Video Article

Intraportal Transplantation of Pancreatic Islets in Mouse Model

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Abstract

Pancreatic islet transplantation to reduce hyperglycemia is highly successful in rodents with chemically-induced diabetes. The most common transplantation site in experimental islet transplantation is the kidney capsule. However, as less is known about the interaction of pancreatic islets with blood constituents, it also makes sense to utilize the portal vein approach in experimental islet transplantation.

This protocol demonstrates an intraportal islet transplantation technique in NMRI nude mice. Streptozotocin (180 mg/kg) is injected intraperitoneally to induce hyperglycemia in recipient mice. They are considered as diabetic at a non-fasting blood glucose level greater than 20 mmol/L. One day prior to transplantation, mouse pancreatic islets are isolated from the donor pancreas by collagenase digestion; a minimum of 350 islets are utilized per diabetic recipient. Depending upon the islet isolation yield, two or more donor mice are utilized per recipient. After overnight culture at 37 °C, islets are administered into the recipient liver via the portal vein. After surgery, the mice are protected in red Makrolon houses and observed until are awake. This protocol maintains glycemic control for 120 days in syngeneic mice and 15 days in allogeneic mice.

Video Link

The video component of this article can be found at https://www.jove.com/video/57559/

Introduction

Islet transplantation is a promising approach to treat type 1 diabetes mellitus[1]. The first attempt to transfer a sheep pancreatic fragment into a diabetic patient was performed by Watson Williams in 1893. However, a major breakthrough in clinical islet transplantation was achieved with the Edmonton protocol, and thereafter, a series of national programs were developed[2]. In the past, several sites, such as the bone marrow, omental pouch, intramuscular region, gastric mucosal surface, spleen, and kidney capsule, were explored in preclinical models, but the portal venous system is considered as one of the suitable and effective site for clinical programs[3,4,5,6].

Exogenous administration of insulin can be substituted by islet infusion into the portal vein. This is considered a preferred site because the oxygen supply is comparable to that in the native pancreas due to the location downstream of the confluence of the portal artery and vein. Moreover, there is a large surface area, so that the three-dimensional structure of the islets may be preserved, and vascularization could be facilitated[5]. In a recipient diabetic mouse, 2,000 human or porcine islet equivalents or 350 mouse islets are adequate to reverse the hyperglycemic state[7,8]. Euglycemic levels were reported for 15 days in the mouse recipient model of xenogeneic islets and in the allogeneic mouse-to-mouse model, and for more than 120 days in the syngeneic mouse-to-mouse transplantation.

Factors pertinent to the efficacy of islet transplantation via portal vein are appropriate anesthesia, method of puncture, and hemostasis[9]. Anesthesia can be induced by inhalation (5% isoflurane) or injection (ketamine, xylazine, or pentobarbital), and the substances are often combined. For control of the depth and time of anesthesia, attention should be paid to the state of the mouse, such as the color of the mucous membrane, eye lid, corneal reflex, respiratory rate, and body temperature. It is important to ensure that the animal is not struggling and that it survives the operation. The temperature can be maintained by different heating devices, such as heating pads, red light bulbs, *etc.* A warm thermal plate has been used to maintain a temperature of 25-30 °C between the mouse and the operation table, which prevents the occurrence of hypothermia. The dosages of the anesthetics are important, as all of them are metabolized by the liver, and hepatic function may be transiently disordered by the infusion of the islet suspension. The ideal puncture point for the portal vein is the position between the first and second tributary vein.

The number of islets and the intended injection volume also influence the outcome of the transplant, as an excessive volume may increase the shear stress. A 0.2-mL volume is considered appropriate for islet transplantation into the portal vein. The puncturing wound (26-gauge needle) induces bleeding from the portal vein, which needs to be stopped in a timely and effective manner. Sterile gauze or a finger can be applied with minimal pressure at the site of the puncture for about 6 min to stop the bleeding. Taken together, portal islet injection is effective and provides regulation over blood glucose levels in chemically induced diabetic mouse models.

