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ORIGINAL ARTICLE

Clinical islet isolation and transplantation outcomes with deceased cardiac death donors are similar to neurological determination of death donors

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Summary

In islet transplantation, deceased cardiac death (DCD) donation has been identified as a potential extended source. There are currently no studies comparing outcomes between these categories, and our goal was to compare islet isolation success rates and transplantation outcomes between DCD and neurological determination of death (NDD) donors. Islet isolations from 15 DCD and 418 NDD were performed in our centre between September 2008 and September 2014. Donor variables, islet yields, metabolic function of isolated isled and insulin requirements at 1-month post-transplant were compared. Compared to NDD, pancreata from DCD were more often procured locally and donors required less vasopressive support ($P < 0.001$ and $P = 0.023$, respectively), but the other variables were similar between groups. Pre- and postpurification islet yields were similar between NDD and DCD (576 vs. 608 $\times$ 10^3 islet equivalent, $P = 0.628$ and 386 vs. 379, $P = 0.881$, respectively). The metabolic function was similar between NDD and DCD, as well as the mean decrease in insulin requirement at 1-month post-transplantation (NDD: 64.82%; DCD: 60.17% reduction, $P = 0.517$). These results support the broader use of DCD pancreata for islet isolation. A much larger DCD islet experience will be required to truly determine noninferiority of both short- and long-term outcomes.

Introduction

Clinical islet transplantation is a highly effective method to stabilize glycemic control in selected patients with type 1 diabetes complicated by refractory hypoglycaemia or glycemic lability. While in the future, alternative stem cell or xenograft islet sources may be considered, islet transplantation is currently dependent upon the scarce organ donor pool [1]. While variable, most centres are only able to transplant sufficient islet yield from a processed pancreas in approximately half of occasions. Additionally, most recipients require more than one intraportal islet infusion to achieve and sustain periods of insulin independence. At a point where the indications for islet transplantation become less selective, the donor pool will be unable to meet the potential supply. To maximize currently available organs, transplant centres routinely process extended criteria donor organs, and more recently deceased cardiac death (DCD) donation has been identified as a potential extended source. In kidney transplantation, DCD donation has been associated with increased delayed graft function but similar long-term survival [2,3]. In liver transplantation, DCD donation is associated with inferior graft survival and increased risk of ischaemic cholangiopathy [4,5]. In whole pancreas transplantation, graft and patient survival have been reported similar in several series comparing DCD to

neurological determination of death (NDD) donation [6,7].

In islet transplantation, the procured pancreas is retrieved and stored for variable periods in cold preservation solution to accommodate transfer from donor to isolation centre and then followed by a complex digestion, purification and culture process. Intraportal transplantation can then only occur if there is sufficient islet yield, provided that all product release criteria are met. Previous studies have demonstrated that islet isolation from DCD donors yield similar numbers of functional islets compared to NDD donation in nonclinical setting [8,9]. The largest experience in clinical DCD pancreas donation for islet isolation comes from the Japan Islet Transplantation Registry, with approximately half of processed pancreata found to be suitable for islet transplantation. However, graft function in a series of 18 subjects was judged to be suboptimal as evidenced by a 5-year c-peptide positive rate of only 22% [10,11]. In the Western world, only a single case of successful islet transplantation from a DCD donor associated with a prolonged period of insulin independence was reported [12]. Due to only limited sporadic islet transplant experience with DCD pancreas donation in the Western world, and a paucity of NDD donors in Japan, there are currently no single-centre studies that directly compare outcomes between these categories. The goal of this study therefore was to compare islet isolation success rates and transplantation outcomes between DCD and NDD pancreas donors at a single centre.

