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Islet Cell Yield Following Remote Total Pancreatectomy With Islet Autotransplant is Independent of Cold Ischemia Time

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Abstract

OBJECTIVES—Total pancreatectomy with islet autotransplantation is increasingly being performed remotely – i.e. removing the pancreas in one location, isolating the islet cells in another location, then returning the islets to the original location for re-implantation into the patient. We determined the influence of extended cold ischemia time on key clinical outcomes in remote islet autotransplantation.

METHODS—We evaluated patients who underwent remote islet autotransplantation at two centers from 2011 – 2014. Patients were divided into two groups; those with and those without a decrease in c-peptide greater than 50% from baseline. The primary clinical outcome was the quantity of isolated islet equivalents per kilogram body weight (IEQ/Kg).

RESULTS—Twenty-five patients met inclusion criteria; 15 had a decrease in c-peptide greater than 50% from baseline and had lower corresponding IEQ/Kg compared to those without a

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SJ Kesseli, KD Smith and TB Gardner: Study concept, design, obtained funding, acquisition of data, analysis, interpretation, drafting and editing of manuscript, study supervision.

All authors: Acquisition of data and critical revision.

decrease greater than 50% (4045 vs. 6654 IEQ/Kg, $p=0.01$). There was no difference in cold ischemia time between the two groups (664 vs. 600 minutes, $p=0.25$). Daily insulin use at 1-year nearly met statistical significance (25.3 U vs. 8 U, $p=0.06$), as did Hemoglobin A1c (8.07 vs 6.69 mmol/L, $p=0.06$).

CONCLUSIONS—Cold ischemia time does not influence islet yield in patients undergoing pancreatectomy with remote isolation.

Keywords

total pancreatectomy with islet cell transplant; cold ischemia; islet shipment; islet isolation; c-peptide; insulin independence

INTRODUCTION

Total or partial completion pancreatectomy with islet autotransplantation (TP-IAT) is increasingly being performed to treat patients with refractory pain from recurrent acute and/or chronic pancreatitis.^{1–4} Because islet cell autotransplant requires an islet isolation facility, centers without these facilities are now performing remote islet cell isolation in which the explanted pancreas is transported to a separate location for isolation, the islets are processed remotely, and then the islets are transported back to the patient where they are infused.^{5–7} Remote isolation therefore allows centers without isolation facilities to perform TP-IAT.⁸

One concern about performing remote isolation is the effect of cold ischemia time on the islet preparation, as the procedure requires extended tissue ischemia in three distinct phases; the first as a solid organ in University of Wisconsin (UW) solution, the second while undergoing digestion, and third as an islet suspension.⁴ While initial studies from the islet allotransplant literature suggested solid organ ischemia time would diminish islet isolation yields, more recent data suggests there is no significant impact.^{9–12} However, in the post-isolation phase, the shipment of islets in liquid suspension has been shown to result in a significant loss of islet equivalents.¹³ While some degree of islet cell attrition may be tolerable for the purpose of allogeneic transplantation, isolations for TP-IAT patients typically yield fewer islet equivalents due to preexisting pancreatic disease and/or subtotal resections, and thus extended ischemia time may have a greater impact on their clinical outcomes.

Given this concern, we performed a multicenter, retrospective cohort study to determine if the extended, multi-phase ischemia time associated with remote isolation is a barrier to successful islet engraftment in TP-IAT patients. *A priori*, we hypothesized that longer cold ischemia times would not lead to a decrease in islet cell yield.

MATERIALS AND METHODS

Study Design and Patient Population

We performed a retrospective, multicenter cohort study on patients who underwent pancreatectomy with remote IAT between 2011 and 2014 at two academic medical centers -

Dartmouth Hitchcock Medical Center in Lebanon, NH (remote isolation in Boston, MA) and Cleveland Clinic in Cleveland, OH (remote isolation in Pittsburgh, PA). Included patients were those who underwent either complete or partial pancreatectomy for recurrent acute or chronic pancreatitis that was unresponsive to maximal medical therapy. All patients were either non-diabetic or had C-peptide positive diabetes, and had diminished quality of life as determined by a multidisciplinary case review prior to operative intervention. Excluded patients were those in whom outcome data was not available and/or did not have recorded islet cell transport times.

