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Islet oxygen consumption rate (OCR) dose predicts insulin independence for first clinical islet allotransplants

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Abstract

Human islet allotransplant (ITx) for the treatment of type 1 diabetes is in phase III clinical registration trials in the US and standard of care in several other countries. Current islet product release criteria include viability based on cell membrane integrity stains, glucose stimulated insulin release (GSIR), and islet equivalent (IE) dose based on counts. However, only a fraction of patients transplanted with islets that meet or exceed these release criteria become insulin independent following one transplant. Measurements of islet oxygen consumption rate (OCR) have been reported as highly predictive of transplant outcome in many models. In this paper we report on the assessment of clinical islet allograft preparations using islet oxygen consumption rate (OCR) dose (or viable IE dose) and current product release assays in a series of 13 first transplant recipients. The predictive capability of each assay was examined and successful graft function was defined as 100% insulin independence within 45 days post-transplant. Results showed that OCR dose was most predictive of CTO. IE dose was also highly predictive, while GSIR and membrane integrity stains were not. In conclusion, OCR dose can predict CTO with high specificity and sensitivity and is a useful tool for evaluating islet preparations prior to clinical ITx.

Introduction

Islet cell allograft transplant (ITx) is a promising form of treatment for type 1 diabetics suffering from severe hypoglycemia [1–4]. Following the publication of the Edmonton Protocol[5], this therapy has been the subject of multiple clinical trials and is currently in phase III trials in the United States and standard of care in several other countries [6]. Prior

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to transplant, islet preparations are evaluated by several product release criteria assessments in order to ensure that recipients are receiving products that have a high potential of reversing diabetes and to satisfy federal regulation requirements. These assays commonly include islet dose based on the number of islet equivalents (IE) transplanted normalized to recipient body weight (IE/kg), fractional viability as measured by fluorescence-based membrane integrity stains, and glucose stimulated insulin release (GSIR) [7,8].

Islet dose has been reported to correlate with CTO in both allo- and autotransplant recipients [9–13]. This has allowed a cutoff for favorable outcome to be established in ITx, generally set at 5,000 IE/kg [14]. Fluorescence based membrane integrity stains have been purported to be inconsistent between dyes and very limited in their accuracy and prediction capabilities [15–17]. Nevertheless, they are still used as release criteria, with preparations reporting 70% viability as acceptable. Measurements of insulin release after introduction in basal and stimulated levels of glucose (GSIR) would hypothetically assess the functional capabilities of the islet preparation. Current standards call for an acceptable stimulation index (ratio of stimulated insulin release to basal) of 1. However, this method has its limitations as well and does not correlate well with CTO [8, 18–19]. Despite having these release criteria, <50% of recipients that receive products that meet or exceed the cut-offs become insulin independent (II) [13]. There is a need for a more reliable assay that can accurately predict clinical transplant outcome (CTO) for ITx recipients.

Oxygen consumption rate (OCR) is a real-time, operator-independent method of assessing fractional cell viability [8,15,20]. When normalized to the cellular DNA content (OCR/DNA), the assay has shown to correlate with transplant outcome in the nude mouse bioassay, in which distinct regions of function and non-function could be associated with OCR/DNA values and OCR dose (or OCRtx/kg recipient body weight, the product of islet dose and OCR/DNA) [21]. Furthermore, OCR dose has proven to be highly predictive of outcome in the clinical islet autotransplant model [22]. We hypothesized that OCR dose would be highly predictive of CTO in the clinical islet allotransplant model and set out to measure standard release criteria and OCR dose for a series of first transplant recipients.

Methods

Study design

Over a three month period at the University of Alberta, Canada, clinical islet isolations were performed on pancreata using defined standard of care or CIT protocols (n=30). Only those recipients in which the transplant was their first were included in the analysis (n=13) due to multiple confounding factors including possible function from previous transplants. Isolations that yielded a transplantable islet mass were further assessed for clinical suitability as well as OCR measurement.

