Luminescence technology in preservation and transplantation for rat islet

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Key words: firefly rat, islet, preservation solution, transplantation, diabetic mice

Abbreviations: Luc-Tg, luciferase-transgenic; STZ, streptozotocin; IVIS, in vivo imaging system; POD, post-operation days; PDX-1, pancreatic and duodenal homeobox protein-1; UW, university of wisconsin solution; ET-K, ET-kyoto solution; EC, euro-collins; HTK, histidin-tryptophan-ketoglutarate solution; LPDS, low-potassium dextran glucose solution

The development of organ preservation solutions and associated technology has been a major effort in tissue transplantation recently. However, this research takes a great deal of time and resources. In this study, a novel method for the evaluation of preservation solutions was established by using islet cells. Primary islets were obtained by hand-picking method from the luciferase transgenic (Luc-Tg) rat pancreas. The viability rate and living condition of islets preserved with several solutions were evaluated by relative photon intensity. Preserved islets were transplanted to the renal capsule of streptozotocin (STZ)-induced type 1 diabetic NOD-scid mouse, and the intraperitoneal glucose tolerance test (IPGTT) and histology were analyzed. The Luc-Tg rat islet viability was increased in a relative photon intensity-dependent manner. In the recipients of ET-Kyoto (ET-K) or University of Wisconsin (UW) solution preserved Luc-Tg rat islet at 1 day, hyperglycemia induced by glucose injection declined to the normal range. In conclusion, this study demonstrates that the ET-K preservation method allowed tissue ATP synthesis and amelioration of cold ischemic tissues damage during extended 24 h isolated-islet preservation. This simple method will be adapted easily to the clinical setting and used to maximize the utilization of islet transplantation as well as for pancreas sharing with remote centers.

Introduction

The introduction of cyclosporine to clinical transplantation in the early 1980s has dramatically improved transplantation results for experimental organs such as the liver, pancreas and heart, and more recently, for the lung. Additionally, the development of the organ transplantation preservation solutions supported tissue transplant therapy that was a major medical revolution of the 20th century. Organs removed from donors must be preserved to suppress deterioration of the organ until the transplant is completed.

The implantation of functional pancreatic islet cells is a potential cure for diabetes, but the availability of high quality islets for transplantation is critical for success.1–9 Islet transplantation can control blood sugar level effectively, reduce the complication of diabetes mellitus and prevent hypoglycemia and insulin resistance caused by ectogenic insulin.10 Thus, islet transplantation is an effective means for the treatment of type 1 diabetes mellitus. The ability to maintain islets of Langerhans in tissue preservation solutions provides a method of storage for islets after isolation from the pancreas but before clinical transplantation. However, one major disadvantage of islet culture is the loss of tissue mass that occurs over time.11 Technical and immunological challenges, however, remain prior to larger scale, cost-effective application of islet transplantation. Technical challenges remain, including a limited human islet supply from cadavers, a low islet yield and quality associated with donor brain death, and long cold-ischemia times during organ procurement, storage and transportation.12–15 Thus, development of preservation solutions is important for future breakthrough to address these problems.

Glucose-sensing and insulin-signaling pathways have been shown to play important roles in insulin secretion as well as β-cell growth and survival. Glucose plays an essential role in control of the secretory activity of β-cells. Metabolism of glucose leads to an increase in the ATP-to-ADP ratio, membrane depolarization, Ca2+ influx and stimulation of insulin secretion.16 Recently, the luciferase based viability assay described here utilizes firefly luciferase to detect intracellular ATP levels in viable cells. We have already established that the luciferase-transgenic
rat (Luc-Tg rat) system with modern optical imaging offers a new platform for a better understanding of transplantation.\textsuperscript{17}

STZ has been used as a reagent, which induced diabetes in rodents by functioning as a β-cell toxin.\textsuperscript{18} Although the effect of STZ on β-cells seems to be mediated by nitric oxide (NO), it is generally accepted that the mechanism for STZ toxicity is via alkylation, DNA damage and poly-ADP ribose polymerase (PARP) activation.\textsuperscript{19–21}

Here we report the development of a multi-well whole cell Luciferase based viability assay for the detection of the viability of islets. Importantly, transplantation of preserved islets improved pancreas function in this diabetic mouse model using STZ.