Islet Cell Transplantation

To minimize the cold ischemia time, the splenic and gastroduodenal vessels were preserved throughout the pancreas dissection to maintain arterial and venous flow until explant. Once the pancreas was mobilized, these vessels were ligated and the organ was removed and placed into an ice-cold UW solution bath supplemented with cefazolin. This solution was flushed through the splenic and gastroduodenal arteries and excess fat and tissue were dissected away with care not to disrupt the pancreatic capsule. The organ was then submerged in UW solution at 4°C for ground transport to the isolation facility. The isolated islet suspension was shipped in gas-permeable tissue culture bags in 5% human albumin solution. Bags were laid flat to minimize the height of culture medium to promote oxygen diffusion. None of the organs were subject to complications of transport en route to or from the isolation facilities. Upon returning to the operating room, all islets were infused into the portal or splenic vein. Figure 1 represents a flowchart schematic of the remote IAT process.

Outcome Measures

The primary study outcome was the number of isolated islet equivalents per kilogram body weight (IEQ/Kg). Patients were stratified into two groups based on their one year fasting c-peptide levels: 1) those with a decrease in c-peptide greater than 50% from pre-operative value, and 2) those without a decrease in c-peptide greater than 50% from pre-operative value. Secondary outcomes included insulin use, hemoglobin A1c and opiate use (based on morphine equivalents) at one year post-transplant.

Cold ischemia time was defined as follows: Solid Organ Ischemia Time: time from the pancreas leaving the patient to arrival at the islet isolation facility; Isolation Time: time from pancreas arrival at isolation facility to departure of islet suspension from facility; Islet Shipment Time: time from islets leaving isolation facility to initiation of portal vein infusion; Total Cold Ischemia Time: time from the pancreas leaving the patient to the time of initiating infusion.

Statistical Analysis

Data were obtained by chart review and/or telephone interview. Baseline demographic, procedural, and outcome data were available on all included patients and were available within 2 months of the one-year transplant anniversary. In patients reporting a range of daily insulin use, the mean was used, and only baseline insulin use was considered (i.e. sliding scale correction was considered '0')

Continuous variables were evaluated using the student's t-test and categorical variables using the Fisher's Exact test. Exploratory subgroup analysis of the different time components of cold ischemia was performed. An additional subgroup analysis was completed with patients stratified by insulin independence at 1 year. Statistical analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA) and Graphpad QuickCalcs (GraphPad Software Inc., La Jolla, CA).

RESULTS

57 patients underwent remote IAT during the study period at the two centers. Twenty-five patients met inclusion criteria and were included in the analysis; 16 patients from Dartmouth-Hitchcock and 9 from the Cleveland Clinic. Baseline characteristics were similar between the two groups and are shown in Table 1. Of the 25 patients, 21 underwent total pancreatectomy, while 4 had a distal completion procedure. Procedural characteristics between the two groups are shown in Table 2. There was no difference between groups in regard to pancreas weight, isolation time, islet purification, or total cold ischemia time.

Figure 2 demonstrates that the number of isolated IEQ/Kg predicted whether patients would have a decrease in c-peptide greater than 50% at 1 year (4045 vs. 6654 IEQ/Kg, $p=0.01$). A multiple linear regression analysis showed no relationship between isolated IEQ/Kg and the solid organ ischemia time ($R^2=0.03$, $p=0.50$, $n=16$), nor the isolation time ($R^2=0.05$, $p=0.41$, $n=16$). A regression comparing IEQ/Kg with islet shipment time was not performed, as the determination of IEQ/Kg occurred prior to the shipment of islets from the lab facility.

Figure 3 shows a regression analysis of the percent change (%) in C-peptide compared with total cold ischemia time in minutes. To adjust for the IEQ/Kg isolated, patients were stratified as having greater or less than the median value of 4300 IEQ/Kg. There was no significant correlation between % C-peptide and total cold ischemia time among those with ≤ 4300 IEQ/Kg ($R^2=0.067$, $p=0.39$, $n=13$) or those with >4300 IEQ/Kg ($R^2=0.20$, $p=0.15$, $n=12$).

Figure 4 outlines the clinical outcomes between the two groups. The daily insulin use and HgA1c at 1 year approached significance between the two groups ($p=0.06$, $p=0.06$). Daily morphine use at 1-year was similar between groups ($p=0.61$).

The subgroup analysis of ischemia time, stratified by each phase of transit, is presented in Table 3. The solid organ ischemia time, isolation time, islet shipment time and total cold ischemia time were all comparable between groups ($p=0.69$, 0.09 , 0.45 , and 0.15 respectively).