Protocol

All procedures in this protocol have been approved by German Animal welfare law and guidelines. Consider using 10- to 12-week-old NMRI nude mice and C57Bl/6 mice (donor: old female; recipient: male) throughout the experiments. Use NMRI nude mice for islet isolation. Keep the mice under defined conditions according to local animal facility regulations.

1. Induction of Diabetes by Streptozotocin

1. On day -4, measure the body weights of 10 to 12 week-old NMRI nude mice and C57Bl/6 mice using a weighing machine.
2. According to the measured body weight, prepare a 180 mg/kg dose of streptozotocin in 0.1 mL of saline per mouse.
   NOTE: Streptozotocin should be freshly prepared before use and covered with aluminum foil, as it is light-sensitive.
3. Inject the syngeneic and allogeneic mice each with a single dose of 0.1 mL of streptozotocin (180 mg/kg) intraperitoneally using a 26-G needle.
4. On day -3,-2 and -1 collect 2-4 µL of blood by puncturing the tail veins of non-fasted mice.
5. Use 2-4 µL of the blood sample per test strip to measure the blood glucose in NMRI nude mice and C57Bl/6 mice
   NOTE: At this point, 80% of the mice should become diabetic (20 mmol/L).
   1. Use mice for islet transplantation when their blood glucose levels reach 20 mmol/L.

2. Isolation of Pancreatic Islets from Mice

1. Anesthetize the mice with ketamine (100 mg/kg body weight) and xylazine (20 mg/kg body weight) by intraperitoneal injection with a 26-G needle.
   NOTE: The volumes of ketamine and xylazine were determined according to the body weights of the animals (0.10 mL/10 g).
2. Keep the mice in supine position and disinfect with povidone-iodine solution. Confirm proper euthanasia by checking the pedal reflex.
   NOTE: In case of C57Bl/6 shave the abdominal region.
3. Hold the skin with fine forceps and make a midline incision with scissors to expose the abdominal cavity. Place the intestine situs to the left and outside of the abdominal cavity on a sterile gauze compress.
4. Adjust the stomach in another direction to locate the pancreas and other associated tissues.
5. Reach the aorta and make a small cut to drain the blood. Use sterile gauze for blood absorption.
6. Carefully remove the intestine. Separate the pancreas from the stomach and spleen.
7. Excise the pancreas and place it in a fresh Petri dish. Remove all the unwanted materials, such as fat, using forceps.
8. Fill a 5 mL syringe with 4 mL of collagenase and inject into the pancreas with a 26-G needle (stock solution: 30 mg of collagenase in 12 mL of Hank's solution).
9. Mince the pancreas with scissors to increase the surface area for an improved digestion and transfer to a 12 mL red cap tube.
   NOTE: Hank's balanced salt solution: HBSS 10x (100 mL), Penicillin-Streptomycin (10 mL), Gentamycin (1 mL), Ciprofloxacin (10 mL), HEPES (35 mL), distilled H_2O (900 mL)
10. Perform a digestion for 10 min at 37 °C in a shaking water bath. Vortex the sample thrice at a regular interval for 15 s.
11. Mix the digested pancreas vigorously by hand for 3 min and place it on ice for 10 s.
12. Add 6 mL of cold Hank's solution to stop the digestion reaction and centrifuge at 560 x g for 3 min at room temperature.
13. Remove the supernatant and dissolve the pellet in 10 mL of Parker/fetal calf serum (P/FCS) medium at room temperature.
    NOTE: P/FCS medium: TCM-199 10x (100 mL), FCS (50 mL), Penicillin-Streptomycin (10 mL), HEPES (20 mL), distilled H_2O (900 mL)
14. Identify the islets based on their morphology (spheroid, golden-brown color) under a light microscope. Avoid islets with dark centers. Check for purity and viability by performing dithizone staining under a light microscope.
    NOTE: Dithizone binds to zinc ion and provides red-colored stain to the islets.
15. Use a 1-mL pipette to count and handpick islets under a light microscope and transfer them to a fresh Petri dish (60 mm x 15 mm) containing 4 mL of P/FCS medium.
    NOTE: The volume of *P/*FCS medium in the Petri dish should not exceed 4 mL in order to ensure oxygen availability.
16. Keep the isolated islets in 4 mL of P/FCS medium overnight at 37 °C in an incubator.

