Metabolic Control After Autotransplantation of Highly Purified Canine Pancreatic Islets Isolated in UW Solution

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TECHNICALLY, pancreatic islet transplantation as a therapeutical approach to human diabetes has become more realistic. The lack of efficient means of purifying islets from contaminating exocrine tissue however remains a major impediment to safe islet transplantation. Recently, we demonstrated that the use of UW organ preservation solution for the isolation of canine islets consistently results in >90% purified islets.[1,2] In order to test the viability of UW-isolated islets, we introduced this new approach to islet isolation in our current study of metabolic control after autotransplantation of canine islets.

MATERIALS AND METHODS

In 5 normal dogs (adult partially inbred beagles, 11 to 15 kg; Central Institute for the Breeding of Laboratory Animals, Zeist, The Netherlands), general anesthesia was induced with sodium thiopental (Nesdonal, Rhône-Poulenc, Paris, France) 25 mg/kg body weight IV, and maintained with a N_2O/O_2 (1:1) halothane (1% to 2%) mixture following intubation. The pancreas was mobilized with its major vascular connections preserved, and two Luer 20- or 23-gauge stub adaptors were inserted into the main pancreatic duct, one directed to the left, and the other to the right lobe. The blood vessels were clamped, the gland was removed, weighed, and immediately (warm ischaemia time <90 seconds) processed for islet isolation by intraductal infusion of a high-volume (3 to 4 mL/g tissue) cold collagenase solution (Sigma, St Louis; 1600 U/mL Hanks' solution) using a peristaltic pump. The dogs were maintained under general anesthesia with the abdomen provisionally closed while the pancreas was processed, ~4 hours. Subsequently (cold ischaemia time <30 minutes), the pancreatic tissue was processed by stationary digestion at 38°C and after addition of ice-cold isolation medium, either the UW solution supplemented with 0.4% bovine serum albumin (n = 3) or RPMI tissue culture medium supplemented with 10% newborn calf serum (n = 2), gentle teasing using forceps, discarding ducts and large blood vessels, syringing (14 G), and filtration (mesh 400 μm). The dispersed tissue was spun in discontinuous dextran density gradients (31%, 25%, 23%, and 11% in HBSS) and purified islets were collected from the uppermost 2 interfaces. Finally, the islet suspension was washed using plain RPMI, resuspended in 20 mL (highly purified islets) to 50 mL (partially purified islets) RPMI with 10% dog's own serum, and autotransplanted into the spleen of the dog by reflux via 1 to 2 splenic vein tributaries, while clamping the splenic pedicle and short gastric veins with prior systemic injection of 100 U/kg body weight heparin. Islet yield was determined from the number and volume (calculated from the mean diameter) of freshly isolated dithizone-stained islets. Purity was estimated from amylase recovery in sonicated samples, and expressed (assuming 98% acinar volume in the pellet of tissue prior to purification) as the volume fraction of islet tissue. Postoperatively, the regular diet of semiliquid dog food (Complete Dog Food D-B, Hope Farms, Woerden, The Netherlands) was supplemented with ~2 g/d protease-lipase-amylase pellets (Pancreas Granulaat, Organon, Oss, The Netherlands) for exocrine substitution. Graft function was assessed up to 3 months by determining the peripheral glucose and insulin response to (1) fasting 18 hours overnight; (2) an IV glucose bolus injection (0.5 g/kg glucose; IVGTT); (3) IV arginine bolus injection (2 g) during steady state 35 mmol/L glucose, clamped using a variable glucose infusion (AT); and (4) regular mixed meals consumed within 15 minutes.

RESULTS

The islet dose at transplantation ranged from 3500 to 13,000 islets (Ø > 75 μm)/kg body weight. Islet counts during islet isolation using RPMI as the isolation medium (n = 2) indicated, in both cases, an inadequate islet mass for density gradient purification, which invariably reduces total islet yield by ~50%. In these 2 cases, only part (12% and 50%, respectively) of the digest was used for purification. Thus graft volume (purified plus nonpurified islets) could be reduced to 14 and 4 mL, respectively, while maintaining the islet dose at >6000 islets/kg body weight. Over 90% of purified islets resulted in a calculated graft volume ≤0.3 mL by using the UW solution as the isolation medium prior to dextran purification (n = 3). One animal became overtly hyperglycemic within 7 days after receiving 3500 UW-isolated islets/kg, although well-preserved islets could be demonstrated by immunostaining for insulin. All other grafts (>6000 islets/kg body weight) were successful [normal fasting glucose; Fig 1(D)] but demonstrated, compared to preoperative values, a 50% reduced glucose tolerance (K value 1.2 ± 0.1 vs 2.2 ± 0.4), a 70% reduced integrated insulin response [Fig 1(A)] to IV glucose bolus injection (IVGTT), and a 90% reduced insulin secreting capacity as assessed by arginine injection [AT; Fig 1(B)] during a 35 mmol/L glucose clamp (P < .01). Postprandially moderate hyperglycemia [~10 mmol/L; Fig 1(D)] and in contrast to the response following IV glucose and arginine, a normal integrated insulin response (3705 ± 624 vs 3896 ± 508 120 min · mU/L), was observed [Fig 1(C)].

DISCUSSION

In dogs, "one-to-one" transplantation was successful in recipients of >6000 isolated islets/kg body weight. In 1 dog

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Supported by the Diabetes Fonds Nederland, and Dupont de Nemours (UK).