DISCUSSION

This study demonstrated that cold ischemia time does not influence the quantity of IEQ/Kg isolated, nor the post-operative change in c-peptide in patients undergoing remote TP-IAT. We found that the isolated IEQ/Kg were a significant determinant of the change in c-peptide at 1-year ($p=0.01$), which is consistent with other studies that have observed the correlation of islet yield and positive functional outcomes.^{14, 15} Additionally, although our outcome

comparisons of insulin use and HbA1c at 1-year fell short of our p-value cutoff of 0.05, (Figure 4, $p=0.06$, $p=0.06$) it is reasonable to conclude that both these results are in fact related to the change in c-peptide, a marker of successful islet engraftment.

We did observe the total cold ischemia time to be greater among insulin dependent patients, however we suspect that this finding is confounded by several variables. First, the isolation time (which accounted for the largest fraction of total ischemia time) may have a confounding relationship with islet yield, as longer pancreas digestion times may lead to islet fragmentation and thus lower yield.¹⁶ Additionally, insulin independence is a subjective outcome, as it may be affected by both patient and physician preference. Furthermore, we included patients in our insulin independent group whom still required small doses of sliding scale correction. Lastly, some patients included in the study had c-peptide positive diabetes prior to transplant and thus insulin independence would not be an appropriate outcome for these patients. Given these variables, we are hesitant to conclude that cold ischemia time was truly predictive of insulin independence at one year.

Based on our subgroup analysis in Table 3, we are encouraged that the islet shipment time did not appear to influence patients' change in C-peptide, as two studies have demonstrated that IEQ recovery is significantly diminished with prolonged islet shipment times. Ichii *et al* reported that after an average 4.57 hours of transport time, roughly 70% of the pre-shipment IEQ were recovered.¹³ In addition, Ikemoto *et al* shipped islets an average of 48.2 hours and found a post-shipment IEQ recovery of 56.4% if gas permeable bags were used.¹⁷ Of note, these studies were performed for purposes of allotransplant using cadaveric donor pancreas, and had average post isolation yields upwards of 500,000 IEQ. Considering that isolations for TP-IAT provide lower yields (about 300,000 in our cohort), it is plausible that islet shipment could appreciably influence TP-IAT outcomes; however, we did not observe such an effect with our patients. With a mean islet shipment time of 215 minutes, perhaps shipment times in our cohort were below the threshold to produce any observable impact. In addition, because we measured IEQ following islet isolation but not prior to islet infusion, we are unable to quantify the impact of our shipment specifically on total islet yield or viability.

Overall, we believe that our study validates remote TP-IAT as an acceptable alternative to TP-IAT with on-site isolation. With 9 out of 25 (36%) patients having >5000 IEQ isolated, and 50% being insulin free or on minimal insulin at 1 year, our results are comparable to those of the landmark University of Minnesota series that established TP-IAT as viable treatment for chronic pancreatitis.¹ These outcomes are also comparable with two recently published remote TP-IAT series from the Cleveland Clinic and UCLA.^{18, 19}

In the future, we believe there is a need for further exploration into the effects of prolonged shipment of islets, and perhaps guidelines for centers considering remote isolation for TP-IAT at distances greater than those in this study (about 134 and 125 miles for Cleveland and Dartmouth, respectively). In addition to the physical transport, islet shipment time is also determined by logistical barriers such as the time to prepare the patient and the operating room for a second operation. Anecdotally, some of the longer islet shipment times in our group occurred due to logistical factors rather than delayed transport. Therefore, both

distance and timing should be considered by new centers to ensure that shipment time will not detract from the islet recovery and potentially patient outcomes.

Our study is limited by its small sample size and retrospective analysis, which may have contributed to type II error. This limitation was particularly exemplified in our regression analysis in Figure 3, where nearly all patients with islet yields above the median (>4300 IEQ/Kg) had total ischemia times in the lower half of our series (range = 511 – 681 minutes) – thus it remains difficult to infer how this particular subgroup would perform over a larger range of ischemia time.