3. Preparation of Animals for Islet Transplantation

1. Remove the isolated islets from the 37 °C incubator and place them under a flow bench. Center the islets with a circular swinging motion of the Petri dish.
2. Fill a 1-mL syringe (blue 23-G needle) with 0.1 mL of P/FCS medium. Then, add 350 islets in 0.3 mL of Hank's solution. Keep the syringe in a vertical position to allow the islets to settle.
3. Decrease the volume to 0.05 mL and replace the blue 23-G needle with a brown 26-G needle. To the same syringe, add 0.15 mL of Ficoll (density gradient medium) to the top to reach a final volume of 0.2 mL in the syringe. Avoid air bubbles.
4. Keep the syringe in a vertical position and rotate it slightly to avoid the clumping of islets. The islets will settle at the cone of the syringe within 2-3 min.
5. Measure the body weight and blood glucose levels of the recipient mice; the blood glucose levels should be around 20 mmol/L.
6. Anesthetize the mice with ketamine (100 mg/kg body weight) and xylazine (20 mg/kg body weight) by intraperitoneal injection with 26-G needle.
7. Place the mice in supine position on a warm thermal plate (37 °C). Check the depth of anesthesia by pinching the toe.
8. Clean the abdominal region with povidone-iodine solution (skin disinfectant). To prevent the eyes from drying, keep them moist with eye ointment.

NOTE: In case of C57Bl/6 shave the abdominal region.

4. Islet Transplantation via the Intraportal Route

1. On day 0, start with a laparotomy by holding the skin with forceps and making a 2-3 cm-long midline incision with a scissor. Place a sterile wet gauze compress around the wound edges to keep it moist.
2. Place the intestine situs to the left and outside the abdominal cavity on the sterile gauze compress.
   NOTE: Keep the intestine moist throughout the procedure with pre-warmed 0.9% NaCl solution or sterile gauze dipped in normal saline.
3. Move the duodenum and locate the pancreas. Locate the portal vein situated at the ventral site by pushing the pancreas upward. Use a finger and thumb to expose the portal vein and, once exposed, puncture it near to the liver.
4. Within 1 min, slowly and steadily inject the islets (0.2 mL from step 3.4) into the portal vein under the inspection of magnifying glass to avoid any leakage of islets.
   NOTE: After injecting, always check for remaining islets in the syringe. Take 1 mL of P/FCS media in a syringe, flush into a fresh Petri dish and count under a microscope.
5. Place a sterile gauge at the puncture site and apply pressure with the index finger (penetration depth: 0.5-1 cm) for 6 min to stop the bleeding.
   NOTE: The volume injected should not exceed 0.2 mL. Excessive volume and pressure can damage the islets due to shear stress.
6. Carefully place the intestine situs, peritoneum, abdominal muscle layer, and fascia back into position.
7. Using forceps, lift the skin, close the muscle incision with absorbable silk suture, and upper skin layer with 2-3 clips.
8. Clean the surface with skin disinfectant, apply a wound healer, and place the mice in individual cages. Wound healer provides a water-resistant acrylate layer and protect from maceration and secondary infection.
   NOTE: Observe the mice until they have regained sufficient consciousness, provide tramadol (0.2 mg per mouse) along with fluids. In this protocol, mice recovered from anesthesia quickly and tramadol administration reduced the pain. After 7 days, the wound should heal.
9. Feed the mice with regular food and water. Food and water consumption and urine excretion will increase after the streptozotocin injection.
10. Measure the blood glucose levels every day for the first week and then once a week in syngeneic mice. In allogeneic mice, measure the blood glucose levels thrice a week for up to 15 days.

