

An Ex-Vivo Model for Hypothermic Pulsatile Perfusion of Porcine Pancreata: Hemodynamic and Morphologic Characteristics

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Abstract

Objectives: Hypothermic machine perfusion is a well-established preservation method for kidneys that allows for better preservation over longer periods and pretransplant assessment of graft viability. This technique has only sporadically been used for pancreatic grafts. The aim of this study was to establish a hypothermic machine perfusion model for porcine pancreas perfusion.

Materials and Methods: Fifteen porcine pancreata were subjected to 25 minutes of warm ischemia and 149 minutes of cold ischemia before undergoing meticulous bench work preparation and perfusion, via an aortic segment, on the RM3 perfusion machine with University of Wisconsin (Barr Laboratories Inc., Pomona, NY, USA) solution. Perfusion variables (°C, temperature; mm Hg, systolic perfusion pressure; mL/min, flow volume; mm Hg/mL/min, resistance) were recorded every 30 minutes. Tissue samples were assessed for each pancreas preperfusion and postperfusion using a semiquantitative scoring scale to grade histopathologic changes: acinar cell damage (0-4), islet cell damage (0-3), inflammation (0-3), and edema (0-3).

Results: Hypothermic machine perfusion time was set at 315 minutes, and all grafts were maintained between 4-10°C. The results were as follows (range, mean $\pm$ SD): systolic perfusion pressures were

5-13 mm Hg (9.61 ± 3.25 mm Hg) during the first 60 minutes (priming), and 15-23 mm Hg (21.07 ± 4.26 mm Hg) during the maintenance period. Target flow volumes reached 141-152 mL/min (147.6 ± 8.969 mL/min) at 60 pulses per minute. Intrapaneatic resistance decreased throughout priming to 0.03-0.09 mm Hg/mL/min (0.083 ± 0.042 mm Hg/mL/min), and remained unchanged until completion of perfusion. Pancreatic weight increase varied from 3.2% to 18.3% ($13.36\% \pm 4.961\%$). There was significant postperfusion reduction in islet and acinar cell damage ($P = .001$ and $P = .01$ respectively).

Conclusions: We have developed a model of machine perfusion for porcine pancreata which is simple, reliable, and protects graft histopathologic integrity. The model can be used in further studies to improve the quality of pancreas preservation, and assess and improve the viability of the condition of borderline pancreatic grafts.

Key words: Machine perfusion, Pancreas transplant, Pancreas preservation

A small number of pancreata from deceased donors are suitable for transplant. This is because the pancreas is a low-flow organ (1) and more vulnerable and fragile compared with the kidney. It often is seriously damaged during the events of brain death and organ retrieval (1).

Graft thrombosis is one of most severe problems, representing more than 70% of all technical failures (2, 3). The incidence of thrombosis is 1.4% to 19% (4-8). Multiple risk factors have been implicated in the development of graft thrombosis: low blood flow within the pancreas graft (5-7), overperfusion with preservation solutions (9), retrieval and reperfusion injury (5), cold ischemia time over 12 hours (10),

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donor age over 45 years (10), cardiovascular cause of donor death (10), use of a portal vein extension graft (10), left-sided implantation into recipient (10), the requirement of high-dose dopamine (10 mg/kg/min), or 2 or more vasopressors at the time of retrieval (10).

In the field of renal transplantation, especially in the context of donation after cardiac death, hypothermic machine perfusion has been shown to be an excellent method of preservation (11-14). It yields the longest periods of preservation, and allows pretransplant assessment of graft viability (11-12, 14). However, it has only sporadically been used for the preservation of pancreatic grafts (15). This may be due to its complexity, and the lack of development of ideal perfusion conditions for the pancreas.

The major aim of our study was to develop a reproducible, *ex vivo*, hypothermic perfusion model that will reliably preserve pancreatic grafts by improving their intraparenchymal integrity, thereby increasing the chances for successful engraftment, as well as the number and quality of donor pancreata available for transplant.

Materials and Methods

Fifteen porcine pancreata were obtained from Landrace-cross pigs weighing 60-90 kg (65 ± 11.87 kg) from the Northwick Park Institute of Medical Research. All animal procedures were approved by the animal research ethics committee of the source institution, and the experiments were conducted in unison, with the conclusion of several unrelated studies, thereby limiting unnecessary organ retrieval. The care and handling of the animals was in accordance with the Animals (Scientific Procedures) Act of 1986.