Patients and methods

Between September 2008 and September 2014, 487 pancreata procured from both DCD and NDD donors retrieved across Canada were processed for islet isolation in a single common good manufacturing practice islet isolation facility at the University of Alberta. We started accepting DCD donors for islet isolation since 2008, usually restricted to local donor, and broadened the acceptance criteria to whole Canada in 2014. Acceptance criteria of pancreas for islet isolation were similar for DCD and NDD. Over the observed period, 54 islet isolations were performed in the context of experimental trials using different isolation methods and were therefore excluded from evaluation. Thus, the remaining 433 pancreata formed the basis of our study.

Pancreas procurement after DCD donation followed standard local practice at each of the Canadian donor institutions and was procured in the context of a multiorgan procurement. At our own institution, no treatment modifications were initiated prior to declaration of death. An asystole period of 5-min observation was performed to rule out auto-resuscitation before death declaration, and an additional transport period of approximately 5 min was required to move the donor to the operating room. The warm ischaemic period in our centre is calculated from the time the mean arterial pressure <5 mmHg, and/or the arterial blood oxygen saturation <70% to the moment of cold perfusion with chilled histidine ketoglutarate (Methapharm, Coral Springs, FL, USA) containing 30 000 units heparin, through a surgically placed aortic cannula [13]. The acceptable limits of tolerable warm ischaemia for pancreata procured for islet isolation remain unknown, but we generally applied a maximal warm ischaemia limit of <30 min. The cold ischaemic period was defined as the period of aortic cannulation for DCD and aortic cross-clamp for NDD to the initiation of islet isolation. No intraductal preservation solution was injected.

Donor information and islet isolation outcomes were reviewed from all 433 islet isolation batch files. The methods for islet isolation have been described previously [14], but in brief, the pancreas weight was documented before digestion, two cannulae were inserted in the main duct at the mid-pancreas level, and a cold collagenase solution was perfused under controlled pressure for 10 min. Three type of collagenase were used for pancreata in DCD and NDD groups: Serva GMP (Serva Electrophoresis, Heidelberg, Germany) in two and 101 cases, respectively, Clzyme (VitaCyte LLC, Indianapolis, IN, USA) in seven and 127 cases, respectively, and Liberase MTF (Roche Diagnostics, Indianapolis, IN, USA) in six and 190 cases, respectively. The choice of the collagenase was not influenced by the DCD/NDD status. The cut pancreas was introduced in a Ricordi chamber whose content was warmed to 37 °C. After digestion, the pancreas remnant was weighted, and islet yield, expressed as islet equivalent (IEQ), was assessed before purification. Islet yield, purity and viability were further assessed after purification using continuous density gradient centrifugation, and again after culture, just prior to transplantation.

In vitro islet metabolic function was analysed by oxygen consumption rate (OCR) introduced in September 2012, and by dynamic glucose-stimulated insulin release (d-GSIR) using a continuous islet perifusion assay introduced at our centre in February 2013.

Oxygen consumption rate measurements were performed as described previously [15]. OCR was normalized to the DNA content resulting in OCR/DNA (nmol O_2/min × mg DNA). Samples for OCR were taken postpurification and measured in triplicate promptly without culture. Additional islets were sampled for OCR after culture prior to transplantation.

For d-GSIR assay, islet samples in triplicate were perfused with low-glucose Krebs solution (2.8 mM) followed by high-glucose (28 mM) Krebs solution at a flow rate of 100 ml/min at 37 °C using a perifusion apparatus

(Biorep Technologies, Miami, FL, USA). The perfusate was sampled periodically for insulin assay (Alpco, Salem, NH, USA). The d-GSIR index was calculated as the ratio between peak insulin concentration during high-glucose exposure to a peak insulin during low-glucose exposure, as well as the ratio between an area under the curve (AUC) for insulin during high-glucose exposure to an AUC during low-glucose exposure.

