Hypothermic machine perfusion in liver transplantation

Schlegel, Andrea ; Kron, Philipp ; Dutkowski, Philipp

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Andrea Schlegel, Philipp Kron, and Philipp Dutkowski

Purpose of the review
The purpose of the review is to report recent human application of hypothermic machine liver perfusion, and to discuss potential protective mechanisms.

Recent findings
Human application of hypothermic machine liver perfusion is still very limited. Currently, three transplant centers apply this novel treatment in donation after cardiac death (DCD) or donation after brain death (DBD) liver grafts. In all cases, endischemic perfusion was performed after initial cold storage for organ transport. Perfusion conditions differ slightly in terms of oxygenation (pO_2 15–60 kPa), perfusion route (dual vs. portal), perfusion time (2–4 h), and perfusate.

Summary
The current data support the hypothesis that applying endischemic hypothermic machine liver perfusion protects extended criteria DBD and DCD livers from initial reperfusion injury, with better graft function and less biliary complications. Hypothermic machine perfusion may therefore offer revitalization of liver grafts before implantation by a simple and practical perfusion technique with a high impact on enlarging the donor pool. Multicentric phase III randomized control trials in DBD and DCD liver transplantation have been initiated to further test this strategy, which may establish machine liver perfusion in the clinical setting.

Keywords
danger-associated molecular patterns, mitochondria, oxygen, reactive oxygen species

INTRODUCTION

Organ perfusion at normothermic conditions has been explored very early in the history of extracorporeal perfusion, compared with hypothermic perfusion [1]. However, the technical challenges of providing sufficient oxygen without vascular damage and infection prohibited any clinical application in the first part of the last century. In contrast, it has been recognized that lowering the temperature during organ procurement was key to induce low oxygen demand in cells, with consecutive longer viability *ex vivo* [2]. Subsequently, cold flush preservation and ice cooling permitted in the 1960s, the establishment of first clinical transplant programs [3], and were also the conceptual basis for cold perfusion in renal transplantation [4]. After pioneering work, it became quickly clear that an idealized perfusion circuit for extracorporeal organ perfusion depends on four key components, for example, pumping, sufficient supply of oxygen, a filter system, and a reservoir [5–7]. It was also reported already in the 1970s that high perfusion flow at low temperatures increases the risk of vascular endothelium injury by abnormal shear stress [8]. Despite these important and still true findings, hypothermic perfusion preservation gradually lost favor in the following 2 decades for several logistical and economic reasons. However, as the continuous shortage of organs caused a worldwide increase in the use of compromised donors, a significant proportion of transplanted organs are currently taken from extended criteria donors, and also after circulatory death [donation after cardiac death (DCD)] [9▪]. These organs generally suffer from a higher risk of dysfunction after implantation, which trigger acute rejection and impaired long-term graft survival [10,11]. Therefore, the

Department of Surgery and Transplantation, Swiss HPB and Transplant Center, University Hospital Zurich, Zurich, Switzerland

Correspondence to Philipp Dutkowski, MD, Department of Surgery and Transplantation, University Hospital Zurich, Swiss HPB and Transplant Center, Raemistrasse 100, CH-8091 Zurich, Switzerland. Tel: +41 44 255 4236; fax: +41 44 255 4999; e-mail: philipp.dutkowski@usz.ch

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KEY POINTS

- Hypothermic machine liver perfusion is protective after initial cold ischemia in human liver transplantation.
- The presence of oxygen in the perfusate is a key factor.
- The underlying mechanism is related to mitochondrial repair and endothelial effects.
- Prediction of liver viability and further application in steatotic livers are future-promising targets.

concept of machine perfusion instead of conventional static cold storage has been nowadays revisited to improve graft viability [12]. The concurring machine perfusion strategies for livers differ in perfusate conditions, including particularly temperature and timing of perfusion [13,14]. In this review, we summarize recent developments in the field of hypothermic machine liver perfusion, and focus on clinical application in humans.

