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SUCCESSFUL CLINICAL ISLET ISOLATION USING A GMP-MANUFACTURED COLLAGENASE AND NEUTRAL PROTEASE

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Abstract

In 2007, the islet community was notified that the collagenase product most commonly used for human islet isolations contained bovine neural tissue contaminants. To minimize this potential hazard, we adapted our human islet processing procedure to utilize a GMP-manufactured, bovine neural tissue-free collagenase blend. Here we describe the factors that we consider most important for achieving reproducible and clinically useable islet isolations using this product.

Keywords

Islet Transplantation; Islet Isolation; Collagenase; Pancreas Procurement

MINI-REVIEW

Pancreatic islet transplantation offers a specific, minimally-invasive approach to restore normoglycemia and insulin independence in patients with type 1 diabetes (1), but its clinical applicability is limited by the complexity of the islet isolation process. The digestion of the pancreas with collagenase is an important but particularly unpredictable step since many of the currently available enzyme formulations have batch-to-batch variability and narrow activity parameters outside which islets are degraded along with acinar tissue. In 1995, a highly purified formulation of collagenase and protease (Liberase™ HI) manufactured by Roche (Indianapolis, IN) became available and was found to be very effective in dispersing acinar tissue without injuring the islets (2,3). This product quickly became the preferred blend for clinical islet isolation by many islet transplant centers (4,5,6). Unfortunately, the enzyme was manufactured using neural tissue and other specified risk materials from cattle, and its use in clinical isolations was discontinued in 2007 due to concerns about potential transmission of Bovine Spongiform Encephalopathy (7,8). The enzyme formulation that subsequently became

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available and is now used by many centers, including those in the Clinical Islet Transplantation (CIT) consortium (9), is a mixture of a current Good Manufacturing Practice (cGMP) grade collagenase (Collagenase NB1) and cGMP neutral protease (Neutral Protease NB) that is manufactured by SERVA Electrophoresis GMBH (Heidelberg, Germany). Although this product (SERVA blend) is manufactured with animal products, it does not contain bovine neural tissue contaminants or other specified risk materials (7,10). According to the certificate of analysis, the biochemical activity of this product is similar to Liberase™ HI, but its parameters for optimal use are different and many experienced centers have had inconsistent results using this product for clinical islet isolation. At the University of California, San Francisco, we have made several changes to the islet isolation protocol to account for these differences, and can now routinely obtain high quality islets suitable for transplantation using this enzyme blend. Our protocol changes and experience are summarized below.

Donor Selection

As shown in Table 1, the SERVA blend is effective in isolating islets from a wide range of donor ages, including younger (<45 years old) donors. This is different from our experience with Liberase™ HI (Table 3), and may be related the newer product's relatively low levels of tryptic-like activity and the use of neutral protease in place of thermolysin (11). As with previous isolation protocols, donor size remains an important consideration. Our best yields have come from male donors who weigh more than 90 kg and are more than 180 cm tall, and we use these guidelines to select donors rather than relying on BMI alone (Table 1). Many of our donors are on insulin infusions due to the administration of corticosteroids and thyroid hormones and we do not consider this an exclusion criterion unless there is a history of diabetes or the hemoglobin A1c is $\geq$ 6.0%.

Pancreas Procurement and Preparation

Proper procurement and pre-digestion preparation of the pancreas are very important components of the manufacturing process that can have significant effect on islet quality and yields. In order to optimize this step, all pancreases intended for clinical islet transplantation are procured by our own transplant fellows using a protocol that is identical to pancreas procurement for solid organ transplantation. University of Wisconsin (UW) solution is used to preserve all organs. Particular attention is paid to keeping the organ cold after circulatory arrest and removing the organ from the body early during the procurement. We do not use the 2-layer preservation method to store the organ, but do initiate processing immediately so that our cold ischemic times are consistently less than 8 hrs (Table 1; 12, 13). Upon receipt, the organ is immediately trimmed and prepared for digestion by one of the transplant surgeons (AMP) in an operating room in order to minimize cold ischemia and ensure that sterility and cold conditions are maintained at all times.

Enzyme Preparation

The Collagenase NB1 and Neutral Protease NB are reconstituted separately and mixed immediately prior to use to minimize degradation of the collagenase by the neutral protease. We have found that 1600 Units collagenase/100g pancreas and 200 Units neutral protease/100g pancreas reconstituted in a total volume of 350ml produce the best digestion of the pancreas and result in the highest islet yields. These enzyme quantities are significantly lower than those recommended by the manufacturer and other centers, and are lower than those used with Liberase™ HI (Table 3, 14).

