IN VIVO ASSESSMENT OF HUMAN ISLET POTENCY

PROCEDURE OVERVIEW

Transplantation of human islets into immunodeficient mice represents an important tool for the assessment of viability and function of human islet preparations. It allows for the assessment of the ability of transplanted human islets to restore and maintain euglycemia.

Procedures involving animals are performed under protocols approved and monitored by the University of Miami IACUC under an Animal Welfare Assurance on file (A-3224-01, effective 12/4/02) with the Office of Laboratory Animal Welfare (OLAW), National Institutes of Health, and full accreditation by the *Association for Assessment and Accreditation of Laboratory Animal Care*.

Nude mice (20-25 g) receive an intravenous injection of Streptozotocin in order to induce diabetes. Blood glucose is then monitored in the following days. Established hyperglycemia is defined as at least 3 blood glucose readings >350mg/dl. Only mice with established diabetes will be used as recipients of human islet transplants.

When possible two mice will be transplanted: One mouse will receive 2,000 IEQ and one will receive 1,000 IEQ under the kidney capsule.

Transplants will be performed on the same day that the clinical transplant is performed (generally 1-3 days post isolation). The islets utilized for the transplant are an aliquot of the final preparation that is to be infused in the patient, therefore purity and volume of the tissue to be implanted will be variable. Islets will be counted and an aliquot will be prepared for each transplant to be performed. Islet aliquots are plated in Petri dishes in 4 ml of culture medium and maintained in the incubator at 37ºC with 5% CO_2 until transplant.

Buprenorphine will be administered as analgesic on the day of transplant and BID on the 2 days following transplantation.

Blood glucose and weight of the transplanted mice will be monitored for the first week after transplant and 1-5 times a week thereafter.

Animals will be monitored for at least 30 days after transplant. Animals achieving stable normoglycemia (blood glucose < 200 mg/dl) will be nephrectomized to confirm graft function and then euthanized. Animals that remain hyperglycemic after transplant and show chronic weight loss, signs of discomfort, and do not respond to supportive fluid therapy will be euthanized.

The grafted kidney and the pancreas of the transplanted mice will be collected for histological analysis in either 10%buffered formalin or OCT.

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INDUCTION OF DIABETES BY STREPTOZOTOCIN ADMINISTRATION IN MICE

1.0 OBJECTIVES

Streptozotocin (STZ) is a β-cell toxin used for the induction of diabetes in several animal models. STZ is toxic and potentially carcinogenic and has to be handled using personal protective clothing (*i.e.*, gown, gloves, shoe covers and mask) and weighed in a chemical hood.

2.0 REAGENTS

2.1 Animal Model: 20-25 g Athymic nu/nu (nude) female mice

2.2 Equipment:
- Chemical hood
- Precision scale
- Laminar flow hood
- Ice bucket
- Mouse restrainer
- Portable Glucometer (OneTouch Ultra 2, Life Scan)

2.3 Personal Protective Equipment:
- Gloves
- Mask and bouffant cap
- Protective gowns
- Face mask
- Shoe covers

2.4 Materials & Reagents:
- Sterile 0.5 ml disposable syringes
- 4x4 sterile gauze pads
- Ice
- 5 ml polystyrene round bottom tubes
- Streptozotocin (Sigma Cat#: S-0130)
- Citric Acid (Sigma Cat#: C-0759)
- Sodium Citrate (Fisher Cat#: BP327-1)
- 0.9% Sodium Chloride solution
- Aluminum foil
- Blood glucose strips

3.0 SOLUTION PREPARATION

3.1 Stock Buffer Solution:

3.1.1 Prepare buffer mix as follows:
27 ml of 2.1% Citric Acid solution
23 ml of 2.94% Sodium Citrate solution

3.1.2 Titrate solution to pH of 4.5 with citric acid

3.1.3 Prepare aliquots and store at 4°C until use.

3.2 STZ Buffer Working Solution:

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3.2.1 Mix the following:
1 ml of Stock buffer solution
10 ml of 0.9% Sodium Chloride (saline) solution
3.2.2 Sterilize solution using a 0.22 µm filter and syringe.

3.3 STZ Aliquots:

NOTE: STZ is highly toxic and should be handled with care, and following manufacturer instructions.

3.3.1 Weigh 30 mg of STZ in a 5 ml polystyrene round bottom tube covered with aluminum foil. Prepare one tube for approximately five mice to be injected.
3.3.2 Place tubes containing STZ on ice and take them to the VAF animal housing room.

4.0 STREPTOZOTOCIN TREATMENT

Personal protective clothes are used to enter the VAF animal room (*i.e.*, gowns, gloves, caps, masks and shoe covers) and all procedures are performed in a bio-safety cabinet.

4.1 Streptozotocin Dose:

4.1.1 Add 3 ml of the STZ buffer Working solution to a polystyrene round bottom tube containing 30 mg of STZ to obtain a final concentration of 10 mg/ml.
4.1.2 Close the tube and mix gently until powder dissolves completely.
4.1.3 Estimate volume of STZ solution to be injected. Give 20 µl of solution per gram of body weight, for a final dose of 200 mg/kg administered per mouse.
4.1.4 Load the appropriate amount of STZ solution in the 0.5 ml syringe.

