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Temperature Profiles of Different Cooling Methods in Porcine Pancreas Procurement

Brad P. Weegman¹, Thomas M. Suszynski¹, William E. Scott III^{1,2}, Joana Ferrer¹, Efstathios S. Avgoustiniatos¹, Takayuki Anazawa¹, Timothy D. O'Brien⁴, Michael D. Rizzari¹, Theodore Karatzas³, Tun Jie², David ER. Sutherland¹, Bernhard J. Hering¹, and Klearchos K. Papas^{1,2}

¹Schulze Diabetes Institute, Department of Surgery, University of Minnesota, Minneapolis, MN

²Institute for Cellular Transplantation, Department of Surgery, University of Arizona, Tucson, AZ

³Second Department of Propedeutic Surgery, University of Athens, Athens, Greece

⁴College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Abstract

Background—Porcine islet xenotransplantation is a promising alternative to human islet allotransplantation. Porcine pancreas cooling needs to be optimized to reduce the warm ischemia time (WIT) following donation after cardiac death, which is associated with poorer islet isolation outcomes.

Methods—This study examines the effect of 4 different cooling *Methods* on core porcine pancreas temperature (n=24) and histopathology (n=16). All *Methods* involved surface cooling with crushed ice and chilled irrigation. *Method A*, which is the standard for porcine pancreas procurement, used only surface cooling. *Method B* involved an intravascular flush with cold solution through the pancreas arterial system. *Method C* involved an intraductal infusion with cold solution through the major pancreatic duct, and *Method D* combined all 3 cooling *Methods*.

Results—Surface cooling alone (*Method A*) gradually decreased core pancreas temperature to < 10 °C after 30 minutes. Using an intravascular flush (*Method B*) improved cooling during the entire duration of procurement, but incorporating an intraductal infusion (*Method C*) rapidly reduced core temperature 15–20 °C within the first 2 minutes of cooling. Combining all methods (*Method D*) was the most effective at rapidly reducing temperature and providing sustained cooling throughout the duration of procurement, although the recorded WIT was not different between *Methods* ($p=0.36$). Histological scores were different between the cooling *Methods* ($p=0.02$) and the worst with *Method A*. There were differences in histological scores between *Methods A* and *C* ($p=0.02$) and *Methods A* and *D* ($p=0.02$), but not between *Methods C* and *D* ($p=0.95$), which may highlight the importance of early cooling using an intraductal infusion.

Corresponding Author: Klearchos K. Papas, Ph.D., Professor of Surgery, Institute for Cellular Transplantation, Dept. of Surgery, University of Arizona, 1656 E. Mabel Street, Room 121, Tucson, AZ 85724, Phone: (520) 626-4494, Fax: (520) 626-7779, kkpapas@surgery.arizona.edu.

Disclosures

The authors have no conflicts of interest.

Conclusions—In conclusion, surface cooling alone cannot rapidly cool large (porcine or human) pancreata. Additional cooling with an intravascular flush and intraductal infusion results in improved core porcine pancreas temperature profiles during procurement and histopathology scores. These data may also have implications on human pancreas procurement since use of an intraductal infusion is not common practice.

Keywords

pancreas procurement; organ cooling; cold preservation; islet xenotransplantation

Introduction

Widespread clinical application of islet transplantation is currently limited by the shortage of human organ donors (1). Porcine islet xenotransplantation (2–4) provides an opportunity to obviate the inadequate supply of human organs by isolating islets from a practically-unlimited supply of porcine donors. Just as with human islet isolation, the outcome of porcine islet isolation is dependent on the quality of the donor pancreas (5), which is in large part affected by the procurement and cooling methodologies used. This is especially true in porcine islet transplantation since the preservation periods are typically short and limit the negative impact that cold ischemia time may have on islet quality.

