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Prediction of Marginal Mass Required for Successful Islet Transplantation

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Abstract

Islet quality assessment methods for predicting diabetes reversal (DR) following transplantation are needed. We investigated two islet parameters, oxygen consumption rate (OCR) and OCR per DNA content, to predict transplantation outcome and explored the impact of islet quality on marginal islet mass for DR. Outcomes in immunosuppressed diabetic mice were evaluated by transplanting mixtures of healthy and purposely damaged rat islets for systematic variation of OCR/DNA over a wide range. The probability of DR increased with increasing transplanted OCR and OCR/DNA. On coordinates of OCR versus OCR/DNA, data fell into regions in which DR occurred in all, some, or none of the animals with a sharp threshold of around 150-nmol/min mg DNA. A model incorporating both parameters predicted transplantation outcome with sensitivity and specificity of 93% and 94%, respectively. Marginal mass was not constant, depended on

OCR/DNA, and increased from 2,800 to over 100,000 islet equivalents/kg body weight as OCR/DNA decreased. We conclude that measurements of OCR and OCR/DNA are useful for predicting transplantation outcome in this model system, and OCR/DNA can be used to estimate the marginal mass required for reversing diabetes. Because human clinical islet preparations in a previous study had OCR/DNA values in the range of 100–150-nmol/min mg DNA, our findings suggest that substantial improvement in transplantation outcome may accompany increased OCR/DNA in clinical islet preparations.

Keywords

marginal graft; oxygen consumption rate; transplantation outcome; predictive assay; mouse bioassay; rat islets

INTRODUCTION

The success of islet transplantation for treating diabetes [1–6] depends on the quality of islets, and meaningful and standardized methods for islet assessment are needed [7, 8]. The multicellular nature of islets complicates use of most assays employed with single cells. Because pancreata are procured from brain dead donors [9–11], exposed to ischemia [12–14], and subjected to stressful mechanical and enzymatic procedures during isolation [4], organ donor characteristics alone do not necessarily predict isolated islet quality. Recent studies to develop methods for prospectively predicting transplantation outcome [15–22] have focused on mitochondrial function, which provides an early indication of entry into the cell death process [23]. These assays include concentration of reactive oxygen species, mitochondrial membrane potential, ADP/ATP ratio, oxygen consumption rate (OCR), and related indices [15–22]. Diabetes reversal (DR) by islet transplantation under the kidney capsule of mouse is a benchmark for validating islet potency tests [15–21] that correlate with clinical outcome but can only be used retrospectively.

We previously used the mouse bioassay to study the use of OCR and OCR/DNA to predict DR with high purity human islet preparations [20]. Here, we examine in more detail the relationship between these parameters and transplantation outcome, especially the impact of islet quality on marginal islet mass required for DR. In previous studies [e.g., 24, 25], marginal mass has been assumed to be a constant but with different values between studies. By using systematically prepared mixtures of healthy and purposely damaged rat islets to achieve a large range of OCR/DNA values, we demonstrate here that marginal mass is not constant and varies over a wide range as islet quality changes. These novel observations indicate that OCR/DNA is a useful islet quality parameter for predicting marginal islet mass for DR.

MATERIALS AND METHODS

Islet isolation and culture

Islets were isolated from male Sprague-Dawley rats, 10–12 weeks old, by collagenase digestion/ficoll purification [26] and cultured as described previously [27]. For the isolation procedure, the animals were given an overdose of Nembutal for the anesthesia and sacrifice. The islets were isolated under the Joslin Clinic IACUC protocol 99–01 as presented ahead. The procedure was done under aseptic techniques.

