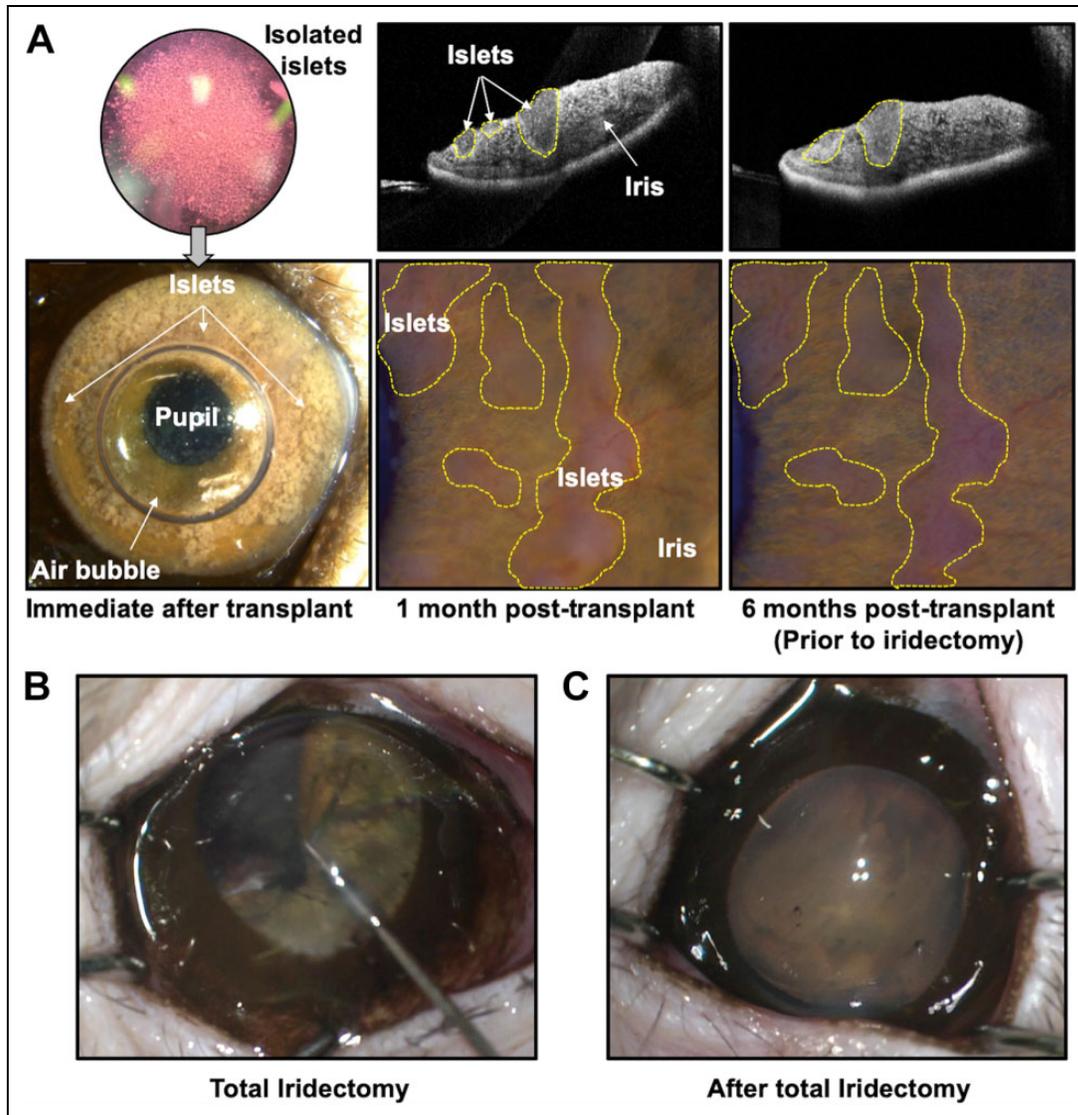
Figure 2. Visualization of islet graft and total iridectomy. (A) A total of 1,500 islet equivalents/kg were transplanted into the anterior chamber of the eye (ACE) of a high-fat-diet-induced type-2 diabetic monkey. The engraftment was confirmed by direct slit-lamp examination and OCT imaging of the iris at 1 month and 6 months (before removal of the graft) post-transplantation. The areas indicated by yellow dotted line are the engrafted islets on the iris in the ACE of the monkey. (B) The whole iris engrafted with transplanted islets was removed on POD 186 to show the effect of the graft on blood glucose homeostasis. (C) After the removal of the iris showing the absence of engrafted islets. POD: post operation day.

vascularization interval, and to exclude results from the unexpected tail injury period.

