Advances in Machine Perfusion Graft Viability Assessment in Kidney, Liver, Pancreas, Lung, and Heart Transplant

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Abstract

Solid organ transplant constitutes the definitive treatment for end-stage organ failure. Better organ preservation methods have enabled use of marginal grafts, thereby expanding the donor pool to meet the growing demand for organs. Static cold storage as a preservation method has been superseded largely by machine perfusion in kidney transplant, with work regarding its use in other organ transplants ongoing. We hope that machine perfusion will allow better graft preservation, and pretransplant assessment, and optimization. The most extensive laboratory, preclinical, and clinical research into machine perfusion organ preservation has focused on kidneys. Successful outcomes in its use in renal transplant have sparked interest for its development and application to the liver, pancreas, heart, and lungs. This article reviews the current state of machine perfusion in abdominal and thoracic organ transplant, focusing on the recent developments in assessing graft viability.

Key words: *Biomarkers, Flow rate, Perfusion pressure*

Introduction

Since the 1960s, the most commonly used method of preserving organs for clinical transplant has been static cold storage (SCS). Several preservation solutions have been used to limit ischemic damage and preserve cellular integrity and organ function. The current widespread demand for organs for transplant, secondary to the stagnation of deceased donors, has led to increasingly longer waiting lists. Attempts to expand national donor pools have focused on the use of "marginal" or expanded criteria donors. Static cold storage, with conventional preservation solutions is believed to have reached its plateau in preserving marginal allografts, and has led to re-emergence of machine perfusion (MP) as an increasingly popular and potentially superior method of preservation.

Machine perfusion of an organ traditionally generates a controlled recirculating flow of preservative solution at hypothermic temperatures in the 0°C to 4°C range. This continuous flow permits perfusion of the organ promoting a thorough washout of blood and equilibration of the interstitium with the perfusate medium, delivery of oxygen and nutrients, and removal of toxic metabolites. In addition, it allows for real-time assessment and provision of metabolic support during preservation.

Kidneys

Machine perfusion

Grafts from non-heart beating donors (NHBDs) are increasingly being used. Added to the risk of suboptimal donor characteristics (advanced age, preexisting medical conditions) is the exposure of these kidneys to prolonged warm ischemia as well as prolonged anoxia/hypoxia. These grafts are at a greater risk of delayed graft function and more importantly—of primary nonfunction.[1] This has led to an increased awareness of the importance of managing the quality of grafts and has sparked interest in optimization of pretransplant graft preservation of marginal and nonmarginal kidneys.

While there are recognized donor risk factors (donor age older than 50 y, serum creatinine values

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over 150 μmol/L, warm ischemic times over 45 minutes, and cold ischemia times over 22 hours),[2] donor grafts cannot be rejected based solely on these parameters. This has led to the need to develop organ preservation systems, which allow for preservation and assessment of kidneys before the transplant. The 2 main preservation systems are cold storage (CS) and hypothermic MP. Both are based on the principle that lower temperatures result in lower tissue oxygen demands and in slowing of the chemical and physical processes; and hence, generating fewer toxic metabolites and better preservation of organs pretransplant.[3-4] Compared to CS, MP has the disadvantage that it is more expensive, it requires an operating technician, and it has the risk of failure.[5] However, it has the benefits of being associated with reduced risk of delayed graft function[6-8] and with enhanced graft survival. The existence of such benefits has been controversial, with conflicting evidence.

Research in the United States by Burdick and associates, who analyzed United Network for Organ Sharing data, highlighted the superiority of MP by showing its beneficial effect in reducing the need for first-week dialysis, especially in high-risk groups.[9] Similarly, a 2003 meta-analysis by Wight and associates concluded that MP resulted in a 20% decrease in the rate of delayed graft function (DGF),[5] and though based on potentially underpowered retrospective and nonrandomized studies (level B, National Health Service evidence grading), they also presented anecdotal evidence in favor of MP.

Schold and associates' retrospective review of 25 000 deceased-donor kidney transplants from United States (US) registry data reported both improved rates of DGF and graft survival with MP.[10] However, in a more-recent systemic review, Bond and associates concluded (based on available evidence at the time, predominately from unpublished level A and level B studies) that MP preservation improved only 1-year graft survival compared to CS, with no effect on DGF.[11] Only with the first large multicentered randomized control study (RCT) of MP versus CS, from the Eurotransplant region by Moers and associates, has level A evidence shown that MP is associated with a shorter duration of DGF and better graft survival at 1 year.[12]

More recently, contradictory evidence has emerged from an RCT by Watson and associates comparing MP to CS that no observable benefit was found regarding DGF or graft survival for MP kidneys.[13] An important limitation in comparing the Moers and the Watson studies is the difference in timing to the start of MP. In the United Kingdom (UK), RCT of kidneys randomized to MP underwent an initial period of CS of several hours for transport to the perfusion laboratory before start of MP; while in the Eurotransplant trial, kidneys underwent immediate perfusion after retrieval—suggesting the clinical benefits of MP may require immediate, rather than delayed, use of MP in allografts.

