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Will the machine perfusion of the liver increase the number of donor organs suitable for transplantation?

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Worldwide, there is a trend towards an increase in the number of patients waiting for liver transplantation, despite an increase in the total number of liver transplantation operations. Solving the problem of donor organ shortage is possible through the use of organs from marginal donors: organs removed after cardiac arrest, organs with a high percentage of steatosis, organs from donors over the age of 60 years. The main reason for refusing to use them is the risk of severe complications and an unfavorable outcome of the operation. Static cold preservation today is the main method

of protecting donor organs from thermal damage, which possesses rather effective protective properties. At the same time, the duration of cold preservation has a limited time interval. There is always uncertainty about the viability of the organ. Modern methods for assessing donor organs such as donor history, laboratory data, visual examination and morphology, do not reliably predict liver function after transplantation. In this regard, the development of methods for preserving the organ after removing, which do not degrade the quality of the organ or even are capable of restoring the lost functions, is relevant. The machine perfusion of the liver is one of the new concepts aimed at solving this problem. The article highlights the international experience of using the machine *perfusion of the donor liver over the past 15 years. Machine perfusion is a promising trend of transplantation development, which allows reducing the shortage of donor organs and improving their quality.*

Keywords: machine perfusion, donor organs, liver transplantation

ALT, alanine aminotranstransferase

AST, aspartate aminotranstransferase

ATP, adenosine triphosphoric acid

DAMPs, proteins released from the cytoplasm when a cell is damaged

ECD, expanded criteria donor

GDHG, glutamate dehydrogenase

HBD, heart-beating donors

HMP, hypothermic machine perfusion

ICAM-1, intercellular adhesion molecule 1

IL-13; IL-17, T-cell cytokines

IRI, ischemic and reperfusion injury

LT, liver transplantation

MELD, Model for End-stage Liver Disease scoring system

MMP, matrix metalloproteinases

MMP, medium-thermic machine perfusion

MP, machine perfusion

NHBD, non-heart-beating donors

NMP, normothermic machine perfusion

NO, nitric oxide

NrMP, normothermic regional machine perfusion

O_2CC, oxygen-carrying component

PBG, peribiliary glands

PGNF, primary graft non-function

ROS, reactive oxygen species

SCS, static cold storage

SnMP, sub-normothermic machine perfusion

SrMP, sub-normothermic regional machine perfusion

TLR-4, Kupffer Cell Receptors

TNF, tumor necrosis factor

TNF-α; IL6; IL8, proinflammatory cytokines

VWF, von Willebrand factor

Organ transplantation was the impetus for the development of new medical technologies. After earlier experimental studies, including those in the USSR, T.Starzl (USA) performed the first liver transplantation (LT) to a human in 1963 [1]. LT became the main treatment of patients with end-stage liver disease. Thus, in 2012 alone, 23,986 LTs were performed in 68 countries of the world. In recent years, the mortality rate after LT has

significantly decreased, and this operation has become the “gold standard” treatment for decompensated liver diseases [2]. Currently, there is an increase in the number of patients awaiting surgery, and the difference is constantly growing between the number of the people in need of a transplant and the organs available for this. For example, in 2014, 6729 LTs were made in the United States while the waiting list increased by 10,648 people during that year [3]. Mortality of those on the waiting list makes from 11.1% to 30% [4].

The need to increase the donor pool contributed to the use of marginal donors, namely, the donor organs harvested after cardiac arrest, organs with a large percentage of fatty hepatosis, and organs from donors over the age of 60 [5]. According to the 2014 Report, there were nearly 15% of potential donors registered after cardiac arrest, but only 6% of them became actual donors. About 20% of the transplanted organs in the United States were obtained from donors older 60 years of age [6].

In liver transplants from non-heart-beating donors (NHBD) with prolonged warm ischemia, the incidence of primary graft non-function (PGNF) and biliary complications has been significantly higher [7–9]. Macrovesicular hepatosis of over 30% also negatively affects the outcome of surgery, as a whole. We should note that the hepatosis prevalence is from 6% to 33% (mean 20% in general population) [10, 11].

Static cold storage and its drawbacks (0–4 ^{o}C)

Static cold storage (SCS) currently forms the basis for protection of donor organs against thermal injury. However, even the best preservative solutions do not protect the graft from damage. This is due to two factors that are closely related both to the ischemia time and cooling per se [12].