Results

Changes in Glycemia in a High-Fat-Diet-Fed Macaque

The plasma glucose level consistently rose above 5 mmol/l for three consecutive time points during monthly surveillance. Subsequently, the plasma blood glucose level was monitored twice a day by tail prick method using an Accu-Chek hand-held glucometer (Roche, Indianapolis, IN, USA).

The monkey was not under any diabetic medication or insulin therapy throughout the experiment. Following transplantation, there was a nontransplantation-related inflicted tail injury from POD 60 to POD 96, and the glucose level became erratic during this period, most likely due to the influence of pain and adrenaline. The monkey was given more recreational tools to reduce stress. The tail injury was fully recovered at POD 96 with appropriate wound and pain management by qualified veterinarians. There was a slight bodyweight drop by 350 g after the transplantation from 8 kg, which was further decreased to 6.7 kg after removal of the graft.
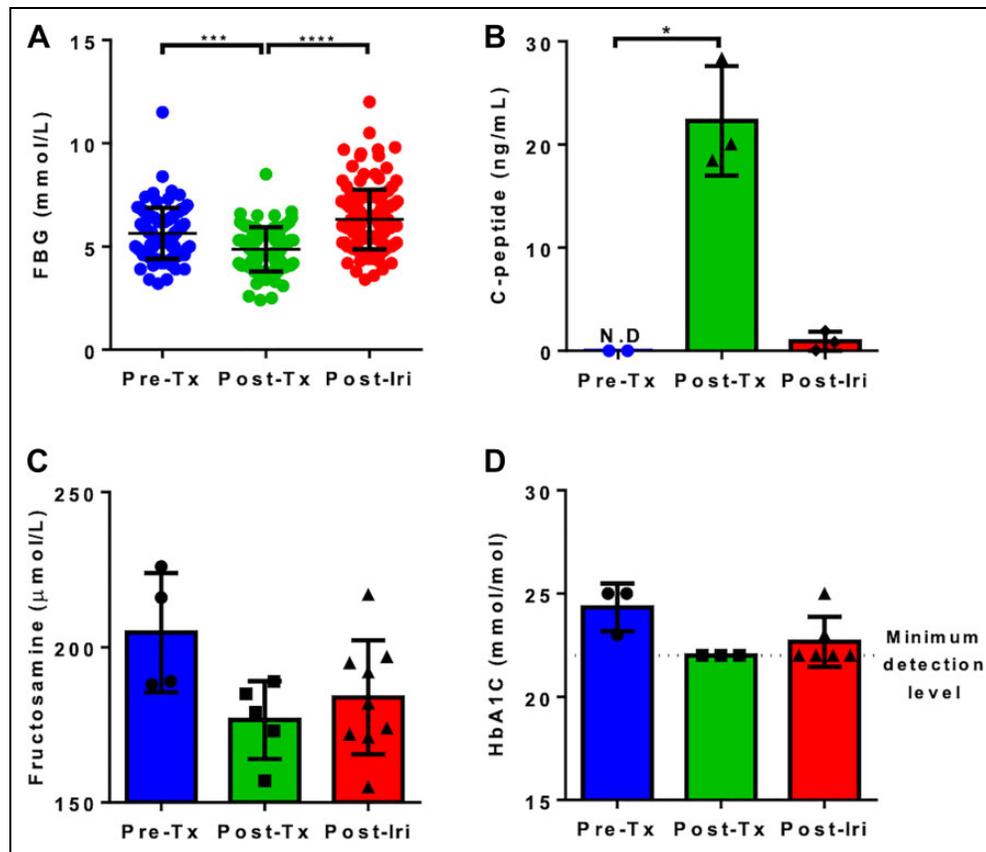
Figure 3. Daily blood glucose levels and metabolic parameters. (A) The mean fasting plasma glucose level 5.6 ± 0.1 mmol/l pre-transplantation was significantly dropped (***, $P = 0.0001$) to 4.8 ± 0.1 mmol/l post-transplantation, which was increased significantly (****, $P < 0.0001$) after the removal of the graft, post-iridectomy to 6.3 ± 0.1 mmol/l. (B) C-peptide was not detected in the aqueous humor prior to transplantation but increased to 22.30 ± 3.07 ng/dl (*, $P < 0.05$) while the graft was in place. After the removal of the graft, the C-peptide level dropped to 0.94 ± 0.54 ng/dl. C-peptide was not detected in the control, nontransplanted eye at any of the time points. (C) The fructosamine level showed a decrease from 204.80 ± 9.60 to 176.60 ± 5.60 μmol/l after transplantation. (D) HbA1C level dropped from 24.33 ± 1.16 to 22 ± 0.00 mmol/mol after transplantation, which was subsequently increased back to 25 mmol/mol at the end-point. (The values are mean ± SD.)

