Clinical intraocular islet transplantation is not a number issue

A. Shishido1,2, A. Caicedo1,4, R. Rodriguez-Diaz4, A. Pileggi1,2,3, P.-O. Berggren1,5, M. H. Abdulreda1,2

1Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL, USA
2Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, USA
3Departments of Microbiology and Immunology and Biomedical Engineering, University of Miami, Miami, FL, USA
4Department of Medicine, Division of Endocrinology, University of Miami Miller School of Medicine, Miami, FL, USA
5Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska Institutet, Stockholm, Sweden

Corresponding Author: Midhat H. Abdulreda, Ph.D; e-mail: mabdulreda@miami.edu
Per-Olof Berggren, Ph.D; e-mail: Per-Olof.Berggren@ki.se

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Abstract

It is now well established that beta cell replacement through pancreatic islet transplantation results in significant improvement in the quality-of-life of type 1 diabetes (T1D) patients. This is achieved through improved control and prevention of severe drops in blood sugar levels. Islet transplant therapy is on the verge of becoming standard-of-care in the USA. Yet, as with other established transplantation therapies, there remain hurdles to overcome to bring islet transplantation to full fruition as a long-lasting therapy of T1D. One of these hurdles is establishing reliable new sites, other than the liver, where durable efficacy and survival of transplanted islets can be achieved. In this article, we discuss the anterior chamber of the eye as a new site for clinical islet transplantation in the treatment of T1D. We specifically focus on the common conceptions, and preconceptions, on the requirements of islet mass, and whether or not the anterior chamber can accommodate sufficient islets to achieve meaningful efficacy and significant impact on hyperglycemia in clinical application.

Introduction

Recent results from the Clinical Islet Transplantation (CIT) Consortium trials showed that pancreatic islet transplantation in the liver improves blood sugar control significantly and reduces severe hypoglycemia episodes in type 1 diabetes (T1D) patients. This leads to significant improvement in the patients’ quality-of-life. However, it has also become evident that due to the immediate blood-mediated immune reaction (IBMIR) and other liver-related issues (e.g., hypoxia, highly enzymatic and inflammatory environment, high drug levels, etc.), the benefits of intrahepatic islet transplantation may be limited on the long-term.

Another concern, which is common to transplantation therapies in general, is about the serious and potentially life-threatening side effects associated with the required life-long systemic immunosuppression to avoid graft rejection. Clinical evidence also shows that long-term success of islet transplantation therapy may be hindered by recurrent autoimmunity. Therefore, to realize the full benefits of islet transplantation there remain two unmet critical needs to: 1) establish new islet transplantation sites with no added “strain” on the islet grafts as has been shown in the liver; and 2) achieve long-term efficacy and survival of islet grafts without the need for life-long systemic immunosuppression or its complications through induced graft immune tolerance.

New approaches to minimizing and/or eliminating immunosuppression are under investigation; however, such approaches must be paired with new transplantation sites to ensure better engraftment and long-term function of transplanted islets. Consequently, different new sites for islet transplantation including but not limited to the omentum, subcutaneous, intramuscular, and the bone marrow have been investigated.
tent with these efforts, we have been investigating the immune privileged anterior chamber of the eye as a potential site for clinical islet transplantation, where transplanted islets thrive during early engraftment due to high oxygen tension and can potentially survive long-term with minimal to no immunosuppression.

Our extensive studies have demonstrated the feasibility and efficacy of intraocular islet transplantation in preclinical models, and this has recently led to a significant first step in the clinical implementation of this novel approach to islet transplantation. We have obtained FDA approval (IND 017007) to conduct a pilot clinical trial to assess primarily the safety and secondarily the efficacy of pancreatic islet transplantation into the anterior chamber of the eye of legally blind T1D patients with a stable kidney transplant (i.e., already on immunosuppression). The purpose of this article is to discuss in the context of preclinical and clinical evidence whether the human eye anterior chamber is big enough to accommodate sufficient islet mass in clinical application.

MATERIALS AND METHODS

All animal procedures were performed under protocols approved by the University of Miami IA-CUC.

MICE

C57BL/6J (B6) and AKITA mice were purchased from Jackson Laboratories (JAX) and housed under the supervision of the University of Miami’s Department Veterinary Resources (DVR).

DIABETES INDUCTION

Acute induction of diabetes in B6 mice was achieved via single intravenous injection (150-220 mg/kg) of Streptozotocin (STZ). Frank diabetes was defined as 3 (three) consecutive readings of nonfasting glycemia ≥300 mg/dL. Heterozygous male AKITA mice developed severe hyperglycemia spontaneously during the first 3-6 weeks of life and no induction of diabetes was necessary.

ISLET ISOLATION AND TRANSPLANTATION INTO THE EYE ANTERIOR CHAMBER

Pancreatic islets were isolated from B6 donor mice as previously described. Islet transplantation into the eye anterior chamber of was performed as previously described in details.

GLYCEMIA AND BODY WEIGHT MONITORING

Glycemia was measured using portable glucometers (OneTouchUltra2; LifeScan, CA, USA) using a drop of blood from the tail vein.

RESULTS

Based on clinical and preclinical experience in islet transplantation in the liver and other sites, research investigators have gravitated towards transplanting large numbers of islets to restore euglycemia in animal models of diabetes. Interestingly, an islet dose of 5,000 IEQ/kg under the kidney capsule, which corresponds to ~100 islets in an average mouse weighting 20 g, has been widely accepted as sufficient islet mass to restore euglycemia in mouse models of islet transplantation. Moreover, since our introduction in 2008 of islet transplantation in the anterior chamber of the eye, we collected anecdotal evidence that even fewer islets may be sufficient to restore euglycemia in diabetic mice transplanted either under the kidney capsule or in the anterior chamber of the eye.