**Results**

Detection of luciferase assay signal from Luc-Tg rat’s islets. Islets were isolated from Luc-Tg rat pancreas at 382.5 ± 34.27 IEQ (n = 12, body weight: 371.5 ± 33.27 g). Isolated islets were observed in culture with luminescence using the in vivo imaging system (Fig. 1). To determine the viability of Luc-Tg rat islets, cells were exposed to luciferin-containing medium at various relative photon intensities and stained with trypan blue solution after the measurement of photon intensity. Figure 2 shows that the Luc-Tg rat islet viability was increased in a relative photon intensity-dependent manner (R = 0.95). These results show that the amount of relative photons reflects the viability rate of the Luc-Tg rat islets.

Effect of preservation solution on islet viability. Luc-Tg rat islets preserved in a variety of preservation solutions were evaluated for viability by measuring the relative photon intensity (Table 1). The effect of preservation for Luc-Tg rat islets was divided into two groups—an effective group (ET-K, UW and HTK) and non-effective group (EC, LPDS and saline) (Fig. 3). After 24 h, the relative photon intensity in samples preserved in saline and LPDS solutions had decreased to 20% of their initial level, while the corresponding samples preserved in ET-K and UW solutions maintained over 70% of their initial level. Luc-Tg rat islet preserved in the non-effective group solutions demonstrated a greater degree of injury than effective group solutions, which appeared to be apoptosis, rather than necrosis (data not shown). Additionally, when relative photon intensity was measured POD7 after preservation, the amount of light emitted was only slightly detectable (POD2; ET-K: 61.9 ± 5.4%, UW: 52.9 ± 3.9%, POD7; ET-K: 14.2 ± 1.5%, UW: 13.6 ± 1.1%). Thus, ET-K and UW were superior for the long-term preservation effect of islets.
Transplantation of islets into STZ-induced diabetes mice. A dose of 50 mg/kg consistently succeeded to induce overt hyperglycemic blood glucose concentration (>400 mg/dl) in the NOD-scid mouse up to 3 weeks following treatment. Within 48 h of 100 and 150 mg/kg STZ injections, all of the NOD-scid mice had dead (data not shown). Pathological findings showed that the islet population decreased in STZ-treated mice, whereas the non-treated mice had normal numbers of islets. Varying numbers of islets were transplanted into to STZ-induced diabetic mice, and the therapeutic effect assessed by blood glucose levels (Fig. 4). Transplantation of 150 and 200 islets (about 100 and 150 IEQ) into STZ-induced diabetic mice led to an immediate decline in blood glucose within 2 days. These data demonstrate that in order to be effective for the therapy of diabetes in this experimental model, more than 150 islets must be transplanted.

Functionality of the preserved islet in STZ-induced diabetic mice. Finally, to address the ultimate goal of determining whether preserved islets are therapeutically applicable, we transplanted Luc-Tg rat islets into the kidneys of mice with STZ-induced diabetes. In vivo imaging analysis of Luc-Tg rat islets showed that transplanted islets adhere to the injected kidney (Fig. 5A and B). Histological sections in the transplanted Luc-Tg rat islet group revealed that Luc-Tg rat islets grafted in the kidney capsular space (Fig. 5C and D). We then ascertained whether transplanted Luc-Tg rat islets exhibit islet-marker gene expression profiles. Transplanted Luc-Tg rat islets were collected by surgical resection and RT-PCR analysis of isolated transplanted Luc-Tg rat islets confirmed that the population expressed the pancreas-specific marker, enzyme and transcriptional factor genes: Insulin 2, GLUT2, PDX-1, Ngn-3, NeuroD1 and PAX-6 (Fig. 5E). No amplified Insulin 1, Insulin 2 and PDX-1 were detected in the resected kidney. This expression pattern is similar to that of primary islets. Thus, the preserved Luc-Tg rat islets revealed the same characteristics as normal islets.