The animals first were premedicated with ketamine (5 mg/kg IM) and xylazine (1 mg/kg). After intubation and artificial ventilation, induction of inhalational anesthesia was maintained by the continuous infusion of isoflurane—and a gas mixture of nitrous oxide (66%) and oxygen (33%). All animals were maintained during the operation under standardized and controlled conditions: body temperature was $37 \pm 1^\circ\text{C}$, the electrocardiogram showed a pulse rate between 80 and 120 bpm, and blood pressure was 90-160 mm Hg systolic and 40-80 mm Hg diastolic. Arterial blood pH was kept between 7.25 and 7.5, and the arterial pO_2 above

90 mm Hg. Analgesia was provided in the form of carprofen (4 mg/kg). Furthermore, an antibiotic, ampicillin LA (25 mg/kg), and an antihelminthic—ivermectin (1 mL)—were administered intravenously. The animals were finally killed using sodium pentobarbitone (200 mg/mL [140 mg/kg]) under previously induced, full, general anaesthesia.

After declaration of cardiac death, pancreata were exposed for a period of 1.25-1.75 minutes (1.501 ± 0.159 min) of *in situ*, warm ischemia, during which time the animal carcass was transferred to the dissection table. The abdomen was opened by a median laparotomy. After a splenectomy, the segment of the aorta with the celiac and upper mesenteric artery was stripped bare of all surrounding tissue, and snared after ligation of the lumbar arteries. The common hepatic artery was ligated and transected behind the outlet of the gastroduodenal artery close to the liver. After ligation of the left gastric artery and vein, the pancreas was separated from the duodenum and the surrounding vessels. The portal vein was ligated above and below the pancreas, after insertion of a catheter for collecting the venous effluent.

The pancreatectomy procedure for each pancreas was satisfactory, with minimal, observable, physical damage, and took approximately 15.7-24.2 minutes (20.328 ± 2.676 min). After their retrieval, pancreata were transferred to a bench, and placed in a kidney dish filled with ice and University of Wisconsin solution. A 20-French, Foley catheter was inserted into the preserved aorta and kept in position with a silk tie. The aorta was tied distally with another silk tie, and the tributaries ligated. Five hundred milliliters of University of Wisconsin solution was then allowed to pass through the Foley catheter, into the aorta, with a hydrostatic pressure of 90 mL/min. The bag of perfusate was placed approximately 1 meter above the bench. Perfusion was confirmed when blood-stained fluid was noticed draining from the portal vein, and continued until clear fluid drained from the portal vein. Perfusion, via the aorta, was used to avoid any damage to the splenic arteries, which would bias the study. The warm ischemia period from the impact of the lethal injection, to cannulation of the aorta and beginning of perfusate flow (flush-out), varied between 23.2 and 27.5 minutes (25.13 ± 1.328 mins). Each flushed pancreas was then placed in a plastic bag, prefilled with University of Wisconsin solution and preserved on ice, for transportation to the laboratory. This subjected pancreata to a cold

ischemia period of 135-165 minutes (149.6 ± 8.982 mins) before laboratory perfusion was commenced.

All 15 retrieved pancreata were included in the study, and each was perfused using a RM3 perfusion machine (Waters Medical Systems, Rochester, MN, USA) with nonoxygenated University of Wisconsin solution. The total perfusion period for each experiment lasted 315 minutes. Each experiment in the laboratory started with a second blood wash-out of the pancreatic graft, with meticulous control of any leaks. A disposable cassette—including the reservoir, pump heads, oxygenator, and tubing—were simultaneously attached to the RM3 control unit. The pancreas was submerged in the reservoir of the cassette containing University of Wisconsin solution, and the proximal aortic segment was cannulated to the tubing of the cassette via a plastic adaptor (Henleys Medical Supplies Ltd, Hertfordshire, UK).

Systolic perfusion pressure (mm Hg), flow volume (mL/min), intrapancreatic resistance (mm Hg mL/min), and temperature ($^{\circ}\text{C}$), were continuously recorded and analyzed in 30-minute intervals. Data were transmitted from the RM3 perfusion machine to a host computer, using HyperTerminal software (Hilgraeve Inc., Detroit, MI, USA). Trend data were subsequently imported and plotted using Microsoft Excel.

