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Improved Functional Outcome in Transplantation of Dispersed Pancreatic Islet Tissue by Intraductal Collagenase Perfusion for Islet Isolation

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Dispersed islet autografts are able to ameliorate pancreatectomy-induced diabetes. The aim of this study was to compare the two major islet-harvesting techniques usually performed in large experimental animals, in terms of islet yield (rendement), degree of purification (insulin-to-amylase ratio), functional outcome (dogs normoglycemic/dogs transplanted), and percentage engraftment of the originally transplanted tissue. The pancreases of 44 dogs (group 1) were intraductally distended by manual injection of Hanks' balanced salt solution. Thereafter, each organ was mechanically disrupted, and the tissue pieces were subjected to collagenase digestion. The pancreases of 20 dogs (group 2) were intraductally distended and subsequently perfused with collagenase by a roller pump. The organ was then mechanically disrupted and filtered through a screen. The resulting suspension was transplanted into the spleen of each animal as an autotransplant in both groups. Four to 6 wk posttransplant, splenectomy was performed; the spleen was homogenized, and insulin content was measured. The functional outcome was better in group 2 than in group 1 [15/20 (75%) vs. 14/44 (29.5%); $P = .0025$]. The islet yield was similar in both groups (27 ± 17 vs. 34 ± 13%; $P = .12$). The degree of islet purification measured as an increase in insulin-to-amylase ratio was higher in group 2 than in group 1 (12.7 ± 14.4 vs. 3.9 ± 2.2, $P = .011$), and it was higher in normoglycemic than in hyperglycemic animals in both groups. The percentage engraftment, i.e., the amount received from the spleen after splenectomy as a percent of tissue transplanted (29% in group 1 and 36% in group 2) or as a percentage of the original pancreas (4.9% in group 1 and 4.4% in group 2), was low in both groups but again was higher in normoglycemic than in hyperglycemic animals within each group (13.8 ± 7.2 vs. 1.5 ± 2.9, $P < .001$, and 6.5 ± 4.1 vs. 25 ± 37, $P < .01$, respectively). In conclusion, both the degree of engraftment and purification influence the functional outcome after dispersed pancreatic islet autotransplantation to the spleen of totally pancreatectomized dogs with purified functioning better than unpurified tissue, which is accomplished with intraductal collagenase perfusion rather than with collagenase digestion of pancreatic fragments.

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Quantification of Canine Pancreatic Islet Isolation

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In this study, we have concentrated on quantification of canine islet isolation from the left and right lobe separately, behind the background of the continuing debate on segmental- as opposed to whole-pancreas transplantation in diabetic patients. Ten left or right segments were processed with stationary digestion and dispersion according to Gray. Next, the suspension was passed over a cascade of mesh filters (1000–350 μm) and purified with Ficoll gradients. The final suspension contained islets cleanly separated from exocrine tissue. Neutral red indicated >90% viability, and glucose stimulation by perifusion elicited a biphasic insulin response with a tenfold increase over basal release ($P < .001$). Major differences between left and right segments were found by calculating total islet yield from the volume of each counted neutral red–stained particle. Small islets, rarely >75 μm, were obtained from the right lobe ($n = 3$). Mean islet yield amounted to 0.1 μl/g processed tissue (PT), and large islets (75 μm diam) amounted to 182 ± 63 islets/g PT. However, islets from the left lobe ranged from 25 to 250 μm diam; isolated islet volume was 0.9 ± 0.2 μl/g PT ($n = 6$, $P < .01$), and islet counts ranged from 3000 to 11,000/g PT, whereas large islets (75 μm) amounted to 1042 ± 223/g PT ($P < .01$). These data are in keeping with our data on the insulin distribution in the canine pancreas ($n = 9$). The right lobe contained 121 ± 10 and the left lobe 319 ± 43 μg insulin/g tissue ($P < .001$). The endocrine volume of the intact normal left lobe ($n = 4$) has been morphometrically (with immunohistochemical staining of all 4 major islet cells) calculated as 1.4 ± 0.1%, corresponding to a mean of 14 μl islet volume/g of tissue. The filtrated digest of left segments contained 2.0 ± 0.7 μl islets/g PT, so it can be concluded that 14% of pancreatic islets had been isolated. However, Ficoll purification further reduced islet yield ($P < .02$) to 5–10%, and because islet size distributions on purification shifted to smaller diameters ($P < .08$), it is inferred that Ficoll purification may lead to fragmentation of islets. Islet yield from left segments amounted to 14% of pancreatic islet volume. Because Gray's method cleanly separated canine islets, yield on purification may further improve. Volume measurement of islet yield, also recently advocated by Alderson and co-workers, apart from being more accurate than islet counts or insulin recovery, seems especially apt to evaluate the yield of different islet populations. Exclusively small islets as well as far lower yields were obtained from the right lobe.