4.2 Injection:

4.2.1 Weigh the mouse and place it in restrainer.
4.2.2 Warm up the tail by using warm water or a lamp prior to injection to cause vasodilatation and to facilitate the insertion of the needle into the tail vein.
4.2.3 Inject STZ solution into lateral tail vein according to mouse weight.
4.2.4 Remove mouse from restrainer and place in a clean sterile cage.
4.2.5 Cages containing mice injected with STZ will be labeled: "Potentially Biohazard: STZ and the date of injection".
4.2.6 Dispose of remaining STZ solution as per regulations.

5.0 ANIMAL FOLLOW-UP

5.1 Monitoring: Nonfasting blood glucose and body weight of the treated mice is monitored 2-3 days after STZ injection and every 1-3 days thereafter to confirm diabetes induction.

5.2 Definition of Hyperglycemia: At least 3 consecutive readings over 350 mg/dl on different days are required to consider successful diabetes induction.

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5.3 Animal Care:

5.3.1 From the onset of diabetes on, cages will be changed more frequently (daily) to prevent hypothermia and discomfort due to the wet bedding. Water supply should be closely monitored and intensified too.

5.3.2 Mice have to be closely monitored 2-3 times a week. Severe dehydration and weight loss may occur due to diabetes induction.

5.3.3 In order to prevent and treat weight loss and/or dehydration, Saline solution (0.5-2 ml) can be injected subcutaneously.

5.3.4 Animals showing signs of distress and profound body weight loss (≥ 20%) not responding to treatment are humanely sacrificed according to IACUC guidelines.

5.4 Insulin Therapy: The availability of human islets is unpredictable and diabetic immunodeficient animals for islet assessment should be available at all times. To reduce morbidity and mortality associated with diabetes, animals may be treated with exogenous insulin, after having confirmed sustained hyperglycemia.

5.4.1 *Insulin glargine* (Lantus® 1-4 IU/day) injected subcutaneously.

5.4.2 Slow release *insulin pellets* (LinBit; LinShin Canada) may be implanted subcutaneously for continuous slow release.

5.5 Data Recording: Data relative to blood glucose, body weight, general condition, treatments, and procedures should be recorded in the daily follow up forms.

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HUMAN ISLET TRANSPLANTATION IN MICE

6.0 OBJECTIVES

Diabetes reversal after human islet implantation into chemically diabetic mice allows for the assessment of islet cell potency in an in vivo diabetic environment. Nude mice are used after chemical induction of diabetes only if at least 3 daily blood glucose readings > 350 mg/dl are obtained after streptozotocin administration. Whenever possible and depending on the availability of adequate islets, one or more mice will receive 2,000 IEQ and one or more will receive 1,000 IEQ under the kidney capsule.

On the day of implant, human islets are counted and independent aliquots will be prepared for each transplant to be performed. Islet aliquots are plated in Petri dishes in 4 ml of culture medium and maintained in the incubator at 37º or 22º with 5% CO_2 until transplant.

In vivo assessment is done on the same day the clinical transplant is performed (generally within 72 hours post isolation). The islets aliquots utilized for the mouse transplant are representative of the final preparation that is infused in the patient, therefore purity and volume of the tissue to be implanted may vary between preparations.

7.0 REAGENTS

7.1 Animal Model: 20-25 g Athymic nu/nu (nude) mice of either sex

7.2 Equipment:

- Laminar flow hood
- Tabletop centrifuge
- Portable scale
- Anesthesia machine (Isoflurane vaporizer)
- Anesthesia exhaustion system
- Dissecting microscope and light source
- Portable Glucometer (OneTouch Ultra 2, Life Scan)

7.3 Surgical Instruments:

- Small forceps
- Jeweler forceps
- Dissecting scissors
- Needle holder
- Electric cautery

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- Surgical stapler & staples
- Surgical staple removal
- 1-ml precision syringes
- Polyethylene (PE)-50 tubing
- PE-60 tubing
- Silicon connectors
- Scalpels

7.4 Personal Protective Equipment:

- Gloves
- Mask and bouffant cap
- Protective gowns
- Face mask
- Shoe covers

7.5 Materials & Reagents:

- 0.5 ml disposable syringes
- 4x4 sterile gauzes
- 4 cm diameter petri dishes
- 10 ml disposable syringes
- Sterile pipette tips
- 0.9% Sodium Chloride solution
- Blood glucose strips
- 50 ml conical tubes
- Small sterile metal wires
- 4x4 sterile gauze pads
- 5-0 sutures (PDS)
- Chlorhexidine Solution
- Isofluorane
- Hanks' Buffered Salt Solution (HBSS)
- Buprenorphine
- Oxygen tank
- CO_2 tank
- Clavamox Antibiotic Solution

8.0 EQUIPMENT PREPARATION

8.1 Tubing preparation: A 10 cm piece of sterile PE-50 tubing is cut and connected to a 20 cm piece of sterile PE-60 tubing using a piece of silicone connecting tube. On the other extreme of the PE-60 tube, another piece of silicone connecting tubing is used to connect the tube to the micropipette tip attached to the threaded plunger syringe.