Intraocular Islet Transplantation Had No Impact on the Eye Anatomy in the Anterior or Posterior Segments

Consistent with our prior observations[11,12,15,16], the transplanted islets were visually confirmed to be engrafted and revascularized on the iris 1 month after transplantation. The graft was checked again by direct slit-lamp examination and optical coherence tomography imaging 3 and 6 months (before the iridectomy) after transplantation (Fig. 2A). There was a cicatrization of the iris near the edge of the pupil at the 6 o'clock position due to islet aggregation, but no synechia was formed. We performed a brief cage-side assessment for vision using a flashlight to the transplanted eye to check the behavioral response with no indication of affected vision. We did not observe any change in IOP in both eyes throughout the experiment (Table S1). There was no cataract formation, ophthalmitis, or retinal vessel deformation, and the retinal thickness profile was within the normal range in the transplanted eye. There was no apparent change in RNFL thickness (Table S1).

Glycemic Control Was Improved Following Intraocular Islet Transplantation in the Type-2 Diabetic Macaque

The mean fasting plasma glucose level at pre-transplantation (5.6 ± 0.1 mmol/l; $n = 96$ measurements) was significantly decreased ($P = 0.0001$; 4.8 ± 0.1 mmol/l; $n = 88$ measurements) post-transplantation. After the removal of the islet graft by iridectomy, it was significantly increased to 6.3 ± 0.1 mmol/l until the end of the experiment ($n = 144$ measurements; $P < 0.0001$; Fig. 3A). We also observed an increase in the frequency of lower plasma glucose values following transplantation (Fig. 4A–C). There was an evident left-shift in the frequency distribution curve to lower blood glucose levels (<5 mmol/l) after transplantation, and this was reversed by the removal of the graft (Fig. 4D).
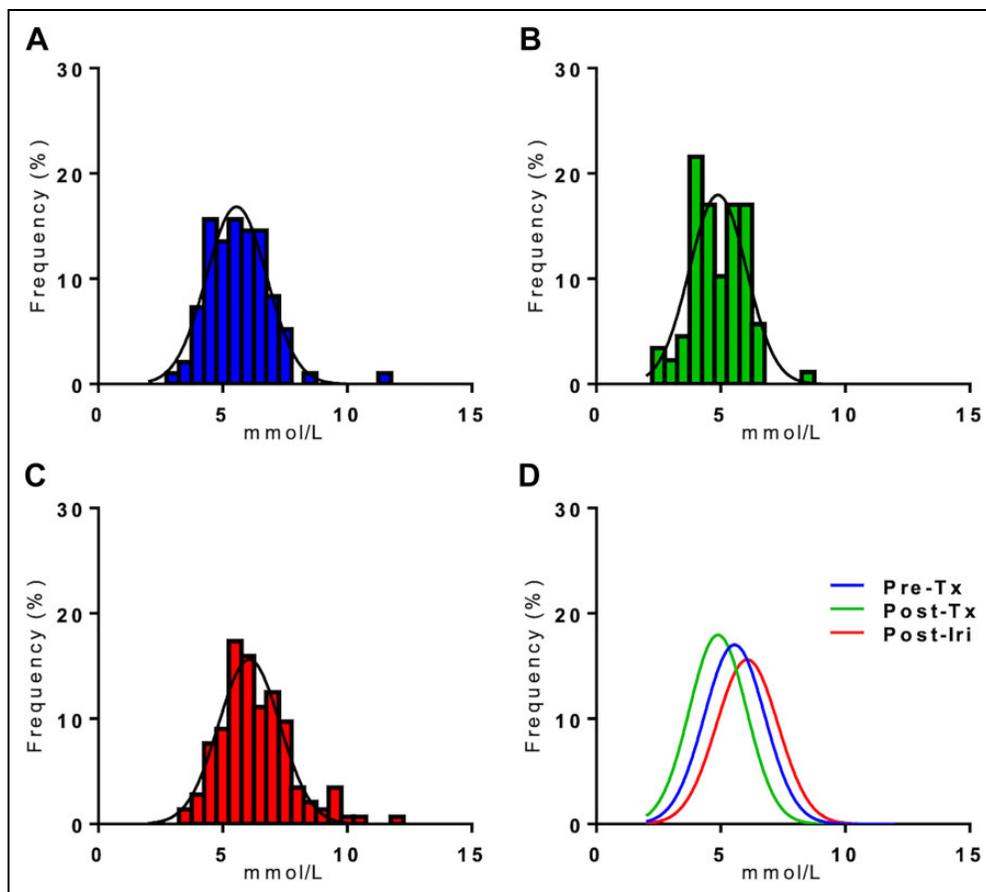
