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Preclinical Foundation for Normothermic Machine Liver Preservation

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Abstract Liver transplantation is the primary treatment for end-stage liver disease, but demand exceeds supply due to the shortage of available healthy donor grafts. To increase the supply of useable grafts for transplantation, innovative and better preservation methods are needed to enable the use of livers from extended criteria donors. Normothermic machine perfusion (NMP) has been extensively studied in the laboratory and holds great potential for expanding the donor pool. This review summarizes the progress that has been made in NMP over the last two decades, with emphasis on the recent studies preparing the method for clinical trials.

Keywords Organ preservation · Liver transplantation · Normothermic machine perfusion · Donation after cardiac death · Extended criteria donation · Ischemia-reperfusion injury · Ischemic cholangiopathy · Hepatic steatosis

This article is part of the Topical Collection on *Machine Preservation of the Liver*

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Introduction

A variety of factors have fueled increased demand for liver transplants, exacerbating the already existing shortage of donor livers. In the USA, an estimated 15,000 individuals are on the waiting list annually. As many as 2,500 of these candidates die each year before receiving a liver [1], with waiting list mortality rates approaching 20 % in some regions of the country [2]. Unfortunately, the donation rate has remained static in recent years. This persistent shortage represents one of the greatest challenges facing the field today.

An important way to address this donor shortage is to expand the donor pool through the use of livers from donors defined as 'extended criteria'. However, organs from extended criteria donors (ECD) currently are associated with poor outcomes and are often discarded. Typically, ECD livers are those from the elderly, the obese, and from donors after cardiac death (DCD).

DCD organs represent the largest proportion of underutilized livers. Such organs undergo prolonged periods of warm ischemia, exposing them to a greater ischemic injury than donation after brain death (DBD) organs. Often, these organs are irreversibly damaged during preservation, leading to higher rates of graft failure and biliary complications [3–5]. To ameliorate the problems associated with these marginal grafts in clinical practice, new techniques of preservation are needed. The most promising of these preservation techniques is normothermic machine perfusion (NMP). NMP has already seen success in lung [6] and kidney preservation [7]. This review examines the experience of the past two decades, which has built a foundation to apply this method to human liver transplantation.

Organ Preservation Today

Cold Storage and its Limitations

The success of liver transplantation depends on an effective means of preserving donated grafts. For decades, cold storage

(CS) has been used to preserve organs after procurement. CS slows metabolic processes, increasing the amount of time the graft can spend outside of the body. Although slowed, these processes cannot be halted, and damage to the organ invariably occurs during preservation and reperfusion [8, 9]. While CS produces acceptable results for normal organs, it fails to adequately preserve marginal grafts, as evidenced by elevated rates of dysfunction, failure, and ischemic cholangiopathy in such transplantations [10]. Machine perfusion aims to solve these problems by providing substrates for metabolism and by removing harmful by-products, thereby attenuating ischemia–reperfusion injury and prolonging preservation time [11].

Hypothermic Machine Perfusion

Currently, machine perfusion is an active area of research, but no consensus has been reached on how it should be carried out. Hypothermic machine perfusion (HMP) involves perfusion at a low temperature with enough oxygen in the perfusate to maintain the reduced metabolic rate. HMP has been shown to protect against mitochondrial and nuclear injury by establishing reduced mitochondrial activity prior to reperfusion, as well as to lessen endothelial injury [12]. Recently, eight patients received DCD grafts that had undergone 1–2 h of HMP following standard procurement procedures [13]. These patients had similar post-operative liver enzymes, kidney function, and intensive care unit and hospital stays to those of matched DBD patients. Most importantly, after a median follow-up of 8.5 months, none of these patients had biliary complications. While HMP is promising, low flow rates must be maintained to avoid damaging the hepatic sinusoids and Kupffer cells [14], requiring a fine balance between adequate perfusion and prevention of further injury.

Subnormothermic Machine Perfusion

Subnormothermic machine perfusion (SMP) has recently been proposed as an alternative to HMP and NMP. The first study involving SMP showed that perfusion of healthy rat livers at 20 °C resulted in significantly improved preservation compared with CS upon simulated transplantation [15]. The same group used a similar perfusion model for steatotic rat livers, and found that preservation and function of these livers was improved when SMP was used rather than CS or HMP at 4 or 8 °C [16]. In 2012, SMP at both 20 and 30 °C was investigated in a rat DCD model, and was directly compared with both CS and NMP [17]. After transplantation, livers preserved with SMP showed high bilirubin levels and low bile production compared with NMP, while showing similar outcomes in every other regard. Thus, due to the simplicity and feasibility of SMP compared with NMP (demonstrated in discarded human livers [18]), further comparative studies are warranted to determine whether these shortcomings can be addressed.

Normothermic Machine Perfusion (NMP)

Unlike CS and other machine perfusion techniques, NMP aims to maintain normal metabolic rates in an attempt to approximate physiology. The potential benefits of re-establishing a physiological environment include enabling natural repair of injury sustained during procurement, lengthening the time grafts can be preserved, and easily testing tissue viability before transplant [19]. Such possibilities were responsible for spurring research into NMP over the last two decades.