UPFRONT VS. ENDISCHEMIC PERFUSION

The key element of machine perfusion of organs is to maintain viability and aerobic metabolism before implantation. As any static cold storage leads to anaerobic metabolism within short time, because of the lack of oxygen and substrates [15], machine perfusion has traditionally been designed as a continuous approach, starting directly after organ procurement. In the past 10 years, it became, however, clear that instead of continuous cold perfusion upfront, endischemic perfusion after cold storage is an attractive option [16–19]. Apart from additional logistic advantages, endischemic perfusion implies also a lower risk of shear stress, because of shorter perfusion time [20]. The idea behind such endischemic machine perfusion after initial cold storage relies on the assumption that metabolic and structural changes during the ischemic period may not be irreversible. In fact, delivery of oxygen under cold conditions turned out to be very effective in uploading cellular energy with only minor oxidative stress, in sharp contrast to exposure of any ischemic tissue to oxygen at normothermic conditions [21]. The underlying mechanism involves predominantly a mitochondrial repair [22,23]. Accordingly, short periods of hypothermic oxygenated perfusion (HOPE) or hypothermic oxygen persufflation increase ATP significantly in several tissues within 1–2 h [24,25▪], and decrease radical oxygen species (ROS) and danger-associated molecular patterns (DAMPs) subsequently (High-Mobility-Group-Box Protein 1, DNA fragments, and histones) that release during implantation [21,22,26]. Subsequently, several Toll-like receptors 2,4,9 and also receptor for advanced glycation end products on membrane surfaces become less activated [26–28], and lead to less release of chemokines (TNFα, CXCL2, and CXCL10) with less immune response [21,23,27,29▪,30,31].

Consistent to these experimental results, hypothermic perfusion has recently been shown to be also effective in human. Hypothermic dual (portal vein and hepatic artery) perfusion of 20 standard DBD human livers was first reported in 2010 by Guarerra *et al.* [32]. Machine perfusion was applied after previous cold storage and transport of organs without additional oxygenation, but reasonable oxygen availability in the perfusate (pO_2 15–20 kPa). Machine-perfused livers showed significantly less peak enzyme release and a shorter hospital stay, as well as less early graft dysfunction compared with a nonrandomized control group [32]. In a further report from 2015, the same investigators showed less biliary complications after application of hypothermic perfusion to marginal DBD organs [33▪]. Our group applied HOPE for 1–2 h only through the portal vein in cold-stored human DCD livers (Fig. 1a) [34]. A recent comparison of matched unperfused and HOPE perfused extended DCD livers indicate significantly improved graft survival because of less occurrence of intrahepatic biliary complications (Table 1) [35▪▪]. The Groningen group applies HOPE through the portal vein and the hepatic artery dual HOPE (DHOPE) in extended DCD livers. The results confirm a greater than 10-fold ATP increase by HOPE treatment and less nonanastomotic biliary strictures in recipients (Table 1) [36]. Randomized trials are initiated to further evaluate the effect of hypothermic oxygenated liver perfusion on DBD and DCD liver grafts (hope-liver.com – Zurich, Groningen Institute for Organ transplantation – GIOT).

DUAL VS. SINGLE PORTAL VEIN PERFUSION

In the context of the abovementioned experiences in human, there is a current debate whether cold machine perfusion should be best performed by dual perfusion through the hepatic artery and the portal vein, or by single portal vein access. Importantly, while dual perfusion under normothermic conditions is essential for biliary function and epithelial viability, hypothermic perfusion fundamentally differs from physiological conditions.

First, during cold machine liver perfusion, oxygen consumption of all liver cells, including the extrahepatic bile duct is dramatically reduced

