Morphometry of Native and Isolated Canine Islets: A New Approach to Isolation Assessment

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A MAJOR PROBLEM hampering large-scale programs of human islet transplantation is the variability of isolation outcome. Due to the intertwined effects of numerous variables, such as predonation events, donor and pancreas characteristics, organ preservation conditions, and isolation methods, the variability of isolation outcome is difficult to analyze. Knowledge of the variability attributable to intrinsic factors such as the islet and insulin content of the individual pancreas is essential for analysis of the relative importance of the many extrinsic variables that can be controlled. We therefore studied the impact of interindividual differences in islet and insulin content of the canine pancreas and other donor characteristics on isolation outcome and compared morphometric and biochemical assessment of isolation efficacy.

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Supported by the Diabetes Research Fund, The Netherlands, and Dupont de Nemours (UK).

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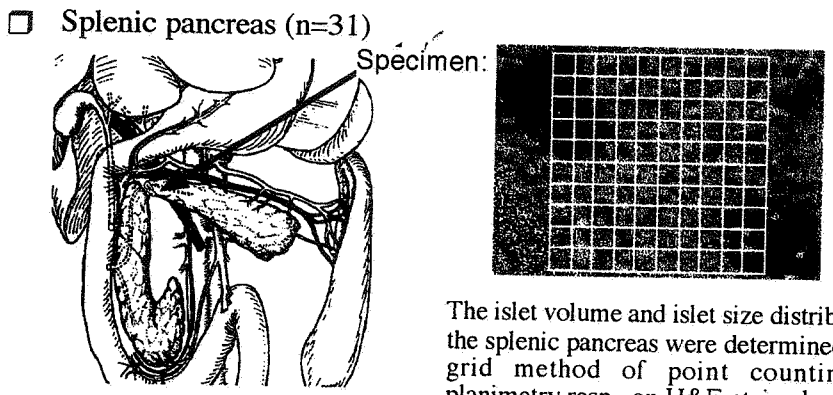

Fig 1. Canine islet isolation and assessment. Samples from the pancreas, the digest suspension, and the purified (Pure) and non-purified (Rest) fractions of the gradients were compared by morphometry and extraction of insulin and amylase.

MATERIALS AND METHODS

We report the results from 31 consecutive islet isolations from the splenic segment of the dog pancreas. Methods are illustrated in Fig 1. Briefly, the pancreas was divided where it overlies the portal vein, and a specimen from the cut end of the splenic pancreas was taken for assessment. Within 90 seconds from the onset of ischemia, islet isolation was performed by intraductal stationary collagenase (Sigma type XI) digestion. Tissue was dispersed by syringing and screening in cold (4°C) University of Wisconsin (UW) solution, and the resulting digest was purified by density gradient centrifugation. We used different density gradients for nonrelated studies and pooled the results in this study because a similar purity and similar distributions of islets and insulin were observed in the purified (pure) and nonpurified (rest) fractions of these gradients. Samples from the pancreas, the digest suspension, and gradient fractions were compared by morphometry of the islet volume and size distribution, by insulin and amylase extraction, and by microscopy to assess β-cell granulation.

RESULTS

Islet volume of the pancreas averaged 16 μL/g and varied by threefold. Digest islet yield averaged 8 μL/g and varied by ninefold. Animals too varied in age by eightfold and body weight by twofold. Differences in body weight and age explained 60% of the variance in the fractional islet volume of the pancreas and 50% of the variance in islet yield ($P < .001$). Fractional islet volume and insulin content of the pancreas also explained 50% of the variance in islet and insulin yield, respectively ($P < .001$). Both insulin (89%) and amylase (95%) recovery reflected tissue recovery (79%) in the digest; in contrast, islet recovery averaged only 49% ($P < .001$). Islets entrapped in acinar tissue were not included in our counts. Subjectively, however, the volume of entrapped islets was estimated to amount to 20%—at most—of the total volume of dithizone-stained tissue. Recovery, expressed as a percentage of digest values, in the pure fraction vs the combined pure and rest fractions of the gradient amounted to 36 vs 83% for insulin, 53 vs 64% for islet volume, 0 vs 80% for tissue volume, and 0 vs 64% for amylase. Purity was nearly 100%. Digest islet volume correlated well with islet and insulin values in the pure fraction ($r = .7$; $P < .001$). However, digest insulin did not correlate with islet volume or insulin in the pure fraction, but did correlate with insulin in the combined pure and rest fractions of the gradient ($r = .6$, $P < .001$). Comparison of native and isolated islets demonstrated a similar size distribution, insulin content, and degree of β-cell granulation.

DISCUSSION

Our findings demonstrate that the variability of islet and insulin yield may be attributed to a large extent to the variability of the native endocrine pancreas. Isolation efficacy was best documented by morphometry of the isolated and native islet population, which demonstrated a yield of 50% of the islets from the dog pancreas and no fragmentation of islets during isolation by the gentle single-endpoint collagenase digestion technique. Although insulin extraction does not discriminate between free and entrapped islets, assessment by both morphometry and extraction allowed the quantitation of entrapped islets, which demonstrated that we subjectively had underestimated the proportion of entrapped islets during islet sizing, and further documented islet integrity by demonstrating preservation of the insulin content of isolated islets. Similar studies should facilitate the analysis of other factors affecting the outcome of islet isolation for transplantation in humans.