A theoretical advantage of the SERVA blend is that the amounts of collagenase and neutral protease can be adjusted independently to match the type of pancreas being processed. We tested this possibility by varying the relative proportions of these enzymes according to donor characteristics such as age and organ consistency, but we were unable to identify any consistent

trends and found that the standardized concentration described above produced the best islet yields from the majority of organs. We also evaluated several lots of enzyme to determine whether there was significant variability between different lots. Our 14 clinical islet transplants were processed using 4 different lots of collagenase NB1 and 4 different lots of neutral protease NB that had been reconstituted according to the protocol described above. All of the lots produced similar digestion times, islet yields, viability, survival in culture, and post-transplant function. This limited series suggests that the SERVA blend lots are relatively consistent, but lot-to-lot variability remains an important obstacle in islet transplantation and larger studies with additional batches and preparations of the enzyme are needed to fully evaluate this issue.

The SERVA enzyme blend is available as a premium version and a cGMP version. The two versions have similar composition and activity, but only the latter is produced in compliance with the European Union guide to cGMP and is recommended by the manufacturer for use in clinical islet transplantation (15). In order to comply with our islet facility's cGMP regulations and the requirements of the CIT, our group has only used the cGMP blend, but there have been reports of good results using the premium grade of the enzyme. More testing is needed to determine whether this version is significantly different from the cGMP enzyme.

Pancreas Processing

After arrival in the cGMP islet isolation facility, the pancreas is bisected, the pancreatic duct orifices are cannulated, and the pancreas is distended in a controlled manner using a pressure sensor and pump. Once distention is complete, the organ is cut into pieces and placed into a Ricordi chamber as previously described (16,17). Digestion progresses rapidly: the temperature of the digestion solution is raised to 37°C within five minutes and the digestion time from this point on rarely exceeds 15 minutes. Digestion is stopped when more than 40 islets are seen in the 2 mL sample aliquots, both free and embedded islets are present, and the acinar tissue is less than 300μm in size. The decision to stop digestion is made by the same person (GLS) during each isolation. The duration of digestion and stopping criteria are similar to what we used with the Liberase™ HI enzyme. Digestion is stopped by flushing the chamber with room temperature RPMI and lowering the chamber temperature to 30°C. The digest is quickly harvested into flasks containing cold RPMI, human serum albumin, insulin, and heparin and then centrifuged. The tissue pellets are then reconstituted in Cold Storage Solution (Mediatech, Inc., VA) containing 2% PentaStarch (Mediatech, Inc., VA), human serum albumin heparin, and insulin. Starch is used because it preferentially enters the pancreatic acinar tissue and alters its density, thus making easier to separate from the islets (18,19). Just prior to purification, the digest is washed with Cold Storage Solution containing a lower concentration of PentaStarch (0.2%) to allow better loading onto the density gradient.

Purification and Culture

The islets are separated from non-islet tissue utilizing an isopyknic iodixanol (Optiprep, Axis-Shield PoC AS, Oslo, Norway) gradient on a Cobe® 2991 cell separator (13,20). The gradient densities we use are 1.06 g/mL (light), 1.10 g/mL (heavy), and 1.2 g/mL (super-heavy). If the tissue volume exceeds 25 mL, a second Cobe® bag is used. Fractions with islet purities >80%, 50–80%, and 30–50% are collected separately, washed, and cultured in 175 cm^2 tissue culture flasks in CMRL 1066 (Mediatech, Inc., VA) supplemented with 0.025% human serum albumin. Islets are cultured for 12–24 hours at 37°C and for an additional 24–48 hours at 22° C (13,21). Prior to transplantation, the islets are washed and suspended in 200 mL of room temperature transplant medium (Mediatech, Inc., VA) for transplantation. Heparin is added at 70 U/kg recipient body weight. Yields and metabolic characteristics of the isolations are depicted in Table 2. Using these techniques, the overall results with the SERVA blend were comparable to those observed with the Liberase™ HI blend, although a higher percentage of

'clinical' islet isolations prepared with the SERVA blend were suitable for transplantation (Table 3).