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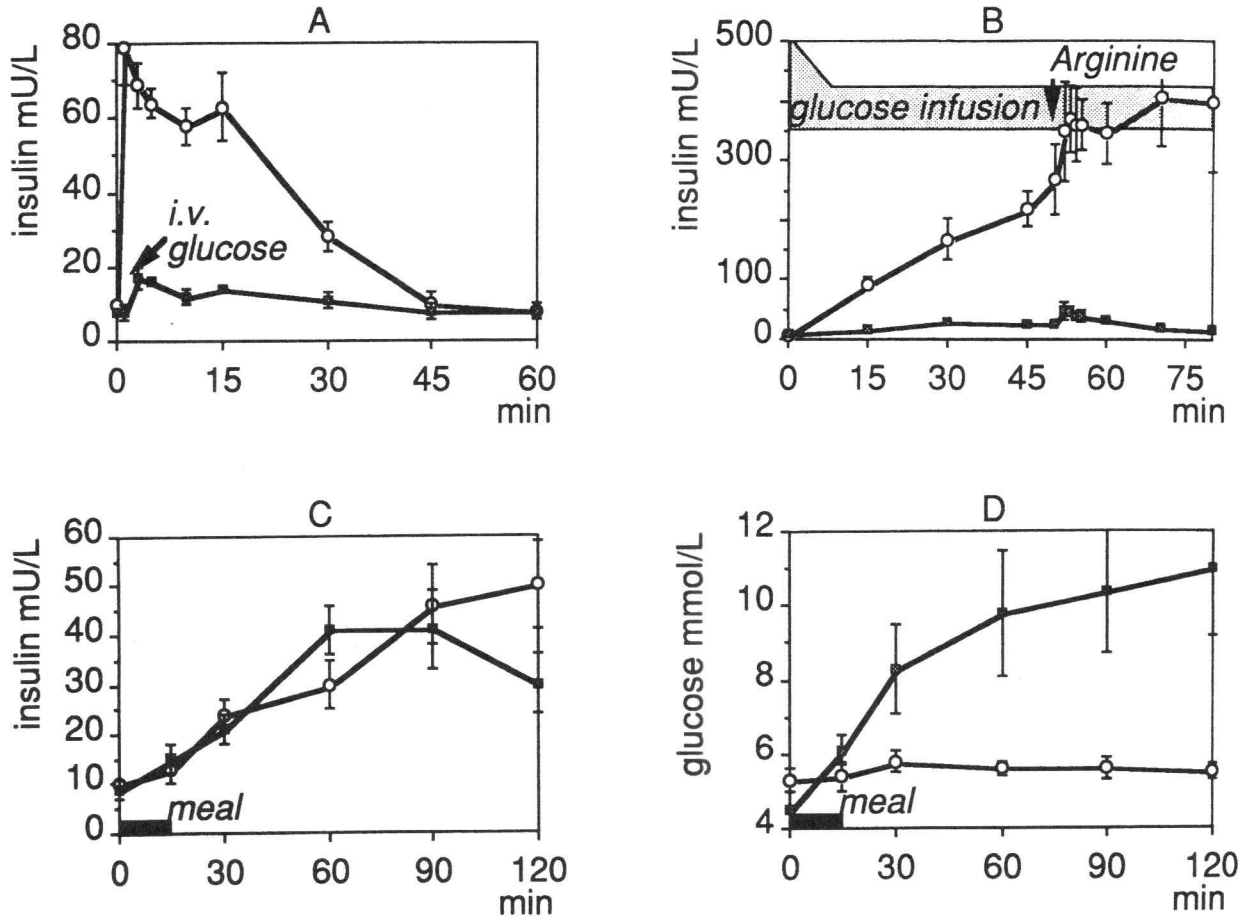

Fig 1. Insulin response to IV glucose **(A)**, arginine injection at 50 minutes during a 35 mmol/L glucose clamp **(B)**, and the insulin **(C)**, and glucose **(D)** response to a mixed-meal, prior to (o) and at 3 months after (■) successful canine islet autotransplantation (mean ± SE; $n = 4$).

who received <4000 islets/kg, hyperglycemia was evident at once after transplantation. These data confirm previous reports[3,4] indicating that ~5000 islets/kg is the threshold-dose to induce prolonged near-normoglycemia in dogs. The difference in the effect of islet transplantation on the insulin response to IV glucose or arginine stimulation and a test meal may be related to the postprandial, hyperglycemia-enhanced activation of the entero-insular axis. Preliminary data from in vitro perifusion of UW-isolated canine islets (not shown) indicate that glucose-dependent insulinotropic polypeptide (GIP) is an important mediating hormone. The cytoprotective properties of UW solution and other organ preservation solutions have been demonstrated in vitro during hypothermic preservation in cellular models for organ preservation such as renal tubule preparations[5] and hepatocytes.[6] So far, no other attempts to improve pancreatic islet preservation during isolation using solutions designed for organ cold-storage preservation, have been described. We recently demonstrated that the use of UW solution as the islet isolation solution prior to density gradient separation markedly improves purification, consistently resulting in >90% purity, as compared to variable results and a mean 30% purity using conventional (tissue culture or physiologic salt) media for islet isolation.[1,2] The present results support the view that use of UW-solution for isolation of viable highly purified islets should promote safe islet transplantation.

ACKNOWLEDGMENTS

The authors acknowledge the skillful assistance of K.H. Van der Nat-Van der Mey, G.M. Van Brakel, J.M.H. Heilen, H.A.M. Holtslag, and W.M. Kloosterman-Boele.

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