Intraportal islet transplantation was performed routinely through percutaneous ultrasound and fluoroscopic-guided access, as described in detail previously [16]. Induction T-depletional or modulatory immunosuppression consisted of alemtuzumab, thymoglobulin, basiliximab or daclizumab, varying based on practice and protocol. Maintenance immunosuppression was based on combined tacrolimus (trough blood levels 8–12 ng/ml) and mycophenolate mofetil (up to 2 g per day in divided dose as tolerated), started on the day of transplant. Standard therapeutic-dose heparin infusion was initiated immediately post-transplant and transitioned to low molecular weight heparin and aspirin for 2 weeks thereafter. Clinical islet transplant outcomes were defined by change in insulin requirement (in units of insulin per kg recipient body weight per day), and metabolic stimulation testing at 1-month post-transplant.

Continuous variables are expressed by mean and standard error of the mean; dichotomous variables are expressed as natural numbers. For analysis between groups, two-tailed *t*-test or ANOVA were applied for continuous variables, and chi-square test was applied for dichotomous variables. A P value <0.05 was considered significant. The statistical analysis was performed using SPSS 21.0 (IBM Corporation, New York, NY, USA).

Table 1. Donors, pancreata and islets characteristics.

	NDD	DCD	*P*
Number of processed pancreata	418	15	
Number of transplanted preparations	196	9	0.309
Age, year, mean (SEM)	48.6 (0.683)	45.3 (3.677)	0.362
Gender (M/F)	224/194	11/4	0.131
Body weight, kg, mean (SEM)	80.5 (0.934)	82.3 (6.638)	0.714
Height, cm, mean (SEM)	171.4 (0.55)	168.1 (3.497)	0.275
Body mass index (kg/m^2) (SEM)	27.4 (0.284)	29.2 (1.147)	0.245
Cold ischaemia time, h (SEM)	9.8 (0.179)	8.7 (1.147)	0.267
Vasopressor requirement	No 132 Yes 283 (68.2%)	No 9 Yes 6 (40%)	0.0231
Donor location (local/distant)	30/388 (92.8% distant)	5/10 (66.7% distant)	0.00026
Pancreas weight, g, mean (SEM)	93.8 (1.389)	96.8 (7.125)	0.692
Undigested pancreas weight, g (SEM)	16.3 (0.512)	20.2 (3.661)	0.162
Islet yield prepurification, $\times 10^3$ IEQ (SEM)	576 (12.439)	608 (76.311)	0.628
Islet yield postpurification, $\times 10^3$ IEQ (SEM)	386 (9.380)	379 (61.415)	0.881
Islets purity % (SEM)	52.6 (0.787)	51.7 (5.578)	0.822
Viability % (SEM)	83.5 (0.360)	81.5 (2.704)	0.317

NDD, neurological determination of death; DCD, deceased cardiac death; IEQ, islet equivalent.

Results

Over the course of this study, we processed 15 pancreata from DCD and 418 from NDD. Donor demographics were similar between DCD and NDD except for the location of the donors, most of the pancreata from DCD being procured locally, and vasopressor requirement was significantly more frequent in NDD (Table 1).

Islet isolation processing of pancreata gave similar pre- and postpurification islet yields for NDD and DCD (Fig. 1a). Purity and viability were also similar for NDD and DCD [purity 52.6% (SEM 0.787) and 51.7% (SEM 5.578), respectively, $P = 0.822$; viability 83.5% (SEM 0.360) and 81.5% (SEM 2.704), respectively, $P = 0.317$].

Results of d-GSIR and OCR are shown in Fig 1b,c. OCR of pre-and postculture islet preparations used for transplantation, and preculture values for nontransplanted preparations, were not significantly different between NDD and DCD. Likewise, both NDD and DCD islets released insulin responding to glucose stimulation to a similar degree as determined by no significant difference in d-GSIR index for AUC and for peak insulin.

Transplantation rates were similar between NDD and DCD islet preparations (196/418 (46.9%) and 9/15 (60%), respectively, $P = 0.309$). Transplant recipients had similar body weight between the two groups [NDD: 72.7 kg (SEM 0.866); DCD: 75.8 kg (SEM 4.037), $P = 0.462$].