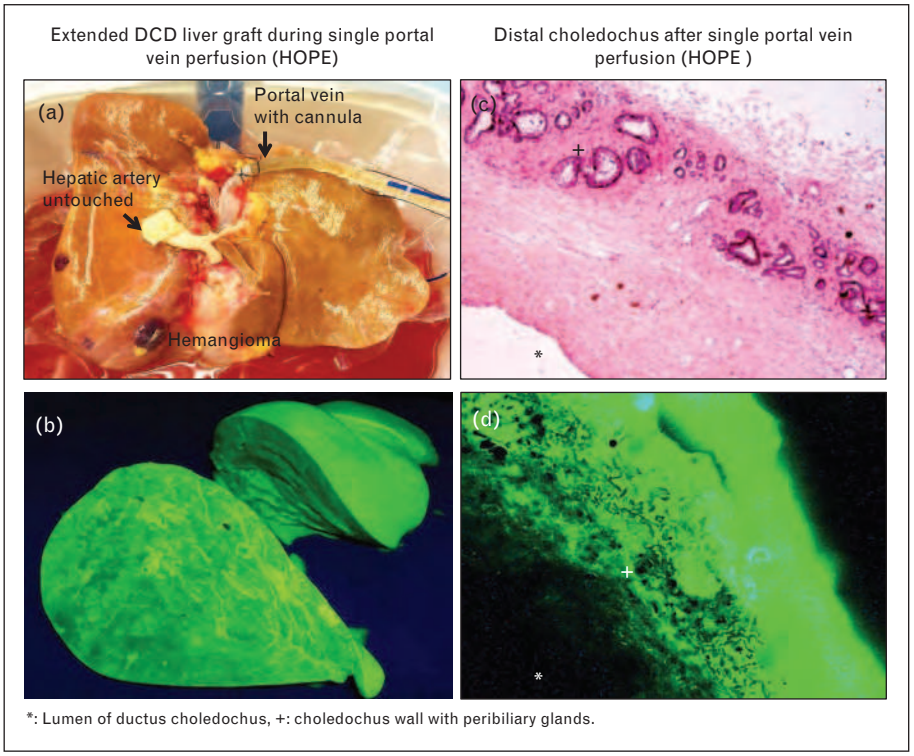

FIGURE 1. HOPE of human liver grafts: (a) HOPE through the portal vein of a human DCD liver (liver assist). (b) Fluorescence under dark light confirmed complete liver perfusion within 10 min during single portal vein HOPE (fluorescein in the perfusate). (c) Hematoxylin and eosin staining of distal extrahepatic bile duct with healthy peribiliary glands (+) after HOPE in DCD livers. (d) Fluorescence under dark light confirmed the presence of fluorescein in the complete biliary duct wall (+) after HOPE through the portal vein alone. HOPE, hypothermic oxygenated perfusion.

[37]. Second, oxygen saturation is largely different between hypothermic oxygenated perfusate (pO2 >80 kPa) [13,34,35▪▪] and normothermic portal pO_2 *in vivo* ($pO_2 < 10$ kPa) [38]. Third, we show in a recent study in pig and discarded human DCD livers that single portal vein perfusion resulted in complete graft supply (Fig. 1b), with simultaneous perfusion of the entire extrahepatic choledochus by small portal branches (Fig. 1c and d) [39]. This finding correlates well with healthy bile duct epithelia in the distal choledochus 2 weeks after transplantation [40]. Single portal vein approach during cold machine perfusion appears therefore as excellent method delivering oxygen and substrates to the entire biliary epithelia, which has been very recently shown to be of utmost importance for regeneration and protection against later cholangiopathy [30,41].

PERFUSATE ASPECTS

Human application of hypothermic liver perfusion is currently done using Belzer's machine perfusate [34,35▪▪] or its modifications, including ketoglutarate, nitroglycerine, L-arginine, N-acetylcysteine, and prostaglandin E1 (Vasosol) [32,33▪]. No conclusive comparison of machine liver perfusates remains yet available. In principal, solutions with low potassium and without starch appear advantageous, as low potassium concentrations decrease vascular resistance in the cold, whereas the presence of starch increases viscosity [17]. Of note, perfusate analysis during hypothermic machine liver perfusion allows prediction of subsequent peak liver enzymes after transplantation [32]. No clear thresholds, however, exist when to discard pumped livers based on perfusate analysis.

OXYGEN

The delivery of oxygen under cold conditions to different types of tissues has been tested long before the development of hypothermic machine perfusion [42]. In this context, gaseous oxygen persufflation was beneficial against warm and cold ischemia in various organs and achieved better organ quality compared with cold storage [43]. Based on these experiences on the key role of oxygen and hypothermia, perfusates are actively oxygenated (pO2 >80 kPa) in two human applications of hypothermic liver perfusion (Table 1), whereas in

Table 1. Hypothermic machine perfusion of human liver grafts

Author	Year	Donor characteristics	*n*	Perfusion device	Donor age (median; years)	Donor functional warm ischemia time (min)[c]	Graft steatosis (>20%)	Cold storage (h)	Perfusion duration (h)	Perfusion control	Perfusate	Temperature (°C)	Perfusate oxygenation	Perfusion pressure (mmHg)
Guarrera [33▪]	1/2015	DBD (ECD)[a]	31 vs. 30	Medtronic analog life port transporter	57.5	n.a.	n.a.[a]	9.3	3.8	Flow (0.667 ml/g liver/min)	Vasosol	4–8	No[d] (30 kPa)	Portal vein: 2.9, hepatic artery: 5.1
Dutkowski [35▪▪]	11/2015	Extended and high-risk DCD[b]	25 vs. 50	Liver assist (organ assist)	54	183 136	27%	3.5	2	Pressure	KPS–1	10	Active (>80 kPa)	Portal vein: 3
Van Rijn [36]	Upcoming 2016	Extended DCD	10	Liver assist (organ assist)	n.a.	n.a.	n.a.	5.2	2	Pressure	KPS–1	10	Active	Portal vein: 5, hepatic artery: 25