The observed clinical benefits of MP are presumed to be due to maintenance of vascular bed patency, provision of nutrients and oxygen to support tissue metabolism, removal of toxic metabolites, and enhancing expression of endothelial protective genes.[4, 14] Machine perfusion compared to CS also allows for pharmacological intervention, and more importantly, for graft viability assessment through the use of parameters such as perfusion dynamics (machine flow rates, vascular resistance) and perfusate biomarkers.[3]

Machine perfusion pressures

One of the first problems that became apparent during development of organ preservation methods was the rising perfusion pressure in the presence of a falling flow rate, which could eventually lead to graft failure.[15] Further research demonstrated that while low pressures can lead to underperfusion, high perfusion pressures are associated with shear stress and endothelial damage.[15]

Doorschodt and associates showed that porcine kidneys exposed to higher pressures before transplant have higher endothelial expression of von Willebrand factor, a marker that is found in renal endothelial cells of patients with hypertension and acute or chronic renal failure.[14, 16] Furthermore, porcine kidneys exposed to 25 mm Hg, as opposed to 30 mm Hg, had perfusion pressures that were superior in terms of preserving their renal structural integrity and faster recovery of function, as shown by serial urea and creatinine measurements after the transplant.[16]

Maathius and associates used a porcine transplant model to demonstrate that kidneys exposed to high perfusion pressures (60/40) were more likely to sustain diffuse vascular damage with recipient renal failure. Lower pressures were associated with a more favorable posttransplant outcome.[14]

Much work on perfusion pressures has been based on porcine models, which are known to be more sensitive to higher perfusion pressures than are human renal grafts.[14] It is thought that these principles may be extrapolated and applied to clinical renal transplant. From their own clinical experience, the Newcastle group developed their 2001 viability criteria for renal allograft MP and recommended perfusion pressures of less than 60 mm Hg.[17] Importantly, in the recent Eurotransplant RCT, all MP kidneys underwent perfusion at pressures of 30 mm Hg.[12]

Machine perfusion flow rate

Matsuno and associates examined the effects of different perfusion flow rates (0.4 to 0.65 mL/min/g, 0.65 to 0.9 mL/min/g, and more than 0.9 mL/min/g) on immediate function, DGF, duration of acute tubular necrosis, postoperative creatinine, and on a number of postoperative days required to reach a urine output greater than 2000 mL/d. They showed that kidneys with the highest perfusion flow rates were superior in all 5 fields.[18] Further research by the group confirmed the correlation between flow rates and immediate function and primary nonfunction.[18-19] Both studies showed high rates of acute tubular necrosis, but this resolved faster in kidneys with higher flow rates.[18-19]

The importance of flow rates was highlighted further by Balupuri and associates, with a case of immediate nonfunction in a graft with acceptable perfusate biomarkers and perfusion pressures, but a low flow rate (26 mL/min/100 g). Of the 13 transplants, this was the only one to not work, suggesting that flow rates may be one of the most important variables.[20] Based on similar research, models have been developed with renal flow rate recommendations for donor grafts. The Newcastle viability criteria recommend a flow rate over 25 mL/min/100 g of tissue.[17]

Resistance indices

Increasing resistance to flow with time during MP indicates a poor outcome.[21] This was confirmed by Reznik and associates who looked at the resistive index in human kidneys with immediate function compared to DGF.[22] They showed in the former group, the decrease in the resistive index (a measure of resistance to arterial flow within the renal vascular bed) occurred more rapidly, suggesting that ischemically damaged kidneys require prolonged pump perfusion time.[22] Use of renal resistance to determine graft suitability for transplant is variable, with published cutoff values determined empirically at individual institutions. Analyses from the recent Eurotransplant trial showed renal resistance to constitute a predictor of graft outcome and suggested it may be of prognostic value.[23] Specifically, renal resistance was found to correlate with DGF and graft survival, but not primary nonfunction, with a threshold of renal resistance of 0.28 at the end of perfusion correlating with a higher 3-month serum creatinine level and 17% poorer graft survival.[23]

Machine perfusate biomarkers

Research has focused on the use of machine perfusate biomarkers for pretransplant in vitro assessment of kidney viability.[12, 24-28] Evidence remains controversial and based largely on animal models. Biomarkers most likely will be incorporated in clinical viability scores, rather than be used in isolation. Some of the more-established work has focused on glutathione S-transferase (GST), lactate dehydrogenase (LDH), N-acetyl-β-D-glucosaminidase, alanine aminopeptidase, aspartate aminotransferase (AST), and heart-type fatty acid binding protein.