The location of the procured pancreata (local vs. distant) did not affect the transplantation rate after islet isolation for both NDD and DCD. For NDD, 180 of 388 (46.4%) pancreata procured in remote centres were transplanted, versus 16 of 30 (53.3%) pancreata procured locally ($P = 0.463$). For DCD, five of 10 pancreata procured in remote centres were transplanted, versus four of five pancreata procured locally ($P = 0.263$).

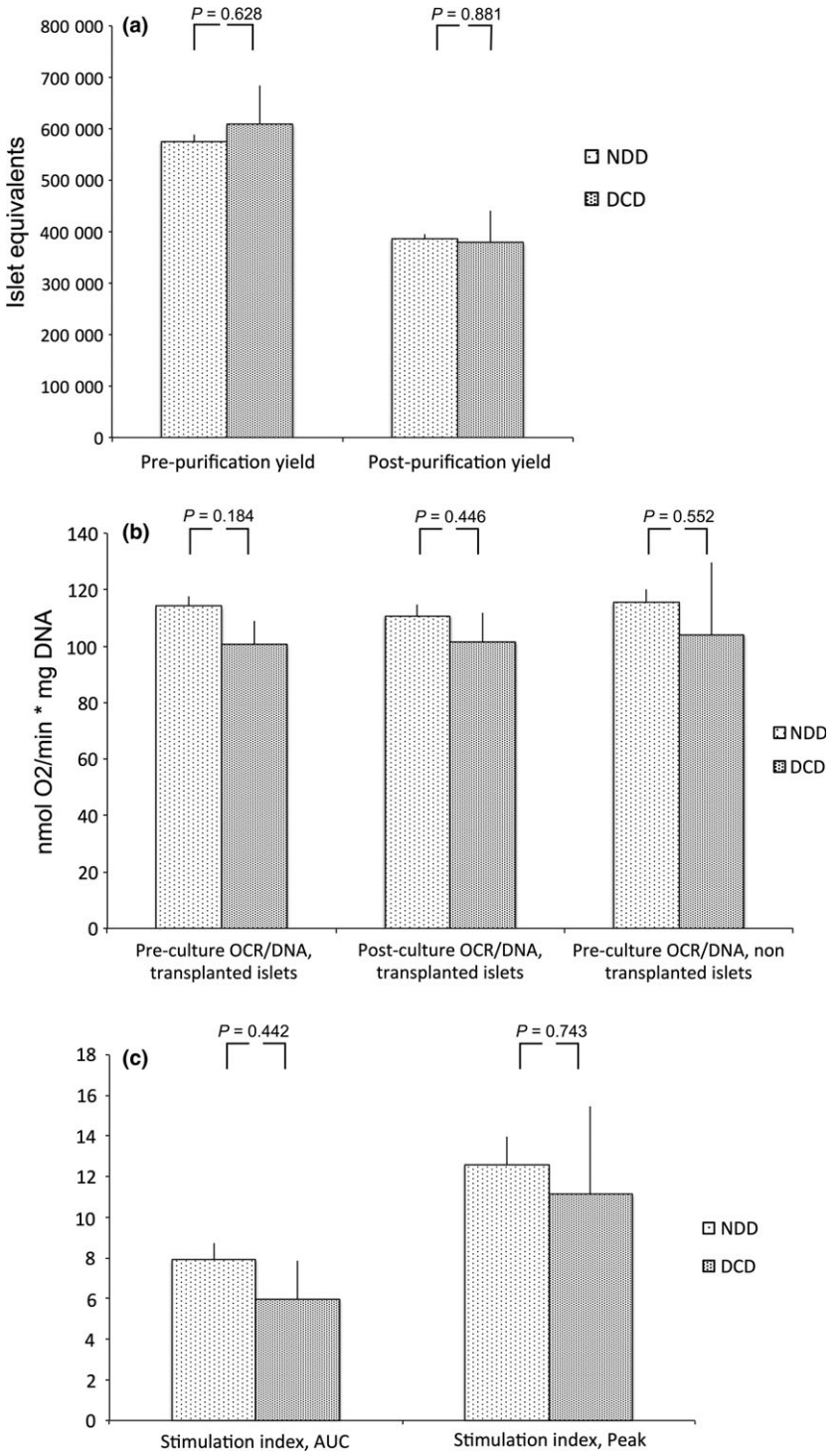

Figure 1 Islet yield and metabolic assessment. (a) Pre- and postisolation islet yields expressed in islet equivalent (IEQ). No difference was reported between neurological determination of death (NDD) and deceased cardiac death (DCD) groups for both prepurification and postpurification yields. (b) Oxygen consumption rates (OCR) in NDD and DCD groups. OCR was similar between the two groups prior and after culture (NDD $n = 64$, DCD $n = 7$) of transplanted islets as well as prior culture for nontransplanted islets (NDD $n = 45$, DCD $n = 3$). (c) Glucose-stimulated insulin release index was similar between NDD and DCD groups for both AUC (NDD $n = 48$, DCD $n = 5$) and peak values (NDD $n = 47$, DCD $n = 5$).

Recipients often required more than one transplant, and allocation to receive islets from NDD or DCD was conducted by random assignment, with no attempt to bias for first or subsequent transplant. In the NDD group, the islet transplant was initial in 77 cases, second in 74 cases, third in 32 cases and fourth onward in 13 cases. In the DCD group, the transplant was initial in four cases, second in four cases and the third in one case. The total number of cumulative transplants was similar between groups ($P = 0.901$).

Baseline insulin requirement before each transplant and insulin requirement at 1-month post-transplant were similar between groups as shown in Fig. 2. Mean decrease in insulin requirement at 1-month post-transplant was similar between groups (NDD: 64.82%; DCD: 60.17% reduction, $P = 0.517$). In the NDD group, 54 of 196 procedures resulted in insulin independence at 1-month post-transplant versus three of