Donor porcine pancreata are frequently procured following cardiac death to save on the additional cost, complexity, and logistical challenges associated with performing a heart beating procurement with an adult porcine donor. However, donation after cardiac death (DCD) is accompanied by warm ischemia time (WIT), which is known to be detrimental to the quality of a donor pancreas (6). The current procurement method involves dissecting the pancreas out as quickly as possible, while the core pancreas temperature is reduced by diffusion-based surface cooling with crushed ice and cold irrigation. The WIT is traditionally defined and recorded as the time from cardiac death until cooling is initiated. In a large organ, like a porcine or human-sized pancreas, diffusion-based cooling is not effective at rapidly reducing the core temperature below 8 °C and thus the WIT is underestimated. In this study, we evaluate 4 different methods of donor porcine pancreas cooling performed during the procurement: surface cooling alone (*Method A*), an intravascular arterial flush (*Method B*), an intraductal infusion (*Method C*), and combining an intravascular flush with an intraductal infusion (*Method D*). The primary outcome of this study was to establish temperature profiles for the core of the donor porcine pancreas for each of the cooling methods and to determine which cooling method most rapidly cools a large organ. Secondary outcomes include correlating the cooling method that was employed with core pancreas tissue quality as assessed by histology. The main objective of this study was to optimize the DCD porcine pancreas cooling protocol used during procurement, which may also have implications on cooling protocols used during human pancreas procurements.

Methods

Porcine Pancreas Procurement

In this study, pancreata (n=94) were harvested following *en bloc* viscerectomy from DCD Landrace pigs. Subgroups of donor pancreata were designated for direct core pancreas temperature monitoring during procurement (n=24) and histological evaluation (n=16). All other donors (n=56) presented in this study were included to report statistics on the surgical procurement (e.g., mean WIT) but were used as part of other studies. All work involving animals was approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

The porcine pancreas procurement methods used in this study have been described previously (7). Briefly, donors were given intravenous heparin (100,000 U bolus) 5 minutes prior to sacrifice, which was done via an overdose with sodium pentobarbital (Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI) followed by exsanguination. *En bloc* viscerectomy was performed and the abdominal viscera were transferred to a surgical dissection table. Care was taken to preserve the vasculature for all organ procurements regardless of cooling method used.

Different cooling methods were used for each pancreas during the remainder of the procurement. All cooling methods involved standard surface cooling with crushed ice and irrigation with chilled (refrigerated) lactated Ringers solution (LRS). Unless designated for surface cooling alone (*Method A*), organs were randomized to undergo enhanced cooling with additional methods described below. Some of the organs underwent direct core pancreas temperature measurements throughout the procurement. Following organ cooling and dissection, each pancreas was massed and processed as indicated. If pancreas was designated for histological assessment (n=16), following procurement, the entire pancreas was flushed through the vasculature with 10% buffered formalin solution and biopsies from the core of the pancreas were taken for evaluation. If the pancreas was designated for islet isolation (n=18), the pancreas was placed on CPS and transported to the isolation facility.

Porcine Pancreas Cooling

All donor pancreata were surface-cooled with crushed ice and chilled LRS. Pancreata were then randomized to undergo cooling using 1 of 4 cooling *Methods*:

Method A—This *Method* only involved surface cooling with crushed ice and irrigation with chilled LRS (n=8), with no additional cooling technique used. This was considered the standard cooling methodology used during DCD porcine pancreas procurement. Two or more liters of crushed ice and 2 liters of chilled LRS were applied to the surface of the donor pancreas throughout the duration of the procurement. Isotonic ice was used for organs designated for histology or islet isolation. Active cooling began as soon as the pancreas was located within the viscera (typically within 2 minutes of evisceration), and was completed at the time of total pancreatectomy.

Method B—This *Method* involved additional arterial intravascular flushing which was conducted as quickly as possible (typically between 1–3 minutes) following viscerectomy

via cannulation of the aorta (*Method B1*, n=11) or individual cannulation of both the celiac trunk (CT) and superior mesenteric artery (SMA) (*Method B2*, n=10). For *Method B1*, the supraceliac aorta was flushed antegradely after the infrarenal aorta was clamped. For *Method B2*, all major branches from the CT that did not supply the pancreas and the intestinal branches from the SMA were ligated prior to flushing. *Method B1* was initially used during this study, which then evolved to *Method B2*. The methodology was changed because it was observed that substantial amounts of flush solution were lost through the numerous lumbar arteries, and needing to ligate these branches prolonged the procedure. It was determined that individually cannulating the CT and SMA did not increase WIT, so *Method B2* replaced *Method B1*. Regardless of which vessels were cannulated, 3–5L of heparinized (1,000 U/L) CPS (modified Cold Storage/Purification Stock Solution from Mediatech, Inc., Manassas, Va) or LRS were flushed at a rate of approximately 0.5L/min until flush effluent appeared clear of blood. CPS was used instead of LRS for pancreata designated for histology or islet isolation.