While the body is deprived of oxygen delivery, cooling slows down the metabolic processes without the loss of viability. At the same time, the energy consumption and metabolic activity do not stop, but decrease (approximately by 12 times); the depletion of adenosine triphosphoric acid (ATP) and mitochondrial damage occur. Cooling directly damages the plasma membrane, cytoskeleton, microtubules [12], blocking ion-exchange pumps, and leading to cell lysis during cell membrane edema. The main damage-causing component is the reactive oxygen species (ROS) that are formed during ischemia. The mechanisms underlying the ROS formation include the production of hypoxanthine (the metabolic product of ATP), the appearance of excess calcium in mitochondria [13], the activation of neutrophils, release of cytokines, and stimulation of complement [14]. It was suggested that under hypothermia, the release of intracellular proteins (DAMPs) being the direct initiators of the inflammatory response, is initiated [15].

When the venous blood supply (reperfusion) of the graft is restored, mitochondria abundantly consume oxygen during the first 10 minutes with a significant release of ROS [16]. The excessive intracellular ROS cause the hydroxylation of deoxyribonucleic acid molecule in the nucleus and contribute to the release of the HMGB-1 nuclear sequence. The Kupffer cells activated through the receptor (TLR-4) become the main targets for HMGB-1. The reperfusion injury of the organ leads to the activation of endothelial cells and T-cells by cytokines (IL-13 and IL-17), which additionally cause infiltration of neutrophils, stimulate the development of graft fibrosis and the proliferation of intrahepatic cholangiocytes [16, 17].

SCS has quite effective protective properties. Meanwhile, the duration of the cold storage is limited in time, and the use of marginal donor organs is

associated with a high risk of severe complications and a poor outcome of surgery [18]. Current (often subjective) methods of organ evaluation, including donor medical history, preoperative biochemistry study, visual examination, and morphology can not reliably predict the posttransplant liver function [19]. The use of organs from NHBD or other marginal donors requires more reliable methods for predicting postoperative function. There is always an uncertainty about the organ viability. In this regard, the development of methods for preserving the organ after removing, which would not degrade the quality of the organ but would be capable of restoring the lost functions, is relevant.

The history of machine perfusion

Machine perfusion (MP) is not a new concept. This attractive idea brings us back to the 1930s, when Alexis Carrel and Charles Lindberg first infused normothermic blood serum into the organs of animals in pressure-controlled glass vessels, providing recirculation, filtering and oxygenation of the solution, and demonstrated the tissue viability for several days [20]. As the transplantation was coming closer to the clinical practice in the 1960s, their system failed to maintain human liver function even for a few hours, but the dog organs were preserved at 12-15°C in autologous blood with using extracorporeal femoral-femoral perfusion [21]. The infusion was made after the cessation of natural blood circulation without flushing the organs in situ, and the existing warm ischemia was the likely cause of subsequent cytolysis, hyper bilirubinemia, and coagulopathy [21]. In 1966, Kestens et al. [22] used oxygenated blood and successfully preserved the dogs' liver at 10–18° C for 5 hours for transplantation. Pulsed perfusion through the liver artery only, using a hyperbaric oxygenated solution, was performed in 1967

with unsatisfactory results [23]. Brettschneider et al. [24] perfused the portal vein and artery with a mixture of preservative solution and autologous blood in an experiment on dogs, and all 7 animals underwent surgery and survived the first week after surgery.

The introduction of effective preservative solutions (University of Wisconsin [UW], Custodiol [HTK], Celsior) from 1970 to 2000, ease of SCS use led to the refusal from the MP development [25]. SCS was introduced into clinical practice as the only option for organ protection from the moment of its removal till the reperfusion. By that time, the technique of using plasma or blood-based solutions had been ready for clinical use [26].

In recent years, there has been a renaissance of the interest in dynamic methods of preservation. A lot of reports on the use of donor organs after MP have been published, in which they note significant success both in the experiment and in clinical setting. Currently, MP is considered as a promising alternative to the SCS. The MP provides protection of the donor organ, ensuring the delivery of oxygen and nutrients; in addition, it allows restoring and optimizing the liver functional capacity, provides the opportunity to test the quality and viability of the organ ex situ prior to transplantation. The method of "degreasing" in MP has also been reported, which reduces the degree of steatosis [27].