Early Studies of NMP

The development of the current NMP techniques began in the early 1990s, as groups searched for new ways to resuscitate livers damaged by prolonged warm ischemia [20, 21]. The first study demonstrating the potential of NMP was published in 2001 [22]. In a series of experiments, Schon et al. demonstrated that when livers were exposed to 1 h of warm ischemia time (WIT) and then preserved with either NMP or CS, animals transplanted with CS-preserved livers developed primary graft non-function within 1 day of the transplant, while animals transplanted with NMP-preserved livers survived for the length of the experiment (7 days). Soon after the publication of this study, the preservation of both DBD and DCD livers for 24 h was reported [23, 24]. In both studies, livers preserved with NMP showed significantly better function than CS-preserved livers. Another study demonstrated the ability of NMP to maintain graft viability for 72 h [25].

Later findings indicated the need for bile salt supplementation [26] demonstrated the ability of the perfused organ to maintain its own acid–base balance [27], and identified viability markers for monitoring the health of the perfused liver [28]. Additionally, it was discovered that cold ischemia prior to NMP increased biomarkers of liver injury [29, 30], indicating the importance of developing a mobile perfusion machine to obviate the need for CS. In 2009, researchers demonstrated over 80 % survival for transplantation with porcine DCD and DBD livers, both preserved for 20 h with NMP, providing further evidence of NMP's promising role in liver preservation.[31].

The porcine model provides the closest approximation to human physiology and has been invaluable in the investigation of NMP. However, small animal models have also contributed to the current understanding of the mechanisms of NMP. In a rodent model [32], NMP has been shown to be able to resuscitate DCD grafts [33, 34] and provide a means to

reduce steatosis in fatty livers [35]. In the future, the rodent model will be especially important for sophisticated mechanistic studies of NMP.

Recent Developments in NMP

Research conducted over the last four years shows that NMP has the potential to make considerable contributions to the clinical practice of liver transplantation. Studies have investigated NMP in porcine and discarded human liver models with clinically-oriented objectives, the results of which illustrate the effectiveness and feasibility of NMP in both DCD and marginal grafts.

NMP in Donors after Cardiac Death *Grafts*

The potential benefit of NMP in the preservation of DCD grafts has been an object of intense investigation. In a 2011 study performed by Fondevila et al., the group examined the addition of NMP to their current clinical protocol for DCD liver transplantations [36••]. The protocol established a normothermic extracorporeal membrane oxygenation (NECMO) circuit in patients who had suffered cardiac arrest prior to preservation with CS. Using a porcine DCD transplantation model, porcine livers underwent 90 min of WIT, followed by 60 min of NECMO and then 4 h of preservation with either CS or NMP. After transplantation and 5 days of observation, livers preserved with NMP had significantly decreased hepatocyte, cholangiocyte, and endothelial injury, as well as decreased inflammatory markers, compared with those preserved with CS.

In 2013, Boehnert et al. expanded on this study using acellular NMP and investigated the effect of NMP on livers first preserved with CS, modeling the required refrigeration period during transit from donor to recipient hospital [37•]. In the first part of this study, porcine livers exposed to 1 h WIT were used as a DCD model. These livers were preserved with 4 h of CS followed by 8 h of acellular NMP and compared with livers preserved only with 4 or 12 h of CS. After preservation, transplantation was simulated by reperfusing the livers with dilute blood for 12 h. Upon reperfusion, livers preserved with NMP showed dramatically decreased ALT levels and hepatocyte necrosis compared with those livers preserved with CS. Hepatic arterial perfusion in CS livers (measured with computed tomography) was decreased almost twofold compared with livers preserved with NMP. Additionally, bile composition and intrahepatic biliary histology was significantly improved in the NMP livers after reperfusion when compared with CS livers, suggesting that NMP has the potential to reduce the ischemic cholangiopathy that plagues DCD grafts. The second part of their study compared DCD porcine livers preserved with 4 h of CS and 4 h of acellular NMP with livers preserved with 8 h of CS. These livers were orthotopically transplanted into recipient pigs and serum AST levels were measured for 8 h. As occurred with the simulated transplantation, transplanted livers preserved with NMP showed significantly lower AST levels than those preserved with CS.

The cellular mechanisms underlying the superior preservation results of NMP over other methods are also being investigated. In 2012, the group at the Massachusetts General Hospital subjected 12 porcine livers to either 0 or 60 min of WIT, followed by 2 h of CS and 4 h of sanguineous NMP [38]. Initially, the organs that experienced 60 min of warm ischemia showed increased liver enzyme levels and decreased bile production compared with controls with no WIT; however, after 1 h of NMP, liver enzyme levels in the ischemic livers stabilized and bile production increased to levels similar to those of the controls. The most striking results were seen in the histology of the ischemic livers. After 60 min of WIT and 2 h of CS, light microscopy revealed fatty degeneration as well as patchy necrosis and apoptosis of hepatocytes. Notably, these changes were almost completely reversed after 4 h of NMP. The authors hypothesized that these changes occurred through the improved energy status of the grafts as a result of NMP, as indicated by the increase of tissue ATP levels by the end of perfusion. During the 60 min of warm ischemia, ATP levels decreased by 70 %; after 4 h of NMP, these levels increased to 80 % of the pre-ischemia levels. This suggests that the ability of NMP to restore energy levels to the organs may be important to the superior preservation of these grafts.