Figure 4. Frequency distribution of fasting plasma glucose level. (A–C) A higher frequency of lower plasma glucose levels occurred during the post-transplantation period compared to both pre-transplantation and post-iridectomy period. (D) There was a left shift in the frequency distribution curve after transplantation, which was reversed after the removal of the graft. (A: Pre-Transplantation period, B: Post-Transplantation period; C: Post-Iridectomy period; D: Frequency distribution curves)

C-peptide Levels Were Increased in the Aqueous Humor of the Eye Where Islets Were Transplanted

C-peptide was not detected in the aqueous humor of both eyes before transplantation. It was, however, significantly increased in the transplanted eye to 22.30 ± 3.07 ng/dl ($n = 3$ measurements; $P < 0.05$) while the graft was in place. Following iridectomy, the aqueous humor C-peptide in the transplanted eye was reduced to 0.94 ± 0.54 ng/dl ($n = 3$ measurements; Fig. 3B).

Long-Term Metabolic Parameters Showed Improved Type-2 Diabetes in the Recipient Macaque Following Intraocular Islet Transplantation

Fructosamine (glycosylated protein) level showed a decrease from 204.8 ± 9.60 μmol/l ($n = 3$ measurements) pre-transplantation to 176.6 ± 5.60 μmol/l ($n = 3$ measurements) post-transplantation. It subsequently increased to 183.9 ± 6.13 μmol/l ($n = 6$ measurements) following graft removal by iridectomy (Fig. 3C). We also observed a measurable reduction in HbA_{1C} (glycosylated hemoglobin)

levels from 24.33 ± 1.16 mmol/mol ($4.33\% \pm 0.07\%$, $n = 3$ measurements) pre-transplantation to 22 ± 0.0 mmol/mol ($4.2\% \pm 0.0\%$, $n = 3$ measurements) post-transplantation. At all time points during the post-transplantation period, HbA_{1C} remained at 22 mmol/mol (4.2%), which is the minimum detection range of the assay. After the graft removal by iridectomy, it eventually increased to 25 mmol/mol (4.4%) at the end-point of the experiment (Fig. 3D). The AUC of the blood glucose excursion during IVGTTs showed that there was an improvement of glucose tolerance after transplantation compared to before transplantation, and this was reversed after the iridectomy (Fig. 5).