Dynamic methods of donor organ preservation

Dynamic preservation is being implemented into clinical practice. The currently offered techniques include hypothermic machine perfusion (HMP), subnormothermic machine perfusion (SnMP), subnormothermic regional machine perfusion (SrMP), normothermic machine perfusion (NMP) and normothermic regional machine perfusion (NrMP). In hypothermic dynamic

preservation, low temperatures (4–10° C) are used and the perfusate proposed by Belzer et al [28]. During the dynamic preservation, the perfusate is continuously infused through the vessels into the graft. A heat exchanger regulates the temperature of the perfusate from hypothermia to subnormothermia or normothermia. Before removing the organ, they restore the recirculation of donor blood or solution, thereby protecting the organ from hypoxia and warm ischemia. MP is started during a donor operation by injecting blood or solution through the cannula installed in the femoral artery or aorta (a regional abdominal perfusion). But most often the MP is used after a period of SCS.

MP can be performed at three main temperature modes: hypothermic 0–10° C, subnormothermic (20–33° C), normothermic (35–38° C); a medium-temperature mode (13–20° C) is rarely used. HMP (0–12° C) is more often made at a temperature of 10° C and below, which is associated with metabolism and enzymatic reactions that are reduced to 20% or even lower. Since the rates of numerous energy-dependent mitochondrial reactions slow down significantly at 12.5° C [29], this temperature is considered the cut-off point for HMP. SnMP is performed at 20–22° C in most cases. NMP (35–38° C) is increasingly seen as the most attractive alternative to SCS, as it effectively reduces hepatocellular damage and improves the allograft function [30].

Oxygenated hypothermic machine perfusion (1–13 ^{o}C)

The first devices for MP provided a constant or pulsating pressure with limited oxygenation [31]. The ATP decomposition products are normally converted to xanthine dehydrogenase and uric acid, and under ischemia conditions they are converted to xanthine oxidase that, in the next

phase, is converted to xanthine and free radicals in the presence of oxygen, causing a lipid peroxidation and a further cell destruction [32].

The significance of HMP becomes obvious when bearing in mind that mitochondria are the central link in the mediated damage to the ischemic cell [33]. However, mitochondrial and other cellular processes do not function normally in a hypothermic state. SCS leads to decreased cellular metabolism and a decreased consumption of ATP. Thus, for every temperature drop by 10° C, metabolism slows down by 1.5–2 times. Meanwhile, anaerobic metabolism and ATP consumption continue even at 1° C; and during reperfusion, a cascade of ischemic and reperfusion injury (IRI) develops. Early HMP at low temperatures did not involve the use of O_2CC, since the oxygen fraction in the perfusate was sufficient to maintain adequate metabolism under hypothermia for a short period [34]. Guarrera et al. showed that even in an open system without an oxygenator, it is possible to achieve the oxygen saturation of > 120 mm Hg in the perfusate [41]. Short periods of HMP with perfusate oxygenation after SCS were shown to significantly improve the restoration of the cell energy charge, to record the increase in liver ATP and glycogen content [35]. In this regard, the authors concluded that the HMP to be successful requires a constant supply of oxygen.

In addition, the optimal duration, the mode of liver MP to be used (continuous or intermittent perfusion), the level of the perfusate pressure, and the route of the perfusate delivery are under investigation. Experimental studies of liver HMP performed over the recent 15 years have shown an improvement in the safety of hepatocytes and endothelial cells compared to the SCS effect. HMP does not increase the preservation time compared to SCS. The advocates of MP through the hepatic artery only have emphasized

the improved oxygen supply to the peribiliary vascular plexus, unlike the perfusion through the portal vein. Meanwhile, most of the interlobar bile ducts are accompanied by the portal vein branches, and the perfusion through the portal vein only is also effective. Recent clinical studies have shown the appropriateness of a short-term perfusion simultaneously through the artery and the portal vein at 4–8° C [36]. Sinusoidal endothelial cells comprise the main area of damage in terms of HMP or SCS. An increase in the perfusion pressure at HMP though led to a good perfusion, but also increased the damage to these cells [37].

High infusion rate also damaged sinusoidal endothelium, increased the expression of von Willebrand factor (VWF) and the tumor necrosis factor (TNF) followed by the activation of Kupffer endothelial cells [38]. It has been found that the pressure values of 3-5 mm Hg in the portal vein and 20–30 mm Hg in the artery were the most effective.

