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A Comparison of Pancreatic Islet Purification using Iodixanol with University of Wisconsin Solution and with Na-Lactobionate and Histidine Solution

Yoshiki Nakashima[1], Chika Miyagi-Shiohira[1], Nana Ebi[1], Eri Hamada[1], Yoshihito Tamaki[1], Kazuho Kuwae[1], Naoya Kobayashi[2], Issei Saitoh[3], Masami Watanabe[4], Takao Kinjo[5], and Hirofumi Noguchi[1]

Abstract

Purification of pancreatic islets is an important step in islet isolation for islet transplantation. In this study, to investigate how a solution composed mainly of Na-lactobionate and histidine (HL) influences the purification of islets, iodixanol was added to a purified solution for porcine islet isolation. A solution (IU) made by adding iodixanol to University of Wisconsin solution and a solution (IHL) made by adding iodixanol to HL solution were used to evaluate the islet isolation performance. We noted no significant differences between the two purification methods with regard to the islet yield, survival rate or purity, score, or stimulation index. These results show that IHL solution is as useful as IU solution for islet purification.

Keywords

Islet transplantation, islet isolation, islet purification, bottle purification, continuous density gradient

Introduction

Pancreatic islet transplantation is an alternative to pancreatic transplantation for achieving tight glucose control in type 1 diabetic patients who experience hypoglycemic unawareness despite the best care[1–5]. The pancreatic islet isolation process consists of collagenase injection, pancreatic digestion, and islet purification and is one of the most important and difficult procedures in islet isolation.

The most common method of islet purification is density gradient centrifugation based on different densities between pancreatic islets and acinar tissue[6–9]. Ficoll solution is the most common solution used for the purification of pancreatic islets[10]. We previously reported that an iodixanol-controlled density gradient with iodixanol and modified Kyoto (MK) or University of Wisconsin (UW) solution during islet purification improves the recovery rate in human islet isolation[11]. However, in studies of organ preservation using not only the pancreas[12] but also other organs (e.g. heart, liver)[13–15], there have been reports that Na-lactobionate and histidine (HL)

[1] Department of Regenerative Medicine, Graduate School of Medicine, University of the Ryukyus, Okinawa Prefecture, Japan
[2] Okayama Saidaiji Hospital, Okayama Prefecture, Japan
[3] Division of Pediatric Dentistry, Graduate School of Medical and Dental Science, Niigata University, Niigata, Japan
[4] Department of Urology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama Prefecture, Japan
[5] Division of Morphological Pathology, Department of Basic Laboratory Sciences, School of Health Sciences, Faculty of Medicine, University of the Ryukyus, Okinawa Prefecture, Japan

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Corresponding Author:
Hirofumi Noguchi, Department of Regenerative Medicine, Graduate School of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa Prefecture 903-0215, Japan.
Email: noguchih@med.u-ryukyu.ac.jp

solution has a better tissue preservation ability than UW solution. This HL solution has a high sodium content and a low potassium content, while the UW solution has a high viscosity, a high potassium content, and a low buffering capacity.

Sumimoto et al.[12] proposed the use of a new solution composed mainly of HL solution to resolve issues associated with UW solution. In the present study, we used purification solutions combining iodixanol with UW solution (IU solution) and with HL solution (IHL solution). These IU and IHL solutions were then compared with determine their respective efficacies in islet purification. As the use of human pancreatic tissue from cadaveric donors for research is unlawful in Japan, we used porcine pancreatic tissue in this study.

