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REVIEW

Role of hypothermic machine perfusion in liver transplantation

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Summary

Machine liver perfusion has significantly evolved during the last ten years to optimize extended criteria liver grafts and to address the worldwide organ shortage. This review gives an overview on available *ex vivo* and *in vivo* data on hypothermic machine liver perfusion. We discuss also possible protective pathways and show most recent clinical applications of hypothermic machine liver perfusion in human.

Introduction

More than 5500 liver transplants (LT) per year are performed in Europe and in the US [1,2] with 5-year patient survivals over 70% in most centres. The success of LT has, however, resulted in a substantial worldwide organ shortage. As a response, to reduce the gap between the need and availability of donors, many liver grafts, previously considered unsuitable for transplantation, so-called extended criteria donor (ECD) grafts, are now used by several teams. Key risk factors, associated with higher liver graft dysfunction and postoperative complications, have been defined as follows: Donor age above 70 years, duration of cold storage of more than 12 h, macrosteatosis of more than 30%; mixed steatosis of more than 60%, and additional donor warm ischaemia of more than 30 min due to reanimation or donation after cardiac death (DCD) [3–7].

Optimizing techniques for those liver grafts would help to increase the donor pool and machine liver perfusion before transplantation has therefore gained interest during the last 10 years. This review focuses on hypothermic machine liver perfusion. We provide a short historical overview and a summary on current relevant research in the field. We will also show underlying mechanistical views and the most recent clinical applications.

History of organ machine perfusion

Ex vivo organ perfusion for improving organ quality has been a fascinating goal for researchers and returns back to the 19th century. The first organ perfusion machine was designed by Alexis Carrel and Charles Lindbergh already in 1935 who successfully perfused small organs normothermically in a glass device with a pressure-controlled, recirculated, filtrated and oxygenated

perfusate [8]. However, despite the technical advances since that time, no system has been able up to now to maintain function of human livers for more than several days outside of the human body. In addition, machine perfusion of livers attracted less attention between 1970 and 2000, mostly due to its high technical requirements, in contrast to the simple cold storage principle introduced by Geoffrey Collins in 1969 [9]. Accordingly, for standard liver grafts, cold storage with modern preservation solutions, such as University of Wisconsin (UW), Histidine-Tryptophane-Ketoglutarate (HTK) and Celsior, has remained highly successful up to now [10–12]. With the increasing need for organs over the past years, an increasing interest in the use of novel preservation techniques has currently emerged due to the search for novel preservation approaches for the safer use of ECD grafts.

The main debate on machine liver perfusion relates to three different approaches, differing in perfusate temperature and the degree of oxygenation: normothermic, subnormothermic and hypothermic liver perfusion. Normothermic machine liver perfusion simulates *in vivo* conditions and therefore needs dual perfusion through the portal vein and the hepatic artery at physiological flow and temperature with oxygenated diluted blood or red blood cells and nutritional compounds as perfusate. In contrast, both subnormothermic and hypothermic machine liver perfusion rely on the physical solved oxygen in a blood cell free perfusate at temperatures of 20–25 °C (subnormothermic) or 2–10 °C (hyopothermic). Besides the perfusate temperature and oxygenation, the optimal duration of machine liver perfusion has been repeatedly discussed with either votes for continuous perfusion until implantation, or only short perfusion intervals either before (pre-ischaemic perfusion) or after organ transport (end-ischaemic perfusion).

Continuous hypothermic liver perfusion (*ex vivo* studies)

Numerous *ex vivo* experiments in rats and pigs have been published during the last 15 years on continuous hypothermic machine liver perfusion, and predominantly demonstrated improved hepatocyte and endothelial cell viability compared with simple cold storage (Table 1). Perfusion conditions in the cold, however, vary highly in terms of oxygenation, perfusion route, and perfusate composition (Table 1) and hypothermic machine perfusion never exceeded preservation times of cold storage. Of note, most studies analysed healthy livers and used enzyme release as marker of injury. In addition, reperfusion experiments were either omitted [13–25] or reperfusion periods remained short (30 min–2 h) [26–48], and conditions of reperfusion appear un-physiologic in most reports (perfusate without any blood cells [27–48], or with isolated red blood cells [26,49]).

With these significant limitations, we summarize current knowledge regarding perfusion conditions during continuous hypothermic machine liver perfusion and refer also to recent reviews [50–52].

Perfusion route

Advocates of perfusion through the hepatic artery repeatedly emphasize better supply of oxygen to the peribiliary vascular plexus [53–55]. Most of the interlobular biliary branches are, however, also reached by portal branches and it remains unclear, how much oxygen is needed in the cold.