The perfusate viscosity increases at low temperatures, and depending on the perfusion duration, the resistance in the vessels increases, which determines the risk of damage to the sinusoidal endothelium and glycocalyx, especially in cold perfusion exceeding 18 hours [39]. Pienaar et al. [40] reported a 3-day continuous liver HMP in dogs without damaging the organ and it was only through the portal vein; but that was the only study of such duration. The temperature of the preservation during the continuous HMP varies between 1° C and 18° C, so most of the experiments are limited to perfusion intervals from 2 to 24 hours.

The most commonly used perfusion solution is based on the UW solution. To improve the quality of perfusion, it was proposed to include spasmolytics, antioxidants, and amino acids in the perfusate [41].

The HMP relies on the physical dissolution of oxygen in the free blood perfusate at a temperature of 2–18° C, which allows the cell to recover a sufficient energy charge. Oxygen does not increase the number of free radicals, but in its absence, the growth of DAMP is provoked [42]. Achieving the balance between the positive effects of oxygenation and the ROS formation is important. The absence of oxygen during MP leads to reperfusion injury and contributes to the damage to mitochondria and the cell nucleus with the release of HMGB1 and 8-OHdG proteins. The HMGB1 release from the necrotic cell nucleus activates TLR4 in Kupffer cells as an initiator of the innate immune response [43, 44]. We should note that the recorded level of HMGB1 was the highest after SCS, and the lowest in the combination of SCS with HMP.

The first clinical trials of HMP in heart-beating marginal donors (HBD) were reported in 2010. Further studies were conducted on the organs from NHBD, and their results did not differ from those of the standard donors in SCS [34].

Ischemic and reperfusion injury (PRI) causes a cascade of injuries that develop under the conditions of anaerobic metabolism, cold storage and subsequent entry of oxygenated blood in the donor organ at a normal body temperature. This leads to the dysfunction of the organ that functioned normally before its removal. In case the organs have been obtained from NHBD, warm ischemia causes a catastrophic decrease in intracellular ATP even before SCS. The absence of ATP along with the low-temperature mode disrupts the function of the Na/K pump, a key mechanism protecting against swelling and death of cells [45]. An impaired ATP regeneration results in a slower recovery of the liver function [46].

HMP of the liver obtained from NHBD protects against a significant DAMP release, reduces the electron leakage from mitochondria. The technique reactivates mitochondrial respiration, oxidizes mitochondrial electron complexes before warming and reperfusion [43]. The MP continued for more than 90 minutes leads to an almost complete cessation of the electron loss; a unique slowing down of mitochondrial respiration occurs, regardless of the previous warm ischemia. Due to the decreased rate of the mitochondrial electron transfer, less ROS and nuclear DAMP proteins are released; the further activation of Kupffer cells and damage to the endothelium are prevented [47].

ATP deficiency contributes to the release of Ca^{2+} from the endoplasmic reticulum into the cytosol, causing an increased calpain activity, the actin dissociation, and the release of matrix metalloproteinases (MMP). This, in turn, leads to the expression of the VWF and intercellular adhesion molecules 1 (ICAM-1) on the sinusoidal surface [48].

HMP with UW solution or Custodiol includes several defense mechanisms: first, the cellular energy charge increases due to the oxidative phosphorylation; second, the calcium content in cells increases, the protection against calpain and MMP is provided, which is believed to be associated with the specific action of calcium lactobionate and histidine (components of preservative solution), third, the overvoltage of the respiratory chain decreases [49].

Experimental and clinical studies of oxygenated hypothermic machine perfusion

The theoretical advantages of MP were confirmed in experimental studies and clinical practice. Thus, in experiments on rats, the liver obtained

from NHBD at 30–60 minutes after cardiac arrest and after further SCS for 4 hours and HMP was transplanted with a good outcome [50]. Guarrera et al. [41, 51] successfully performed 20 operations after the combination of SCS and HMP. In 2015, the same authors reported 31 LT from marginal donors after HMP. And no significant differences were noted in the number of developed bile duct strictures, days of hospital stay, and early allograft dysfunction compared to standard donors [51, 53]. In another study, HMP was performed on 8 organs obtained from NHBD (Maastricht category III). Perfusion was performed for 1–2 hours before transplantation. After surgery, a good graft function was noted with low serum aspartate aminotransferase and alanine aminotransferase in all the recipients. The post-transplant hospital stay and expenses were comparable to the recipients who had received the organ from standard HBDs. A six-month follow-up of the recipients showed no difference in the development of the choledochus-choledochus anastomosis incompetence or the strictures of the bile ducts [52, 54].