Materials and Methods

Reagents

UW solution (Viaspan™) was obtained from Astellas Pharma Inc. (Tokyo, Japan). Extracellular-type trehalose-containing Kyoto (ETK) solution was obtained from Otsuka Pharmaceutical Factory (Naruto, Japan). ETK solution contains trehalose and gluconate and it has a high sodium-low potassium composition with low viscosity. Iodixanol (Optiprep®) was obtained from AXIS-SHIELD PoC AS (Oslo, Norway). The Gradient Mixer and Ricordi Isolator Tubing Set were obtained from Biorep Technologies (Miami, FL, USA). Roswell Park Memorial Institute (RPMI)-1640 medium was obtained from Nacalai Tesque (Kyoto, Japan). Dithizone was obtained from Sigma-Aldrich Co., LLC (St. Louis, MO, USA). All other materials used were of the highest commercial grade.

Pancreas Procurement and Islet Isolation

Three-year-old porcine pancreata (female, $n = 5$) were obtained from a local slaughterhouse. We used 3-year-old pigs since the pancreas size is large and easy to control (weighing about 100 g), and it is also easy to cannulate into the main pancreatic duct. The operation was started about 10 min after the cessation of the heartbeat. All pancreata were procured using a standardized technique to minimize the warm ischemic time (WIT). After removing the pancreas, we immediately inserted a cannula into the main pancreatic duct. The pancreas was weighed, and 1 ml/g pancreas weight of MK solution (ETK solution with ulinastatin) was infused through the intraductal cannula[16]. The pancreata were placed into the MK solution container at 4°C for about 18 h until the islet isolation procedure[17]. The 'operation time' was defined as the time from the start of the operation until the removal of the pancreas. The WIT was defined as the time from the cessation of the heartbeat until the placement of the pancreas into the preservation solution. The cold ischemic time (CIT) was defined as the time from the placement of the pancreas into the preservation solution until the start of islet isolation.

Islet isolation was conducted as previously described[4]. In brief, the ducts were perfused in a controlled fashion with a cold enzyme blend of Liberase MTF (1.0 mg/ml) with thermolysin (0.075 mg/ml; Roche Diagnostics Corporation, Indianapolis, IN, USA). The distended pancreas was then placed in a Ricordi® Chamber and gently shaken. While the pancreas was being digested by recirculating the enzyme solution through the Ricordi® Chamber at 37°C, we monitored digestion with dithizone staining (2 mg/ml final concentration; Sigma Chemical Co.) by taking small samples from the system. Once digestion had been confirmed to be complete, the dilution solution (Center for Promotion of Education and Science, Hiroshima, Japan) was introduced into the system. The system was then cooled to stop further digestive activity. The digested tissue was collected in flasks containing 5% fetal bovine serum (FBS; GIBCO-Invitrogen, Carlsbad, CA, USA). The phase I period was defined as the time from the placement of the pancreas in the Ricordi® Chamber until the start of digested pancreas collection. The phase II period was defined as the time between the start and end of collection. The tissue was collected and washed with fresh medium to remove the enzyme after the digestion, and the digested tissue was then incubated in UW solution for 30 min before purification[18].

Islet Purification

Islets were purified with a continuous density gradient of iodixanol-UW solution as previously reported[11,19–21] or iodixanol-HL solution (Center for Promotion of Education and Science). We combined iodixanol with UW solution (IU solution) and HL solution (IHL solution) to generate new purification solutions. Low-density (1.075 g/cm^3) and high-density (1.085 g/cm^3) solutions were produced by changing the volumetric ratio of iodixanol and UW/HL solution, as reported previously[8]. Before purification by IU or IHL solution, we calculated the density of the digested tissue. During this step, digested tissue (0.2 ml) (after incubation in UW solution and prior to purification) was added to six 5-ml test tubes (Corning Japan, Tokyo, Japan) of different densities (1.085, 1.090, 1.095, 1.100, 1.105, 1.110 g/cm^3), and these tubes were centrifuged at 235×g (1000 rpm) for 5 minutes. The density at which most of the digested tissue floated was defined as the density of the digested tissue. According to the outcome of the density determination step, we determined the necessary density of the high-density IU or IHL solution and added an appropriate amount of iodixanol to the solution. Islet purification was performed by digested tissue (≤20 ml of tissue/run) with continuous gradients using low-density and density-adjusted high-density solutions in bottles (size 500 ml; NALGENE, Rochester, NY, USA). The gradient was produced with a gradient marker (Biorep Technologies) and candy cane-shaped stainless-steel pipes (length 30 cm; UMIHIRA, Kyoto, Japan). The theoretical density on each continuous gradient is shown in Fig. 1B. The bottles were centrifuged at