Islet samples for transplantation

Seven rat islet preparations were isolated at the Joslin Diabetes Center. Three preparations (1A-3A) were transplanted as received. To systematically investigate a wider range of OCR/DNA values, four of the preparations were divided in two. One portion was cultured under normal conditions. The other portion was cultured under stressful conditions to damage the islets: Islets were placed in 15-ml centrifuge tubes containing 15-ml medium and the tubes were sealed, islets settled to form a loose pellet overlain by 11 cm of medium, and were cultured for 18 hr at 37°C. OCR/DNA of islets in pellet cultures decreased to 10–25% of their initial value. Mixtures having intermediate values of OCR/DNA were obtained by mixing islets from each portion in different proportions. Samples having various OCR/DNA values and OCR doses were prepared and transferred to Massachusetts General Hospital (MGH) for immediate transplantation into immunosuppressed mice. Use of these mixtures allowed the preparation of islet samples from a single preparation in which the OCR/DNA of each sample was independently varied. Properties of samples are summarized in Table 1. The relationship between the number of islet equivalents (IE, the volume of a sphere with a diameter of 150µm), OCR/DNA, and OCR dose of a sample is given by

$$IE = \frac{OCR}{N_{IE} DNA_c \left(\frac{OCR}{DNA} \right)}, \quad (1)$$

where $N_{IE} = 1,560$ cells/IE is the average number of cells in an IE, and $DNA_c = 6.4$ pg/cell is the DNA content of a single rat islet cell [23].

OCR

OCR and DNA measurements were performed at MIT 4 hr after isolation and again after culture. OCR and DNA were measured as described previously [27]. Measurements were made with three islet samples, each containing about 500 islet equivalents, which provided a coefficient of variation of approximately 10%. Each measurement took 20–50 min.

Mouse bioassay

All procedures employing laboratory animals were approved by the Institutional Animal Care and Use Committee at MGH (accredited by the American Association for the Accreditation of Laboratory Animal Care).

Four week old female Balb/C mice (Jackson Laboratory, Bar Harbor, ME), were rendered diabetic with a single dose of 275 mg/kg streptozotocin. Mice with non-fasting blood glucose concentrations greater than 350 mg/dl for at least three consecutive measurements were used as transplant recipients.

Islet transplants

Recipients were anesthetized by i.p. ketamine (100 mg/kg) / xylazine (10 mg/kg); level of anesthesia was determined by response to toe-pinch. If necessary, anesthesia was maintained during the transplant procedure by isoflurane (1.5%) delivered by a precision vaporizer. The operative site was prepared using standard sterile surgical procedures, and a small incision was made in the animal's left flank. The kidney was carefully exposed and islets injected into the kidney capsule. After the kidney was returned to position, the wound was closed with wound clips. Before waking, mice prophylactically received buprenorphine (0.05–0.10 mg/kg) for analgesia. Buprenorphine (0.05 mg/kg SQ) was administered as needed during the immediate postoperative period.

$$\text{DR probability} = 1 - \frac{1}{1 + \exp[a + b(\text{OCR}) + c(\text{OCR}/\text{DNA}) + d(\text{OCR})(\text{OCR}/\text{DNA})]}, \quad (2)$$

A small quantity of blood was collected from the tail vein and glucose concentration measured every 1–2 days for the first two weeks after transplantation, and every 2–3 days thereafter (Accu-Check blood glucose monitor, Roche, Indianapolis, IN). Successful DR was defined as blood glucose concentrations less than 200 mg/dl for a minimum of seven consecutive days. If mice lost greater than 10% of their pre-surgical weight or the surgical site appeared purulent (showing signs of infection), the animal was immediately euthanized.

At the conclusion of the study, mice were euthanized by CO₂ inhalation, consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia.

Statistical analysis

Data analysis was performed using JMP Statistical Discovery Software (SAS Institute, Cary, NC). DR outcomes were fitted using a logistic regression model of the form where a , b , c , and d are regression coefficients. The model was then used to predict outcome as a function of OCR and OCR/DNA using a probability cutoff point of 0.5. Model predictions were compared to observed outcomes to generate sensitivity and specificity values.