Method C—This *Method* involved intraductal infusion of CPS (n=6). Following the viscerectomy, the head of the pancreas was quickly (typically 2–3 minutes) separated from the bowel, and the main pancreatic duct was identified and directly cannulated with an angiocatheter (18–20 gauge depending on size). After the catheter was placed, 60 mL of CPS was infused at a rate of 1 mL/sec.

Method D—This *Method* combined an arterial intravascular flush (*Method B*) and an intraductal infusion (*Method C*) (n=59). Some of the pancreata were flushed via the aorta (n=4), but most via the CT and SMA (n=55). Immediately following transfer of the viscera to the surgical table, the vessels were cannulated and the flush was started. Next, the main pancreatic duct was located, cannulated, and infused as described above in *Method C*. Typically, 2 or more surgeons worked concurrently to expedite the cannulation and ligation procedures, and all cooling methods were initiated within 4 minutes of viscerectomy. The total flush and infusion volumes of CPS or LRS, and flow rates were the same as used in *Methods B* and *C*.

Temperature Measurements

As mentioned previously, some of the pancreata were designated for direct core pancreas temperature monitoring (n=24), and involved organs cooled by *Method A* (n=4), *Method B* (n=13), *Method C* (n=4) and *Method D* (n=3). Individual 33-gauge needle temperature probes were placed into the core of the distal splenic lobe, the proximal splenic lobe, the duodenal lobe and the connecting lobe (7) as soon as the viscera were transferred to the surgical table and the pancreas was identified. Temperature measurements were recorded in real-time (every 30 seconds) and throughout the duration of the procurement. Care was taken to ensure that the probes remained within the core of the pancreas as the organ was dissected. The mass of each pancreas was recorded following procurement and the mass of the ductal infusion solution was subtracted for relevant pancreata. The temperature measurements were used to observe different trends in core temperature profiles between groups and to calculate the amount of energy removed from the donor pancreas during cooling. These energy values were calculated for each pancreas by multiplying the

temperature change during a 10 minute period by the pancreas mass and an estimate of the specific heat of pancreas tissue. The specific heat for pancreas tissue was approximated assuming the pancreas has a density and thermal conductivity equal to that of water at the average observed temperature during the procurement. The average energy removed was also converted to a corresponding expected temperature drop (ΔT) for a representative and standardized 350-gram pancreas during the first 10 minutes of cooling.

Histology

As mentioned previously, some of the pancreata were designated to undergo histological evaluation ($n=16$), and included organs cooled by *Method A* ($n=4$), *Method C* ($n=4$), and *Method D* ($n=8$). Histology was not done for any organ cooled by *Method B* due to budgetary constraints and also because significant differences in the histological scores between organs cooled by *Methods C* and *D* could be attributable to the use of an intravascular flush (which indirectly reflects the impact of *Method B*). Approximately 240 mL of chilled 10% buffered formalin solution was infused intravascularly following the surgical procurement, and multiple core biopsies were then taken and placed into formalin. The biopsies were then processed by embedding in paraffin, sectioning and staining with hematoxylin and eosin (HE). Histological evaluation was done in blinded fashion by an experienced histopathologist. Samples were systematically scored for the degree of necrosis and autolysis observed in each histological section; scoring was graded as follows: 0=none, 1=10%, 2=11–25%, 3=26–50%, 4=50–75%, 5=76%. Necrosis and/or autolysis was defined by the presence of nuclear pyknosis or karyorrhexis, cytoplasmic hypereosinophilia, loss of intercellular junctions, loss of tissue integrity, cellular lysis, dystrophic mineralization, and disintegration of tissue structures sometimes with associated extravasation of blood.