DBD, donation after brain death; DCD, donation after cardiac death; ECD, extended criteria donors; KPS: kidney perfusion solution.
[a]ECD defined as one of the following criteria: (1) donor age greater than 65 years; (2) hepatitis C virus (HCV) positive with 15% macrosteatosis; (3) greater than 25% macrovesicular steatosis by biopsy; or (4) evidence of significant donor ischemic injury (donor serum aspartate aminotransferase or alanine aminotransferase >1000 IU/l at the time of organ offer [33▪].
[b]High risk according to British Transplant Society (BTS) guidelines [11]: 32% extended DCD (donor age >50 years, donor ICU stay >5 days, functional warm ischemia >20–30 min, and steatosis >15%) and 68% high risk (donor age >60 years, donor ICU stay >7 days, functional warm ischemia >30 min, and steatosis >20%) [35▪▪].
[c]Total donor warm ischemia time: time from withdrawal and organ flush (first time); donor functional warm ischemia time: time from MAP less than 50 mmHg to liver flush (second time); and donor asystolic time (third time): time between cardiac arrest and flush.
[d]No active oxygenation.

another series, surgeons rely on dissolved oxygen in the perfusate (pO2 30 kPa), which may be sufficient at 4 °C for standard livers (Table 1) [32]. Previous work, however, confirmed that hypothermic liver perfusion with completely deoxygenated perfusate failed to protect from reperfusion injury [22,29▪]. Of note, the rate of oxygen consumption during HOPE is not stable but decreases rapidly during the first hour, and ceases after 90 min at a low baseline level [13,22,34]. This effect relates to a decrease of electron-rich substrates during hypothermic oxygenation [22]. HOPE may, therefore, provides a reversible downregulation of mitochondrial chain carriers, which leads to slow 'switching on' of mitochondrial electron transfer during normothermic reperfusion [15,22].

MECHANISM

During ischemia, aerobic respiration is disabled by the lack of oxygen and nutrients.

As a result, cellular energy reserves are depleted, cytosolic ion concentrations are changed, and cellular membranes become instable [44,45]. Liver endothelial cells are particularly vulnerable to ischemia/reperfusion injury and develop serious alterations during cold storage, such as retraction, cell body detachment, and apoptosis, whereas hepatocytes appear to be mostly unaffected [46,47]. During warm reperfusion, early sinusoidal endothelial cell (SEC) necrosis is followed by delayed hepatocyte apoptosis [47]. However, severe changes in SECs, resulting in disappearance of the SEC lining do not decrease the overall viability of rat livers after transplantation, since they are similar under both nonlethal and lethal conditions [47]. In contrast, the main pathological event was shown to be the profound alteration of hepatic microcirculation with loss of the extracellular matrix and finally interruption of sinusoidal flow leading to hepatocyte necrosis (Fig. 2) [47]. Hypothermic liver perfusion potentially addresses at least three different protective mechanisms in parenchymal and nonparenchymal cells against lethal impairment of hepatic flow (Fig. 3).

Endothelial cell effects

Flow cessation per se results in a significant reduction of several endothelial vasoprotective pathways leading to cell activation and apoptosis. The negative effects of cold storage conditions are partly because of the loss of expression of the vasoprotective transcription factor Kruppel-like factor 2 (KLF2) [48]. Machine perfusion may trigger endothelial protection because of upregulation of shear