An additional drawback is that our outcome of having a greater or less than 50% decrease in c-peptide is not a direct measurement of islet engraftment, and this designation may have been influenced by regression to the mean if patients had unusually elevated c-peptide levels at baseline, potentially due to preexisting insulin resistance or failure to fast prior to testing. Indeed, the group with a greater decrease in c-peptide had average baseline values approaching the upper limit of normal, 3.08 ng/mL (vs. 2.30 ng/mL, $p=0.26$), and thus may have been more likely to see a drop simply because their baseline value was higher. Regarding our ischemia time subgroup analysis, although we had a range of total ischemia times ranging from 553 to 965 minutes, this was again limited because nearly half (9/20) fell between 600 and 660 minutes, which prevented us from effectively stratifying outcomes by ischemia time. An ideal study to validate remote TP-IAT would compare outcomes at a single center that used both remote and on-site isolations. As Dartmouth has recently started to perform islet isolation intra-operatively on-site, we anticipate that we will soon be able to compare our future outcomes with those from our remote TP-IAT experience.

This retrospective study was conducted to determine if the overall extended cold ischemia time associated with remote islet isolation impacted islet engraftment and outcomes. We have shown that the IEQ/Kg isolated is a significant predictor of c-peptide at 1 year, but that cold ischemia time did not affect success in our remote TP-IAT cohort.

Thus, we conclude that remote TP-IAT is an effective method to treat unremitting pancreatitis at centers without on-site islet isolation facilities.

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Abbreviations

TPIAT	Total Pancreatectomy with Islet Autotransplant
IEQ	Islet Equivalents
IAT	Islet Auto Transplant
UW	University of Wisconsin

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Figure 1.
The Remote TP-IAT process with definition of isolation stages

Figure 2.
Isolated IEQ/Kg based on 1-year c-peptide

Figure 3.
Linear Regression: percent change c-peptide vs. total cold ischemia time

Figure 4.

Clinical outcomes based on 1-year c-peptide. Insulin, U=Mean daily number of units of insulin used. MME=Morphine equivalents/day.

Table 1

Baseline Patient Characteristics

	> 50% Drop in C-Peptide (n=15)	< 50% Drop in C-Peptide (n=10)	P=
Age (years)	43.2 ± 12.5	41.9 ± 11.2	0.79
Sex			
Female	9	4	0.43
Male	6	6	
Body Mass Index	26.3 ± 5.8	24.4 ± 6.2	0.44
Etiology[†]	CFTR (4)	CP, idiopathic (4)	n/a
	CP, alcohol (4)	RAP, idiopathic (1)	
	CP, Idiopathic (3)	DPDS (1)	
	SOD (2)	SOD (1)	
	PD (1)	PD (1)	
	RAP, triglycerides (1)	CP, triglycerides (1)	
		CFTR (1)	
Type of Surgery			
Total Pancreatectomy	13	8	1.00
Completion Distal	2	2	
Location			
Dartmouth Hitchcock	10	6	1.00
Cleveland Clinic	5	4	
Baseline Morphine Equivalents[*]	109 ± 102	78.1 ± 76.2	0.46
Baseline c-peptide	3.08 ± 1.32	2.30 ± 2.02	0.26
Baseline HgA1c	5.72 ± 0.99	5.25 ± 0.24	0.16

^{*} Data available only for 22 of 25 patients

[†] Etiology as follows: CFTR=Cystic Fibrosis Transmembrane Conductance Regulator Gene; CP=chronic pancreatitis; SOD=Sphincter of Oddi Dysfunction; DPDS=Disconnected Pancreatic Duct Syndrome; RAP=Recurrent Acute Pancreatitis; PD=Pancreas Divisum

Table 2

Islet Transplant Characteristics

	> 50% Drop in C-Peptide (n=15)	< 50% Drop in C-Peptide (n=10)	P=
Pancreas Weight (g)	75.2 *	67.5	0.55
Isolation Time (min)	300 ± 99.3	268 ± 62.2	0.37
Islet Purification (n)	9 †	7	1.00
Total Cold Ischemia Time	664 ± 153	600 ± 89	0.25

* Data available on 13 of 15 patients

† Data available on 14 of 15 patients

Table 3

Subgroup Analysis of Stages of Remote Isolation Time (Dartmouth Data Only)

	> 50% Drop in C-Peptide (n=10)	< 50% Drop in C-Peptide (n=6)	P=
Solid Organ Ischemia Time	154 ± 37.6	147 ± 21.3	0.69
Isolation Time	355 ± 63.9	298 ± 50.7	0.09
Islet Shipment Time	229 ± 101	187 ± 110	0.45
Total Cold Ischemia Time	737 ± 134	641 ± 92.6	0.15