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A Prospective Study of the Efficacy of Human Pancreas Hypothermic Oxygenated Machine Perfusion for Islet Isolation in Clinical Settings

Marjolein Leemkuil*

Department of Gastroenterology and Hepatology, Mayo Clinic, Rochester, USA

Introduction

Allogeneic transplantation of pancreatic islets can be beneficial for a select group of people who have been living with type 1 diabetes for a long time. Modern immunosuppressive regimens can improve glycemic control in the majority of transplanted patients. In the Eurotransplant region, where vascularized pancreas transplantation is the preferred option, pancreases with less favorable donor characteristics are left for islet isolation. As a direct consequence of this, a greater number of pancreases have been approved for isolation from donors who meet extended criteria (ECDs), such as donors who donated following a circulatory death (DCDs). In the Netherlands, DCD procedures accounted for 34% of all multi-organ donation procedures in 2010, rising to 59% in 2019. The use of DCD organs is thought to carry additional risks due to their increased susceptibility to the adverse effects of both warm and cold ischemia. It has been demonstrated that islet isolation from DBD pancreases yields 87 000–100 000 IEQ more than from DCD donors. DCD pancreases frequently gain more benefit from donor characteristics as a result: After islet isolation, similar levels of viability and function were observed in donors who were younger, more frequently male, and had no prior history of cardiac arrest. It has been demonstrated that prolonged cold ischemia time (CIT) is an independent risk factor for technical failure following pancreas transplantation. It is hypothesized that these negative effects are even more pronounced in DCD pancreases. In addition to cold ischemia, multiple physiochemical stress factors cause islet loss when islets are isolated from a donor pancreas. One way to extend the CIT without sacrificing quality is to minimize damage during preservation. Hypothermic machine perfusion (HMP) has been proposed as an alternative method for completing this task. It has been demonstrated that HMP decreases delayed graft function (DGF) and enhances the quality of higher-risk livers prior to transplantation after kidney transplantation. Recently, it was demonstrated that oxygenated HMP can effectively preserve pancreatic tissue. However, the effects of oxygenated HMP on human islet isolation and subsequent days in culture have not yet been thoroughly investigated. We hypothesized that we could safely extend the CIT of human DCD pancreas by using oxygenated HMP for six hours [1].

Description

Ten pancreases were taken from human multi-organ donors in the Netherlands for this study. The University Medical Center Groningen's Medical Ethical Committee issued a statement saying that it had no objection to this study. The clinical and research activities adhered to the Declaration of Istanbul's guiding principles, according to the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism." As was mentioned earlier, the regional multi-organ recovery teams used the no-touch method to transport the pancreas. Before storing the pancreases in the University of Wisconsin Cold Storage Solution, SCS initially flushed them all. The DCD pancreases were transported in UWS under hypothermic conditions to the machine perfusion facility at the University Medical Center Groningen (UMCG) to prepare for perfusion. Oxygenated HMP was performed during transport to the islet isolation facility at the Leiden University Medical Center (LUMC). The DBD pancreases were brought directly (SCS) to the islet isolation facility at the LUMC. The time between the donor's aortic cold flush and the pancreas' initiation of ductal enzymatic perfusion, which included oxygenated HMP, was used to calculate CIT [2].

Oxygenated hypothermic machine perfusion When the patients first arrived at the UMCG, they were getting ready to be connected to the portable pressure-controlled dual perfusion device. To put it succinctly, the spleen was taken out, the gastroduodenal artery was ligated, and the superior mesenteric and splenic arteries were cannulated in order to receive separate 25 mmHg perfusions. The portal vein was left open to ensure passive perfusion fluid drainage into the reservoir. One liter of the University of Wisconsin's Machine Perfusion Solution, which was oxygenated by delivering 100 percent oxygen through hollow fiber oxygenators at a fixed flow rate of 100 milliliters per minute. The pancreas was placed in a plastic organ holder with a net inside an insulated box that was covered with melting ice. The ambient temperature remained between 4 and 7 degrees Celsius throughout the entire transportation [3].

Upon arrival at the isolation facility, independent researchers examined edema and apoptosis markers for visible macroscopic edema in the HMP and SCS pancreases. Tissue samples (ventral head and tail) were taken from the HMP pancreases shortly before the start of HMP and from both the HMP and SCS groups shortly before islet isolation using a 12G needle biopsy (Bio-feather). Medax, San Possidonio, Italy) The examples were embedded in paraffin, embedded in 4% paraformaldehyde, and cut into 4 m-wide sections. Light microscopy of H&E-stained sections was used to evaluate morphological shifts. Edema was measured by comparing the area of interstitial tissue to the area as a whole using a custom logarithm in Image-Pro Premier 9.1 (Media Cybernetics, Silver Springs, MD). The immunofluorescent staining of insulin was carried out [4].