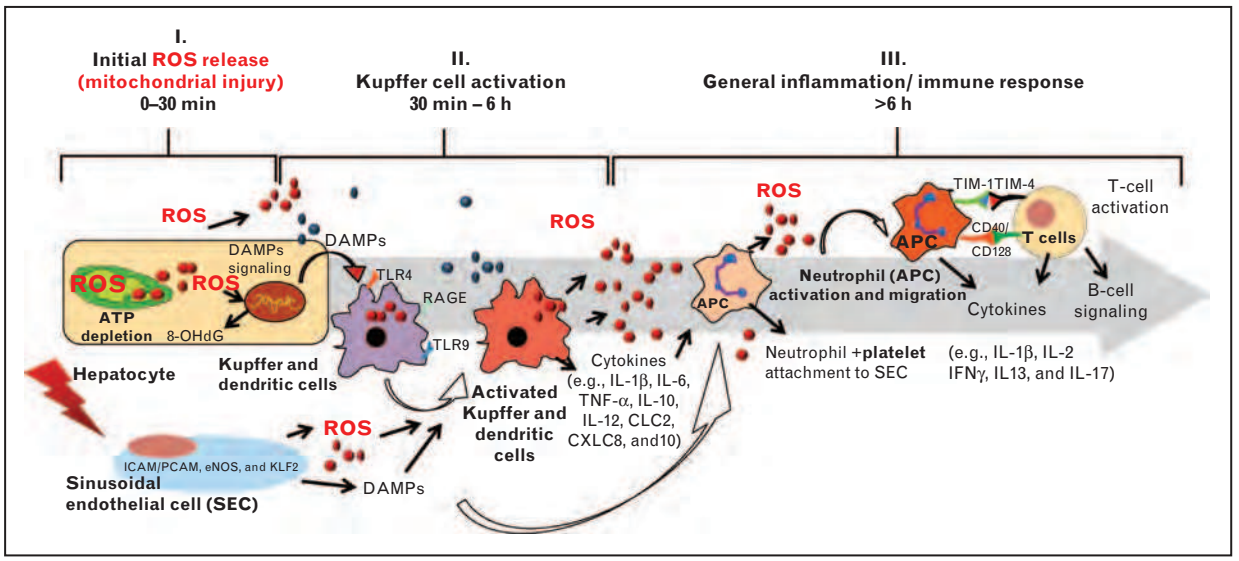

FIGURE 2. Time frame and cascade of reperfusion injury as well as link to immune response. eNOS: endothelial nitric oxide synthase.

stress-sensitive protective genes (Fig. 3) [46]. Of note, however, a recent study failed to prove alteration of KLF2, in neither cold storage or machine perfusion compared with controls in porcine DCD kidneys, but showed increased activation of eNOS [49]. Based on this, the impact of biomechanical sinusoidal stimulation during machine perfusion appears currently unclear, and needs further clarification. Importantly, however, perfusion at high pressures in the cold (>4 mmHg) has been shown to exert severe injury in livers [22]. This fact points to the importance of endothelial effects during hypothermic liver perfusion.

Hepatocyte effects

In parallel to endothelial damage, current research indicates a key role of hepatocyte released DAMPs