Statistical Analysis

All data were reported as mean values and standard errors. Overall differences between groups were compared using a one-way analysis of variance (ANOVA, if normally-distributed) or the Kruskal-Wallis test (if not normally-distributed). Mean values between all groups were compared using Tukey's multiple comparison post-test (if normally-distributed) or the Mann-Whitney test (if not normally-distributed). Statistical significance corresponded to p -values <0.05 using a 95% confidence interval. All statistical analyses were performed using Graphpad Prism Version 5.03 (Graphpad Software, Inc., La Jolla, CA).

Results

Donors had a mean age of 22 ± 2 months ($n=67$) and a mean weight of 505 ± 10 pounds ($n=87$), in those donors for which data was available. Mean time required to perform the *en bloc* viscerectomy was 6.0 ± 0.3 minutes. Mean time to complete the arterial intravascular flush was 31 ± 1 minutes. There were overall differences in the mean donor weight between *Methods* ($p = 0.008$) and total procurement time ($p < 0.001$), but no differences in the other metrics (Table 1). When comparing individual *Methods*, there were differences in the total procurement times between *Method B1* and every other *Method* ($p < 0.05$). This difference is likely explained by the increased time needed to ligate numerous lumbar arteries for organs procured using an arterial flush through the aorta (*Method B1*). This difference was

not observed with *Method B2* or *D* because these organs were primarily procured using direct intraarterial flush via the CT and SMA. All other *Methods* took a statistically similar total procurement time ($p > 0.05$). There were no differences in the recorded WIT between groups ($p = 0.36$), and no difference when comparing *Methods A* and *D* ($p = 0.50$), which represented the most different cooling methodologies.

The core temperature profiles were recorded for pancreata procured with each cooling *Method* and representative profiles are illustrated in Figure 1. The representative illustration was generated based on the actual data obtained from the recorded temperature profiles. Illustrative profiles were used to present this data because they accurately reflect the nature of the cooling observed with respect to the *Method* used. This interpretation of the data allows for direct visual comparison between cooling methods without the confounding differences that the organ variability introduces to the individual organ temperature measurements. *Method A* gradually decreased the core temperature over a 30 minute period. *Method B* decreased the organ temperature more rapidly than *Method A*. *Method C* decreased the core temperature of the pancreas rapidly (15–20 °C during the first few minutes of cooling), but the rate of cooling immediately slowed after completion of the intraductal infusion. *Method D* provided the most optimal cooling, including both rapid cooling in the first few minutes of the procurement and sustained cooling throughout the procurement.

When temperature data was used to calculate the average thermal energy removed during a 10 minute period and then standardized to a 350-gram pancreas, *Method A* removed an average of 10 ± 1.5 kilojoules (KJ) of energy, which corresponded to a ΔT of 10°C. *Method B* removed an average of 18 ± 2.8 KJ of energy, corresponding to a ΔT of 22°C. *Method C* removed an average of 16.8 ± 3.9 KJ of energy, corresponding to a ΔT of 20°C. *Method D* removed an average of 34 ± 4.8 KJ of energy, corresponding to a ΔT of 37°C, and was the *Method* which provided the most rapid cooling (Figure 2). These calculations enabled more accurate comparisons between cooling *Methods* in the setting of confounding variables such as different starting core pancreas temperatures and organ masses.

Biopsies obtained from pancreata cooled with *Method A* exhibited moderate necrosis/autolysis, while biopsies from pancreata cooled with *Method C* exhibited mild necrosis/autolysis. Biopsies obtained from pancreata cooled with *Method D* exhibited the least (or minimal) necrosis/autolysis (Figure 3). Histological scores were overall different between the cooling *Methods* used ($p=0.04$). When compared individually, there were differences in the histological scores between *Methods A* and *C* ($p=0.03$) and *Methods A* and *D* ($p=0.03$), but not between *Methods C* and *D* ($p=0.91$) (Figure 4).