Glutathione S-transferase

Glutathione S-transferase is found in the kidneys and is involved in detoxifying metabolites and conjugating glutathione.[12, 24-25] The 2 subtypes, α-glutathione and π-glutathione, found in proximal and distal tubules are released during ischemia. Proximal tubules, being metabolically more active, are more vulnerable to hypoxia, making α-glutathione a more-sensitive marker,[24] and a potential tool for pretransplant assessment of NHBD kidney viability. Quantification of total glutathione is technically easier and often is used as a surrogate marker of α-glutathione levels.[6, 20]

During MP, α-glutathione levels correlate with warm ischemia time; with prolonged ischemia being associated with higher levels.[26-27] Similarly, levels are lower in functioning, compared to nonfunctioning, grafts from NHBDs.[26-27] Further research into the functioning NHBD kidney group has demonstrated that GST levels are significantly higher at the end of MP in kidneys with DGF compared with kidneys with immediate function.[6]

Moers and associates, were the first to show that GST levels, although related to warm ischemia time,[6, 26] also represent an independent risk factor for DGF.[6]

Other research has shown isolated cases of high GST levels occurring in functioning NHBD grafts, showing that GST levels cannot necessarily be considered in isolation.[26] Similarly, based on their experience with a case of immediate graft nonfunction in a kidney with normal GST but low flow rates, Balupuri and associates also suggested other factors should be considered in addition to GST levels.[20] They postulated that GST may represent capillary beds, and that low levels of GST may be related to small capillary beds secondary to low flow rates, not necessarily reflecting a lack of ischemic damage. They recommended that a flow rate of at least 50 mL/min/100 g kidney is required for GST levels to be clinically relevant.

The above research suggests α-glutathione is the most-reliable pretransplant renal perfusate marker for transplant outcome based on its potential to predict graft function and nonfunction and even immediate versus DGF.[26] The Newcastle clinical viability protocol recommends a GST content at 4 hours of less than 200 IU/L/100 g of renal mass.[17]

Lactate dehydrogenase

Lactate dehydrogenase is a nonspecific marker of cellular injury. It was hoped that its level in kidneys undergoing MP would reflect the extent of ischemic injury. However, research by Daemen and associates did not confirm a significant correlation between LDH and warm ischemia time or graft function/nonfunction.[27] In the group of NHBD functioning grafts, this biomarker was found to be a good discriminator of immediate versus DGF,[6, 27] but did not have an independent predictive value of graft failure in the first year posttransplant.[6] Therefore, its use as a biomarker may be of value in the context of other determinants of graft function, but it is unlikely to have much independent predictive value.

N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase is a lysosomal enzyme. There is limited work looking at N-acetyl-β-D-glucosaminidase levels in renal perfusate of MP grafts and their correlation with ischemic damage. Research by Moers and associates showed that like LDH, N-acetyl-β-D-glucosaminidase levels constitute a risk factor for DGF but have no independent predictive value of graft failure in the first year posttransplant.[6]

Alanine aminopeptidase

Alanine aminopeptidase is a peptidase located in renal cells and involved in cell regulation.[6, 25] Like GST, it is excreted in the urine secondary to renal tubular damage and has been investigated as a pretransplant marker of posttransplant renal viability. Models of non-heart beating porcine donors have demonstrated a significant correlation between warm ischemia time and levels of alanine aminopeptidase in the urine of renal grafts subjected to ischemic conditions.[25] However, research in human NHBD kidneys, did not demonstrate a correlation of alanine aminopeptidase with primary nonfunction or with delayed versus immediate graft function. As such, based on current research, alanine aminopeptidase is a poor pretransplant predictor of transplant outcome.[6]

Aspartate aminotransferase

Aspartate aminotransferase is an enzyme seen in hepatic and renal parenchymal cells that has been studied in the context of MP. Most research has focused on its use in liver transplant, but there is also evidence for its applicability in renal transplants.[6] Its presence in the renal perfusate of MP kidneys is thought to represent acute damage to parenchymal cells.[6] Higher levels are associated with an increased risk of delayed, as opposed to immediate, graft function. This relation is thought to be the result of the association of AST levels with prolonged ischemia time.[6] Further research is required regarding its potential as a biomarker of posttransplant graft outcome.

Heart-type fatty acid binding protein

Heart-type fatty acid binding protein is found mainly in the heart, and in smaller amounts, in the small intestine, the skeletal muscle, and the distal tubules of the kidneys.[28] Most research has focused on its use in identifying myocardial injury, and work in MP of renal grafts is still in the early stages. In the kidneys, it is located in the distal tubule cells and is involved in the uptake of fatty acid from the cytosol into the mitochondria.[6] Significantly higher levels of heart-type fatty acid binding protein have been found in the renal perfusate of MP-NHBD grafts that have developed DGF. Here, heart-type fatty acid binding

protein levels were found to be independent risk factors for DGF, much like GST and N-acetyl-β-D-glucosaminidase.[6]

Current application of machine perfusion in kidney transplant

An important question in the MP versus CS story is: Which kidneys are preferable to undergo perfusion? It is generally accepted that expanded criteria donors kidneys are liable to draw the greatest benefit from MP[29]; thus, many centers routinely pump expanded criteria donors kidneys before transplant.[30-31] Standard criteria donor kidneys are likely to be less sensitive to ischemic insults during retrieval and therefore, may not draw as great a benefit from MP.

Donation after cardiac death (DCD) kidneys experience lengthier warm ischemia times during retrieval and subsequent higher DGF rates compared with donation after brain-dead kidneys. The only modifiable risk factors are cold ischemic preservation time and the use of MP. Shorter cold ischemic preservation times are preferable to limit further preservation injury, but the benefit of MP is not as well established. Consequently, its use in this donor group varies greatly.