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8.2 **Anesthesia Machine Preparation:** Make sure that the level of anesthetic in the machine is sufficient for the needs of the procedure. Turn on the oxygen tank and exhaustion system, if available. Isoflurane is generally administered at 2-3% and adjusted based on plane of anesthesia. Anesthesia depth is assessed and documented every 15 minutes.

9.0 ISLET TRANSPLANTATION IN THE SUBCAPSULAR KIDNEY SPACE

9.1 Precision Syringe Preparation:

9.1.1 Using sterile gloves and sterile technique, assemble the sterile precision syringe connecting a micropipette tip to it and fill it with Hanks,or Saline solution avoiding bubbles.

9.2 Islet Graft Preparation:

9.2.1 Under the dissecting microscope, use the precision syringe to collect the islets from the Petri dish.

9.2.2 With a gentle circular motion, bring the islets to the center of the Petri dish.

9.2.3 Aspirate islets with syringe into the pipette tip. Maintain the syringe in the vertical position using a 50 ml Falcon tube as a stand.

9.2.4 Adapt polyethylene tubing PE-50 to pipette tip on the precision syringe. Slowly carry the islets to about 1 cm distance to the end of the tubing.

9.2.5 Bend the tube and tie it using a small piece of sterile metal wire.

9.2.6 Place the tube inside a 15 ml conical tube and spin at 1,000 RPM (200xg) for 1 minute.

9.2.7 Adapt polyethylene tubing PE-50 to pipette tip on the precision syringe.

9.2.8 Remove the wire and measure the distance (in cm) that the islet pellet occupies in the PE-50 tube and record it on the transplant record form.

9.2.9 Release the bent on tubing slowly.

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9.3 Animal Preparation:

9.3.1 Anesthetize mouse by placing it in the induction chamber connected to the anesthesia machine. Use anesthetic at 2-3%. Verify depth of anesthesia by observing respiratory rate and abrogation of reflexes (*i.e.*, toe pinch).

9.3.2 Place the mouse in right lateral recumbence position maintaining anesthesia mask connected to its face at all times. Disinfect the abdominal skin with chlorhexidine.

9.3.3 Perform skin and abdominal muscle incision in the left flank with scissors to expose the kidney. Maintain the kidney wet with saline solution at all times.

9.3.4 Under the microscope, using jeweler forceps or a needle, make small puncture on the kidney capsule at the cranial pole.

9.4 Islet Implant Procedure:

9.4.1 Introduce tip of the PE-50 tubing (connected to the precision syringe) carrying the islets under kidney capsule and push it gently toward the caudal pole of kidney. Avoid trauma and bleeding of the kidney parenchyma during the procedure.

9.4.2 Slowly inject islets, while holding tip of tubing in the subcapsular space.

9.4.3 Remove tubing and gently spread islets using the forceps to separate them.

9.4.4 Seal the tear on the kidney capsule by using an electrical cautery.

9.4.5 Reposition the kidney into the abdominal cavity.

9.4.6 Suture the muscular layer with 5-0 PDS suture.

9.4.7 Suture the skin using surgical staples. Clean the surgical wound with chlorhexidine.

9.5 Completion of Procedure:

9.5.1 Turn off anesthesia vaporizer and remove mask.

9.5.2 Clean up working area and discard disposables.

9.6 Postoperative Management:

9.6.1 Give buprenorphine 0.05 mg/Kg subcutaneously and place mouse in clean cage over heating pad to allow recovery.

9.6.2 Allow free access to food and water.

9.6.3 Give buprenorphine 0.05 mg/Kg subcutaneously BID on the next two days after transplantation.

9.6.4 Prophylactic antibiotic therapy may be administered during the two first weeks of follow-up in the drinking water. Clavamox solution (i.e., 140 mg/L in drinking water) should be changed twice a week.

10.0 ANIMAL FOLLOW-UP

10.1 Monitoring: Nonfasting blood glucose and body weight of the treated mice is monitored daily for the first week post transplant, every other day, or more frequently on the second week and at biweekly thereafter.

10.2 Definition of Hyperglycemia: At least 3 consecutive nonfasting readings ≥ 350 mg/dl on different days are required to consider successful diabetes induction.

10.3 Definition of Normoglycemia: The first of 5 consecutive nonfasting glycemic values ≤ 200 mg/dl over different days.

10.4 Animal Care:

10.4.1 In the postoperative period, mice will be observed daily

10.4.2 Severe dehydration and weight loss may occur due to diabetes. In order to prevent and treat weight loss and/or dehydration, Saline solution (2 ml) can be injected subcutaneously.

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10.4.3 Animals showing signs of distress and severe body weight loss (≥ 20%) not responding to treatment are humanely sacrificed according to IACUC guidelines.

10.5 Data Recording: Data relative to blood glucose, body weight, general condition, treatments, procedures and any adverse event should be recorded in the daily follow up forms.

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