Subnorothermic machine perfusion (20–33° C)

It is assumed that the gradual warming of an organ to a body temperature can reduce the post-reperfusion injury thanks to a more balanced replenishment of cell metabolic requirements. Slow warming of the liver to 20° C for 3 hours with the perfusion through the hepatic artery and portal vein improves metabolic processes, functional, biochemical parameters, and the histological pattern [55, 56]. At the same time, the recovery of the mitochondria respiratory function and the improvement of the energy deficit play a key role in ensuring the liver viability during IRI [57]. Especially attractive in this method is the possibility of a controlled

improvement of the liver energy balance by increasing the ATP level [49, 58]. The cell transition onto the anaerobic energy pathway increases the lactate level and leads to acidosis. Under conditions of adequate oxygenation, ATP returns to the baseline level after 3 hours of perfusion, the same as other markers do, including the synthetic function and bile production function.

Perk et al. [59] showed that measuring glucose, urea, lactate and albumin levels during perfusion provides a good predictor of the surgery outcome.

SnMP and other HMP systems have shown their efficacy in reducing the damage to the biliary tract. The main mechanisms include improving the imbalanced composition of bile salts, phospholipids and bicarbonates, reducing toxicity and increasing the stability of the bile composition [60]. SnMP at ambient temperature gives a technical advantage, eliminating the need for temperature control; and the reduction of metabolic status eliminates the need for more complex oxygenators.

Berendsen et al reported good clinical results of SnMP in 6 marginal ("refused") liver allografts showing a normal post-transplant function in a 3-month follow-up period [61].

Normothermic machine perfusion (35–38° C)

NMP provides a logical approach to the problems that are inherent in organ transplantation. The basic concept of the technique is to maintain normal liver function during the entire preservation period and to ensure rapid recovery after implantation. The temperature of +37° C provides for a complete metabolism that supports normal homeostasis and other processes, including the ATP content. The technique opens up the opportunities for

more accurate monitoring of the viability and function of the graft: assessing the secreted bile amount, the urea production, the cytolysis level, etc. NMP provides the delivery of nutrients and oxygen to hepatocytes and has anti-inflammatory properties [62, 63].

Spetzler et al. [64] compared NMP of the liver with the organs after SCS on pig model ex vivo. After transplantation, the similar peak AST values were obtained, with no differences in survival or postoperative complication rates. Imitating the NHBD conditions by 60-minute warm ischemia in situ, the liver was connected to NMP for 24 hours. A good synthetic function of the liver and less cellular damage were recorded, as compared with the SCS technique [64]. In recent years, they have noted the efficacy of using NMP for marginal human organs obtained from NHBD. NMP also reduces IRI and protects the bile ducts from damage, but so far there have been no convincing results that this technique can effectively prolong the time of organ preservation.

Authors from Columbia University (USA) were the first to report a successful liver transplantation in 20 recipients. The organs were harvested from NHBD using the NMP technique. And meanwhile, the biliary complication and the hospital length of stay (in days) decreased compared to those in the standard comparison group [50].

In another center, 4 LTs from NHBD were performed after 12–17 minutes of warm ischemia followed by a 4.5–9.5-hour SCS, and further NMP for 6 hours. There were no significant differences in the levels of AST, ALT, and hyaluronic acid compared to the standard preservation technique [65].

At the University of Zurich (Switzerland), they performed 8 transplants from NHBD after NMP with perfusion through the portal vein

only and also showed no signs of ischemic cholangiopathy after 8 months of follow-up, despite the marginality of the organs. Dutkowski et al [53] transplanted 25 organs from NHBD after NMP and compared them with a group of 50 liver transplants after SCS. A decreased intrahepatic cholangiopathy rate and an improved graft survival were reported compared to the organs after SCS.

After conducting numerous preclinical studies, the advantages of continuous NMP were defined. Thus, a Phase I study in England showed that a prolonged continuous NMP using the Meter portable device (Organ Ox, Oxford, UK) was convenient and safe [67]. Twenty high-risk organs were successfully transplanted with the results comparable to 40 control ones [68]. A "refused" liver was used, which was successfully implanted after evaluating the parameters while on NMP, after the normalization of lactate (<2 mmol/L) and bile production [69]. The system is under clinical trials in Europe with promising early clinical results. Of the 6 "refused" liver grafts from NHBD with warm ischemia in situ for 36 minutes to 109 minutes, in NMP conditions, after control, five of them met the criteria of viability assessment and were successfully transplanted; and after 6-month follow-up, their good function was seen [67].