The Eurotransplant study has been the only prospective randomized trial to examine the potential effect of MP in controlled DCD kidney preservation, and though it had only a few kidneys, it did show that MP improves DGF rates in this group.[32] More specifically, US registry data on 6057 DCD kidneys indicated that MP may be beneficial only in certain DCD subsets; reducing DGF in DCD donors younger than 60 years old and 1-year survival in donors older than 50 years. However, thought must be given to weighing the potential benefits of MP to the drawbacks of increasing cold ischemic preservation time. In these sensitive kidneys, all attempts should be made to ensure cold ischemic preservation time is less than 30 hours, with more than 30 hours of cold ischemic preservation time associated with a higher risk of DGF and 1-year graft loss.[33] The National Institute for Clinical Excellence (NICE) recognizes the potential benefit MP could afford this donor group and thus, they recommend MP as an option for DCD kidney preservation in the UK.[34]

With more evidence accumulating in favor of MP, it is emerging as the preferred means of preserving expanded criteria and DCD kidneys. The strongest evidence is for the use of flow rates and resistance indices as viability measures, but interest in perfusate biomarkers as prognostic indicators during the postoperative period is intensifying with GST, LDH, and heart-type fatty acid binding protein identified as biomarkers of clinical significance. Clinically, several different parameters are taken into account, collectively, rather than individually, to assess graft viability, and protocols differ between centers worldwide. One of the most established criteria in the UK is from the Newcastle group. These include a flow rate greater than 25 mL/100 g, with a GST less than 200 IU/100 g, a surface temperature ideally lower than 14°C, a perfusion flow index greater than 0.4 mL/min/100g/mm Hg,[17] and a corresponding decreasing resistive index. Ideal perfusion of allografts is through low pressure pulsatile flow in the 30- to 40-mm Hg range, attempting to limit edema and barotrauma.[12]

Though perfusion dynamics may provide an indication of the risk of postoperative complications and graft survival, the determinants of transplant outcomes are multifactorial and predicting outcomes based on isolated perfusion dynamics is not without error. Risk scores for DGF and graft survival based on donor, procurement, and recipient factors have been proposed,[35-36] and it would seem useful to add perfusion dynamics and perfusate biomarker levels[6] to these criteria to improve predictive accuracy and provide a multidimensional assessment of kidney viability. Further research could focus on developing these scoring systems and determining the most-useful perfusion parameters and biomarkers to be included.[37]

Liver

Machine perfusion, although integrated into clinical practice in kidney transplant, has only recently come under closer scrutiny in transplant for other organs. Monbaliu and associates[38-39] summarize the advantages of using MP, which are common to kidney and liver transplant, but also highlight the distinctions. These differences mean that renal MP protocols cannot be applied directly to liver transplant, but need to be adapted to account for these distinctions, which include hepatic and portal systems flow competition, hepatic sinusoidal endothelial cell susceptibility to damage, high liver metabolism, the MP effect on preventing biliary tree injury, and Kupffer cell activation.

Hepatic MP has demonstrated some worth in animal models, particularly porcine models, by demonstrating reduced cellular necrosis and hemodynamic stability.[40-42] However, the optimal protocol has not been determined; thus, there are no commercially available or FDA-approved devices. Currently, all systems are purpose-built, such as the Groningen Liver Perfusion System and the Organ Recovery Systems Device. The question of optimal temperature (normothermic, hypothermic or subnormothermic), optimal flow rates and perfusion pressures (high or low, pulsatile or continuous), single or dual vessel (hepatic artery and/or portal vein) perfusion, perfusate oxygenation, and different perfusate compositions are still under investigation. These are reviewed well in several places.[40-48]

Only human clinical trial

Clinically, static hypothermic CS is the criterion standard preservation method for liver transplant. Since the late 1960s, when Brettschneider and associates[49] and Starzl and associates[50] trialed MP, there has been little use of MP for human livers. The recent success of MP in renal transplant, and use of MP in animal models, has led to growing interest in MP application in liver transplants.[51-56]

In particular, Guarrera and associates undertook a phase I prospective cohort study of 20 patients, having successfully carried out preclinical studies.[57] They compared 20 adults who received hypothermic MP (HMP)-preserved livers and a matched group transplanted with SCS livers. University of Wisconsin solution was used for SCS and Vasosol R solution for MP. The different solutions may have contributed to any beneficial effects of perfusion over CS, as Vasosol has added antioxidants, vasodilatory and metabolic support. Notwithstanding this, the study found early allograft dysfunction rates were 5% in the HMP group versus 25% in controls ($P = .08$) and serum liver injury markers were significantly lower in the HMP group. Although a small study, its results definitely give reason for further larger studies.

Perfusate viability markers

It is without question that viability markers are one of the great advantages, which MP can bring to liver transplant. Investigation into viability markers has predominantly focused on traditional enzymatic markers of hepatic damage such as aspartate transaminase (AST) and alanine transaminase (ALT). However, several other markers have been investigated. These include lesser-known markers of hepatic damage such as liver fatty acid-binding protein (L-FABP), glutamate dehydrogenase (GLDH), α-glutathione-S-transferase (α-GST), hyaluronic acid, and β-galactosidase. Furthermore, bile markers such as glutathione and apparent diffusion coefficient also have been suggested as potential surrogate markers of hepatic damage, and almost all have shown some promise.