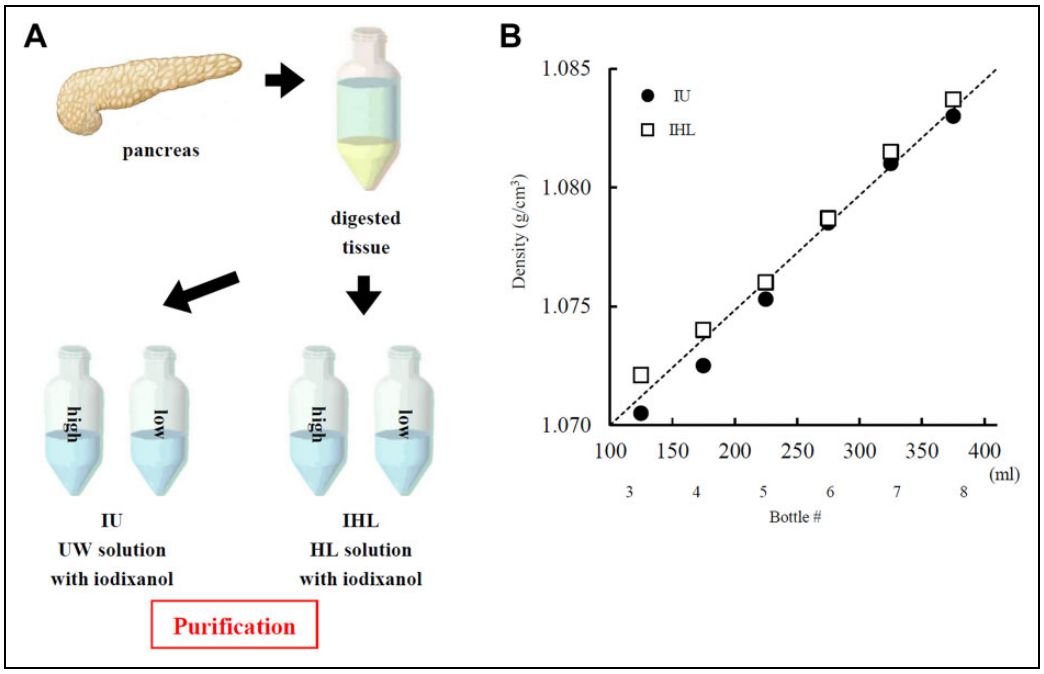

Fig. 1. Density of the IU and IHL gradients. (A) A schematic drawing of the islet purification method. (B) Theoretical density of the IU and IHL gradient. Low-density (1.075 g/cm^3) and high-density (1.085 g/cm^3) solutions were produced by changing the volumetric ratio of iodixanol and UW/HL solution.
HL: Na-lactobionate and histidine; IHL: iodixanol and HL; IU: iodixanol and University of Wisconsin solution; UW: University of Wisconsin solution.

235 × g (1000 rpm) for 5 min at 4°C. After centrifugation, approximately nine fractions (50 ml each) were collected and examined for purity.

