Comparison of Islet Isolation Techniques in Dogs: Over 90% Purified Islets Using UW Solution

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OVER the last several years, various techniques have been described for the isolation of islets of Langerhans from large mammalian pancreases. The efficacy of islet isolation in humans, however, must improve to allow clinical islet transplantation. The lack of an efficient means of purifying islets from contaminating exocrine tissue remains a major impediment to safe islet transplantation.[1]

The two major isolation techniques usually performed in large experimental animals, intraductal infusion of collagenase by either manual injection or use of a roller pump, were compared in terms of islet yield. In addition, encouraged by the recent demonstration of successful long-term cold storage of the canine pancreas using UW solution,[2,3] we tested the cytoprotective properties of UW solution under hypothermic conditions during islet isolation, as compared with currently standard isolation solutions, such as RPMI tissue culture medium and Hank's balanced salt solution (HBSS).

MATERIALS AND METHODS

In 14 dogs (adult inbred beagles, 9-15 kg), the left pancreatic limb was removed. General anesthesia was induced with sodium thiopental (Nesdonal, Rhône-Poulenc, France) 25 mg/kg body weight intravenously, and maintained with a N_2O/O_2 (1:1) halothane (1-2%) 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 HBSS with 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 HBSS) using a peristaltic pump (group IIa, $n = 4$, and group IIb, $n = 4$). Subsequently, the tissue was processed by stationary digestion at 38°C and, at 0-4°C, by 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. In group I and IIa for all steps before and after Dextran purification, RPMI1640 supplemented with 10% newborn calf serum was used. In group IIb, instead of RPMI before density gradient purification, the UW solution supplemented with 0.4% bovine serum albumin was used. 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 pancreatic islet content. Purity was estimated from amylase recovery in sonicated samples and expressed (assuming 98% acinar volume in the pellet of tissue before purification) as the volume fraction of islet tissue.

RESULTS

Before 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 with the manual injection method, distention was more uniform, dispersion of tissue after digestion occurred more spontaneously, and less tissue was trapped and discarded on screening. Isolated islets ranged from 25-400 μm in diameter. Using the duct injection method (group I), total islet yield (mean ± SE) amounted to 14 ± 5% of pancreatic islet content. 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, similar results were obtained with respect to islet yield in group IIa as compared with group IIb. Islet yield averaged 73 ± 8% of pancreatic islet content before ($P < .01$ vs group I) and 40 ± 8% after Dextran purification ($P < .02$). Moreover, the use of UW solution as the isolation medium markedly improved density gradient purification, resulting in 91 ± 3% purity (group IIb) as compared to 31 ± 9% using tissue culture medium (group IIa) for isolation ($P < .005$). Immunoperoxidase staining for insulin of sections of tissue pellets confirmed these results. Dextran-purified islets maintained overnight in tissue culture demonstrated a biphasic insulin response with a 4.7-fold increase over basal release following glucose stimulation (from 3 to 10 mmol/L) by perifusion with RPMI ($P < .001$).

DISCUSSION

As compared with manual duct injection of collagenase, isolated islet yield increased 5-fold using duct infusion. Key elements of the superior technique appeared to be a

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more uniform distention of the gland and the higher volume ratio of collagenase to tissue. It is suggested that the markedly improved efficacy of density gradient purification using UW solution as the isolation medium, resulting in 90% purity as compared with 30% using tissue culture medium for isolation, may be attributed to UW preservation of normal islet and acinar tissue density. We conclude that the use of UW solution for isolation of pancreatic islets, a new approach to islet isolation, consistently results in highly purified islets and should promote safe islet transplantation.

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