Pancreas procurement, islet isolation, purification, and culture

Pancreas for islet donors were managed prior to harvest according to local hospital protocols, following aortic cross clamp the organ was flushed using cold organ preservation solution (HTK or UW) packaged in solution and ice and transported to our facility. Pancreas

dissociation was carried out as previously described [23] using a collagenase/thermolysin enzyme mixture and the resulting digest suspension was purified using a modified COBE 29991 cell processor with UW/Ficoll density gradients [24]. The purified islet fraction(s) were assessed for clinical suitability and cultured in CMRL based medium at 22°C (5% CO₂) for up to 72 hrs.

Islet product characterization

Following the defined culture period, islets were assessed using the standard release criteria techniques and OCR. Islet dose was calculated using standard methods [7,25]. Briefly, islets >50µm in diameter were enumerated by manual count with an inverted light microscope and classified into size ranges in increments of 50 µm. The number of islets in each size range was converted to IE to account for size difference. The total IE was then divided by body weight of the recipient on the date of hospital admission (IE/kg).

Membrane integrity staining was performed by sampling a small aliquot of islets from the final product and stained either SYTO Green/ Ethidium Bromide (n=10) or Fluorescein Diacetate/ Propidium Iodide (n=3). Samples were manually assessed using fluorescent microscopy and reported as a percentage of viable to dead cells.

Glucose stimulated insulin release (GSIR) is not a release criteria at the University of Alberta; however, values were available for these isolations. The assay was performed by introducing an aliquot of islets in triplicate to a low glucose solution (2.7mM) and subsequently a high glucose solution (25.0mM). The amount of insulin released was measured using an ELISA (Mercodia Insulin ELISA, Mercodia, Uppsala, SE) and the ratio of insulin released from high glucose over low glucose was reported as the stimulation index.

OCR was performed as described previously [20]. Briefly, a small aliquot of islets (3,000 IE) was split into triplicate samples and introduced into pre-calibrated, water-jacketed, titanium chambers outfitted with fiber optic patches (175µl FOL oxygen monitoring system, Instech Laboratories Inc., Plymouth Meeting, PA, USA). OCR was normalized to the DNA content per chamber by collecting the islets and assessing for DNA by using a dsDNA fluorescent dye (Quant-iTPicoGreendsDNA Assay Kit, Invitrogen, Life Technologies Corporation, Grand Island, NY, USA) resulting in OCR/DNA (nmol O₂/min•mg DNA). To calculate OCR dose(nmol O₂/min•kg), the OCR/DNA was multiplied by the IE dose normalized to recipient body weight as indicated below:

$$\frac{\text{nmol O}_2}{\text{min} \cdot \text{mg DNA}} \times \frac{10.4 \text{ ng DNA}}{\text{IE}} \times \frac{\text{mg}}{1 \times 10^6 \text{ ng}} \times \frac{\text{IEtx}}{\text{kg}}$$

Islet allotransplantation

Immunosuppressive induction therapy was dictated by the specific protocol requirements, n=10 recipients received induction with alemtuzumab, anakinra and etanercept whereas n=3 recipients received thymoglobulin and etanercept. Maintenance immunosuppression for all

protocols was tacrolimus and mycophenolatemofetil with n=3 also remaining on previous immunosuppression regimens from their kidney transplant.

Prior to transporting islets to the transplant site, the islets were loaded into a Ricordi Infusion Bag (Biorep Tech, Miami, FL, USA) suspended in 100mL of CMRL based transplant media supplemented with HSA and HEPES buffer. The islets were infused following catheter placement in the portal vein, performed under local anesthetic in radiology using transhepatic approach. Track ablation with avitene paste was done following the procedure. Peritransplant management of patients included insulin-heparin infusions as per Koh et al [26].

Patient follow-up

Following transplant, insulin requirements were managed in clinic as required. Successful short-term transplant was defined as having complete, 100% insulin independence (II) within 45 days.

Statistical Techniques

Receiver operating characteristic (ROC) curve analysis was used to examine the relationship between CTO and islet product characterization assessments. The area under the ROC curve (AUC) was calculated for each method. Statistical analyses were completed using SAS statistical software package (v9.3, SAS institute Inc., Cary, NC, USA) or GraphPad Prism (v5.03, GraphPad Software Inc., La Jolla, CA, USA).