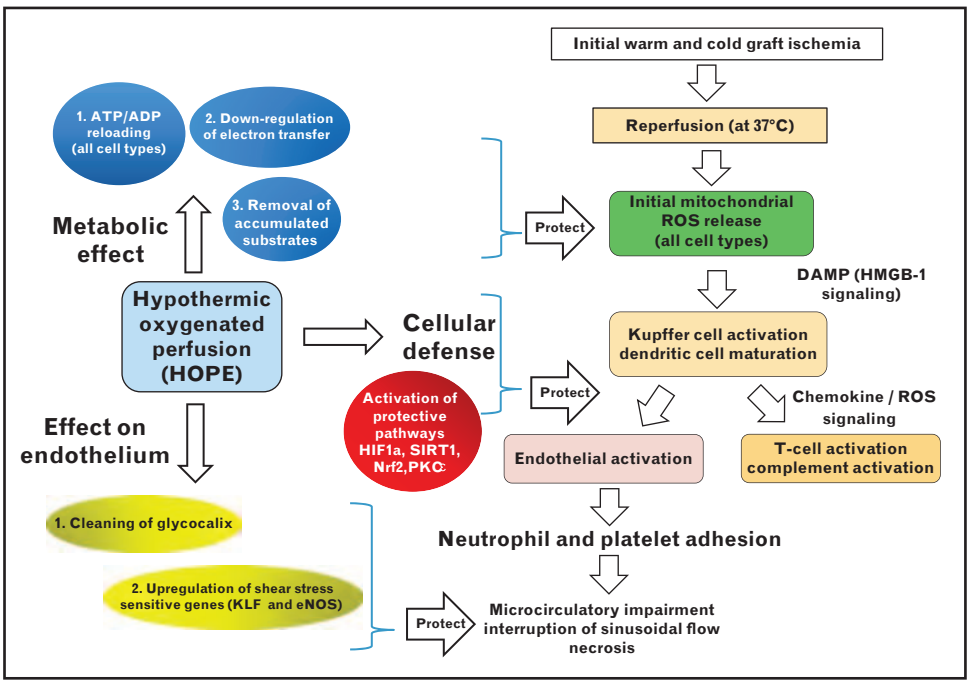

FIGURE 3. Potential protective mechanisms of hypothermic oxygenated perfusion. HIF: hypoxia-inducible factor; KC, Kupffer cell; Nrf2: Nuclear factor (erythroid-derived 2)-like 2; PKCε: protein kinase C epsilon; SIRT1: SIRT-1: gene of NAD-dependent deacetylase sirtuin-1.

[26,27,44,50] during early reperfusion, with a steep arise during the first 4–6 h [26,27,51]. From this point on, reperfusion injury shifts from pure metabolic distress to a potentially lethal innate immune response [27], involving several nonparenchymal cells (SEC, antigen-presenting cells [Kupffer cells and dendritic cells (KC and DC)], leukocytes (T cells and neutrophils) and chemokines, as well as additional ROS (Figs. 2 and 3). HOPE triggers a unique decrease of DAMPs during early reperfusion of DCD liver grafts, as shown in several transplant models (Fig. 3) [16,21,22,29▪,30].

Mitochondrial effects

Metabolic pathways are responsible for mitochondrial ROS production in a large range of tissues, including livers, kidneys, hearts, and brain [52–54]. Selective accumulation of the citric acid cycle intermediate succinate has been shown recently as a universal signature of ischemia, and is responsible for mitochondrial ROS production in all cell types during reperfusion [55▪▪]. Ischemic succinate accumulation arises at least partly from reversal of succinate dehydrogenase (complex II). Upon reperfusion, the accumulated succinate is rapidly reoxidized, driving extensive ROS generation by reverse electron transport at mitochondrial complex I [55▪▪,56]. Decreased succinate load before reperfusion is therefore sufficient to improve I/R injury [55▪▪]. These findings are particularly relevant for hepatic I/R injury as the extent of succinate accumulation was more pronounced in ischemic livers (20-fold increase) than in other organs (four-fold increase) [55▪▪]. Hypothermic oxygenation before reperfusion appears as a novel treatment to shift anaerobic metabolism to aerobic metabolism under cold conditions, together with huge ATP reload (Fig. 3). Whether citric acid metabolite accumulation is reversed effectively by cold oxygenated machine perfusion in human, remains to be investigated.

Cellular defense effects

Hyperoxidation of respiratory chain proteins is characteristic for mitochondria during reperfusion after ischemia and mitochondrial dysfunction is the primary site of ROS release leading to damage [26,51]. However, not all ROS production is detrimental. Low levels of ROS are protective and may serve as a trigger for activation of numerous pathways (PKCε, SIRT1, Nrf-2, and HIF-1) [57,58]. These pathways increase the activation of antioxidant enzymes (glutathione synthase, heme oxygenase, catalase, glutathione, and manganese superoxide dismutase), expression of angiogenic (erythropoetin), and survival proteins (MAPK) [57]. Hypothermic machine perfusion may also exert upregulation of defense pathways through minor ROS release during cold perfusion, especially in livers exposed to warm ischemia before perfusion (DCD grafts) (Fig. 3). Further studies need to address this issue.

FUTURE APPLICATIONS

Based on the available data in human application and on the proposed mechanism, the potential of hypothermic machine liver perfusion appears high. As it addresses ischemia reperfusion at its roots, in contrast to traditional antioxidative therapies, a number of novel strategies may be developed. These include application of machine liver perfusion in steatotic livers to decrease oxidative stress. Secondly, downstream effects of hypothermic machine perfusion on immune response may be of utmost importance in adapting immunosuppressive therapy for transplanted tumor patients.

CONCLUSION

Safe use of liver grafts with significant hepatic steatosis, or of livers exposed to long warm ischemia, has been a major challenge in the era of high model of end-stage liver disease recipients. Machine liver perfusion offers a new strategy to optimize ex-vivo organs before transplantation. Randomized trials remain needed to identify the best liver perfusion strategy, but endischemic HOPE is very effective. It is currently the simplest machine perfusion approach, with therefore high practicability and low-associated costs. The full potential of this approach should be discovered in the upcoming years.

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Conflicts of interest

There are no conflicts of interest.

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