nine in the DCD group, $P = 0.712$. These procedures represented a 1st transplantation in 12 (22.2%) and 0 (0%) cases for NDD and DCD groups, respectively, a second in 30 (55.6%) and 2 (66.7%) cases, a third in 9 (16.7%) and 1 (33.3%) cases, a fourth in 2 (3.7%) and 0 cases and a fifth in 1 (1.9%) and 0 cases ($P = 0.854$ between NDD and DCD groups). Of the nine patients transplanted in the DCD group, five are currently insulin independent. For the four patients who are currently not insulin independent, the insulin requirements decreased by 95%, 62%, 64% and 23%. However, it is not possible to determine the relative contribution of transplants from DCD to overall insulin requirement, due to the combination of DCD- and NDD-derived donors in these cases. For similar reasons, we do not think that insulin requirements more than 1 month post-transplant (i.e. after potentially receiving a further transplant) would be helpful or relevant in this study.

Discussion

The current series is the first to directly compare results of islet isolation and transplantation between DCD and NDD although there are many reports comparing DCD to NDD in solid organ pancreas transplants.

The results show that pancreata from DCD gave similar islet yield and clinical outcomes to NDD, but also that DCD from remote centres are indeed suitable for islet isolation and transplantation. In Canada, the distances between centres are substantial, and the current study confirms that pancreata procured in distant hospitals should be considered for islet isolation and transplantation, irrespective of the donor type. This is especially relevant in islet transplantation where core islet isolation facilities often serve a larger catchment area for donor hospital procurement, including crossing of national borders on occasion [17–20]. In the current series, DCD represented <5% of all processed pancreata. This low rate is explained by the relative novelty of DCD in Canada for islet transplantation, as well as reluctance of some distant Canadian centres to potentially compromise a right replaced hepatic artery in order to leave the