Therefore, to methodically and unequivocally address this issue we performed titration studies to assess the minimal islet mass required to restore euglycemia following intraocular islet transplantation (Figure 1A). We performed these studies in the streptozotocin (STZ)-induced diabetes C57BL/6 (B6) mouse model and in the spontaneously diabetic AKITA mouse. We transplanted 4-6 week old diabetic male AKITA mice in the anterior chamber of the eye with syngeneic (B6) 100 IEQ (~3,500 IEQ/kg at the onset of euglycemia post-transplant), and we monitored their nonfasting blood glucose levels and body weight longitudinally (Figure 1B). The median time to reverse diabetes (i.e., to achieve euglycemia; defined as 3 consecutive glycemia readings ≤200 mg/dL) following transplantation was 54 days; and 100% of the mice achieved stable euglycemia by 60 days post-transplant (Figure 1C). Notably, steady normalization of glycemia occurred despite a progressive and significant increase in the recipients’ body weight (dashed lines in Figure 1B). Similarly, we transplanted STZ-induced diabetic B6 mice with as few as B6 75 IEQ (i.e., ~2,500 IEQ/Kg) and as many as 500 IEQ (i.e., ~17,000 IEQ/Kg); the mice transplanted with 75 or 150 IEQ had a median diabetes reversal time of 77 and 58.5 days, respectively, compared to 26 days in those transplanted with 300 and 500 IEQ (Figure 1D).
Intraocular islet transplantation in T1D patients

Following transplantation in the liver. Yet, benefits observed following intrahepatic islet transplantation, such as restored hypoglycemia awareness, have been shown to be retained by transplanted patients long after getting back on insulin therapy due to graft failure and/or rejection. Notably, clinical evidence further indicates a lack of correlation between the number of transplanted islets and the clinical outcome. Thus, the number of transplanted islets needed to meaningfully impact on hyperglycemia varies significantly among patients, and may not necessarily be as large as...

**DISCUSSION**

Although extrapolation of preclinical findings, especially from small animals such as rodents, to the clinical setting is not straightforward, the above findings demonstrate that small numbers of islets are capable, albeit with delay, of achieving significant improvements in glycemic control following islet transplantation in the anterior chamber of the eye. This notion was further supported in our previous studies in the baboon. Moreover, clinical experience shows that a substantial amount of islets is lost due to IBMIR, inflammation, and apoptosis following transplantation in the liver. Yet, benefits observed following intrahepatic islet transplantation, such as restored hypoglycemia awareness, have been shown to be retained by transplanted patients long after getting back on insulin therapy due to graft failure and/or rejection. Notably, clinical evidence further indicates a lack of correlation between the number of transplanted islets and the clinical outcome. Thus, the number of transplanted islets needed to meaningfully impact on hyperglycemia varies significantly among patients, and may not necessarily be as large as.

**Figure 1.** Pancreatic islet transplantation in the eye anterior chamber. (A) Photo of AKITA mouse eye transplanted with 100 IEQ showing islets engrafted on top of the iris individually or in clusters. (B) Longitudinal glycemia (solid lines; left Y axis) and body weight (dashed lines; right Y axis) record of diabetic male AKITA mice (n=4) which were transplanted with 100 IEQ B6 islets. Euglycemia was defined as 3 consecutive blood glucose readings ≤200 mg/dL (dotted line). (C, D) Kaplan-Meyer curves summarizing the % normoglycemic mice (expressed as “diabetes reversal rate”) following transplantation in (C) AKITA (n=4) and (D) B6 recipients (n=2 for 75 IEQ and n=3 for 150, 300, and 500 IEQ groups).
commonly assumed. It is likely that the notion that a very large number of islets is needed has been reinforced by the mixed experience with intrahepatic islet transplantation, and it may not hold true in more islet-friendly transplantation sites.

Studies estimate the total number of islets in the human endocrine pancreas between 0.5-1 million islets31. Data from pancreatectomized patients also suggest that only 15-30% of the functional beta cell mass may be needed to maintain glucose homeostasis 32,33. Thus, the required number of islets to maintain euglycemia can be estimated at 125,000-250,000 islets; and in an average 80 kg human subject, this equates to ~1500-3000 IEQ/Kg. This is consistent with what may remain in the liver following a conservative 50% loss of infused islets due to IBMIR and initial inflammation/apoptosis; hence, an effective intrahepatic islet mass may be lower than 1,500-2,000 IEQ/kg with higher loss rates34,35. Importantly, an islet dose of 1,000-2,000 IEQ/kg in an average diabetic subject weighing 80 kg can be accommodated in the anterior chamber of one eye, which has an estimated volume of 200-300 µL in humans36,37. Thus, intraocular islet transplantation can be effective in conveying significant improvement in blood sugar control and prevention of severe hypoglycemia in T1D patients. Moreover, if safety is proven islets can also be transplanted into the second eye of the same individuals.

In summary, experimental evidence demonstrates the feasibility, safety, and efficacy of intraocular islet transplantation in preclinical models22,23,25,38-41. Importantly, the anticipated clinical trial will determine its safety profile and establish unequivocally if the anterior chamber of the human eye can host enough islets to sufficiently impact on hyperglycemia.

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**Conflict of interest**

Dr. Pileggi is currently employed at the National Institutes of Health (NIH). The opinions expressed in this article are the author’s own and do not necessarily reflect the views of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

P.-O.B. is cofounder and CEO of Biocrine AB, an unlisted biotech company that holds a patent on the intraocular transplantation technique and is using it as a research platform. M.H.A. is consultant of Biocrine AB.

**References**