Human models

Aspartate aminotransferase, ALT, and LDH are long-established markers of hepatocellular damage, and therefore, an obvious choice for possible markers of viability. Guarrera and associates are the only group to have examined the possibility of using AST, ALT, and LDH as perfusate viability markers in human models.[57] They found serum injury markers were significantly lower in the HMP group, and also peak serum AST and ALT correlated with measured AST, ALT, and LDH levels in perfusion effluent.[57]

This same group carried out a smaller study[58] on 4 HMP-perfused livers matched with SCS controls, and measured serial perfusate levels of AST by RT-PCR. They found a trend toward lower recipient peak AST in the HMP group ($P = .18$) and perfusate AST correlated closely with postoperative day 1 AST, while other perfusate assays were not useful. These studies only provide an evidence base for examining viability markers in humans more closely. What is needed is investigation into possible correlations between warm ischemia or graft failure and markers. Despite limited evidence for this in humans, there is further evidence in animal models.

Animal models of hepatocellular damage markers

Alanine and lactate are obvious possible markers; during warm ischemia they are products of anaerobic metabolism (pyruvate conversion to lactate, and ultimately, to alanine). There are several hypotheses about why histidine might be a marker, but none is confirmed.

Liu and associates used a porcine HMP model to establish AST, alanine, and histidine as possible markers of viability through biochemical analysis and proton magnetic resonance spectroscopy.[59] Aspartate aminotransferase increased significantly during HMP within groups and discriminated warm

ischemic injury significantly from the start to the end of HMP. Furthermore, alanine and histidine were significantly higher in the warm ischemic group than in the control group at the end of HMP. Changes in alanine, lactate, and histidine levels also correlated strongly with AST levels, a well-known marker of hepatocellular damage, suggesting that these 3 markers could be indicative of hepatocyte integrity like AST.

More recently, the same group used HMP pretransplant to analyze 11 discarded human livers and determine whether the level of steatosis was comparable to AST levels.[60] During HMP of livers that were more than 50% steatotic, AST release was significantly higher compared with those that were less than 50% steatotic. Furthermore, AST also was significantly higher in nontransplantable, compared to potentially transplantable, livers. Further evidence for the use of AST as a marker comes from Liu and associates.[61] They used HMP in a porcine liver model and found β-coefficients calculated from initial AST or L-FABP release during HMP are promising clinical tools to predict viability of ischemic livers and subsequent risk of PNF and further suggested that porcine livers with AST β-coefficients of less than 0.006 or LFABP β-coefficients of less than 0.004 might be safe for transplant.

Liver fatty acid-binding protein is another known sensitive marker of liver damage. It is postulated that it may be superior when compared to other markers such as AST, as it has a faster renal clearance and therefore a shorter half-life.[62] This, in turn, means that the levels postreperfusion would more closely reflect hepatic damage. Promising results for the use of L-FABP have been produced in porcine models.[61-62] The use of ALT[63] and LDH[64] also have been considered, and correlations with graft survival have been shown.

Beta-galactosidase is a lysosomal enzyme, released from Kupffer cells that are macrophages activated after reperfusion of ischemic tissue. St Peter and associates[65] postulated that a rise in this enzyme may be a potential marker of graft viability, because it is thought that Kupffer activation and subsequent beta-galactosidase release precede hepatocellular damage. In their porcine model, they found a sharp increase in beta-galactosidase levels on reperfusion of cold, preserved livers compared with levels in normothermically preserved livers, which remained low. This rise was much earlier and greater compared with transaminase levels in livers injured by ischemia.

Further markers

Another novel marker that has been suggested is the apparent diffusion coefficient (ADC),[66] which is a proven marker for assessing cerebral ischemia.[67] Apparent diffusion coefficient measures the magnitude of diffusion of water molecules within a particular tissue. A low ADC, assessed through MRI, is a sensitive marker of early brain ischemia.[67] Apparent diffusion coefficient has been shown to decrease in vivo during hepatic ischemia, but not following ex vivo HMP.[66] The role of ADC remains unclear.

One of the unique attributes of the liver that so far has not been fully explored is the possibility of bile constituents as markers. Early work with rabbit livers,[68] looking at markers within the bile with proton MRS, has shown some promise for the use of bile acids, lactate, glucose, and phosphatidylcholine.

Despite its success in kidney transplant, work by Derveaux and associates[69] has indicated that vascular resistance does not correlate with warm ischemia in an ex vivo porcine model. Their group postulated this was due to the *unique vascular physiology of the liver, with its double vascular circuit and low resistance of the sinusoidal network*, but underscored that changes in experimental design may uncover a correlation.