Sensitivity is the probability that a noncured mouse is predicted by the model not to cure, and specificity is the probability that a cured mouse is predicted by the model to cure.

RESULTS

Results of the transplantations are summarized in Figure 1, where the ordinate is OCR and the abscissa is OCR/DNA of the transplanted islet sample. The data are segregated into three regions: (1) the upper and right-most portion, where diabetes was reversed in all animals, (2) the lower left, where diabetes was not reversed in any animal, and (3) a narrow intermediate region in which all three categories were represented. The data demonstrated the interplay between OCR and OCR/DNA of the transplanted rat islets in correlating DR in mice and demarcated a relatively sharp boundary between complete success and complete or partial failure in reversing diabetes. This boundary was nearly vertical at OCR/DNA of about 150-nmol/min mg DNA. At higher OCR/DNA, the boundary sloped slightly downward to the right. The overall pattern exhibited a strong correlation between transplantation outcome in immunosuppressed mice and the OCR and OCR/DNA properties of an islet preparation.

The lowest values of OCR that produced 100% DR defined a marginal islet OCR requirement (analogous to marginal islet mass), the magnitude of which depended on OCR/DNA. For example, at OCR/DNA = 401-nmol/min mg DNA, DR occurred at an OCR dose as low as 0.26 nmol/min (Figure 1), which corresponded to a marginal islet mass of 65 IE (Table 1) or 2,600 IE/kg body weight for a 25-g mouse. The marginal OCR dose was similarly determined for each value of OCR/DNA investigated, and the corresponding marginal mass was obtained from Table 1. Marginal mass was also evaluated for each condition, for which the next lowest OCR dose produced less than 100% cure, and both are plotted in Figure 2. As OCR/DNA decreased from 401 to 120-nmol/min mg DNA, marginal mass increased steeply from about 2,600 IE/kg to more than 100,000 IE/kg body weight. The scatter of data reflects the discrete values of OCR dose tested and the low incidence of failures at low OCR dose. For OCR/DNA less than 120-nmol/mm mg DNA, DR could not be obtained, even with very large numbers of islets.

In the previous study [20], high-purity human islets were transplanted into immunodeficient mice using a protocol similar to that used in this study. The response to transplantation has been plotted in Figure 3 in the same manner as in Figure 1 to allow comparison with data from this study. OCR/DNA varied from 87 to 206-nmol/min mg DNA, a much smaller range than employed here with rat islets but nonetheless sufficient to display the different regions of response. Data for human islets in which all transplants were cured or all failed did not segregate as cleanly as with rat islets. In addition, marginal OCR was substantially higher for human islets. For example, at OCR/DNA = 206-nmol/min mg DNA, the required marginal OCR was 2.5 nmol/min. Assuming that islets accounted for about 70% of the cells in the sample [23, 30] and that OCR/cell was about the same for islet and non-islet tissue [23], the marginal mass was about 700 IE or approximately 28,000 IE/kg. This higher requirement for human islets compared to rat islets may reflect a reduced sensitivity of mice to human insulin as compared to rat insulin. Mixed behavior of blood glucose response occurred over roughly the same region of OCR/DNA values for the rat and human islet transplants, and the boundary for marginal OCR became nearly vertical at OCR/DNA = 150-nmol/min mg DNA for both cases. At higher OCR/DNA, the boundary of marginal OCR for human islets would likely slope downward to the right, as was observed with rat islets (Figure 1), so that lower OCR would likely suffice at OCR/DNA values higher than the values that were investigated with human islets [20].

Logistic regression methods were used to fit the data in Figure 1 and predict the probability of DR as a function of OCR and OCR/DNA, as shown in the form of contours of constant probability in Figure 4. Sensitivity and specificity analyses on OCR and OCR/DNA exhibited values of 93% and 94%, respectively, in predicting DR with rat islets transplanted in immunosuppressed mice ($n = 74$). The analysis identified a region in which the probability of DR is 100%. By the use of a model similar to Equation (2) but without the last term in the denominator, sensitivity and specificity analyses of the data in Figure 3 exhibited values of 95% and 71%, respectively [20]. The lower specificity is consistent with the wider range of mixed DR behavior observed with human islets [20] than with rat islets in this study.