The Assessment of the Islet Function

The islet yield was determined with dithizone staining (2 mg/ml). The crude number of islets in each diameter class was determined by counting the islets after dithizone staining using an optical graticule (Olympus, Tokyo, Japan). The crude number of islets was then converted to the standard number of islet equivalents (IEs) with the diameter standardized to 150 μm[22]. The gross morphology was qualitatively assessed by two independent investigators scoring the islets for shape (flat versus spherical), border (irregular versus well-rounded), integrity (fragmented versus solid/compact), uniformity of staining (not uniform versus perfectly uniform), and diameter (least desirable: all cells <100 μm/most desirable: more than 10% of cells >200 μm)[18]. Islet recovery was defined as the percentage of IEs recovered after purification divided by the IEs before purification. The islet viability after purification was assessed using double fluorescein diacetate/propidium iodide (FDA/PI; Sigma-Aldrich) staining to simultaneously visualize the living and dead islet cells[2,22,23]. A total of 50 islets were inspected, and their individual viability was determined visually. The average viability was then calculated[4].

The islet function was assessed by monitoring the insulin secretory response of the purified islets during glucose stimulation using the procedure described by Shapiro et al.[2,23]. In brief, 1200 IEs were incubated with either 2.8 or 25 mM glucose (Sigma-Aldrich) in RPMI-1640 medium (Sigma-Aldrich) for 2 h at 37°C and 5% CO_2. The supernatants were collected, and the insulin levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Insulin ELISA kit; ALPCO Diagnostics, Windham, NH, USA). The stimulation index was calculated by determining the ratio of insulin released from the islets in high glucose to the insulin released in low glucose. The data were expressed as the mean ± the standard error of the mean (SE).

The in Vivo Assessment

The 6-week-old nude mice (male: Charles River Laboratories Japan, Inc., Kanagawa, Japan) ($n = 20$) were rendered diabetic by a single intraperitoneal (i.p.) injection of 220 mg/kg of streptozotocin (STZ; Sigma-Aldrich). Hyperglycemia was defined as when a glucose level of >350 mg/dl was detected twice (consecutively) after the administration of STZ. The 2000 IE porcine islets obtained from each group were transplanted into the renal subcapsular space of the left kidney of a diabetic nude mouse, as described previously[24–26]. During the 30-day post-transplantation period, the non-fasting blood glucose levels were monitored three times per week. Normoglycemia was defined when two consecutive blood glucose level measurements were <200 mg/dl. No statistically significant differences were observed in either the

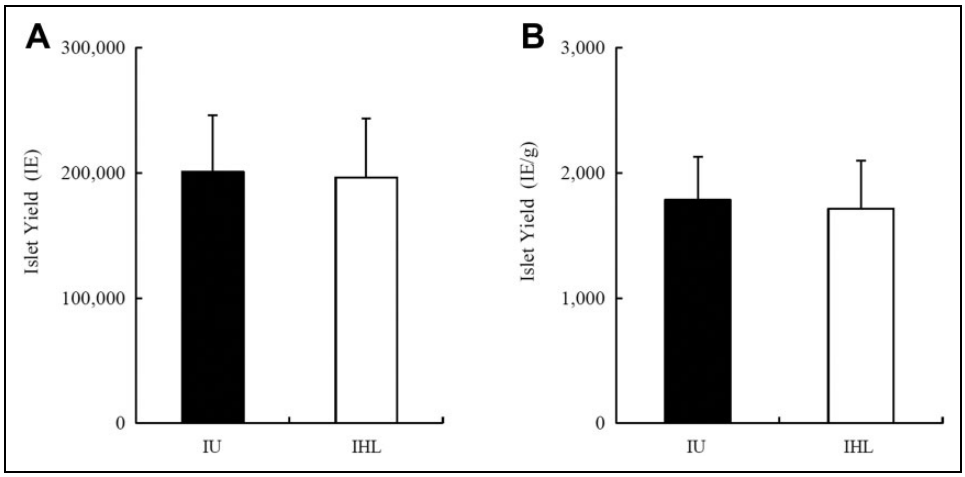

Fig. 2. The islet yields. (A) The islet yield after purification. (B) The islet yield per pancreas weight after purification. IU ($n = 5$). IHL ($n = 5$). IE/g; islet equivalent per pancreas weight. The data are expressed as the mean ± SE.
IE: islet equivalent; IHL: iodixanol and HL; IU: iodixanol and University of Wisconsin solution; SE: standard error.

pre-transplantation blood glucose levels or the pre-transplantation body weight among the two groups of mice. Glucose was measured using an ACCU-CHEK Compact Plus kit (Roche Diagnostics K.K., Tokyo, Japan) in accordance with the manufacturer's instructions. All of the mouse studies were approved by the Institutional Animal Care and Use Committee of University of the Ryukyus, Japan.