Histopathologic analysis

Pancreatic wedge biopsies were taken for histopathologic analysis, before perfusion and immediately after completion of perfusion, when the pancreata had been removed from the RM3 machine. For each case, 1 cm³ of biopsy specimen was obtained from different areas of the parenchyma in the tail of the pancreas to maintain uniformity of the results. All sites of preperfusion biopsies were inspected closely, and there was no evidence of leakage. The samples were preserved in 10% formaldehyde, and kept in cold storage at 0-2 $^{\circ}\text{C}$ for the duration of the study. Tissue blocks were processed in paraffin wax blocks by routine, automated, procedures. Sections were cut at 4 μm and stained with hematoxylin and eosin for evaluation, using light microscopy. The pathologist had no knowledge of the origin of each block. Scoring of sections was over 4 fields, assessing changes in 4 morphologic parameters: acinar cell damage, islet cell damage, inflammation, and edema.

Acinar cell damage, was characterized as no damage (grade 0), mild focal damage (< 5%, grade 1),

moderate sublobular damage (< 20%, grade 2), severe lobular damage (> 20%, grade 3), and global diffuse damage (> 40%, grade 4). For islet cell damage, the degree of injury included no damage (intact islets, grade 0), mild damage (grade 1), moderate damage (grade 2), and severe damage (absent islets, grade 3). In the case of inflammation and edema, each parameter was similarly scored, using the following semiquantitative scale: grade 0 (none), grade 1 (mild), grade 2 (moderate), and grade 3 (severe).

Statistical analysis

All evaluation data is expressed as a range, mean $\pm$ standard deviation (SD). Statistical significance was determined using Minitab Version 15.1.1.0 software (PA, USA). A Pearson chi-squared test was used to compare damaged and nondamaged preperfusion and postperfusion biopsies. Values for *P* less than or equal to .05 were considered statistically significant.

Results

Temperature measurements of the perfusate demonstrated a constant range between 4 $^{\circ}\text{C}$ and 10 $^{\circ}\text{C}$. Pulsatile perfusion was performed at 58-60 pulses per minute for all experiments. Preperfusion pancreatic weights varied between 131.4-216.7 g (187.95 ± 21.115 g). During the first 60 minutes of perfusion (priming), pancreata were perfused at low systolic perfusion pressures ranging between 5 and 13 mm Hg (9.61 ± 3.25 mm Hg). Intrapancreatic resistance was recorded and calculated by the RM3 machine ($\text{IPR} = \text{mean perfusion pressure/flow volume}$).

For each pancreas, intrapancreatic resistance during the period of priming decreased to an optimal resistive index of 0.03-0.09 mm Hg/mL/min (0.083 ± 0.042 mm Hg/mL/min) and was sustained until completion of perfusion. The time to best resistive index was between 3 and 65 minutes (26.26 ± 14.51 min). Pancreatic grafts were then perfused during the maintenance period (255 min), with systolic perfusion pressures between 15 and 23 mm Hg (21.07 ± 4.261 mm Hg). Target maximal flow volumes reached 141-152 mL/min (147.6 ± 8.969 mL/min) (Figure 1). Postperfusion pancreatic graft weights varied between 155.4 and 238.6 g (211.59 ± 22.756 g).

The percentage of pancreatic weight increased after perfusion and varied between 3.2% and 18.3%

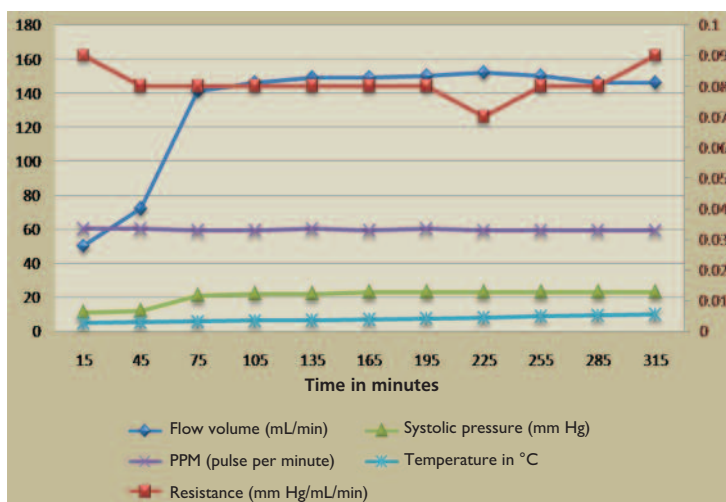


Figure 1. Mean results of flow parameters during 315 minutes of hypothermic machine perfusion preservation. Intrapancreatic resistance is plotted on the secondary Y-axis.