Discussion

Porcine islet xenotransplantation may offer an attractive alternative to human islet allotransplantation if an efficacious immunosuppressant protocol is developed (4) or if the xenoislets can be immunoisolated (9). This would enable access to a practically-unlimited donor pancreas supply. Since the isolation procedure itself is expensive and time-consuming, optimizing isolation outcomes is imperative. Consequently, optimization of

improved porcine pancreas procurement protocols is needed. There may be a substantial difference in the core organ temperature during procurement depending on the cooling method utilized, but also depending on the individual organ size (mass), shape, and condition. There were differences in the average pancreas mass in each group (Table 1), but these differences were not different between groups ($p=0.36$), and are most likely explained by differences in donor mass or age. To effectively compare and interpret the efficacy of cooling methods, the heat removed during the first 10 minutes of cooling was calculated from the temperature data obtained for each individual organ. This calculation can account for the differences in the initial amount of thermal energy in each pancreas due to the observed variability in starting core pancreas temperature and organ mass. In this study, we found that additional cooling via the combination of an intravascular flush and an intraductal infusion of CPS reduced the core pancreas temperature much more rapidly than surface cooling alone. Surface cooling alone is incapable of quickly cooling a large organ (like a human or porcine pancreas). With surface cooling, heat transfer is very inefficient and highly variable, especially for larger organs since it relies on thermal diffusion from the surface of the organ. In many cases, it took as long as 30 minutes for a large pancreas to reach a core temperature of $<15^{\circ}\text{C}$. In these cases, the actual WIT is significantly underestimated. The deleterious effect of increased WIT on organ function following transplantation has been previously recognized (10). Prolonged WIT has also been shown to be a critical factor affecting successful islet isolation (11). Tanioka *et al.* reported that periods of WIT >30 minutes result in significant reduction in islet yields and viability (12).

Although variations in porcine pancreas anatomy and size can affect temperature profiles measured during and after procurement, we have demonstrated that intraductal infusion provided the most consistent and dramatic cooling during the first few minutes of the procedure and can be done quickly. Intraductal infusion was able to consistently cool the pancreas by $>10^{\circ}\text{C}$ during the first 2 minutes of cooling. As shown in Figure 1, a dramatic drop in core pancreas temperature was observed with both *Method C* and *Method D*. However, once the 60 mL of CPS was infused into the pancreatic duct, the rate of cooling was no better than with surface cooling alone. In contrast, the intravascular flush provided a sustained cooling of the pancreas throughout the duration of the procurement. When *Method C* was combined with intravascular flushing (*Method D*), core pancreas temperature could be reduced from 37°C to 15°C in 10 minutes. This dramatic improvement in cooling rate results from the substantial increase in the surface area available for heat transfer, which occurs with flushing CPS into the vasculature and instilling it into the ductal system. *Method D* leveraged both the immediate cooling of intraductal infusion and the sustained cooling of the intravascular flush without increasing the time of procurement when compared with *Method A* ($p=0.31$). Detailed studies focused on the effects of pancreas size and shape on cooling method heat transfer would considerably improve our understanding of these methods, and could potentially suggest further optimized methods for improved organ cooling.

Other factors during procurement and beyond WIT may affect the success of subsequent islet isolation. Increased pancreatic edema has been shown to correlate with decreased islet yields for human islet isolation (13). However, there is some conflicting evidence in the

literature which suggests that some edema may improve islet recovery (14). This may explain why the use of hypothermic machine perfusion (via the CT and SMA) has been associated with greater porcine islet yields as compared to static cold storage (15). We were concerned that flushing and instilling fluid into the pancreas may cause edema, which would be problematic. In our study, no obvious gross pancreatic edema was observed.

In addition to improved cooling, the intravascular flush, either via the supraceliac aorta or directly through the CT and SMA, appeared to completely clear the intravascular space of residual blood (see histology from pancreas cooled by *Method D* in Figure 3). When combined with adequate heparinization, the finding of intrapancreatic blood or clotting is unlikely. This is of particular importance for islet isolation, because the presence of intravascular thrombi within the pancreas has been shown to diminish islet yield (16). The number of recovered islets following enzymatic digestion correlates inversely with the blood content in the porcine pancreas (17).