DISCUSSION

There is a need for reliable assays to assess quality of islet preparations prior to transplantation. Such assays would prevent transplantation of poor quality islet preparations that would fail to reverse diabetes and could be used to improve methods for the preservation of pancreas and islet processing, culture, and shipping. In this study we investigated the ability of two easily measured islet parameters, OCR and OCR/DNA, to predict DR following transplantation of rat islets into immunosuppressed mice. We selected rat islets because they enabled investigations at higher OCR/DNA than previously obtained with human islets [20]. Furthermore, rat insulin is thought to be more readily recognized than human insulin by mice; consequently, smaller volumes of viable rat islet tissue were required for DR.

When plotted on coordinates of OCR and OCR/DNA, transplantation outcome data segregated into regions where all transplants either did or did not reverse diabetes with only a small region of overlap in which all categories of response occurred (Figure 1). Similar threshold behavior in blood glucose control has been described with encapsulated islets [31] and with the loss of beta cell mass in diabetes [32]. As a result of this sharp threshold behavior, OCR and OCR/DNA combine to predict the outcome of rat islet transplantation into immunosuppressed mice (Figure 4) with very high sensitivity and specificity. The only comparable study reported with highly pure islet preparations used measurements of the ADP/ATP ratio to correlate the outcome of transplantations of porcine islets into immunodeficient mice [16]. Sensitivity and specificity of the single-parameter assay, 86%

and 72% respectively, were substantially lower than the values obtained in this study with OCR measurements.

The marginal OCR required to reverse diabetes depended on OCR/DNA. For the preparation of known OCR/DNA, the required OCR was converted to N_{IE} by using reported values for DNA per cell and the number of cells per IE [23, 30] in Equation (1). Although the marginal islet mass is conventionally treated as a fixed number of IE, our results (Figure 2) showed that it depends strongly on the quality of the islet preparation as assessed by OCR/DNA.

In this study an islet preparation of known OCR/DNA was divided; one portion was cultured under stressful conditions to damage the tissue and reduce OCR/DNA, and that portion was mixed with the original preparation in different proportions to generate samples with different values of OCR/DNA. The ratio of OCR/DNA for one of these samples to that of the original preparation is the relative viability of the sample as a fraction of the viability of the original preparation. If islet samples are constructed so that OCR is maintained constant, decreasing values of OCR/DNA reflect a constant amount of viable tissue and increasing amounts of nonviable tissue. If OCR/DNA is held constant and OCR is increased, then successive samples have increasing amounts of viable tissue along with nonviable tissue in constant proportion. In this context, OCR may be viewed as the dose of viable tissue.

Use of OCR and OCR/DNA measurements with high purity human islets transplanted into immunodeficient mice [20] provided a better correlation with transplantation outcome (Figure 3) than that reported in other studies with human islets [17–19], although precise comparison was not possible because sensitivity and specificity were not evaluated. Correlation of transplantation outcome with rat islets was substantially better than with human islet preparations, perhaps because of the absence of non-islet tissue in rat preparations or because the immunosuppressed mice may respond differently from the immunodeficient mice to the large fraction of nonviable tissue at low OCR/DNA. The predicted probabilities of DR with rat islet transplants were sharply defined (Figure 4) with a large domain at 100% cure, whereas the analogous plot for human islet transplants (Figure 3 in Reference [20]) had angled contours of roughly constant slope with virtually no domain of 100% cure, although such a domain might have been attainable if there had been preparations of higher OCR/DNA. The absence of data in the high OCR/DNA range was a limitation of the previous study with human islets [20].