Future markers to be tested

Glutamate dehydrogenase (GLDH) and a-GST have recently been suggested as potential viability markers. They have previously been shown to be markers of acute hepatocellular injury.[70-71] In fact, GLDH has been shown to be a more-effective biomarker of acute hepatic injury than ALT, AST, SDH, or ALP in rat models,[70] and a-GST also has been found to be a sensitive, predictive marker of ischemia/reperfusion-induced hepatocellular injury and postoperative liver dysfunction.[71] Hyaluronic acid (HA) also has proven itself a marker of endothelial cell damage after CS and reperfusion, and is used in ischemia-reperfusion experiments.[72] Itasaka and associates specifically used a rat model to test this, and found a significant correlation between HA clearance in perfusate and sinusoidal cell lining damage.[72]

Golling and associates[73] examined the use of glutathione as a marker through a porcine autotransplant model, and measured serial hepatic perfusion markers, particularly reduced liver glutathione (rGSHL), and oxidized glutathione in the liver. Their group found a cutoff value of 11.5 ng/mmol of rGSHL could distinguish survivors from nonsurvivors, independent of the ischemia time. In conclusion, rGSHL has the potential of becoming an important viability marker, as it could predict survival in auto-transplant NHBD model regardless of the ischemia time.

Pancreas

Pancreatic transplant can broadly be considered in terms of whole pancreas and islet cell transplant and is used to treat type I diabetics. Transplanting is indicated for 2 main subsets of diabetics: those with end-stage renal failure, and *brittle diabetics* who continue to have multiple hypoglycemic or hyperglycemic episodes despite optimal insulin regimens. Simultaneous pancreas-kidney transplant is used in the former group, whereas pancreas transplant alone or islet cell transplant is considered in brittle diabetics.

Whole pancreas transplant compared with islet cell transplant

Historically, the success of whole organ transplant has been considerably greater than islet cell transplant (ICT). Improved immunosuppression regimens have improved whole pancreas postoperative survival rates to around 95% at 1 year, and 90% at 3 years, and more than 30 000 whole pancreas transplants have been performed.[74] Benefits of whole pancreas transplant include good glycemic control, insulin independence, improved hypoglycemia and hypoglycemic awareness, and diabetic complication improvement/nonprogression.[75-76]

Islet cell transplant remains an attractive alternative to whole organ transplant for several reasons. Whole organ pancreas transplant is more invasive and carries greater perioperative risk compared to ICT. Furthermore, isolated islet cells provide the benefit of insulin production but do not have the exocrine cell-related morbidity of whole pancreas transplant. The most-recent increased interest came from the development of the Edmonton Protocol, which has given rise to clinical trials involving deceased-donor pancreata, whereby the islets are infused via the portal vein. These result in persistent insulin secretion, but have the disadvantage that top-up from antihyperglycemic medications is still required.[77-79] Islet cell transplant has shown both reduced frequency of hypoglycemic episodes and reduced microvascular complications.[80-82] It also improves the quality of life and the patients' survival.[83-85] Despite this, persistent insulin independence has not been achieved through ICT, and this remains a drawback. Additionally, ICT often requires multiple transplants to attain enough beta cell mass, unlike whole transplant that requires only 1 transplant.

There is evidence that ICT is a viable treatment and indeed the UK National Health Service now funds ICT, particularly for patients with reduced hypoglycemia awareness or those taking immunosuppressive drugs because of a previous kidney transplant.[86]

Machine perfusion

The preservation method of choice for both ICT and whole pancreas transplant is currently SCS. Despite the definite benefits of transplant, the shortage of organs is a predominant factor in limiting ICT and whole pancreas transplant. This, combined with the increased use of extended criteria donors, has led several groups to attempt to use the success of MP with kidneys, in maintaining and assessing the quality of pancreatic islet cell and pancreatic grafts.

As with other organs, MP confers the advantages of possible viability testing, perfusing the organ with substrates for metabolism, and removing toxic products. Machine perfusion also confers the advantage of ameliorating some pancreatic ischemia, which is detrimental to islet cell yield and insulin release.[3]

Despite this, MP as it stands, would be both complex and costly in preserving a donor's pancreas. It remains a mostly unexplored option, yet it could be most effective if pancreas-specific regimens existed. There are several reasons why renal protocols cannot be directly translated to pancreata. The main physiological difference with the pancreas is its low flow and pressure environment. This means that MP can damage the fragile vascular endothelium leading to platelet activation and thrombosis on graft reperfusion.[87-89]

There were some early attempts in the 1970s to develop a protocol with MP and low perfusion

pressures, but until recently, MP has been out of favor, as results from some early experiments favored cold storage, which was comparably straightforward.[90] Islet yields and quality have improved with the innovation of cold preservation techniques by the use of a 2-layer method that is now widely used in transplant centers, although it has been suggested that MP may further improve yields.[90]

There are few recent studies involving ICT and MP for preservation, and even fewer with whole pancreas transplant. Taylor and associates used a porcine model and transplanted pancreata that were preserved either with 24 hours of SCS at 2°C to 4°C in University of Winsconstin-Viaspan solution, or 24 hours of HMP on the Lifeport Organ recovery system.[91] They reported a statistically significant increase in islet yield in the pancreata that had undergone HMP compared with CS, with function being similar and minimal MP-induced edema.