Our results indicate that there is a correlation between the rate of core pancreas cooling and histological outcomes. Histological scores of the islet tissue sections were compared, and as expected, *Method A*, *C*, and *D* resulted in moderate, mild and minimal autolysis observed, respectively. The necrosis/autolysis score and necrosis/autolysis score per pancreas also inversely correlated with the rate of cooling, and there were differences between *Methods A* and *C* ($p=0.02$) and *Methods A* and *D* ($p=0.02$). Histological studies were not conducted for *Method B*, because of budgetary constraints and because differences between *Method C* and *D* could be in part attributable to the use of an intravascular flush with *Method D*.

Interestingly, there were no differences in the necrosis and autolysis score when comparing samples cooled by *Methods C* and *D* ($p=0.95$). This may suggest that the addition of a selective intravascular flush, though able to clear blood and facilitate sustained cooling of the organ, may provide less benefit as indicated by histology. This study compared the histological necrosis/autolysis score between *Methods* as an early indicator of tissue damage. Tissue biopsies were collected and fixed immediately following completion of the procurement and represent a snap-shot of the tissue quality up to the time of tissue fixation. Although beyond the scope of this study, there may be further divergence in tissue quality following transplantation and reperfusion. However, the observation that combined cooling involving an intraductal infusion may have implications for human pancreas procurement, where an intraductal infusion is not standard practice but may be beneficial (18, 19).

Porcine DCD donors were used to compare cooling methods in this study, but the temperature profiles and energy calculation results can offer insights into cooling methods used for human DCD and donation after brain death (DBD) procurements. DCD organs are being utilized in higher numbers due to an increasing demand for donor organs and improved organ preservation. Enhanced organ cooling may enable even more higher-quality DCD organs to be used for islet isolation. Analysis of the temperature data suggests that it could be advantageous to include an intraductal infusion during human pancreas procurements. For solid pancreas transplant the benefits of the improved cooling would have to be weighed against the potential increased risk of contamination associated with cannulation of the pancreatic duct. For human islet isolation, ductal cannulation is already necessary for the infusion of enzyme, therefore, the added risk of bacterial contamination

may be minimal. In these studies, the risk of contamination is reduced during the cannulation of the duct by avoiding an enterotomy.

Improved cooling results in better porcine islet yields and quality, and these results (from a subset of pancreata studied herein) have been published previously (8). It was reported that when pancreata were cooled with both intravascular flush and intraductal infusion, purified islet yields, and the number of islet equivalents per gram pancreas increased by >150% compared to surface cooling alone. The fractional viability (measured by the oxygen consumption rate normalized to DNA content) of islets after 7 days in culture was also significantly improved for pancreata procured using combined cooling. The rapid, early cooling resulting from using an intraductal infusion may have an effect on islet isolation outcomes (18–20). Noguchi *et al.* found that islet yields were improved, islet apoptosis was reduced and ATP levels in the pancreatic tissue were higher in human pancreata procured with an intraductal infusion prior to cold storage (20). Similarly, Matsumoto *et al.* reported that islet yields and quality were higher in those human pancreata undergoing an intraductal infusion (18). In contrary, Nakanishi *et al.* showed no improvement in rat islet yield and quality (19), which highlights that the greatest benefit of an intraductal infusion may be with larger (human or porcine) pancreata. Cold ischemia time (CIT) is a factor that impacts islet yield after isolation (13). In the present study, CITs recorded for pancreata selected for islet isolation (8) were found to be comparable with CITs recorded for all pancreata cooled by *Methods A, C or D* of this study (data not shown).

In conclusion, using a combined cooling method (*Method D*) can consistently cool the core of the pancreas, in sharp contrast to standard surface cooling alone (*Method A*) which is ineffective at rapidly reducing the core temperature in DCD porcine pancreata and resulted in poorer histological outcomes. In our study, intraductal infusion improved initial organ cooling, but is limited by the volume of CPS that can be used and does not contribute to sustained organ cooling. Alternatively, an intravascular flush can be performed continuously and should be done as it provides sustained cooling during the entire duration of the procurement. Combined surface, intravascular and intraductal cooling, appears to be the optimized and most consistent cooling *Method*. These data also may have implications on human pancreas procurement since intraductal infusion of CPS is not common practice, and should be studied further.

