Comparison of Current Islet Isolation Techniques in Dogs

M.P.M. van der Burg, H.G. Gooszen, M.J. Field, J.P. Scherft, J.L. Terpstra, F.J. van de Woude, O.R. Guicherit, M. Frölich, and J.A. Bruijn

A major reason for failure of clinical islet cell transplantation to induce independence from insulin has been an inadequate mass of purified islets from a single donor pancreas.[1] The islet harvesting process usually involves two essential stages, collagenase digestion of the intrapancreatic connective tissue stroma followed by purification of isolated islets from dispersed acinar tissue. Two recently developed techniques for the large-scale isolation of islets from the large mammalian pancreas, intraductal infusion of collagenase either by manual injection or using a roller pump, were compared in terms of islet yield.

MATERIALS AND METHODS

In 12 dogs (adult inbred beagles, 9 to 15 kg) the left pancreatic limb was removed. General anesthesia was induced with sodium thiopental (Nesodonal, Rhône-Poulenc, France) 25 mg/kg body weight IV, and maintained with a N_2O/O_2 (1:1) halothane (1% to 2%) mixture following intubation.

Next, pancreatic segments were intraductally distended either by manual injection (group I, $n = 6$) of a low-volume (1 mL/g tissue) prewarmed (38°C) collagenase solution (Sigma, St. Louis, USA; type V or XI, 4800 U collagenase/mL Hanks' solution plus 15 mmol/L Ca^{2+}) or by infusion of a high-volume (3-4 mL/g tissue) cold collagenase type XI solution (1600 U/mL Hanks' solution) using a peristaltic pump (group II, $n = 6$). Subsequently the tissue was processed by stationary digestion at 38°C, and (using cold RPMI-1640 tissue culture medium as the isolation solution) teasing using forceps, discarding ducts and large blood vessels, syringing and filtration (mesh 400 μm). Trapped tissue was discarded. In group I the dispersed tissue was spun in Ficoll gradients (25, 23, 20, and 11% in HBSS). In group II the dispersed tissue was spun in dextran gradients (31, 25, 23, and 11% in HBSS). Purified islets were collected from the upper-middle and top interfaces. Islet yield was determined from the number and volume (calculated from the mean diameter) of freshly isolated islets, and expressed as a percentage of the stereologically determined mean pancreatic islet content in control pancreata ($n = 4$).

RESULTS

Prior to purification over 95% of the total number of isolated islets were cleanly separated from dispersed acinar tissue, irrespective of the type of collagenase or method of infusion used. However, following infusion (group II), as compared to the manual injection method, distension was more uniform, dispersion of tissue after digestion occurred more spontaneously, and less tissue was trapped and discarded on screening. Isolated islets ranged from 25 to 250 μm diameter in group I and 25-400 μm in group II. Using the duct injection method (group I) total islet yield (mean ± SE) amounted to 14 ± 5% of the mean pancreatic islet content in control dogs. Ficoll purification further reduced islet yield by ~50% ($P < .02$). In addition on Ficoll- as opposed to dextran-purification islet size distributions shifted to smaller diameters ($P < .08$), suggesting fragmentation of islets to have occurred. Using the duct infusion method (group II) islet yield averaged 76 ± 13% of pancreatic islet content prior to ($P < .01$ vs group I), and 41 ± 14% after dextran purification ($P < .02$). Dextran-purified islets maintained overnight in tissue culture, demonstrated a biphasic insulin response with a 6- to 3-fold increase over basal release ($P < .001$) following glucose stimulation (from 3 to 10 mmol/L) by perifusion with RPMI (Fig 1).

DISCUSSION

As compared to manual duct injection of collagenase, isolated islet yield increased 5-fold and less fragmentation

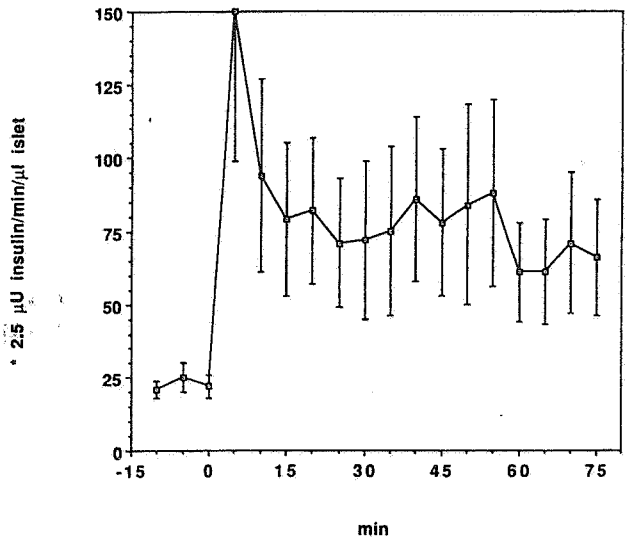

Fig 1. Mean (± SE) insulin response to glucose stimulation from 3 to 10 mmol/L in RPMI obtained by perifusion of dextran-purified isolated canine islets ($n = 5$).

From the Departments of Surgery, Cell Biology, Nephrology, Endocrinology and Pathology, University Hospital Leiden, Leiden, The Netherlands, and the Department of Surgery, University of Minnesota, Minneapolis, Minnesota.

Supported by the Diabetes Fonds Nederland.

Address reprint requests to H.G. Gooszen, MD, PhD, Department of Surgery, University Hospital Leiden, PO Box 9600, 2300 RC Leiden, The Netherlands.

0041-1345/90/$3.00/+0

of islets occurred using duct infusion. Key elements of the superior technique appeared to be a more uniform distension of the gland and the higher volume ratio of collagenase to tissue. We conclude that combining the duct infusion technique for collagenase isolation of canine pancreatic islets and dextran gradients for purification consistently results in high yields of purified islets and should promote islet transplantation studies.

ACKNOWLEDGMENTS

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

REFERENCE

1. Gray DWR, Morris PJ: Transplantation 43:321, 1987