At the LUMC, pancreatic islets were isolated using a modified version of the semi-automated procedure that was previously described. The isolation protocols for HMP- and SCS-preserved pancreases were identical. The mass of the pancreas after peri-pancreas tissue analyzation (pancreas mass), or the tissue mass that remained in the Ricordi chamber after assimilation and was isolated by the pancreas mass, was used to determine the amount of processed tissue. After purification, images of samples (more than 20 islets) from each fraction were used to count the islets that were embedded and those that were not. If exocrine tissue covered more than half of an islet's

***Address for Correspondence:** Marjolein Leemkuil, Department of Gastroenterology and Hepatology, Mayo Clinic, Rochester, USA, E-mail: leemkuil_m@gmail.com*

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perimeter, it was said to be embedded. The fraction of islets was divided by the isolation's total IEQ, multiplied by that fraction's IEQ, and added to all other fractions to determine the percentage of embedded islets. A second, unblinded investigator confirmed the findings [5].

In this study, human DCD pancreases were successfully preserved using hypothermic machine perfusion. This study demonstrates that by isolating a sufficient number of viable islets from discarded human DCD pancreases that are clinically relevant, it is possible to extend the cold ischemia time after oxygenated HMP. Based on the donor's characteristics, these pancreases would typically be rejected for clinical islet isolation. We were, however, able to isolate a sufficient number of viable, functional islets and demonstrate proper functionality both in vitro and in vivo following HMP. Both the outcome following isolation and the function of the graft following transplantation are influenced by a number of factors: characteristics of the donor, the procedure for islet isolation, culture, transplant, and engraftment, and methods of procurement and preservation It is known that pancreatic islet cells are extremely susceptible to oxidative stress due to their lack of endogenous antioxidants. After a period of extreme stress, this stress may eventually lead to the production of reactive oxygen species. There is evidence to suggest that the separation system and the oxidative pressure that occurs during the acquisition of the pancreas can cause ongoing impaired islet capability following transplantation. For the preservation of donor pancreases following multi-organ donation, whether for islet isolation or whole organ transplantation, the current industry standard is SCS. Despite being straightforward and reasonable, prolonged SCS time causes the accumulation of intracellular poisons and ATP exhaustion due to residual metabolic movement without oxygen. A pancreas' CIT is typically caused by the logistical difficulties of an islet isolation center and the location of the donor hospital in relation to the recipient hospital. Islet yield and/or function are decreased by CIT, an independent variable. In our middle, we have discovered a link between a lower islet yield and drawn out CIT in 126 DCD islet detachments. The oxygenated HMP-preserved pancreases in this study were subjected to CIT for an additional six hours [2-4].

In the Netherlands, the number of marginal donors, such as DCD donors, is on the rise. Cold ischemia can have a negative impact on organs from DCD donors in particular, according to research. Due to the cold ischemic insult, the inherent injury caused by the agonal phase following life support withdrawal, and a period of warm ischemia that is common in DCD procedures, DCD organs are less able to deal with this accumulation of stress factors than DBD organs. In three small studies on islet isolation from DCD donors, there were no significant differences in islet yield between DCD and DBD pancreases. However, when compared to DBD pancreas yields, DCD pancrease yields were reduced by up to 100,000 IEQ in two more recent studies, and when beta-cell number was calculated, DCD pancrease yields were reduced by 30%. Recent research suggests that DCD kidneys and livers can be saved by using hypothermic machine perfusion (HMP). In a previous study conducted by our group, oxygenated HMP for six hours using a custom-made, transportable, dual arterial perfusion system at a perfusion pressure of 25 mmHg was technically feasible and safe. A dual perfusion system with two centrifugal pumps can be used to perfuse the superior mesenteric and splenic arteries separately. Because flow in both systems was continuously monitored and potential perfusion issues could be traced and treated, uniform perfusion of the pancreatic tissue was earlier ensured. The addition of oxygen to the perfusion system significantly raised the ATP concentrations in the tissue; We observed a 6.8-fold increase in ATP in DCD pancreases and a 2.6-fold increase in DBD pancreases after 6 hours of oxygenated HMP [1].

