Isolation of Adult Pig Islet
In Vitro Assessment and Xenotransplantation

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Summary
This study was conducted to develop an isolation method for adult pig islets and to investigate in vivo function after xenotransplantation as well as in vitro function of isolated islets. From the splenic portion of the glands (n = 20), 3277 ± 645 islets per gram of pancreas were isolated and recovered by a five-step dextran discontinuous density gradient method. Purity of the final preparation obtained from 20 different consecutive pig pancreata was 80-90%. In vitro incubation study revealed a significant insulin release from isolated adult pig islets in response to glucose stimulation. In vitro perifusion study demonstrated a biphasic insulin response to glucose stimulation from isolated islets. Xenotransplantation of approx 2000 isolated adult pig islets into the portal vein of diabetic Wistar rats (n = 8) significantly reduced serum glucose levels from 431 ± 24 mg/dL to 173 ± 18 mg/dL 24 h after transplantation. This study provides a development of an isolation method for adult pig islets and demonstrates that isolated islets are viable and functioning both in vitro study and in vivo study of xenotransplantation.

Key Words: Isolation of adult pig islet; incubation study; perifusion study; xenotransplantation; diabetic rats.

Introduction
Islet transplantation has long been studied as a possible alternative to whole pancreas transplantation for treatment of insulin-dependent diabetes mellitus. Although experimental islet transplantation has recently enjoyed extensive progress in different mammals, there has been little information on porcine islet isolation and transplantation. Encouraging results in clinical application of human islet transplantation for type 1 diabetes have very recently been demonstrated (1,2). However, broad application of human islet transplantation will be limited in the future because of the difficulty in meeting the requirement of islet tissue on the basis of human organ donation. Alternative sources of donors for islet tissues could be swine (3) chiefly because of their abundance and the structural similarity between human and porcine insulin. This study was
conducted to develop an isolation method for adult pig islets and to investigate in vivo function after xenotransplantation in diabetic rats as well as in vitro function of isolated adult pig islets.

**Materials and Methods**

**Slaughterhouse**

The glands of 5–10-mo-old pigs weighing 90–150 kg (n = 20) were obtained at a local slaughterhouse. The glands were removed from live animals during the exsanguination phase of the slaughtering process. Warm ischemia time ranged from 20 to 25 min. The glands were washed with cold saline (4°C), followed by the perifusion of the glands with 200–250 mL of cold Eurocollins solution (4°C; Midorijoji, Japan). Only the splenic portion of the gland was used for islet isolation. After cannulation of the pancreatic duct with a 3-Fr iv catheter, 10–15 mL of Hanks’ solution containing 0.02% trypsin inhibitor and 10% calf serum, but no collagenase, were injected through the pancreatic duct. In eight pigs, these procedures were performed in the hot season (from June to September). In 12 pigs, these procedures were performed in other seasons (from October to May). In seven other glands of 5–10-mo-old pigs weighing 80–160 kg, pancreatic duct cannulation was not performed at a slaughterhouse in the hot season. Fat tissue, vessels, and membranes were carefully dissected from the gland and discarded. Thereafter, the pancreata (splenic portion) were transported from the slaughterhouse to the islet isolation laboratory in cold Eurocollins solution. The glands were preserved for 40–50 min between procurement in the slaughterhouse and beginning of isolation procedures in the laboratory.

**Laboratory**

Immediately after transportation of pancreata in the islet isolation laboratory, 200 mL of Hanks’ solution containing 2.5 mg/mL collagenase (Nitta; S-1), 0.01% DNase, 0.02% trypsin inhibitor, and 10% calf serum were infused into the pancreatic duct through the catheter for 10 min (n = 20). In seven glands with no cannulation of pancreatic duct at a slaughterhouse in the hot season, a 3-Fr iv cathe-
ter was cannulated into the pancreatic duct. Two hundred mL of the same Hanks’ solution were infused through the cannula. After ductal collagenase distension, digestion was performed at 37°C in the water bath for 20 min. The digested pancreata were teased into small pieces by forceps in Hanks’ solution (4°C) and filtrated through wire meshes (850 and 425 μm).

Isolation of islets from individual pancreata was performed by a modification of dextran discontinuous density gradient method (4, 5) using Eurocollins solution. In this study, a five-step dextran discontinuous density gradient was used for isolation of adult pig islets and details are as follows: Eurocollins solution (T₀, 1.020, 6 mL), and dextran Eurocollins solution (T₁, 1.075, 6 mL; T₂, 1.080, 4 mL; T₃, 1.085, 4 mL; and T₄, 1.094, 14 mL). Purification of the islets was performed by centrifugation on dextran discontinuous density gradients. Islets were collected from the interface between T₀ and T₁ layers. Dithizone (6) was used to optimize identification of islets during the isolation procedure and to determine the purity of the final preparation. Islets were identified by their pink color. Purity was estimated by comparing the relative proportions of dithizone-positive and unstained tissue. The diameter of each islet was measured with an optical graticule incorporated into the eyepiece. Results were also expressed in terms of islet equivalents (islet equivalent, IE: 1 islet with diameter of 150 μm). The isolation procedure was completed within 2 h. Isolated and purified islets were subjected to in vitro and in vivo studies.

**In Vitro Study**

**Incubation Study**

Preincubation of approx 20 isolated pig islets were performed in KRBB solution with 3.3 mM glucose containing 0.2% bovine serum albumin (BSA) for 30 min. After changing solution, the same islets were stimulated with concentration of 3.3 mM of glucose or 16.7 mM of glucose in KRBB solution containing 0.2% BSA for 30 min, respectively.

**Perfusion Study**

Perfusion of isolated pig islets was performed with the technique described by Knudsen et al. (7).