Currently, the clinical experience of using the NMP technique is modest. But the assumptions have been made that the NMP may significantly expand the NHBD pool. Clinical case reports of successful liver transplantation using the NMP technique after prolonged warm ischemia in situ and SCS have been published [68, 69].

A large percentage of liver macrosteatosis entails the risk of early dysfunction, PGNF in SCS, and this is the most common cause of organ discard. Nagrath [70] used NMP, adding "degreasing agents" into the

perfusate. As a result, the author achieved a 65% reduction in triglyceride levels in the experiment. It is noted that a long-term NMP reduces the steatosis degree; the liver fat is quite easily mobilized, thereby NMP reduces the IRI [71].

Given the disastrous consequences of PGNF, the objective evaluation criteria are of paramount importance. The retained metabolic activity prevents further ischemia-caused damage to the graft, and also provides an opportunity for monitoring of the function by assessing biochemical parameters, blood flow, and bile production [72]. AST and ALT, glutamate dehydrogenase are defined as the markers of cytolysis and cholestasis. Beta-galactosidases represent a group of enzymes located inside lysosomes and can be used to assess the damage to Kupffer cells in the analysis of perfusate. It was also noted that when NMP was performed, the VWF level was much higher.

It has been suggested that the graft should be perfused for at least 4 hours for its viability could be assessed. The restored acid-base balance, and increased bicarbonate levels are good predictors of postoperative functioning. The pressure and resistance in the portal vein and hepatic artery during NMP also correlate with the graft function. Their normal values indicate a good organ perfusion [73, 74]. Experimental studies suggest that a functioning liver produces more bile than a non-functioning liver. Assessing the bile production is a simple non-invasive method to determine the graft viability [73].

The first NMP was developed at Oxford University and used in standard criteria donors of the liver without using SCS (see Figure) [75]. In 2014, such organ was transplanted to a human [76].

The results of the conducted studies have shown that NMP normalizes clinically significant markers during reperfusion, improves the outcomes in transplantation of marginal organs, and makes the graft evaluation possible before the graft incorporation into recipient's vascular bed. Fewer biliary complications, severe dysfunctions, and a decrease in the length of hospital stay were recorded when compared to the standard group.