Intraocular Islet Transplantation Associated with Improved Lipid and Liver Profiles in the Type-2 Diabetic Macaque

Total cholesterol (TC) was slightly reduced after transplantation from 3.53 ± 0.41 mmol/l ($n = 4$ measurements) to 3.27 ± 0.23 mmol/l post-transplantation ($n = 3$ measurements) and this was subsequently increased to 4.22 ± 0.43
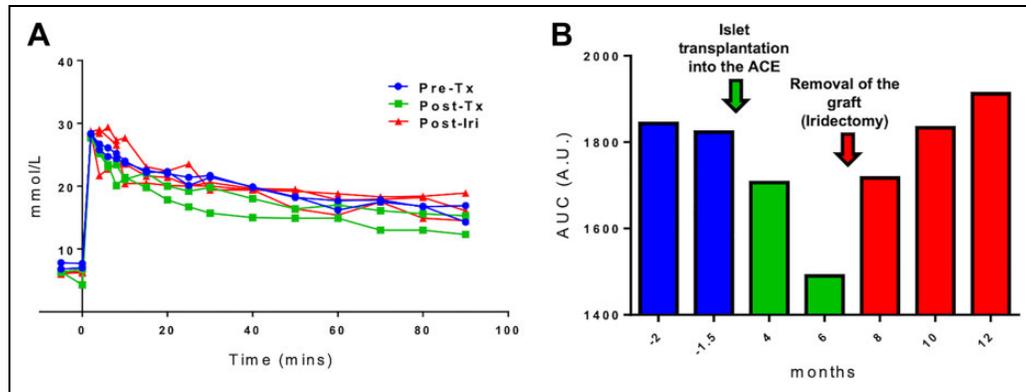
Figure 5. Intravenous glucose tolerance test (IVGTT). (A) IVGTT was performed by giving a 0.5 g/kg body weight bolus dose of glucose intravenously, and the plasma glucose was assessed at predetermined intervals up to 90 min after the glucose injection. (B) The area under the curve (AUC) showed improved glucose tolerance after islet transplantation, which was reversed by the removal of the graft.

Table 1. Lipids Profile and Liver Profile.

Parameters	Normal value[22,23]	Pre-transplant ($n = 4$)	Post-transplant ($n = 3$)	Post-iridectomy ($n = 6$)
Cholesterol (mmol/l)	2.15 $\pm$ 0.02	3.53 $\pm$ 0.41	3.27 $\pm$ 0.23	4.22 $\pm$ 0.43
HDL (mmol/l)	1.14 $\pm$ 0.02	1.36 $\pm$ 0.15	1.66 $\pm$ 0.24	1.90 $\pm$ 0.55
LDL (mmol/l)	0.85 $\pm$ 0.02	2.12 $\pm$ 0.27	1.41 $\pm$ 0.13	2.25 $\pm$ 0.34*
Triglycerides (mmol/l)	0.65 $\pm$ 0.02	1.61 $\pm$ 0.36	1.69 $\pm$ 0.80	1.17 $\pm$ 0.23
ALT (GPT) (IU/l)	48.58 $\pm$ 26.64	56.50 $\pm$ 10.75	48.67 $\pm$ 5.03	49.13 $\pm$ 36.02
AST (GOT) (IU/l)	42.62 $\pm$ 11.75	46.50 $\pm$ 9.95	39.67 $\pm$ 5.51	46.86 $\pm$ 19.70

The values are in mean $\pm$ SD. *, $P < 0.05$, compared with post-transplantation. ALT: alanine transaminase; GPT: glutamic-pyruvic transaminase: AST: aspartate transaminase; GOT: glutamic-oxaloacetic transaminase: LDL: low-density lipoprotein; HDL: high-density lipoprotein.

mmol/l ($n = 6$ measurements) after the removal of the graft by iridectomy. There was a small increase in plasma high-density lipoprotein (HDL) after transplantation, whereas low-density lipoprotein (LDL) was reduced by nearly 1 mmol/l while the graft was present. The plasma triglyceride levels did not show any noticeable difference after transplantation. There was no significant change in plasma alanine transaminase and aspartate transaminase, suggesting that liver damage did not occur at any stage of the procedure (Table 1).