Representative Results

The competency of transplanted islets was studied in chemically induced diabetic mice. The islets were transplanted via the portal venous system in diabetic mice model. The donor-recipient ratio was 2:1. In the syngeneic mouse model, two donor mice were utilized to obtain 350 islets. The islets were then transplanted into diabetic mice. A reduction in blood glucose level was observed on day 1 after transplantation, suggesting a role of transplanted pancreatic islets in reducing the blood glucose level. Streptozotocin increased the blood glucose level up to about 22 mmol/L. A drop in the blood glucose level to 4.6 mmol/L (day 1) was observed after islet transplantation (**Figure 1A**). Normoglycemia was maintained for up to 121 days, as shown in **Figure 1A**. The body weight and blood glucose level roughly followed the same pattern. After the streptozotocin injection and surgery, the body weight decreased. From day 2 onward, it tended to rise towards normal, from 25.3 g (day 4) to 29.7 g (day 121), as shown in **Figure 1B**.

In case of major histocompatibility (MHC) mismatched mice, 350 islets from two C57Bl/6 ($H2^d$) mice were transplanted into diabetic BALB/c ($H2^b$) mice. After streptozotocin injection, the blood glucose increased up to 26.6 mmol/L. After islet transplantation, the blood glucose level dropped to 4.4 mmol/L (day 1; **Figure 2A**). Normoglycemia (4.44 to 7.2 mmol/L) was observed for up to 10 days, but not after 15 days (20.1 mmol/L). The body weight of diabetic mice dropped from 26 g (day -3) to 23 g (day 0) and further to 21.5 g (day 1). After transplantation, the body weight gradually recovered from 23 g (day 2) to 25 g (day 10). Due to MHC-mismatch, the blood glucose level increased and the body weight decreased 10 days after islet transplantation (**Figure 2B**).

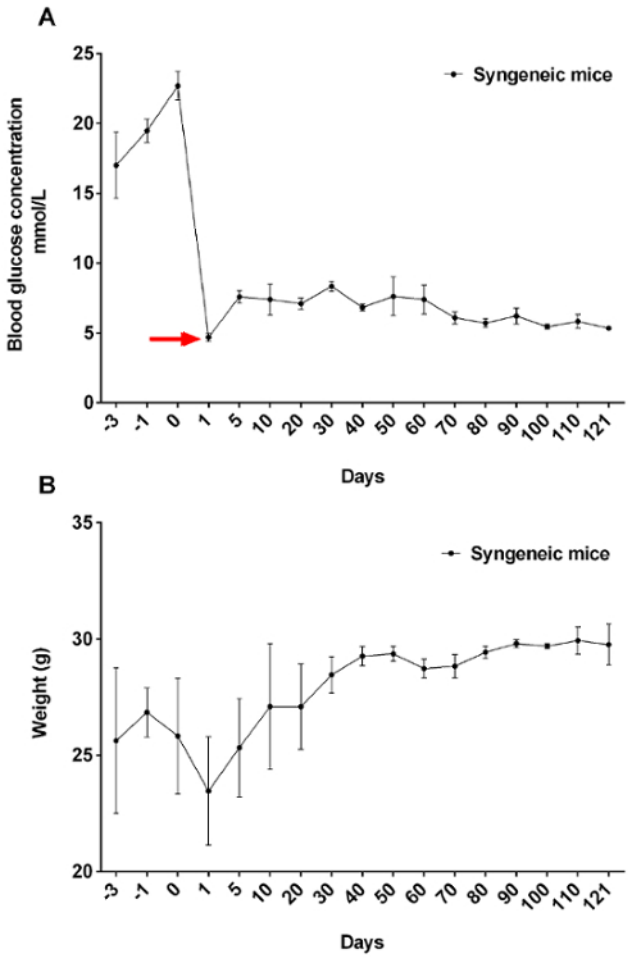

Figure 1: Effect of syngeneic islet transplantation. (**A**) The blood glucose level was measured at different time points in streptozotocin-induced diabetic mice (n = 3). A single dose of streptozotocin (180 mg/kg) was injected before the transplantation of 350 islets to each recipient (day 0). The blood glucose level (mmol/L) was measured for up to 121 days (day -3: 17 ± 2.3; day 0: 22.6 ± 1; day 1: 4.6 ± 0.3; day 5: 7.5 ± 0.4; and day 121: 5.3 ± 0.09). (**B**) Increase in body weight after islet transplantation. The data above represents the mean ± the standard deviation. Please click here to view a larger version of this figure.