Different perfusion routes have been tested for continuous hypothermic machine perfusion in overall 43 experimental studies during the past 15 years (Tables 1 and 2). Single portal perfusion was usually preferred in rat livers (Table 1) [14,21–25,27–45,47,48,56], while seven studies explored liver integrity using dual perfusion approaches via hepatic artery and portal vein in pig livers (Table 1) [13,15–17,19,26,49]. Outcome parameters were most based on hepatocyte viability during isolated perfusion models to test reperfusion injury. Retrograde perfusion via the hepatic veins of rat livers appeared similar effective as anterograde portal vein perfusion, while perfusion via hepatic artery alone was less beneficial [46]. Dual perfusion through the hepatic artery and the portal vein failed to show clear advantages in several *ex vivo* models [13,16,18,26]. In discarded human livers, Jomaa *et al.* showed recently feasibility of short-term machine liver perfusion at 4–8 °C using dual versus portal vein alone versus hepatic artery alone [57]. No difference appeared histologically in these liver grafts after different routes of perfusion [57].

In summary, no conclusive studies are yet available with clear demonstrating the best hypothermic perfusion route. Both, portal vein alone and dual perfusion appear well tolerated.

Perfusion pressure and flow

A critical target of injury by cold preservation, with or without perfusion system, is the sinusoidal endothelial cell [58]. Importantly, 't Hart *et al.* found in rats that increased perfusion pressures during hypothermia resulted in a more complete perfusion, with however also increasing endothelial damage [18,21]. Consistently, high flow rates in pigs were shown to induce detrimental effects due to sinusoidal endothelial injury through overexpression of von Willebrand factor and tumour necrosis factor (TNF) with subsequent activation of Kupffer- and

Table 1. Continuous hypothermic oxygenated perfusion (*ex vivo*).

Author	Year	Species	Asystolic donor warm ischaemia (min)	Perfusion Duration (h)	Perfusion conditions	Reperfusion	Protective (y/n)
Liu *et al*. [26]	2013	Pig	15–120	4	PV flow : 0.5 ml/min/g + HA	2 h, RBC (Hct 60%) + AQIX RS-I	No control group
Dirkes *et al*. [13]	2013	Pig	–	24	HA+ PV	No	No difference
Giannone *et al*. [14]	2012	Rat	–	24	PV only, flow: 1 ml/g/min	No	Y
Luer *et al*. [27]	2010	Rat	–	18	PV only, flow: 0.5 ml/g/min	2 h, Williams E, Albumin	Y
Stegemann *et al*. [28]	2010	Rat	30	18	PV only, flow: 0.5 ml/g/min	2 h, KHB, Albumin	Y
Liu *et al*. [15]	2009	Pig	120	4	PV (flow pv: 300 ml/min) + HA	No	Y
Stegemann *et al*. [29]	2009	Rat	30	18	PV only (flow: 0.5 ml/g/min)	2 h KHB	Y
Jain *et al*. 30]	2008	Rat	60	5	PV only, flow: 0.4 ml/g/min	1 h, KHB	Y
Manekeller *et al*. [31]	2008	Rat	–	18	PV only	2 h, KHB	Y
*Monbaliu *et al*. [16]	2007	Pig	–	24	PV and HA (flow: 600 ml/min)	No	No control group
Vekemans *et al*. [17]	2007	Pig	–	24	PV low and high flow: 0.5–1 ml/g/min, HA	No	Y
Bessems *et al*. [32]	2007	Rat	–	24	PV flow: 3.4–3.8 ml/min/g	1 h, KHB	Y
't Hart *et al*. 18]	2007	Rat	–	24	PV flow: 350 ml/min; HA flow: 80 ml/min	No	Y
van der Plaats *et al*. [19]	2006	Pig	–	24	PV flow: 160–448 ml/min; HA flow: 78–91 ml/min	No	Y
Minor *et al*. [33]	2006	Rat	30	2,18	PV only (flow: 0.5 ml/g/min)	45 min, KHB	Y
Manekeller *et al*. [34]	2007	Rat	30	2	PV only (flow: 0.5 ml/g/min)	45 min, KHB	Y
Bessems *et al*. [35]	2005	Rat	30	24	PV only	1 h, KHB	Y
Bessems *et al*. [36]	2005	Rat	–	24	PV only	1 h, KHB	Y
Jain *et al*. [49]	2005	Pig	–	24	PV flow: 0.3 ml/min/g; HA flow: 0.1 ml/min/g	4 h, RBC in KHB	Y
Xu *et al*. [37]	2005	Rat	–	24	PV flow: 0.4 ml/min/g	30 min, KHB	Y
't Hart *et al*. [21]	2005	Rat	–	24	PV only	No	Y
Bessems *et al*. [39]	2005	Rat	–	24	PV flow: 1 ml/g/min	1 h, KHB	No control group
Bessems *et al*. [38]	2005	Rat	30	24	PV only	1 h, KHB	Y
Xu *et al*. [40]	2004	Rat	–	24	PV flow: 4–15 ml/min	30 min, KHB	Y
Jain *et al*. [22]	2004	Rat	–	1, 24	PV flow: 4 ml/min	No	No control group
Lauschke *et al*. [41]	2003	Rat	60	24	PV flow: 0.5 ml/g/min	45 min, KHB	Y
Dutkowski *et al*. [42]	2003	Rat	–	10	PV only	45 min, Ringer	Y
Olschewski *et al*. [43]	2003	Rat	60	24	PV only	45 min, KHB	Y
Lee *et al*. [44]	2002	Rat	60	10	PV flow: 5 ml/min	1 h, KHB	Y
Minor *et al*. [45]	2002	Rat	60	24	PV flow: 0.5 ml/g/min	45 min, KHB	Y
So *et al*. [23]	2001	Rat	–	24	PV only	No	Y
Compagnon *et al*. [46]	2001	Rat	–	24, 48	PV flow: 0.4 ml/min/g , HA flow: 0.1 ml/min/g	2 h, KHB	Y
Southard *et al*. [47]	2000	Rat	–	24, 48	PV flow: 0.14 ml/g/min	1.5 h, KHB	Y
Dutkowski *et al*. [48]	1999	Rat	–	10	PV only	40 min, Ringer	Y
Dutkowski *et al*. [25]	1998	Rat	–	10	PV only	No	Y
Dutkowski *et al*. [24]	1998	Rat	–	10, 24	PV only	No	y

