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Rapid Quantitative Assessment of the Pig Pancreas Biopsy Predicts Islet Yield

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Abstract

Background—The cost of islet procurement from donor pigs is increased by the use of organs that produce low yields. We developed an assessment system using dithizone-stained pig pancreas biopsies to enable the preselection of donor organs.

Methods—Pig pancreas biopsy slices were soaked in dithizone solution. The islets were evaluated before islet isolation by converting the islet counts (IC) to islet equivalents (IE), then determining the IE/cm², IE/IC, % >150 μm islets, and % >200 μm islets. These parameters were evaluated in 3 different areas of pancreas (duodenal, splenic, and connecting lobe; n = 42 each). Stepwise multivariate linear regression analysis was performed to assess for correlations with islet yield and decide which area of the pancreas had the most predictive value. To identify other predictors, including donor and islet isolation variables, we performed binary logistic regression analysis with significant variables from the univariate analysis (n = 67). For this analysis, the pigs were categorized into high (n = 23) and low (n = 44) yield groups.

Results—Stepwise multivariate linear regression analysis revealed that IE/cm² of the splenic lobe significantly predicted islet yield. Binary logistic regression analysis indicated that the IE/mm² of the splenic lobe was the only parameter that significantly correlated with successful pig islet isolations (*P* = .01; odds ratio 3.605). Variables associated with donor and islet isolation, such as age, gender, ischemic time, or enzyme lot, were not significantly correlated with islet yield.

Conclusion—Our study suggests that islet distribution of splenic lobe biopsies can be a reliable predictor of islet yield from pig pancreata.

Pigs could provide an abundant and ready supply of pancreatic islet xenografts that are potential sources of cellular therapeutic for the treatment of type 1 diabetes.^{1,2} Despite several investigations, the high variability of islet yields from pig pancreas remains a problem. The cost of pancreas procurement and islet isolation from donor pigs is greatly increased by the use of organs that produce low islet yields. There is increased demand for less variability among islet isolations and more economic cost savings per islet isolation to bring this treatment to clinical use. Several variables are known to affect the outcome of pig islet isolation, including the donor characteristics, pancreas procurement, and islet isolation technique.^{3,4} Also, several studies have suggested that islet histomorphology findings can

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possibly predict islet yield from pig pancreas^{5,6}; however, it has not been established how to select the pancreata which provide successful islet yield. In the present study, we developed an assessment system using dithizone-stained pig pancreas biopsies to enable the preselection of donor organs that would have acceptable islet yields.

Materials and Methods

All experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee of the University of Minnesota.

Pancreas Biopsy and Scoring

Donor pigs (Landrace, age 0.5–5.5 y) were given an intravenous injection of heparin (250 U/kg), followed by a lethal intravenous injection of sodium pentobarbital 5 minutes later. Each pig was exsanguinated by cutting the jugular vein. The pig was then eviscerated by dissecting all connective tissue near the backbone of the pig and the thoracic aorta above the celiac trunk (CT). The CT and the superior mesenteric artery (SMA) were cannulated selectively. The pancreas was flushed with 4 L arterial flush solution containing 1,000 U/L heparin. Concurrently, the pancreatic duct was cannulated and injected with 60 mL ductal preservation solution. Pancreatectomy was performed; the pancreas was dissected free of the surrounding vascular and connective tissue. Pig pancreas biopsy slices with 2 mm thickness taken after dissection were soaked in diphenylthiocarbazone (DTZ; Sigma, St Louis, MO, USA) dissolved in dimethylsulfoxide (Sigma) and Hank balanced salt solution (Mediatech, Manassas, VA, USA) for 5 minutes and then photographed under a dissecting microscope (Fig 1). The islets were evaluated before isolation by converting the islet counts (IC) to islet equivalents (IE), then determined the IE/cm² (and IE/mm²), IE/IC, % >150 μ m islets, and % >200 μ m islets. These parameters (DTZ score) were evaluated in 3 different areas of the pancreas⁷: duodenal lobe, splenic lobe, and connecting lobe (n = 42 each). The pancreas was then intraductally distended with cold (4°C–8°C) enzyme solution containing Liberase PI (Roche Molecular Biochemicals, Indianapolis, IN, USA). The distended pancreas was preserved in cold preservation solution. Warm ischemia time was defined as the time from cardiac death to start of arterial flush. Cold ischemia time was defined as the time from end of warm ischemia to start of pancreas digestion.

Isolation of Porcine Islets

Pig islets were isolated by the modified Ricordi method as previously described.⁸ Liberase-PI was used as the digestive enzyme for all isolations. Liberated islets were separated from nonislet tissue on an iodixanol-based continuous density gradient. The islets' yield and purity were assessed by counting duplicate aliquots stained with DTZ.⁹

Statistical Analysis

Data are expressed as mean $\pm$ SD. Statistical comparisons between the groups were performed with 2-sample *t* test. Comparison of categorical variables was performed with a chi-squared test or the Fisher exact test as appropriate. Correlation was expressed as Pearson correlation coefficient. Stepwise multivariate linear regression analysis was performed to assess for correlations with islet yield (IE/g of pancreas) and to determine which area had the most predictive value. To identify other predictors, including donor and islet isolation procedure variables, we performed binary logistic regression analysis with significant variables from the univariate analysis (n = 67). For this analysis, the pigs were categorized into high (n = 23) and low (n = 44) groups according to islet yield (cutoff 2,000 IE/g). All statistical calculations were performed using Statistical Product and Services Solutions version 16.0 (SPSS, Chicago, IL, USA). *P* values of <.05 were considered to be statistically significant.