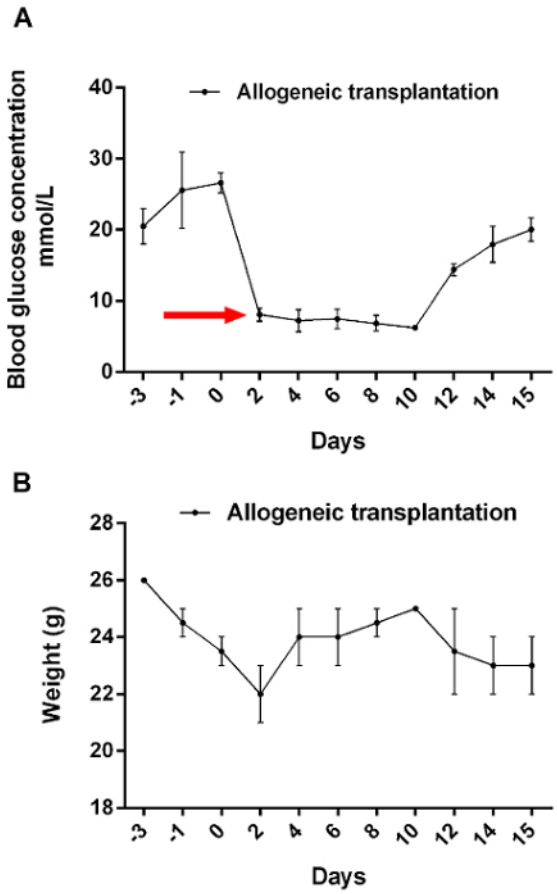

Figure 2: Effect of allogeneic islet transplantation. (**A**) The blood glucose level was measured at different time points in streptozotocin-induced diabetic mice (n = 2). A single dose of streptozotocin (180 mg/kg) was injected before the transplantation of 350 pancreatic islets (day 0). The blood glucose level (mmol/L) was measured for up to 15 days (day -1: 25.5 ± 5.3; day 0: 26.6 ± 1.4; day 2: 8 ± 0.9; day 10: 6.2 ± 0.08; and day 15: 20.1 ± 1.6). Normoglycemic state was maintained for up to 10 days, and subsequently, blood glucose concentrations increased. (**B**) Follow-up of body weight before and after islet transplantation. The data represent the mean ± the standard deviation. Please click here to view a larger version of this figure.