Approximately 150 IEQ were transplanted into the left kidney capsule of newly diabetic NOD-scid mice. Mice receiving islets preserved in ET-K or UW showed better glycemic control than those that received saline preserved Luc-Tg rat islet (Fig. 6). ET-K or UW preserved Luc-Tg rat islet grafts prolonged survival in diabetic recipients (data not shown). Additionally, a recurrence of hyperglycemia was evident in nephrectomized mice, which suggests that the diabetic condition was reversed upon Luc-Tg rat islet transplantation and reappeared when the graft was removed. To evaluate the effective functionality of transplanted preserved islets with glucose stimulation, we performed IPGTT on the 35 POD, and analyzed the serum glucose concentration. In recipient of ET-K or UW preserved Luc-Tg rat islets at one day, hyperglycemia induced by glucose injection declined to the normal range (Fig. 7). Thus, the ET-K preserved islets functioned therapeutically in vivo and their transplantation ameliorated the effects of STZ-induced diabetes in mice.

**Discussion**

Recent progress in clinical islet transplantation resulting from improvements in less diabetogenic immunosuppression and preparation of sufficient quantities of highly viable islets for transplantation now provides an attractive treatment option for selected patients with type 1 diabetes mellitus. Human islet cell transplantation investigations began in the 1970s. However, insulin independence after human islet transplantation did not occur for the first time until 1989. Shapiro suggested that a corticosteroid-free immunosuppression therapy as the “Edmonton Protocol” allows for insulin independence for a median of 11.9 months. However, it is still difficult to obtain sufficient numbers of islets from a single cadaveric donor pancreas to achieve insulin independence after transplantation. Therefore, preservation of the human pancreas or isolated islets has been an important factor in the subsequent success of clinical islet transplantation. At present, the maximum rate of purified islets at leading centers is about 50–70%. 

Pancreatic islet transplantation as a promising treatment for type 1 diabetes has received widespread attention. However, condition of isolated-islet, yield and quality, are collected and considered to determine whether the organ can be used for clinical islet transplantation or research cell materials. In the many case of the preserved islet, one major disadvantage of islet culture is the loss of tissue mass that occurs over time. The ET-K preservation method demonstrated here allows tissue ATP synthesis and amelioration of cold ischemic tissues damage during extended 24 h isolated-islet preservation. Donor pancreas or isolated islets are usually preserved with UW solution. A large number of reports have shown that the two-layer method, which employs oxygenated perfluorochemical and UW solution, is superior to simple cold storage in UW to preserve not only islets but also the whole pancreas transplantation. However, these reports have utilized short-time cold storage of isolated islets before islet transplantation. This data provides a clear demonstration, for the first time, that ET-K solution is superior for the long-term preservation, and that preserved islets function therapeutically in STZ-induced diabetes mice.

<table>
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In conclusion, the data reported here demonstrate that ET-K solution and the novel protocol described may be useful for islet preservation. Optimization of the preservation conditions to minimize islet loss before clinical islet transplantation is a major research objective for improving the outcome of islet transplantation.

Materials and Methods

Animals. All experiments in this study were performed in accordance with the Jichi Medical School Guide for Laboratory Animals. The firefly luciferase-expression transgenic rat (Luc-Tg rat) was established in our laboratory as described previously in reference 17. NOD/SCID mice, eight weeks old, were obtained from Charles River Laboratories (Yokohama, Japan).

Isolation and culture of pancreatic islets. The pancreases of Luc-Tg rat were removed at eight weeks of age, dissected into 2–3 mm segments and islets isolated by the collagenase (Wako, Tokyo, Japan) digestion methods of Lacy and Kostianovsky. The Luc-Tg rat islets were purified for the hand-picking method under the inverted light microscope. Isolated islets were cultured in RPMI1640 medium with 10% fetal calf serum, 1% penicillin-streptomycin solution at 16–24 h (GIBCO, Tokyo, Japan).