We looked into whether the effects of oxygenated HMP remained in diabetic mice's pancreatic islets several days after they were isolated, cultured, and xenotransplanted in this report. Perfusion flow remained constant and there was no visible macroscopic or microscopic edema formation during the 6-hour oxygenated HMP period. Oxygenated HMP did not, as Caspase-3 staining demonstrated, raise apoptosis levels. Comparative findings of low edema and injury markers in trial HMP of pancreases that were not relocated or utilized for islet disconnection have been recorded by various frameworks employing low perfusion pressures. A recent study found moderate and severe edema in porcine pancreases 12 and 24 hours after HMP administration. Histological examination revealed comparable results up to 12 hours of preservation when compared to SCS. In a study of the effects of HMP on isolated porcine islets after 24 hours, it was hypothesized that HMP-induced edema helped the enzymatic digestion process during isolation. Perfused pancreatic tissue digested as well as static-preserved organs in our hands. In addition, the same number of embedded islets demonstrated that HMP did not appear to aid in the separation of islets from the exocrine tissue that was surrounding them. This suggests that additional factors, such as the donor's age and the method of enzyme perfusion, may have a greater impact. Because it has long been a goal of organ preservation in all its forms to prevent the formation of edema due to its detrimental effects, the capability to isolate islets in a normal manner without the inherent risks associated with edema should be viewed as advantageous. According to our findings, the yield, maximum purity, and average purity of HMP and SCS islets were comparable. Our groups' islet yields were comparable despite prolonged CIT, indicating that HMP can mitigate the negative effects of CIT once perfusion has begun. In tests conducted in vitro, the HMP islets displayed a biphasic insulin response to glucose. 55% of the diabetic mice's graft function (blood glucose levels of 10 mmol/L) remained adequate throughout the study. On day 28, IPGTT showed that the normal glucose peak came 30 minutes after the insulin peak. After the transplant, all of the mice experienced a relapse to hyperglycemia. These xenotransplantation results, which support the findings of in vitro studies, suggest that HMP-preserved DCD islets continue to function normally after recovery in culture [2].

There may be some flaws in this study. Isolations of islands were performed in a single location. Due to limited access to discarded donor pancreases, only five HMP (DCD) and five SCS (DBD) pancreases were able to be included in this study. In order to account for differences in cold ischemic injury between DCD and DBD pancreases, DCD pancreases might have been included in a second SCS group. Despite this flaw, we hypothesize that HMP efficiency can be revealed by comparing our results to those of theoretically superior DBD donors. To demonstrate oxygenated HMP's superiority, a randomized controlled trial comparing clinical SCS DCD pancreases to HMP DCD pancreases is required. The purpose of this study was to investigate the biological advantages of HMP for islet isolation and the viability of postprocurement pancreas perfusion in a clinical setting. Although the methodology of the study—a period of SCS during transportation to the HMP center, followed by a period of HMP during transportation to the islet isolation facility—is challenging, the presented results demonstrate that HMP can safely extend CIT without compromising islet yield, function, or viability. This difficult task must be optimized and, ideally, centralized in dedicated perfusion centers before it can be utilized more widely. One strategy that is hypothetically possible is to initiate HMP immediately following procurement at the donor center, which is currently carried out for each and every Dutch kidney: either a short end-ischemic HMP that lasts between two and six hours in the recipient hospital following SCS, or a longer end-ischemic HMP that lasts between six and twelve hours to postpone the isolation procedure for logistical reasons. The study's protocol could theoretically be used with any of the three methods. However, since connecting the pancreas to the device is a complicated surgical procedure that takes a lot of time, the last two are probably the best options. The viability or functionality of ECD pancreases can also be evaluated using normothermic regional perfusion and end-ischemic normothermic machine perfusion in conjunction with HMP. Other stomach organs may be treated with normothermic local perfusion, and islets have been separated from pancreases following these procedures, though the results have not yet been proven conclusive. Following HMP, normothermic machine perfusion (NMP) could be used to test the islet's viability and functionality prior to isolating it. However, there has only been one case of pancreatic NMP that has been documented. Sadly, the perfusion itself may have caused additional necrotic damage to the pancreas, despite

the fact that the endocrine system functioned normally for a brief time during NMP [5].

Conclusion

This study demonstrates that we were able to successfully isolate functional islets from discarded human DCD pancreases after performing six hours of oxygenated HMP, which increased the cold ischemia time.

Acknowledgement

None.

Conflict of Interest

None.

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