Translating Studies to Practice

A number of laboratories [39], including ours at the Cleveland Clinic, have continued investigations into the DCD porcine liver model in order to bring NMP closer to use in clinical practice. Importantly, in one of our studies, we demonstrated the theoretical ability of sanguineous NMP to regenerate the extrahepatic biliary epithelium in a DCD model after 24 h of simulated transplantation [40•]. We reperfused the organs with whole blood after preservation, a method established by Friend and colleagues [23] that is commonly used to simulate clinical transplantation. Interestingly, livers preserved with NMP showed significantly improved histology compared with controls. Furthermore, staining for Ki-67,a protein strictly associated with proliferation of biliary epithelial cells [41], was diffusely present in NMP-preserved biliary tissue and virtually absent in controls. Results from this study suggest that NMP can attenuate the rates of post-transplant cholangiopathy.

In another study, we examined the efficacy of two different vasodilators, adenosine and prostacyclin, during sanguineous NMP in a porcine DCD model [42]. After 10 h of perfusion, the group that was administered prostacyclin showed significantly lower liver enzyme levels than the group given adenosine and the controls that received no vasodilation. Additionally, the group that received prostacyclin exhibited superior bile production and preservation of the cellular architecture, demonstrating the importance of proper vasodilator use in NMP as well as the potential of pharmacological intervention.

The most commonly cited barriers to the translation of NMP to the clinic are the challenges inherent to preservation at physiological temperatures, such as microbial contamination and machine failure. To demonstrate the safety and feasibility of NMP, our laboratory examined safety and performance data on 20 porcine livers preserved with NMP [43•]. Preservation was performed without complications or microbial contamination and target hemodynamic parameters were easily achieved and maintained. This was the first study exhibiting the safety and simplicity of NMP and is encouraging for future clinical application of this modality. The theoretical arguments against the clinical potential of NMP focus on the perceived difficulties of preserving a metabolically active organ, but these recent studies show the ease and safety with which NMP can be performed.

NMP in Steatotic Grafts

In addition to its efficacy in DCD graft preservation, NMP has demonstrated the ability to improve the function of steatotic livers. In a pioneering study, Jamieson et al. induced hepatic steatosis in pigs by damaging islet cells with streptozotocin and subjecting the pigs to a high-fat, high-carbohydrate diet [44••]. This method caused an average baseline steatosis of 28 %. The fatty livers ($n=3$) and control livers ($n=5$) were both preserved for 48 h with sanguineous NMP. Steatotic livers were capable of correcting the perfusate base excess and maintaining factor V and bile production, and showed markers of liver injury comparable with normal livers. NMP was also able to decrease the amount of steatosis present. These results suggest that steatotic livers can be successfully preserved using normothermic preservation for prolonged periods and that normothermic preservation could have a role in the reduction of hepatic steatosis during preservation. The apparent benefit of NMP may stem from the avoidance of CS, and its concomitant destruction of sinusoidal lining cells in steatotic livers [45], from the direct regenerative effects of NMP, or from a combination of the two. With such a small study size, these results require further study to better address the potential of NMP in resuscitating fatty livers.

NMP in Discarded Human Livers

The first study to examine NMP in human livers was performed by op den Dries et al. in 2013 [46••]. The authors obtained four discarded DCD livers, which had been exposed to several hours of cold ischemia. NMP was performed on each organ for 6 h, and markers of cellular injury, synthesis of bile, and histological characteristics were measured throughout. Over the course of the perfusion, biochemical markers of hepatic injury were minimal in the perfusate and bile composition improved, as evidenced by greater concentrations of biliary bilirubin and bicarbonate. Additionally, biopsies taken at the end of perfusion demonstrated no significant differences compared with biopsies taken at the onset of perfusion. These results demonstrated for the first time the feasibility of NMP in human livers and encourage further studies aimed at translating this technology to the clinical setting.

Conclusions

As supported in the literature examining over 20 years of studies, NMP demonstrates significant promise in its ability to improve transplant outcomes and increase the pool of donor livers through improved preservation. The ability of NMP to extend the length of preservation, preserve and repair marginal grafts, and provide therapeutic interventions prior to transplant outweighs its perceived limitations. The established benefits of NMP are further supported by our review, which found no published studies refuting the advantages of NMP. More studies are needed to refine the technical aspects of NMP, as well as investigate its effects on a molecular level. Additionally, clinical trials, some of which are already in progress, are needed to translate the application of NMP from bench to bedside. Regardless, the evidence supporting NMP is encouraging. The clinical potential of NMP, when put to practice, may usher in a new era in liver transplantation.

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Compliance with Ethical Guidelines

Conflict of Interest Daniel Sexton, Sarah Medearis, Qiang Liu, Giuseppe Iuppa, and Cristiano Quintini declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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