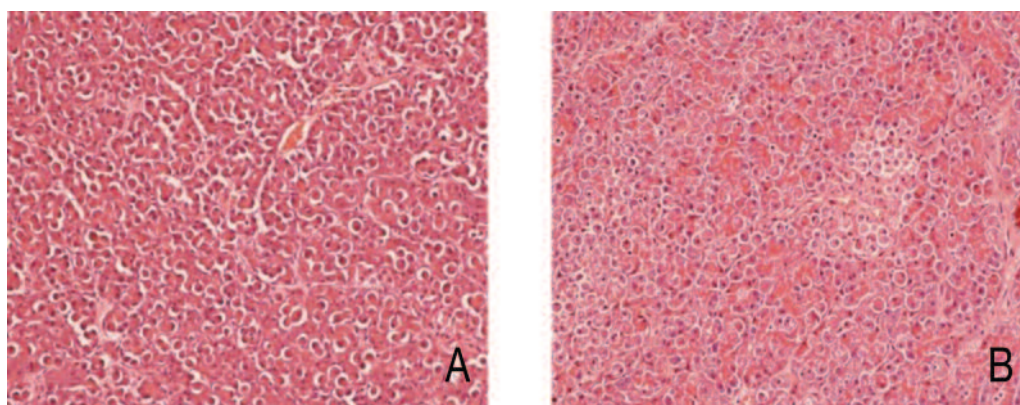


Figure 2. Histopathology (hematoxylin and eosin staining) shows well-preserved parenchymal architecture and improved acinar and islet cell integrity after 315 minutes of hypothermic machine perfusion preservation when comparing preperfusion (A) with postperfusion (B) biopsies. Original magnification ($\times 200$).

($12.715\% \pm 3.613\%$); net weights increased from 5.98 to 31.0 g (23.645 ± 6.2798 g).

The criteria outlined by Talbot and associates (16) for the kidney were used to calculate a perfusion flow index for each pancreatic graft (perfusion flow index = flow volume in mL/min/100 g pancreas/systolic pressure). From these calculations, flow in all the pancreata remained above 65 mL/min/100 g pancreas tissue (72.679 ± 9.8096 mL/min/100 g), and the perfusion flow index stayed above 2.8 mL/min/100 g pancreas/aortic systolic pressure (3.523 ± 1.135 mL/min/100 g/mm Hg). All pancreata showed excellent perfusion characteristics, and would be considered suitable for transplant in a clinical setting.

Histopathology results

Histopathologic examination of preperfusion specimens showed evidence of lobular damage ($> 20\%$, grade 3) and moderate islet cell damage

(grade 2), with moderate degrees of inflammation (grade 2) and edema (grade 2) in all specimens. Postperfusion, pancreatic sections showed significant improvement in the tissue damage with focal ($< 5\%$, grade 1) to sublobular ($< 20\%$, grade 2) acinar cell damage, mild islet cell damage (grade 1), and mild degrees of edema (grade 1) and inflammation (grade 1). These changes were observed for all specimens (Figure 2). The mean damage was statistically significantly different when comparing the 15 pancreatic specimens collectively, preperfusion and postperfusion, for acinar cell damage ($P = .010$) and islet cell damage ($P = .001$), with the calculated Pearson chi-squared equal to 6.652 and 10.800, respectively.

Discussion

Perfusion parameters of flow and resistance are commonly used to evaluate kidney grafts and the

decision whether or not to transplant a marginal kidney allograft (17, 18). We reviewed these perfusion parameters from previous kidney perfusion studies by Talbot and associates (16) and Nicholson and associates (17) to examine the validity of this approach in perfusion of the pancreas. We did this because a better understanding of the correlation of perfusion parameters and subsequent outcome may result in a more-appropriate and more-efficient use of the pancreas. Most viability parameters are determined within the first 4 to 6 hours of perfusion, which is considered to be an optimal time for pancreatic preservation (17). Therefore, the duration of our machine perfusion was not extended beyond this time. This perfusion period was adapted to studies by Nicholson and associates (17), and several other groups during the hypothermic machine perfusion of porcine kidneys (16, 17, 19). Earlier experience with perfusion has shown that vascular resistance falls to a baseline level after 6 hours, and beyond this, perfused organs sometimes have increasing edema (17).