Results

Table 1 shows the correlations between final islet yield (IE/g of pancreas) and parameters obtained from pancreas biopsy slices. The IE/cm² of splenic lobe and connecting lobe were significantly correlated with islet yield. The IE/IC and % >200 μ m islets of connecting lobe were also correlated with islet yield. The parameters obtained from duodenal lobe were not correlated with islet yield. Stepwise multivariate linear regression analysis revealed that IE/cm² of the splenic lobe significantly predicted accurate islet yield (Table 2). Table 3 shows donor and islet isolation parameters in the low- and high-yield groups as based on IE/g with the univariate analysis. Binary logistic regression analysis indicated that IE/mm² of the splenic lobe was the only parameter that significantly correlated with successful pig islet isolation ($P = .01$; odds ratio 3.605 95% confidence interval 1.365–9.523). Variables associated with donor and islet isolation procedure such as age gender ischemic time or enzyme lot were not significantly correlated with islet yield.

Discussion

This study indicates that islet distribution of dithizone-stained splenic lobe biopsies is a reliable predictor of islet yield from pig pancreas. Earlier reports had suggested that morphologic and morphometric features of porcine pancreas are useful in predicting islet yield after isolation, and these features were used to try to select pancreases which would produce better yields in successive islet isolations.^{3,5,6} The present study is the first to demonstrate a significant area of importance for pancreas biopsy. Careful consideration of anatomic differences would be required to establish less variable pig islet isolation. Our study also indicates that this “DTZ score” is the most significant predictor of islet yield out of all variables associated with pig islet isolation. Our comprehensive statistical approach is likely to strengthen the importance of this score and provides an improved approach for rapidly predicting islet yield from porcine pancreas. In conclusion, this rapid assessment facilitates immediate selection of pig donor organs that are more likely to provide acceptable islet yields.

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Fig 1. Porcine pancreas biopsy and digested tissue during islet isolation. Pictures were taken from porcine pancreata that yielded $\sim 2,050$ IE/g (**A, C**) and 600 IE/g (**B, D**). Scale bar: 1000 μm (**A, B**) and 500 μm (**C, D**).

Table 1
Correlation (Pearson Correlation Coefficient) Between IE/g and Each Parameter

	Splenic Lobe		Connecting Lobe		Duodenal Lobe	
	P Value	r	P Value	r	P Value	r
IE/cm ² vs IE/g	.002 *	0.433	.003 *	0.413	.311	0.160
IE/IC	.482	0.007	.044 *	0.266	.864	0.027
% >150 μ m	.321	0.074	.183	0.143	.638	0.075
% >200 μ m	.373	0.051	.032 *	0.288	.952	0.010

* $P < .05$.

Table 2
Predictors of IE/g by Stepwise Multivariate Linear Regression

	B	SE	Beta	P Value
IE/cm ² from splenic lobe sample	11.762	3.870	0.433	.004
Constant	423.4	467.2		

Table 3
Univariate Analysis of Donor and Isolation Characteristics

	High Yield (n = 23)	Low Yield (n = 44)	P Value
Pig body wt (lb)	487.1 ± 116.5	446.7 ± 94.1	.12
Age (y)	2.4 ± 1.8	1.7 ± 1.4	.13
Adult (>1 year old)/Young	13/10	20/24	.45
Male/female	8/15	17/27	.80
DTZ score (splenic): IE/mm ²	1.60 ± 0.69	1.19 ± 0.47	.015*
DTZ score (splenic): IE/IC	1.149 ± 0.493	1.032 ± 0.387	.29
DTZ score (splenic): % >150 μm islets	29.9 ± 10.9	28.4 ± 10.3	.58
DTZ score (splenic): % >200 μm islets	11.7 ± 7.0	11.1 ± 6.2	.71
Warm ischemic time (min)	11.2 ± 5.8	11.2 ± 6.0	.98
Cold ischemic time (min)	151.9 ± 49.6	150.1 ± 49.0	.89
Pancreas weight (g)	187.3 ± 53.2	181.0 ± 48.3	.62
Collagenase lot (14059000/14511700/14572700/14989800)	8/2/8/5	11/7/10/16	.41
Collagenase Wunsch unit/g of pancreas	10.3 ± 1.5	10.5 ± 2.6	.72
Neutral protease caseinase unit/g of pancreas	451.4 ± 68.5	453.2 ± 119.2	.94
Pancreas digest time (min)	19.4 ± 4.8	18.4 ± 3.7	.36
Total postpurification IE/g	2,902 ± 681	1,182 ± 517	.0001*
Total postpurification IE	532,989 ± 155,126	211,335 ± 101,068	.0001*

Data shown as mean ± SD. Cutoff value (high yield vs Low yield): 2,000 IE/g.

* $P < .05$.