Human Islet Isolation and Purification From Pediatric-Age Donors

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The separation and purification of human islets from the pancreas of pediatric-age donors has been considered difficult if not impossible because of easy fragmentation of the islets and/or their incomplete separation from the enclosing acinar tissue (embedding).1,2 The purpose of this study was to develop a method to separate and purify large numbers of viable human islets from pediatric donors. After preliminary studies on swine islet isolation3 which resemble pediatric human isolation in many ways, a modification of the automated method for adult human islet isolation was developed.

MATERIALS AND METHODS

Six pancreata obtained from pediatric donors (age 7 to 18) were distended by intraductal injection of 200 to 250 mL of Hanks’ solution with 1 mg/mL collagenase (Boehringer-Mannheim, Type P) and loaded into a digestion chamber (volume 450 mL) containing a stainless steel screen (400-μm pore size), and 7 solid glass marbles.1 After a first phase of recirculation of the collagenase solution in a closed system (12 to 18") in which intrachamber temperature was progressively raised from 28°C to 37°C (flow rate: 50 mL/min), the islets were collected during a dilution phase of 15 to 30' (flow rate: 300 mL/min). Any mechanical trauma to the islets during separation was carefully avoided.3 Final purification of the islets was obtained by discontinuous density gradient separation with Euro-Collins solution used as vehicle for the Ficoll powder (Sigma, DL-400). Three layers (densities = 1.08, 1.096, and 1.037) have been used.4 After centrifugation on a COBE 2991 cell processor, the islets were retrieved from both 1.037/1.096 (purity 80% to 95%) and from 1.096/1.108 (purity 40% to 50%) interfaces. Morphologic and functional integrity of the islet preparation have been assessed following the criteria recently proposed for islet isolation assessment.5 The functional integrity of the final preparation was tested by transplantation of an aliquot of the final preparation into diabetic nude mice6 and by allotransplantation in patients who underwent total pancreatectomy as part of an upper abdominal exenteration7 for malignancy.

The main modifications from the procedure for adult human islet isolation1 consisted in the absence of a cooling circuit, the absence of shaking during the recirculation phase, the increased volume of the digestion chamber, and the increased pore size of the stainless steel screen.

RESULTS AND DISCUSSION

The results of 6 consecutive pediatric islet isolations are summarized in Table 1. An average of over 430,000 islets were obtained after the purification process, representing over 9500 islets per gram of pancreatic tissue. The purity of the final islet preparation ranged between 40% and 90% islets. Histologic studies showed that the islets obtained by this procedure were well preserved. Significant basal and stimulated C-peptide production have been shown in all patients who received pediatric islet allografts. The results of clinical islet transplantation, described in detail elsewhere,8 demonstrated that this procedure resulted in numbers of viable pancreatic islets from a single pediatric donor sufficient to prevent pancreatectomy-induced diabetes mellitus following intraportal islet allotransplantation.

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Supported by research grants from the Veterans Administration, Project Grant Nos. DK 29961 and DK 25802 from the National Institutes of Health (Bethesda, MD) and the Diabetes Research Institute Foundation (Miami, Fla).

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0041-1345/91/$3.00 + 0

Table 1. Donor Pancreas Data and Postpurification Islet Isolation Results

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<thead>
<tr>
<th>Donor Pancreas</th>
<th>Postpurification Islet Assessment</th>
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<tbody>
<tr>
<td>No.</td>
<td>Age</td>
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<td>-----</td>
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</tr>
<tr>
<td>1</td>
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<td>2</td>
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<tr>
<td>SD</td>
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*CIT = cold ischemia time.
*Estimated number of islets with an average diameter of 150 μm.

Transplantation Proceedings, Vol 23, No 1 (February), 1991: pp 783-784 783
The combination of the automated method for islet separation together with a purification process that involves the COBE cell separator represents a further step toward complete automation of cadaveric pancreatic islet isolation and purification.

REFERENCES