Islet Transplantation and Post-transplant Care

Unless there are medical contraindications such as severe contrast allergy, all islet transplants at UCSF are performed percutaneously by our interventional radiologists (22). Ultrasound is used to establish initial access into a tertiary portal venous radicle, followed by fluoroscopically-guided insertion of guide wires and catheters for the islet infusion. We routinely use large-bore (6-French) catheters which have reduced our infusion times to approximately 25–30 minutes. Portal pressures are monitored periodically and the infusion is temporarily stopped if pressures exceed 20mm Hg. Following the infusion, the tract is occluded using both Gelfoam® (Pfizer, Inc., NY) and occluding spring emboli and a follow-up ultrasound is performed to assess for any evidence of post-procedural intra-peritoneal hemorrhage. We have performed 22 of our 24 allogeneic islet transplants using this technique and have not had any significant procedure-related complications such as bleeding or infection. A follow-up ultrasound is performed 1 day after transplant and most patients are discharged 2–3 days after transplantation.

Summary

After a significant period of trial and error with this new enzyme, we believe that we have successfully adapted our islet isolation protocol to consistently produce high quality islets suitable for clinical use. As this review demonstrates, we have not changed the overall manufacturing and transplantation process but rather have analyzed and refined the individual components to optimize preparation of the pancreas and take advantage of the activity characteristics of the cGMP SERVA blend. Such a systematic approach provides a framework which can be used to guide additional troubleshooting efforts and help determine the optimal conditions for newer enzyme blends that may become available for islet isolation in the future.

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Table 1

DONOR CHARACTERISTICS OF ORGANS SUCCESSFULLY PROCESSED FOR TRANSPLANTATION USING THE SERVA BLEND

Characteristic	Mean ± SD (n=14)
Age (years)	37 ± 12 (Range: 17–53)
Height (cm)	181 ± 7
Weight (kg)	105 ± 15
BMI (kg/m^2)	32 ± 5.7
Cold Ischemic Time (hours)	5.9 ± 1.5
Minimum Blood Glucose (mg/dL)	113 ± 39
Maximum Serum Blood Glucose (mg/dL)	284 ± 98
Hemoglobin A1c (%)	5.5 ± 0.4

Table 2

PROCESSING PARAMETERS, ISLET CHARACTERISTICS, AND CLINICAL OUTCOMES OF ISLET ISOLATIONS USING THE SERVA BLEND

Processing Parameter	**Mean ± SD (n=14)**
Collagenase Dose (U/Pancreas)	1747 ± 212
Neutral Protease Dose (U/Pancreas)	244 ± 21
Digestion Time (minutes)	16.0 ± 2.0
Trimmed Pancreas Weight (g)	102 ± 18
Pancreas Digestion (%)	70 ± 17
Islet Characteristic	**Mean ± SD (n=14)**
Purified IEQ/g Trimmed Pancreas (IEQ)	5,862 ± 1,843
Cultured IEQ/g Trimmed Pancreas (IEQ)	5,458 ± 1,455
Post-Culture Recovery (%)	95 ± 15
Total IEQ at Transplant (IEQ)	544,745 ± 114,862
Islet Viability (%)	98.2 ± 2.3
Glucose Stimulation Index (μLU/ml)	3.15 ± 2.6
Clinical Outcomes	**n**
Pts with C-peptide at 1 month after Transplant	10/10*
Patients Insulin Independent ≥ 30 days	9/10*
Duration of Independence (months)	>24, >24, >21, >18, >13, >3, >6, >3, 4

*6 patients received single islet transplants and 4 patients received 2 islet transplants. All patients produced C-peptide after their first transplant.

Table 3

COMPARISON OF CLINICAL ISLET ISOLATIONS USING TWO DIFFERENT COLLAGENASE ENZYME BLENDS

Characteristic	SERVA BLEND (n=14)	LIBERASE™ HI (n=9)	P Value*
Donor Age (years ± SD)	37 ± 12	45 ± 10	**0.046**
Donor Weight (kg ± SD)	105 ± 15	122 ± 27	**0.049**
Donor BMI (kg/m^2 ± SD)	32.0 ± 5.7	37.7 ± 8.6	**0.036**
Collagenase Dose (U/Pancreas ± SD)	1,747 ± 212	2,420 ± 85	**<0.001**
Digestion Time (minutes ± SD)	16.0 ± 2.0	16.0 ± 1.7	0.5
Purified IEQ/g Trimmed Pancreas (IEQ ± SD)	5,862 ± 1,843	4,888 ± 1,683	0.1
Total IEQ at Transplant (IEQ)	544,745 ± 114,862	492,958 ± 125,477	0.17
Proportion of isolations suitable for clinical transplantation (%)	14/19 (74%)	9/21 (43%)	**0.049**@

*Student's t-test

@Fisher's exact test