Statistical Analyses

The data are represented as the mean ± SE. The differences between two groups were analyzed using Student's *t* test or the Kaplan–Meier log-rank test. *P*-values of <0.05 were considered to indicate statistical significance.

Results

Characteristics of the Isolated Porcine Islets

Porcine islet isolation was performed using IU or IHL solution. The pancreas size (g) was 110.5 ± 5.8, operation time (min) 5.6 ± 0.9, WIT (min) 27.7 ± 1.0, CIT (min) 1089.7 ± 18.7, phase I period (min) 11.4 ± 0.6, phase II period (min) 40.4 ± 3.1, amount of undigested tissue (g) 11.3 ± 1.4, islet yield before purification (IE) 605,779 ± 161,662, and islet yield before purification (IE/g) 5342 ± 1286. There were no significant differences in the islet yield after purification between the two groups (IU group: 201,159 ± 45,272 IE, 1785 ± 343 IE/g, IHL group: 196,535 ± 47,112 IE, 1714 ± 385 IE/g) (Fig. 2A, 2B) or in the post-purification recovery rate, viability, or score (Table 1). Purity of IU group was 50.0 ± 6.9 and purity of IHL was 56.8 ± 7.0. There were no significant differences between the two groups. The two solutions achieved a similar level of efficiency in islet purification.

Table 1. The Islet Characteristics after Purification.

	IU ($n = 7$)	IHL ($n = 7$)
Post-purification recovery (%)*	72.3 ± 6.0	67.5 ± 6.5
Viability (%)	95.8 ± 0.1	95.4 ± 0.3
Purity (%)	50.0 ± 6.9	56.8 ± 7.0
Score	9.1 ± 0.3	9.1 ± 0.3

The data are expressed as the means ± SE.
*Post-purification recovery (%) = IE after purification / (IE before purification/2) × 100
IE: islet equivalent; IHL: iodixanol and HL; IU: iodixanol and University of Wisconsin solution; SE: standard error.

The in Vitro Assessment

To assess the in vitro islet quality in each group, the stimulation index of the isolated islets was measured. There were no significant differences between the two solutions in the stimulation index (IU group: 1.79 ± 0.13, $n = 7$; IHL group: 1.79 ± 0.09, $n = 7$) (Fig. 3). These data suggest that the islets in the two groups were of similar quality in vitro.

The in Vivo Assessment

To assess the islet graft function of each group in vivo, 2000 IEs from each group were transplanted below the kidney capsule of STZ-induced diabetic nude mice. The blood glucose levels of 8 of the 10 mice (80.0%) from the IU group and 8 of the 10 mice (80.0%) from the IHL group decreased gradually after islet transplantation until they reached normoglycemia. The blood glucose levels remained stable thereafter (Fig. 4) and returned to the pre-transplantation levels after the removal of the islet-bearing kidneys (30 days post-transplantation). Post-transplantation normoglycemia was similarly attainable in the two groups. These data suggest that the islets of the two groups were of similar quality.