Results

All 13 transplanted preparations met, or exceeded, current product release criteria. However, only 38% of transplanted recipients were insulin independent within 45 days. One patient required a second transplant after 59 days. No complications were observed from immunosuppression therapy. Comparing patients which achieved II to patients who remained insulin dependent showed no statistical significance at $\alpha=0.05$ when assessing products using GSIR (table 1, $p=0.09$) and membrane integrity stains ($p=0.46$), while IE and OCR dose showed a high statistical significance ($p<0.001$).

ROC analysis showed that GSIR and membrane integrity stains were not predictive of CTO (table 1, AUC: 0.83, 0.63 respectively). IE dose was highly predictive (AUC: 0.98) while OCR dose was most predictive of CTO (AUC: 1.00), with perfect separation between OCR doses that resulted in II and those that did not.

Discussion

In order to be compliant with FDA regulations and to have a better understanding of the quality of islets being transplanted, it is essential to have real-time, *in vitro* characterization assays that can accurately predict CTO. We chose to apply the viable islet dose, a combination of the number of islets transplanted and the mitochondrial function as measured by OCR, to a series of first islet allograft transplants.

Two of the standard release assays measured in the series, membrane integrity staining and GSIR, were not predictive of CTO, which is in support of previous publications [8,18–19]. Interestingly, preparations in which the GSIR stimulation index was higher (higher ratio of stimulated insulin release to basal insulin release) were less likely to reverse diabetes.

All preparations exceeded the 5,000 IE/kg limit, established as the minimum required dose to achieve II [14]. This method of CTO prediction is lacking however, as it doesn't take into account islet viability. If a patient were to receive 10,000 IE/kg of dead islets the probability of reversal would be near impossible. If a patient were to receive 5,000 IE/kg of healthy islets, the probability of II would be much greater. It is imperative that there is a reliable assay to make this distinction as recent reports with sensitive assays have shown a large variation in islet viability [8,16–17]. In this series, IE dose was highly predictive of CTO for high IE doses, but was less so for cases in which a marginal IE dose was transplanted (5,000–7,000 IE/kg). OCR dose was able to correctly predict CTO even when marginal IE doses were transplanted with perfect separation between OCR doses that resulted in II and those that did not for the 13 clinical cases reported in this manuscript.

We conclude that OCR dose normalized to recipient body weight may be a useful tool for evaluating islet preparations and can aid in accurately predicting CTO, especially when a marginal IE dose is transplanted.

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Table 1

Data from n=13 first transplant recipient short-term (<45 days) follow-ups illustrating mean values $\pm$ standard deviation for characterization methods and the corresponding clinical transplant outcomes (CTO; insulin dependent vs insulin independent). Area under the receiver operating characteristics (ROC) curve analysis is also shown and statistical significance was measured with a Student's t-test. Glucose stimulated insulin release (GSIR) and membrane integrity stains were not correlated with CTO while islet equivalent (IE) dose and oxygen consumption rate (OCR) dose were highly correlated.

	Characterization Assay			
	<i>GSIR (stimulation index)</i>	<i>Membrane integrity (% viable)</i>	<i>IE dose (IEtx/kg BW)</i>	<i>OCR dose (nmol/min•kg BW)</i>
Insulin dependent (n=8)	2.2 $\pm$ 1.1	87.4 $\pm$ 6.6	5,452 $\pm$ 560	5.9 $\pm$ 1.0
Insulin independent (n=5)	1.4 $\pm$ 0.6	87.8 $\pm$ 3.2	7,266 $\pm$ 911	10.0 $\pm$ 2.5
<i>p</i>-value	0.09	0.46	<0.001	<0.001
Area under ROC curve	0.83 ^{\$}	0.63	0.98	1.00

^{\$} Note: the area under the ROC curve for GSIR is a paradox, as higher values were seen for insulin dependent patients.