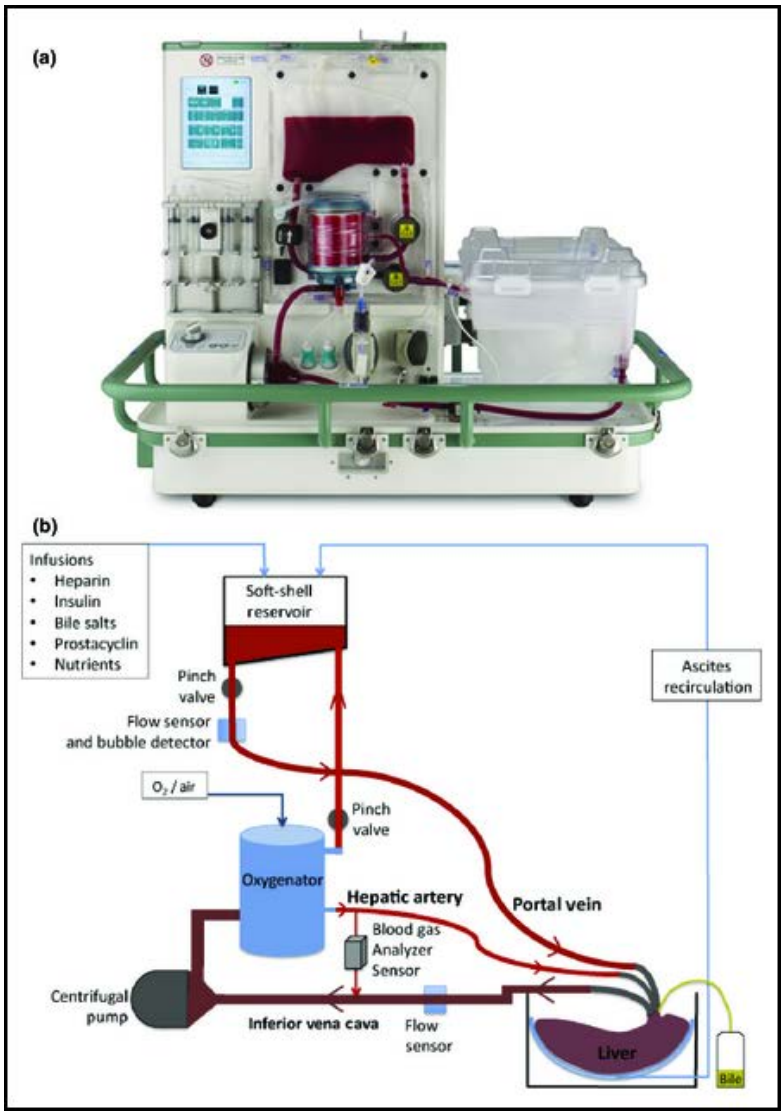

Figure. Normothermic machine perfusion of the liver. (Ravikumar R., Leuvenink H., Friend P. J. Normothermic liver preservation: a new paradigm? *Liver Transpl.* 2015;28:690–699)

Sub- and normothermic regional machine perfusion of the liver

When SrMP or NrMP of abdominal organs is performed, the extracorporeal membrane oxygenators are used for oxygen delivery in NHBD. The abdominal aorta is isolated from the thoracic aorta by using an inflated balloon catheter to block the perfusion of the thoracic organs and the brain. This procedure is also called the abdominal regional perfusion. SrMP reduces the metabolic activity and the need in oxygen, while NrMP can even support the restoration of cellular processes with a constant oxygen supply in an almost physiological way [77].

SrMP provides a fairly high level of kidney graft functioning. So, the transplantation outcomes of 320 kidneys obtained from NHBD showed a 1-year graft survival rate of 87%. In the meantime, a high percentage of delayed graft function was reported [77].

In NrMP of the liver from NHBD, the incidence of PGNF and ischemic cholangiopathy was higher than that in the liver recipients of the comparison group [77, 78, 79].

Combined system

Banan et al. included the MP dialyzer in the circuit and made a combined system. The combined hepatic-renal MP has an additional potential for improving the functional capacity of organs and is significantly superior over a single-circuit infusion of the liver. Liver and 1–2 kidneys can be preserved in the combined MP circuit device. The device can be used to transport the liver and kidneys for a single transplantation center [80, 81]. Op den Dries et al. [82] noted that the biliary epithelium regeneration after ischemic injury is possible only while maintaining the integrity of the

microvascular plexus supplying with blood the bile ducts and the peribiliary glands. Peribiliary glands contain multipotent stem cells that are able to differentiate into cholangiocytes restoring the biliary epithelium both in physiological and pathological conditions. The combined hepatico-renal MP has a potentially regenerating ability, maintains the stability of the internal environment in the circuit, reduces inflammatory injury after transplantation.

The perfusate composition

Currently, the perfusate most commonly used has the following composition: 3 doses of donor Rh-negative red blood cells compatible to the liver by blood-group, 1000 ml of 5% albumin solution, 30 ml of 8.4% sodium bicarbonate, and 10 ml of 10% calcium gluconate. Besides, 10,000 IU of heparin, 500 mg of vancomycin, and 60 mg of gentamicin are added to the circuit; and 8 μg/h of epoprostenol is injected before the connection. To maintain the liver metabolic function, the parenteral nutrition (amino acids, glucose-insulin formula) is used, as well as drugs to prevent thrombosis and improve microcirculation (heparin and prostacyclin), and a number of other drugs to reduce cellular edema, cholestasis and minimize the production of free radicals [83].

Some investigators use for NMP the donated blood obtained during the extraction of organs. Our experience in obtaining blood from HBDs before removing organs showed that taking 3-4 doses is not difficult and safe [84].

Erythrocytes are susceptible to damage and subjected to hemolysis during prolonged ex vivo perfusion; and at lower temperatures, additional rheological deficiencies appear, imposing restrictions on MP. Hemolysis reduces the throughput of the oxygen delivery and currently serves as one of

the main factors limiting the prolongation of organ storage [85]. Those few white blood cells that are present in the erythrocyte suspension can activate pro-inflammatory mechanisms. Fontes et al. [86] reported on the successful use of Hemopure™, a synthetic hemoglobin analogue, in combination with colloids in animal models. The drug exhibits antioxidant activity in vitro, inhibits ROS, and even has a protective effect on the focal IRIs of the brain [87]. Its molecule having the diameter of approximately 1/1000 of the erythrocyte transports more oxygen to the tissues than hemoglobin, and a lower viscosity provides a more homogenous perfusion, which facilitates the diffuse transport of oxygen under microcirculation conditions, improving tissue repair.

