Pancreatic Islet Isolation With UW Solution: A New Concept

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OVER the last years improved techniques developed in the laboratory for isolating islets of Langerhans from the large mammalian pancreas have increased yield to the point where human islet transplantation for the treatment of insulin-dependent diabetes mellitus 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.[1] Recently a new cold-storage organ preservation solution was developed at the University of Wisconsin (the UW solution), demonstrating successful long-term preservation of the canine pancreas.[2,3] Encouraged by these results we tested the cytoprotective properties of UW solution under hypothermic conditions during islet isolation, as compared to currently standard isolation solutions like RPMI tissue culture medium and Hank's balanced salt solution (HBSS).

MATERIALS AND METHODS

Islet isolation from the canine left pancreatic limb (n = 8) was performed by intraductal infusion of collagenase with digestion at 38°C, and (at 0 to 4°C) discarding ducts and large blood vessels, gentle syringing with expulsion over a 400 μm filter. Trapped tissue was discarded. The dispersed tissue was spun in dextran gradients (densities 1.094, 1.085, 1.075, and 1.041 in HBSS) and purified islets were collected from the upper middle (1.085/1.075) and top (1.075/1.041) interface. In group I (n = 4) collagenase in HBSS, and for all following steps, prior to and after dextran purification, RPMI supplemented with 10% newborn calf serum was used. In group II (n = 4) collagenase in HBSS solution, and instead of RPMI 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 stereologically determined mean pancreatic islet content in 4 control pancreata. 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.

RESULTS

We have found similar results with respect to islet yield in both groups, which averaged (± SE) 73 ± 8% of pancreatic islet content prior to and 40 ± 8% after dextran purification. However the use of UW solution as the isolation medium markedly improved density gradient purification, resulting in 91 ± 3% purity as compared to 31 ± 9% using tissue culture medium for isolation ($P < .005$). Immunoperoxidase staining for insulin of sections of tissue pellets confirmed these results (Fig 1). It should be noted that final purity and yield in group I were calculated for tissue obtained from the dextran top interface only, because it contained 92 ± 3% of the islet mass and only about one-third of the acinar mass at both interfaces, whereas final purity and yield in group II were calculated from the tissue collected at both interfaces since 68 ± 16% of these islets were recovered from the upper middle interface.

DISCUSSION

Both islet and acinar tissue were recovered at higher densities using the UW solution as compared to tissue culture medium prior to density gradient purification. This suggests that the virtually complete separation of islet and acinar tissue may be due to prevention of hypothermia-

Fig 1. Section of pellet of density-gradient-purified canine islets stained by an immunoperoxidase method for insulin, demonstrating absence of exocrine tissue after using UW solution as the islet isolation medium. Bar indicates 100 μm.

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induced cell swelling, and thus preservation of normal tissue density by UW solution. 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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