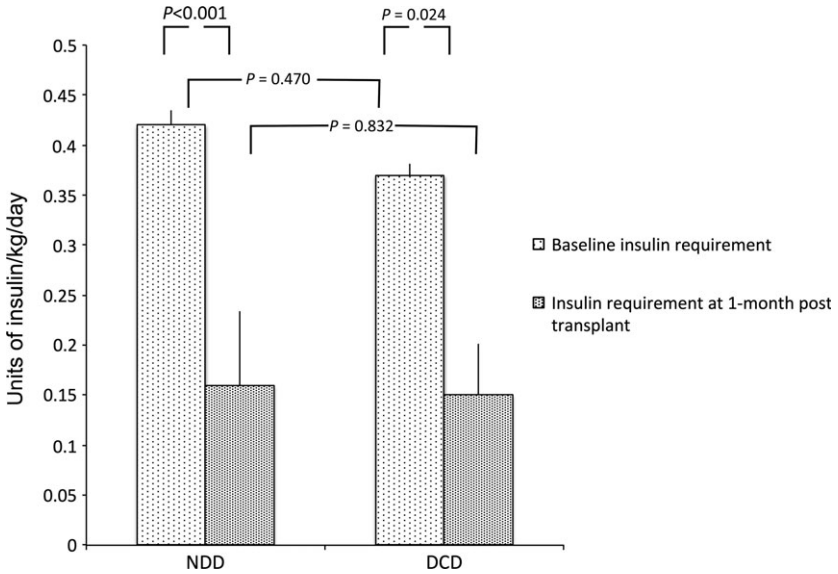

Figure 2 Insulin requirement (units/kg/day) prior and after islet transplantation for both neurological determination of death (NDD) and deceased cardiac death (DCD) groups. Insulin requirements significantly dropped after islet transplantation for both NDD ($P < 0.001$) and DCD ($P = 0.024$) groups. Neither pretransplant nor post-transplant insulin requirements were different between NDD ($n = 196$) and DCD ($n = 9$) ($P = 0.470$, and $P = 0.832$, respectively).

pancreatic capsule intact. Current American Society of Transplant Surgeons [13] guidelines still recommend that the pancreatic head should be routinely transected if the pancreas is not used to avoid an injury of a right replaced hepatic artery, its identification being potentially more challenging in a cold and pulseless field [13]. If the pancreas is transected, this significantly reduces the ability of the islet isolation team to isolate sufficient islets for transplantation [21]. We now advocate that hepatic dissection should routinely maintain the pancreas intact, where there are plans for subsequent islet isolation. We previously showed that minor tears of the pancreas not involving the main pancreatic duct do not contraindicate islet isolation [21].

All efforts should be made to improve the pool of pancreata for islet transplantation, and DCD appears to be an additional suitable source. In Canada, DCD represented 17% of the organ donors in 2012 [22], and in UK, DCD represented 41% in 2014 [23]. Although the ideal DCD profile for islet transplantation remains to be defined, DCD *per se* should not be a reason for declining donor offers for islet isolation.

Acceptable factors for consideration of DCD pancreata offers for islet isolation remain to be defined, and specifically, the maximal agonal warm ischaemic time is as yet unknown. We currently recommend that an agonal warm ischaemic period of up to 45 min be used as a reasonable threshold for accepting pancreata for islets, but this is arbitrary and further data are needed to make such recommendations definitive.

Limitations of the current study include the relatively small number of DCD pancreata used for comparison against NDD pancreata. Relatively small numbers do not allow multivariate analysis of predictive isolation variables presently. Based on the current data, these preliminary results support the broader use of DCD pancreata for islet isolation, irrespective of the donor location. A much larger DCD islet experience will be required to truly determine noninferiority of both short- and long-term outcomes.

Authorship

AA: contributed to research design, data gathering, data analysis and writing of the paper. TK: contributed to research design, data gathering and writing of the paper. DO: contributed to data gathering and writing of the paper. SL: contributed to data gathering and data analysis. DB, NK and PS: contributed to research design and writing of the paper. AMJS: contributed to research design, data analysis and writing of the paper.

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