Hemopure™ can deliver oxygen in a wide range of temperatures used (10–37° C). However, its cost is an order of magnitude more expensive than donor red blood cells, but these costs can be offset by the numerous advantages of using it. The solution is more efficient, rheologically and immunologically better than the packed red blood cells. The use of Hemopure™ ex situ makes it possible to avoid potential systemic complications in vivo and side effects. Histological evaluation showed that Hemopure™ is effectively removed from the liver by NrMP, but its insignificant amount (if it remains) may even improve the microcirculation in the recipient, without causing any damage.

Thus, Hemopure™ as the basis of perfusion fluid improves the logistics, the immune component, provides an effective organ preservation and oxygenation ex vivo in the absence of hemolysis in a wide temperature range.

Disadvantages and benefits of machine perfusion

Considering the pros and cons of MP, one should bear in mind that these devices not only support the viability of the organs, but also ensure the delivery of medicines for the treatment of damaged liver parenchyma. Perfusion circuits make it possible to perform the biochemical testing of organ function and the measurement of hydrodynamic parameters, and also to infuse drugs that improve the cell phenotypes, changing the organ function [68, 90]. Currently, multiple organ harvesting is linked with SCS. One of the options for using MP provides that the warm perfusion should be started immediately before or immediately after the donor liver removal, excluding cooling, in order to avoid significant damage to the graft. This approach is difficult to be used with the simultaneous removal of the heart and lungs [39, 87].

Potential disadvantages of the MP are its high cost, the need for an increase in the number of staff during the organ removal and delivery. The MP device is rather complicated and often heavy, so a specially equipped vehicle may be required for its transportation. A correct cannulation of blood vessels and the prevention of the solution leakage from the organ are the key tasks. With a prolonged period of the organ recovery, a technical failure may occur; abnormal branching of the liver vessels can significantly complicate cannulation and limit the possibilities of MP preservation, as well as subsequent organ transplantation [88].

There are other unsolved problems still remain. For how long can the NrMP extend a safe preservation of the organ obtained after cardiac arrest? Is the organ transplantation safe after the prolonged warm ischemia at NrMP with the subsequent prolonged preservation? How reliably do the

hemodynamic and functional parameters obtained during the NrMP predict liver recovery after transplantation?

In clinical practice, there is still no consensus on the perfusate composition, perfusion parameters and duration; there is no standardized logistics of using NrMP (at donor and transplant centers). Studies on organ MP create a variety of regulatory, legal, ethical, and logistical problems. Using the MP for organ preservation requires a mutual agreement between the regulatory authorities and the transplantation community for the promotion of research. Potential shortages and logistics barriers lead to prolonged operation time both during organ removal and when using the operating donor base. Finally, a technical failure of the system per se is always a risk, which can be detrimental to the graft and to its further use.

Most researchers tend to have a short-term MP for 2–4 hours at the final stage of the back table, using either HMP at a low perfusion pressure or NMP with cell-free perfusate. This is due to the fact that the organ perfusion during transportation has the risk of stopping or ineffective blood supply to the graft. In addition, the organ preparation on a back table is associated with repeated ischemia. Despite the existing technical and logistics difficulties, most clinical data indicate the scientific and practical significance of these techniques. The MP of the liver is now entering the stage of extensive clinical testing [91–94].

Conclusions

1. Static cold storage has proven its efficacy for many years, as a reliable method of preserving organs obtained from standard criteria donors. Good quality grafts tolerate the preservation periods for up to 12 hours.

Meanwhile, the shortage of donor organs can be reduced if marginal organs are used.

2. Machine perfusion is currently being implemented into clinical practice. The technique provides a platform for optimizing the functionality of organs. Currently, many transplant surgeons do not use livers with fatty hepatosis (> 30% macrosteatosis), after a long static cold preservation (> 16 hours), from donors after cardiac arrest (warm ischemia >30 minutes), or from donors of older age groups due to a high risk of primary graft non-function. Machine perfusion provides the assessment of organ viability, thereby reducing the risk of using marginal grafts, and gives an additional potential for increasing the donor organ pool.

3. A widespread implementation of machine perfusion into clinical practice is possible with reducing its cost and overcoming technical difficulties.

4. The device for machine perfusion should be portable, having the capacities for laboratory testing of the perfusate, bile secretion. Procedures should be started at the clinic where the donor operation is performed, and continued during the transportation phase and at the transplant center.

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