The observations with high OCR/DNA values made with rat islets in the current study are novel and important because they demonstrate that DR is achievable with as little as about 2600-IE/kg body weight and marginal islet mass increases dramatically with decreasing OCR/DNA. Furthermore, even small increases in OCR/DNA can lead to substantial reductions in marginal mass and improvements in transplantation outcomes. In the previous study [20], human clinical islet preparations had OCR/DNA values in the range of only 100–150-nmol/min mg DNA, which is at or below the threshold value of 150-nmol/min mg DNA observed in this study. If a similar threshold value is applicable to human islet transplants, as suggested by the data in Figure 3, then modest increases in the quality of islet preparations, as measured by OCR/DNA, may substantially improve success obtained with clinical islet transplantation.

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Figure 1.

Response to rat islet transplants ($n = 74$) in diabetic immunosuppressed Balb/C mice. Each symbol represents transplantations with samples having the same OCR and OCR/DNA in different animals. Numbers represent the number of animals in each response category of blood glucose concentration: (A) normalized within several days, remained below 100 mg/dl, (B) normalized slower, remained in the range of 100–200 mg/dl, and (C) never decreased below 200 mg/dl or did so for only few days. The type of symbol represents the fraction of transplantations that resulted in DR.

Figure 2.

Dependence of normalized marginal islet mass on OCR/DNA. Filled circles correspond to the lowest OCR dose that produced DR in 100% of samples. Open circles correspond to the next lowest OCR dose (at which less than 100% of the sample cured). The straight line portion of the curve was determined by least squares regression of all data for which OCR/DNA $\geq$ 146-nmol/min mg DNA.

Figure 3.

Response to highly purified (85% or more by dithizone staining) human islet transplants in immunodeficient diabetic mice [20]. Symbol format is similar to Figure 1, However, in Figure 3 only two outcome categories are presented (cure / no cure) corresponding to categories (A / C) in Figure 1. Freshly isolated islet preparations (isolated at the University of Minnesota) were cultured for 1 day at 37°C and 1 day at 24°C before assessment and transplantation (these 4 preparations had values of OCR/DNA of 87, 109, 142 and 168 nmol/min-mgDNA). All other preparations were assessed and transplanted following shipment to the University of Minnesota from various Islet Cell Resource Centers within the United States. Diabetes reversal (cure) was defined as blood glucose concentration less than 200 mg/dl for at least 8 out of 10 consecutive days.

Figure 4.

Contours of probability of diabetes reversal in the immunosuppressed mouse as a function of transplanted rat islet OCR and OCR/DNA. Fitted regression coefficients in the logistic regression model, from which the contours were generated, were based on outcomes from all transplants (Figure 1). The form of the model equation is given by Equation (2) in the text.

Table 1

Properties of islet samples transplanted into mice

Sample		OCR dose (nmol/min)				
		0.26	0.52	1.3	2.6	5.2
Number ^a	Type ^b	OCR/DNA Nmol/min mg DNA		Islet equivalents		
7B	Damaged	56		2,325	4650	
6C	Damaged	60	434	868	2,170	
5E	Damaged	64		814	2,035	
6B	Mixture	110	237	473		
5D	Mixture	111		469		
1A	Original	120	434	1,085	2,170	4,340
4B	Damaged	146		892	1,784	
5C	Mixture	216	241	603		
2A	Original	257	203	507	1,013	2,027
5B	Mixture	280	186			
6A	Original	340	77	153	383	
4A	Original	364		358	715	
3A	Original	371	140	351	702	1,404
7A	Original	400		326		
5A	Original	401	65	130		

^a Islet preparations were numbered in chronological order as received.

^b Original refers to the islet preparation as received. Damaged refers to islets in pellet culture. Mixture refers to mixtures of islets from the normal and pellet culture to produce the tabulated OCR/DNA values. From the resulting islet suspensions, aliquots were taken to produce samples having the designated levels of OCR.