Discussion

Islet transplantation in type-1 diabetes patients is a promising therapeutic approach to achieve glycemic control, insulin independence, and a higher quality of life[5–7,9]. One of the major limitations with islet transplantation as a clinical treatment strategy is the shortage of pancreatic donor islets and the survival of the islet graft. These are the challenges for not only type-1 diabetic patients but also insulinopenic type-2 diabetic patients where islet transplantation might be indicated. We have suggested that islet transplantation to the ACE (i.e., intraocular islet transplantation) may be a solution to these problems[12]. We now showed for the first time that transplantation of islets into the ACE of an early type-2

diabetic monkey improved the glycemic parameters and reverted the progression of diabetes.

Following the intraocular islet transplantation, the mean fasting blood glucose level in the type-2 diabetic macaque was significantly lower, and this persisted until the removal of the islet graft. Notably, this improvement in glycemic control was lost after the removal of the graft by iridectomy. In fact, removal of the graft resulted in even further deterioration in glycemic control compared to pre-transplantation, suggesting that the graft was preventing diabetes progression. Moreover, the macaque had an optimal blood glucose level at <5 mmol/l more frequently during the post-transplantation period (Fig. 4B). Together, these results showed that the intraocular islet graft was able to maintain glucose homeostasis. The positive outcome of islet transplantation in the ACE of this type-2 diabetic macaque was further corroborated by the improvements seen in its HbA$_{1C}$, fructosamine levels, and IVGTTs. These observed metabolic improvements were likely the result of insulin production by the intraocular islet graft, which was reflected by the substantial amount of C-peptide ($\sim$22 ng/ml) present in the aqueous humor. We did not observe a significant increase in the plasma/serum C-peptide levels after transplantation, likely due to a substantial dilution of the C-peptide coming from the intraocular islet graft. Interestingly, the observed

benefits by the intraocular islet graft were maintained for 3 months after withdrawing the immunosuppression, consistent with the previous findings of Abdulreda et al., showing that tissue-specific immune tolerance can be established with transient peri-transplantation immune intervention[20], although we did not examine immune tolerance here as it was beyond the scope of the present study. It is also noteworthy that there were no major ophthalmic complications associated with transplanting a relatively high islet mass (1,500 islet equivalents/kg) in this study.

Moreover, lipidemia is a common precursor for cardiovascular disease[24,25], and it has been shown that TC and LDL levels are inversely associated with beta cell function[26]. Our findings showed improved lipid profiles following intraocular islet transplantation in the recipient macaque with high-fat-diet-induced type-2 diabetes (Table 1). It is likely that the reduced TC and LDL levels following islet transplantation further helped in improving beta cell function during the post-transplantation period. Therefore, intraocular islet transplantation into patients with advanced type-2 diabetes may offer benefits directly relevant to glycemic control as well as diabetes-related complications.

In summary, the current preclinical findings in a macaque with type-2 diabetes together with prior evidence in other nonhuman primate models[12,20] supported the clinical application of pancreatic islet transplantation in the ACE as an alternative to intra-hepatic islet transplantation not only in type-1 diabetes patients but also in those with severe insulin-dependent type-2 diabetes.

Acknowledgments

Contribution Statement

P-OB was the originator of the idea transplanting pancreatic islets into the ACE of a type-2 diabetic monkey. SBBT, VAB, and P-OB designed the experiments. SBBT, MC, RH, and VAB conducted the experiments. SBBT, MK, LJ-B analyzed the results. SBBT and P-OB wrote the manuscript. XZ, YA, and MHA helped in the experimental design and edited the manuscript. All the authors commented upon, critically revised, and approved the submission of the manuscript.

P-OB is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data and Resource Availability

The data sets generated from the current study are available from the corresponding author upon request.

Ethical Approval

Ethical approval is not applicable for this article.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC), SingHealth, Singapore (2013/SHS/828).

Statement of Informed Consent

There are no human subjects in this article, and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: P-OB is a cofounder and CEO of Biocrine, a small biotech company that is using the ACE as a screening tool, and MK, MHA, and LJ-B are consultants for the same company.

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Prior Presentation

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Supplemental Material

Supplemental material for this article is available online.

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