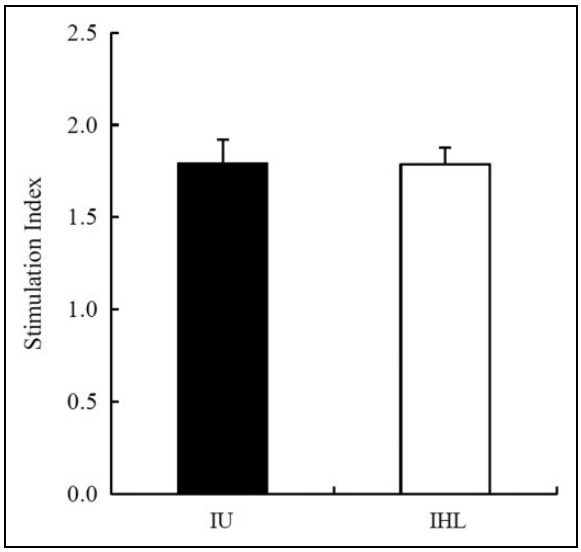

Fig. 3. The stimulation index of the isolated islets. The stimulation index was calculated by determining the ratio of insulin released from islets in high-glucose media to that released in low-glucose media. The data are expressed as the mean ± SE ($n = 5$, each).
IHL: iodixanol and HL; IU: iodixanol and University of Wisconsin solution; SE: standard error.

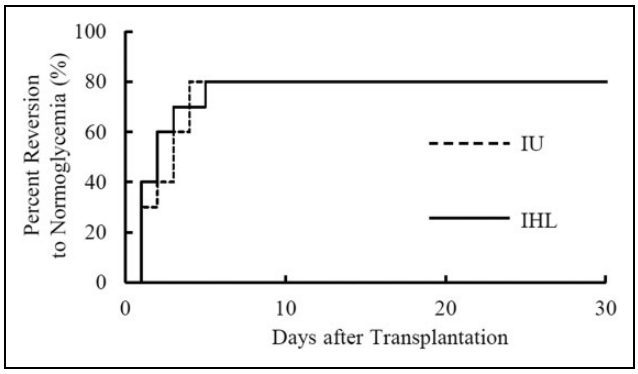

Fig. 4. Islet transplantation into diabetic nude mice. The percentage of STZ-induced diabetic nude mice, in which normoglycemia was achieved after islet transplantation, are depicted. A total of 2000 IEs were transplanted below the kidney capsule in the diabetic nude mice. Normoglycemia was defined as two consecutive post-transplant blood glucose levels of <200 mg/dl (IU group [$n = 10$]; IHL group [$n = 10$]).
IE: islet equivalent; IHL: iodixanol and HL; IU: iodixanol and University of Wisconsin solution; SE: standard error; STZ: streptozotocin.

Discussion

Islet purification using a COBE 2991 cell processor is the gold standard method for clinical islet isolation. However, the high shear force associated with the method causes mechanical damage to the islets[11]. We previously reported that shear stress was substantially reduced by bottle purification, and the size of the islets purified by the bottle method was significantly larger than that of the islets purified by COBE purification[27]. In this study, we compared IU and IHL solutions with different compositions on bottle purification. HL solution has a higher sodium content and a lower potassium content than UW (Na, 90 mEq/l; K, 45 mEq/l: [mEq/l = amount of solute (mg/l) / molecular weight (g) × atomic value]). This solution includes no hydroxyethyl starch, adenosine, dexamethasone, or insulin but does include histidine (90 mM/l) and KH2PO4 (20 mM/l)[13]. UW solution has several disadvantages such as inhibition of collagenase the activity, an enzyme used for pancreatic digestion[28,29]. Although a fact that UW solution has inhibitory activity due to collagenase is a demerit of UW solution for pancreas preservation, it is not a demerit when UW solution is used for purification solution. Indeed, we did not find any inferiority of IU solution when compared with IHL solution.

In conclusion, the IU and IHL solutions had an equivalent capacity for cell preservation when exposed to islets for about 30 minutes. These data suggest that these two solutions may both be used as islet purification solutions for pancreatic islet purification.

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Ethical Approval

All animal studies were approved by the Institutional Animal Care and Use Committee of University of the Ryukyus, Japan.

Statement of Human and Animal Rights

This article does not contain any studies with human subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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