Islets viability assay in preservation solutions. Freshly isolated Luc-Tg rat islets were plated in 96-wells tissue culture plate (50 islets/well: n = 5/each), and incubated in preservation solutions at 4°C for 24 h. The preservation solutions used are shown in Table 1. Detection was performed by addition of 22 μl (2.29 mg/ml) of the Luciferase based reagent (D-luciferin: Wako, Tokyo, Japan). To determine viability, islets were mixed 1:1 with Trypan Blue solution (GIBCO, Tokyo, Japan) and counted within 3 min, under light microscopy using a hemocytometer.

Streptozotocin-induced diabetic mice. STZ (Sigma, Tokyo, Japan) was prepared in citrate buffer (pH 4.5) and delivered by intraperitoneal injection (50 mg/kg) for five consecutive days before transplantation. Mice with blood glucose levels > 400 mg/dl were considered as diabetic.

Analysis of preservation solutions cultured Luc-Tg rat islets for diabetic mice. The Luc-Tg rat islets were cultured in preservation solution at 4°C. After 24 h, preserved islets were injected under the kidney capsule. An incision was made in the renal capsule and advanced in the subcapsular space, to the kidney. Isolated Luc-Tg rat islets were slowly injected and allowed to spread at the pole. The blood glucose level was checked at days 0, 1, 2, 3, 7, 14, 21 and 28. After 35 days, the Luc-Tg rat islet containing transplanted kidney was resected, and monitoring of blood glucose continued 10 days.

In vivo glucose tolerance testing. Glucose tolerance tests were performed by intraperitoneal (intraperitoneal glucose tolerance test [IPGTT]) injection of D-glucose (1 g/kg: Wako, Tokyo, Japan) to recipient mice after a 6 h fast. Blood glucose was monitored for 5, 15, 30, 60 and 120 min after glucose injection.
Reverse transcription polymerase chain reaction analysis. Total RNA was isolated from surgically resected Luc-Tg rat islet-transplanted mouse kidney samples and cultured islets using an RNeasy mini kit (QIAGEN, Tokyo, Japan). First-strand cDNA was synthesized in a volume of 50 μl containing 5 μg total RNA, 1 μl oligo (dT) 18 primer, 10 μl dNTPs (10 pmol/μl), 5 μl DTT (0.5 M), 40 units of RNase inhibitor, 200 units of SuperScript III and 5 μl Reverse-Transcription buffer (Invitrogen, Tokyo, Japan). Synthesis was performed at 36°C for 10 mim, 42°C for 10 min, 56°C for 50 min and 99°C for 5 min. Reverse transcription-polymerase chain reaction (RT-PCR) were performed by using a Ex-Taq system (TaKaRa, Kyoto, Japan). Primers and PCR details are available in Table 2.

Histological analysis of mice kidney sections after islet transplantation. Mouse kidneys were harvested 35 days after Luc-Tg rat islet transplantation, fixed in 10% formalin and embedded with parafin. Histological analysis of kidney tissues were conducted by serial tissue section and stained with hematoxylin and eosin (H&E) for detection of rat islets in the kidneys of immunodeficient mice.

In vivo bioluminescent imaging. In vivo luciferase imaging was performed using the noninvasive bioimaging system IVIS.
Figure 7. The effect of blood glucose clearance (IPGTT) in STZ-induced-diabetic mice after transplantation of preserved islets. IPGTT demonstrates that the in vivo islet function in ET-K and UW solutions-preserved-islets transplanted mice were not significantly different compared with the fresh-islets transplanted-mice. Values are means ± SEM; * p < 0.05.

Table 2. Primers and conditions for RT-PCR

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(Xenogen, Alameda, CA) and IVIS Living Image (Xenogen) software packages. To detect photons from Luc-Tg rat islets, D-luciferin (Promega, Madison, WI) was injected into the tail vein of anaesthetised mice (150 mg/kg/body weight). The signal intensity was quantified as photon flux in units of photons/sec/cm²/steradian in the region of interest.

Statistical analysis. Data are represented as means ± SEM. Results were analyzed using a two-tailed Student’s t-test. A p value of <0.05 was considered significant.

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References