For optimal preservation, we postulate that lower-than-physiologic perfusion pressures should be used during the initial wash-out to minimize pancreatic microvasculature damage due to shear stress (20). During priming, decreased resistance and perfusion pressure serve to optimize perfusion and viability indices. The subsequent target maintains flow volumes of more than 65 mL/min/100 g pancreas throughout perfusion, and a weight increase of less than 20%. This way, we discovered that viability testing using these indices is important, but nevertheless, hypothermic machine perfusion can damage the pancreas. Hart and associates report that hypothermic machine perfusion at approximately 25% of normal physiologic perfusion was optimal, and minimizes endothelial injury during experimental liver perfusion (21).

Based on an educated hypothesis and proven in our experiments comparing different perfusion pressures, we found that the optimal, initial, systolic perfusion pressure during priming was between 5 and 13 mm Hg, followed by a maintenance systolic perfusion pressure of 15 to 23 mm Hg. Our perfusion outcomes show a complete and uniform washout of donor blood from the microvasculature of the porcine pancreas, with minimal endothelial, acinar, and islet cell damage on light microscopy. We suggest that an increase in perfusion pressure is preferable during the maintenance period of perfusion. This is because it improves the effectiveness of the washout of blood

during perfusion, shortens the initial warm ischemia time during pancreatic procurement, and reduces ischemia-reperfusion-related injuries. This is reflected by better hypothermic machine perfusion viability parameter function, with decreased resistances, and stable flow volumes during perfusion. By keeping the maintenance perfusion pressure at 15 to 23 mm Hg and introducing the perfusion flow index, damage incurred from machine perfusion can be minimized while retaining the initial viability. This has the central theme of not forcibly lowering the resistance in the pancreatic tissue, so that acceptable levels of resistance actually may be higher. We defined this level of resistance in our study to be lower than 0.15 mm Hg/mL/min.

Resistance to flow was judged to be one of the most-important perfusion parameters. As long as resistance was decreasing during priming and maintained throughout the remainder of perfusion, this was considered sufficient. Furthermore, by analyzing machine-perfused pancreata, we could assess whether pancreatic weight increase is an independent predictor of graft quality and function. Our pancreata gained between 3.2% and 18.3% of their retrieval weight during perfusion. No simple correlation, however, could be identified between weight gain and the perfusion variables. Weight increase of perfused pancreata is inevitable, and occurs if the pancreatic microvasculature is partially thrombosed and fluid is forced into the interstitium (16). It also occurs as a result of the initial ischemic damage, producing leaky capillaries, which is followed by perfusion with a noncellular solution (University of Wisconsin), which then forces fluid into the interstitium. While this swelling is usually reversible, some long-standing changes can occur (16). These findings satisfy the criteria outlined by Talbot and associates (16), which propose that an acceptable weight increase for kidneys should always be less than 40% (16), and optimally less than 25%.

Preperfusion and postperfusion biopsies are obtained from different areas of the parenchyma in the tail of the pancreas and allow for an overall comparison of the integrity of the pancreatic parenchyma, at the beginning and the end of hypothermic machine perfusion. Morphologic evaluation of pancreata preserved with our low-pressure perfusion technique show that acinar and islet cell integrity are protected after hypothermic machine perfusion. Light microscopy demonstrates intact morphology and no observable alteration in

acinar pattern, nuclei, or cytoplasm. It is assumed that perfusion pressures are injurious, both to parenchymal architecture and the endothelial cell structure. Both acinar and islet cells are more prone to injury, as they become more rigid and less adept to changes in perfusion dynamics at cold temperatures. Hypothermic machine perfusion also may induce shear stress to the endothelial cells that are already more-sensitive at low temperatures and thus prone to injury. We postulate that both cellular and shear stress-induced endothelial cell injury can be prevented by lowering the perfusion pressure during priming.

This model has several advantages, and the potential to be translated to the clinical setting. Porcine pancreata are recognized as having anatomic and physiologic characteristics that closely resemble the human situation, so the data are more representative than findings in small animal models (2). The pig pancreata we used were of a similar size to the adult human pancreata, because they were taken from animals weighing on average 60 kg to 70 kg. This model was reliable, reproducible, and made use of modern perfusion technology, whereby continuous measurement of physiological parameters is possible. It is a versatile method for investigating the pathophysiology of warm or cold ischemia and subsequent pancreatic perfusion injury (1). Furthermore, it permits evaluation of different interventions in the clinical setting to ameliorate ischemic reperfusion injury. And it may prove to be the basis of future pretransplant viability testing for pancreata from marginal donors, thus helping to increase the donor pool safely (1).

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