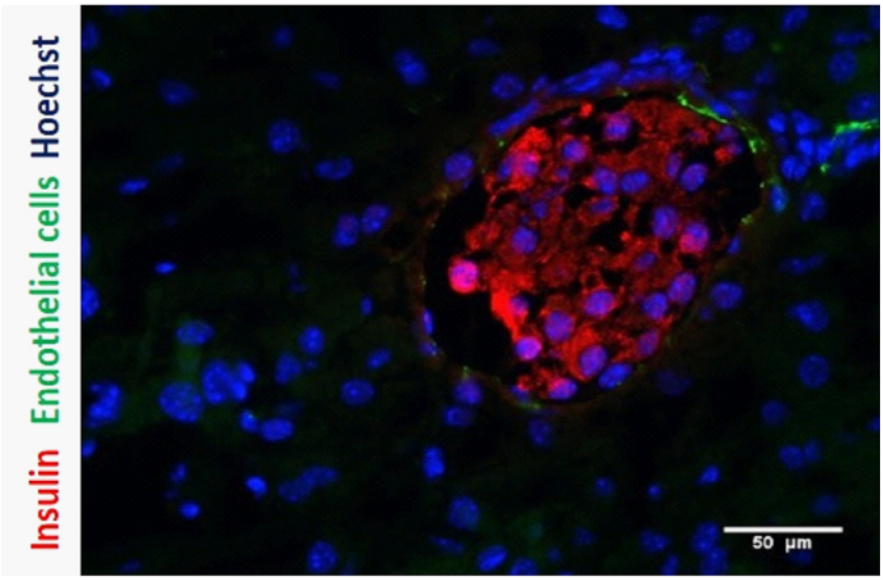

Figure 3: Representative immunostaining picture of transplanted pancreatic islets into liver: Wash the liver section (5 µm) with PBS, block with 10% donkey serum followed by overnight incubation at 4 °C with insulin primary antibody (1:500) and platelet endothelial cell adhesion molecule PECAM-1 primary antibody (1:100). Next day, stain with secondary antibodies for 1 h, wash with PBS, counter stain with Hoechst and capture the images with Leica fluorescent microscope. Please click here to view a larger version of this figure.

Discussion

In this study, the intraportal route of islet transplantation is explored. This protocol is highly efficient in relieving diabetic stress and provides an in-depth opportunity to explore islet transplantation studies. This protocol confirms that about 350 murine islets are capable of reversing the hyperglycemic state. Moreover, none of the mice died during the procedure, and glycemic control was achieved in all recipients. Body weight and blood glucose levels were regularly recorded to reflect the competence, standard, and functionality of transplanted islets in reversing hyperglycemia. In syngeneically transplanted mice, blood glucose levels were measured every day for the first week and once a week thereafter. After islets transplantation, hypoxic condition and others factors trigger the endothelial cells to secrete pro-angiogenic factors such as VEGF after 14 days and thereby increase the vascular engraftment in the liver as shown in **Figure 3**. In allogeneic mice, blood glucose levels were measured thrice a week for up to 15 days. After 15 days, transplanted islets were rejected in the allogeneic model due to an immune attack, unless immunosuppressive drugs are administered

There are several islet isolation protocols published in literature[10,11]. The most straightforward is to pick islets manually on the basis of their size and morphology after collagenase digestion. This is one of the most crucial steps to select only islets and to avoid exocrine cells, debris, *etc.* Selected islets should be maintained at 37 °C overnight in an incubator to allow them to recover from mechanical stress encountered during the digestion procedure. The selection of the streptozotocin dose and the intraportal islet transplantation are also key to this protocol. The body temperature should be regulated and maintained using a thermal plate throughout the surgical procedure. Sterile gauze should be applied with minimal pressure for 6 min at the site of puncture to avoid bleeding. Mice should be kept under observation until they regain sufficient consciousness.

Certain steps in this protocol can be modified. For example, the optimization of the digestion time and the volume of collagenase may increase the yield of islets in different mice strains. Instead of the rapid induction of diabetes (180 mg/kg streptozotocin), slowly increasing hyperglycemia can be induced using a lower dose over an extended period of time (*e.g.,* 40 mg/kg/day streptozotocin for 5 consecutive days). This could provide better engraftment of transplanted islets. Other sites, such as the bone marrow, omental pouch, intramuscular region, gastric mucosal surface, spleen, and kidney can be explored as alternatives to the intraportal vein.

Poor engraftment of transplanted islets is still a major concern in clinical trials. Others include a shortage of donors, inconsistencies in maintaining blood glucose levels after transplantation, immunosuppression, and blood-mediated reactions[12,13]. Lack of methods to evaluate proper engraftment is another obstacle. Tracking transplanted islets by magnetic resonance or bioluminescence imaging could possibly replace classical methods such as the glucose tolerance test or c-peptide. In the future, stem cells transplanted together with islets might provide nutritional and immunomodulating support[14].

Disclosures

All authors declare that they have no conflict of interests.

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