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response to glucose stimulation and have been shown to enhance insulin secretion by β-cells. Accordingly, glucose stimulation decreases TXA_2 production by the islets of Langerhans. The urinary levels of TXB_2, the stable metabolite of TXA_2, have proven to be useful as early indicators of kidney- and heart-allograft rejection. We have also found them to be useful in the early diagnosis of lung-allograft rejection in the dog. We have also previously reported that in diabetic rats, the plasma, lung, liver, heart, and pancreas contain more TXB_2 than control animals. In diabetic (n = 6) and control (n = 6) Wistar rats, in vivo perfusion of isolated pancreases with a solution containing a physiologic glucose concentration (100 mg/dl) resulted in higher TXB_2 secretion by control rats (109 ± 28 vs. 39 ± 7 ng/h in diabetic animals, means ± SE; $P < .05$). Pancreases of normal rats, perfused with varying glucose concentrations, secreted more TXB_2 in response to low (50 mg/dl) than to high (300 mg/dl) glucose levels. Values for these experiments (n = 6) were 117 ± 23 and 34 ± 3 pg · ml^{-1} · min^{-1} ($P < .001$) for low and high glucose concentrations, respectively. The diabetic pancreases showed a low TXB_2 production even with low glucose concentrations, indirectly indicating a possible enhancement in PGI_2 liberation as an attempt to increase insulin secretion. Interestingly, the normal pancreases showed a similar response when stimulated with high glucose concentrations. Mild hyperglycemia can promote PGI_2 secretion with its beneficial antirejection effect. As an autoacoid, PGI_2 can directly enhance insulin secretion as an intracellular modulator. This can be especially useful in the transplanted pancreas, which might have lost part of its paracrine regulation. On the other hand, due to the existing balance between PGI_2 and TXA_2 production, the increased PGI_2 might inhibit TXA_2 secretion, thus avoiding its prorejection effects. The level of hyperglycemia needed to reach these goals must be determined with appropriate dose-response experiments.

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Distribution of Insulin, Glucagon, Pancreatic Polypeptide, and Somatostatin in Canine Pancreas

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As part of our current studies on segmental organ as well as islet transplantation, we have investigated the regional distribution of insulin, glucagon, somatostatin, and pancreatic polypeptide (PP). Hormones were radioimmunoassayed in acid-alcohol extracts of pancreatic tissue sampled from the proximal and distal duodenal segment (ventral lobe) and from the body and distal tail (dorsal lobe) of the beagle pancreas (n = 9). Results (means ± SE) indicated a uniform somatostatin (0.40 ± 0.04 μg/g) distribution. Although insulin, glucagon, and PP values were not significantly different between distal and proximal parts from the ventral or dorsal lobe, values from the ventral lobe (121 ± 10, 0.2 ± 0.1, and 464 ± 29 μg/g, respectively) differed markedly from dorsal lobe values (319 ± 43, 9.9 ± 1.4, and 78 ± 10 μg/g, respectively) ($P < .001$). An inverse distribution of PP and glucagon has been a general finding in the mammalian pancreas. Both a near-uniform distribution in rodent pancreases and an important nonuniform distribution in human and (incidentally) canine pancreases have been reported for insulin. In conclusion, we stress the growing evidence for an important nonuniform insulin distribution, which is especially of interest in studying the segmental pancreas in a dog model, because the canine ventral lobe amounts to 50% of the total pancreatic mass.

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