Using a similar model, the same group found the highest insulin content was in islets obtained from MP-pancreata compared with SCS preserved pancreata.[92] Islet yield was 1.6 to 1.8 times greater in the HMP group than it was in the less than 1-hour SCS group and 2 to 3 times greater than it was in the 24-hour SCS group. One of the previously reported disadvantages of MP was organ edema, thought to lead to bleeding and necrosis.[3] This group found that though they observed moderate edema, there was no associated loss of function. They further suggested that the edema appeared to aid in enzymatic digestion, producing a greater yield and purity of islets compared with pancreas subjected to 24 hours of SCS.[3]

In a small human model (n=4), Leeser and associates compared 13-hour low-flow MP to CS of less than 8 hours in 1 group more than 8 hours in another.[93] They found that MP may be beneficial in maintaining islet yield, viability and function in pancreata with prolonged cold ischemic, which would normally be less likely to meet criteria. They reported islet yields from MP pancreata of 3435 IEQ/g pancreas tissue IEQ/g, compared with 5134 IEQ/g and 2640 IEQ/g for those cold stored for less than 8 hours and more than 8 hours. Mean islet viability after perfusion was 86% (vs 74% and 74% for the < 8- and > the 8-h groups) and insulin secretion index was 6.4 (vs 1.9 and 1.8 for the < 8- and > the 8-h groups).

Karcz and associates also have sought to develop a model with porcine pancreata (n=14) using the RM3 perfusion machine and University of Wisconsin solution based on previous research with kidneys.[94] This model involved pancreata undergoing 25 minutes of warm ischemia and 149 minutes of cold ischemia before 315 minutes of hypothermic MP. The group found postperfusion reduction in islet and acinar cell damage. Furthermore, this was the only group to take the first steps toward examining the possible advantage of perfusate markers. They measured intrapancreatic resistance and flow (both renal perfusate markers) along with the biopsies and used these as markers to optimize conditions used in their model. Despite the sparsity of work in this area, there is promise for using MP and perfusate markers with pancreata.

Lung

As with other solid organ transplants, increased demand for lung grafts has led to alternative sources of organs including marginal donors and more recently, non-heart beating (NHB) donors. In the former group, donor characteristics such as advanced age, smoking, contusion, and infiltrate render these grafts suboptimal.[95]

Regarding the NHB donor group, work in the use of ex vivo perfusion methods will pave the way for introducing methods to assess the viability of these grafts, enable graft MP, and possibly allow for correction of suboptimal characteristics. Studies in this area are limited and mostly involve porcine models. Furthermore, owing to ethical considerations, sample sizes are small, and the method of achieving cardiac death is often variable.

Ex vivo lung perfusion

Lung grafts are classically stored in preservation solutions, with multiple solutions currently available, of which an extracellular solution appears to provide superior results.[96] Ex vivo lung perfusion (EVLP) is currently being explored with attempts in the past largely hindered by edema formation and increased pulmonary vascular resistance secondary to circuit-induced injury of the vasculature and epithelial membranes.[97-98] More recently, Steen and associates developed the Steen solution, a hyperoncotic fluid, with a hematocrit of 15%,[99] and they were the first to create a successful ex vivo lung

evaluation system for donating after cardiac death.[100] In their pioneering case study, they obtained a single lung from a Maastricht Category II NHB donor with a warm ischemia time of 65 minutes and a cold cooling time of 3 hours, assessed it in their ex-vivo perfusion circuit, and successfully transplanted it into a patient with chronic obstructive pulmonary disease.[100] This led to further experimental work in ex vivo lung perfusion models.

Erasmus and associates used the Steen EVLP model to assess donor lung function in pigs in terms of blood gases from the pulmonary artery and the left atrium, mean left atrial pressure, mean pulmonary artery pressure, maximum ventilation pressure, and end-tidal CO_2. They confirmed that this constitutes a sensitive and reliable method for evaluating pulmonary graft function pretransplant.[95]

The group also used the Steen model to assess 6-hour ex vivo perfusion potential in lung preservation. They demonstrated that this is feasible, but that in view of increased pulmonary artery and ventilation pressures during this time, further work is required.[95] While 6 hours may be sufficient for assessing graft function, it is not enough for pretransplant conditioning. To address this issue, Cypel and associates used acellular Steen solution to extend normothermic ex vivo lung perfusion to 12 hours and successfully demonstrated preservation of porcine lung function.[101] The group specifically looked at pulmonary vascular resistance, peak airway pressure, lung oxygenation capacity, and airway plateau pressure. In another study, they also could confirm the superiority of EVLP compared to CS regarding preservation-associated lung injury.[102]

These experimental findings were applied to the clinical setting where Cypel looked at the transplant outcomes of high-risk donors who were stable after 4 hours of normothermic ex vivo lung perfusion and demonstrated results comparable to conventionally selected lungs.[103] The 2 groups were compared in terms of primary graft dysfunction, 30-day mortality, bronchial complications, duration of mechanical ventilation, and length of stay in intensive care unit and hospital.

Ex vivo lung perfusion for lung repair

It is hoped that these successes in EVLP could pave the way for ex vivo lung repair pretransplant and posttransplant. Regarding pretransplant, after a cardiac arrest, lungs are particularly susceptible to injury secondary to aspiration, edema, infection, and contusion.[95, 97] The current challenge posed is whether ex vivo lung perfusion can be used to correct these and improve graft quality. Research in this area is still in the early stages. Ingemansson and associates used extracorporeal membrane oxygenation circuit with the Steen solution, mixed with erythrocytes with lungs that previously had been rejected for transplant.[104] They performed 6 successful, double-lung transplants. The success of reconditioning was thought to be based on the solution's high oncotic pressure, which dehydrated edematous tissues and prevented edema formation in reperfused tissue.