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Abbreviations

T	temperature drop
CIT	cold ischemia time

CPS	cold preservation solution
CT	celiac trunk
DCD	donation after cardiac death
HE	hematoxylin/eosin
KJ	kilojoule
LRS	lactated Ringers solution
SE	standard error
SMA	superior mesenteric artery
WIT	warm ischemia time

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Figure 1.

Illustration depicts the different core pancreas temperature profiles for each cooling *Method*: surface cooling alone (*Method A*, the current practice), intravascular (arterial) flush (*Method B*), intraductal infusion (*Method C*), and combined intravascular flush with intraductal infusion (*Method D*). Note the rapid cooling following an intraductal infusion of cold preservation solution (CPS), and the improved sustained cooling with an intravascular flush with CPS.

Figure 2.

Calculated average amount of energy removed and estimated core temperature drop (ΔT) during the first 10 minutes of cold ischemia time in a standardized 350-gram pancreas procured using surface cooling only (*Method A*, n=4), surface cooling and an intravascular flush (*Method B*, n=13), surface cooling and an intraductal infusion (*Method C*, n=4) and combined method with surface cooling, an intravascular flush, and an intraductal infusion (*Method D*, n=3).

Figure 3.

Representative histology from pancreata cooled by *Methods A, B* and *D*. Pancreatic tissue cooled using *Method A* (surface cooling) exhibited moderate features of autolysis and many of the islet cells have partially condensed nuclear chromatin. Pancreatic tissue cooled using *Method C* (intraductal infusion) showed mild autolysis, nuclear condensation or lysis and some islet cells exhibit cytoplasmic hypereosinophilia. Exocrine cells may present some pyknotic nuclei and cytoplasmic condensation. Pancreatic tissue cooled using *Method D* (surface cooling combined with an intravascular flush and an intraductal infusion) exhibited minimal autolysis. Furthermore, very few of the islet cells exhibited cytoplasmic hypereosinophilia and nuclear pyknosis, and cell morphological details are more easily distinguishable with exocrine granules being well-preserved in samples taken from pancreata cooled by *Method D*.

Figure 4.

Plot of necrosis/autolysis scores from biopsies of pancreata cooled using *Methods A, C* and *D*. The scores were significantly worse in pancreata cooled only with surface cooling (*Method A*) as compared to pancreata cooled by intraductal infusion (*Method C*) or the combined approach (*Method D*). Lines and error bars represent the mean $\pm$ standard errors.

Table 1

Surgical procurement data

	A	Cooling Method			D	p-value
		B1	B2	C		
Number	8	11	10	6	59	
Mean (± SE) donor weight (kg)	189.5±14.3	206.9±10.4	238.4±18.4	215.6±19.0	239.7±5.3	0.008*
Mean (± SE) donor pancreas mass (g)	263.3±30.1	371.9±47.7	383.6±66.1	234.0±4.0	355.4±20.0	0.358
WIT (min) ^a	11.7±1.1	12.7±2.6	10.8±1.0	14.3±2.1	13.0±0.5	0.582
Procurement time (min) ^b	28.8±1.8	39.4±1.4	28.9±3.0	24.6±2.0	31.2±0.9	<0.001**

Abbreviations: SE, standard error (of the mean); WIT, warm ischemia time. Method A: Surface cooling alone; Method B1: Arterial intravascular flush (supraceliac aorta); Method B2: Arterial intravascular flush (celiac trunk, superior mesenteric artery); Method C: Intraductal infusion; Method D: Combined method, including arterial intravascular flush and intraductal infusion.

^aTraditional WIT defined as the duration of time from cardiac arrest to beginning of cooling intervention

^bDefined as the duration of time from cardiac arrest to completion of pancreatectomy.

* $p<0.05$;

** $p<0.001$