Regarding posttransplant complications, it is hoped that EVLP can be used to prevent bronchiolitis obliterans, a manifestation of chronic graft dysfunction.[105] Patients particularly at risk are those with primary graft dysfunction.[106] Van Raemdonck and associates suggest that graft immunotherapy or gene therapy via EVLP may in the future help induce recipient tolerance toward the new grafts.[97]

Biomarkers

The use of biomarkers also has been explored with MP. However, unlike kidneys, where the renal perfusate is examined, cytokines in bronchioalveolar lavage fluids are used to assess the viability of lung grafts. Fisher and associates demonstrated that high IL-8 levels in the donor bronchioalveolar lavage were associated with poor posttransplant outcomes and specifically, with development of severe early graft dysfunction and with early recipient mortality.[107] Similarly, Kaneda and associates found that IL-6, IL-8, TNF-alpha, IL-1beta were risk factors for 30-day mortality, while IL-10 and IFN-gamma were protective.[108]

Heart

Cardiac grafts have traditionally been preserved with CS. While this method has provided good initial results, it is associated with anaerobic metabolism and does not allow for prolonged graft storage and transport, and therefore necessitates short intervals between graft harvesting and transplant.[109] Furthermore, prolonged ischemia time, a consequence of prolonged storage, has been associated with poor 1-year survival with primary graft failure being a leading cause of death within the first month of transplant.[110] As with lung

preservation, it is hoped that MP of heart grafts will allow for prolonged organ storage, improving the quality of the graft, and preventing early posttransplant complications.

Benefits of machine perfusion

Cardiac metabolic processes are complex and remain poorly understood, especially during graft preservation. Machine perfusion provides the graft with continuous supply of an oxygenated solution at room temperature allowing for aerobic metabolism. This is thought to preserve myocardial transmembrane ionic gradients, prevent lactate and adenosine build-up, allow for reparative processes in the ischemic myocardium, and promote excretion of toxic metabolites.[111, 112]

Animal studies looking at lactate and creatine kinase-MB isoenzyme levels in MP compared with CS have shown significantly lower levels in the former group.[112] The effect of continuous washout could be contributory to these results. Low creatine kinase-MB isoenzyme levels imply less myocardial damage, while the lactate levels are suggestive of preserved aerobic metabolism. The significance of lactate has been demonstrated in coronary artery bypass surgery, where lactate release during reperfusion was found to constitute an independent predictor of myocardial dysfunction.[113]

Using a canine model, Ozeki and associates demonstrated continuous perfusion to be superior to CS in terms of postreperfusion myocardial recovery. Grafts preserved with MP also had a higher tissue pH and ATP levels suggestive of less oxidative damage and energy depletion.[114]

Limitations of machine perfusion

Machine perfusion of the heart requires good technical knowledge of the equipment and is associated with higher costs. One of the main reported disadvantages in the animal studies is the association of MP with increased myocardial edema,[115] with some studies showing a variation depending on the preservation solution.[116-119] This association is thought to be mediated by MP-dependent hydrostatic pressure and perfusate colloid oncotic pressure and further compounded by the lack of lymphatic flow to the arrested heart.[118] Collins and associates suggested that lack of physiological variation in coronary artery flow and pressures also may contribute to edema formation,[118] which is thought to be associated with impaired posttransplant diastolic function recovery.[115] More recently, Ozeki and associates suggested that increased edema may only be transient.[114]

Recent work

Progress in organ transplant technologies has led to development of the Organ Care System (OCS) by Transmedics. This is a portable non–FDA-approved warm blood pulsatile perfusion system that allows for organ preservation by pumping warm, nutrient- and oxygen-rich blood through the organ.[120] This is thought to mimic the body's physiological state and allow for prolonged preservation. The PROTECT (Prospective Multi-Center European Trial To Evaluate the Safety and Performance of the Organ Care System for Heart Transplants) trial looked at transplant of 20 grafts maintained with OCS, and showed 100% survival and faster recovery and a shorter ventilation after surgery.[121] There were 2 cases of acute rejection and 2 of transient left ventricular dysfunction, which resolved.[122] PROCEED II (Prospective, Randomized, Multicenter Safety and Effectiveness Evaluation of the Organ Care System Device for Cardiac Use), an ongoing trial in the US, aims to compare traditional CS cardiac preservation to OCS. Its primary endpoints are 30-day patient and graft survival, and its secondary endpoints include incidence of cardiac-related serious adverse events, time in ICU, and incidence of acute rejection episodes.[123] Availability of this new technology is limited by cost: USD $200,000 for the OCS device compared to USD $100 for the CS box.[124]

Conclusions

Organ transplant has evolved significantly, with MP being common practice in the preservation of marginal renal grafts. Current work is focused in establishing the use of perfusate markers as markers of cellular injury. By contrast, work in liver, pancreatic, cardiac, and lung grafts is at much earlier stages with most evidence coming from animal studies and the cost still largely prohibiting its use in the clinical setting. It is hoped that future research in MP will extend its use in solid organ transplant to allow for preassessment and conditioning of grafts, thereby expanding the donor pool.

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