*No active oxygenation.

endothelial cells [59]. Most studies therefore preferred a low portal vein pressure of 3–5 mmHg and low arterial pressure of 20–30 mmHg in case of dual perfusion (Table 1). The resulting total flow rates appear inconsistent for unknown reason with a wide range of 0.14 and 3.8 ml/min/g liver (Table 1).

In summary, pressure-controlled perfusion in hypothermic machine liver perfusion is a key element to minimize

Table 2. Continuous hypothermic oxygenated perfusion (Transplant studies).

Author	Year	Species	Donor warm ischaemia (min)	Perfusion duration (h)	Survival	Protective (y/n)
Fondevila *et al*. [59]	2012	Pig	90 min asystolic	4	5 days	y
*Monbaliu *et al*. [73]	2011	Pig	–	4	3 days	no
Vekemans *et al*. [74]	2009	Pig	–	4	3 days	No control group
*Guarrera *et al*. [75]	2005	Pig	–	12	5 days	y
Lee *et al*. [70]	2003	Rat	30 min asystolic	5	5 days	y
Uchiyama *et al*. [71]	2001	Pig	60 min syst. BP ≤ 60 mmHg	2	≥3 days	y
Iwamoto *et al*. [72]	2000	Pig	60 min syst. BP ≤ 60 mmHg	2	≥2 days	y

h, hours.
*No active oxygenation.

the risk of shear stress, mediator release and sinusoidal damage.

Temperature and duration of perfusion

The reported preservation temperature during continuous hypothermic machine perfusion ranges between 1 and 18 °C [60]. While metabolic activity is more depressed at lower temperature, perfusate viscosity increases in the cold [31]. Accordingly, during cold perfusion, there is a time dependent increase in vascular resistance which bears the risk of damage to the sinusoidal endothelium and glycocalix, particularly when cold perfusion extends beyond 18 h [22,33,40]. Pienaar *et al.* [61] have reported on 72 h continuous hypothermic perfusion of dog livers through the portal vein alone, these results have not been repeated so far up to now. However, most perfusion experiments are therefore limited to relatively short intervals of perfusion from 2–24 h (Table 1).

Perfusate

The majority of studies used a perfusate based on the UW gluconate machine perfusion solution invented by Folkert Belzer and James Southard [62]. The component hydroxyl ethyl starch (HES) in this solution has been a matter of debate with either suggestions to replace HES by polyethylene glycol [36,39] or even to omit any colloid to reduce viscosity [24]. Perfusates consisting of low potassium concentration were shown to be protective in terms of decreased vascular resistance of livers during cold perfusion [22]. Several additives have been suggested to improve perfusion quality, that is, vasodilators, reactive oxygen scavengers and amino acids [82].

Oxygenation and mitochondrial recovery

Hypothermic machine perfusion relies on physical solved oxygen in a blood free perfusate at temperatures of 2–18 °C [60]. Reported perfusate oxygenation in the cold ranged between 10 and 106 kPA (Table 1) [32] and enables the graft to restore sufficiently tissue energy charge [63–65] due to decreased metabolic needs and simultaneous superabundance of oxygen. Therefore, also brief periods of hypothermic perfusion after long-term storage of rat livers significantly improved recovery of cellular energy charge [64–67]. Glycogen depletion occurred not to a higher degree during machine perfusion as compare with cold storage [25]. In spite of hyperbaric oxygen levels, several studies have confirmed no increase of free oxygen radicals during hypothermic oxygenated perfusion [20,25,27,42,65,68].

The number of studies comparing oxygenated against deoxygenated cold liver perfusion is very limited [27,65,69], as deoxygenated perfusates are only achievable by N_2 insufflation. These studies found that protective effects of hypothermic oxygenated perfusion are lost in the absence of oxygen [27,65].

In summary, oxygenation appears as key of success in hypothermic machine liver perfusion due to mitochondrial mechanisms. The optimum level of oxygen needed, however, is unclear. Grafts exposed to ischaemia before procurement (DCD) are likely to need increased oxygen during machine liver perfusion.

Continuous hypothermic liver perfusion (transplant studies)

Seven studies are available using transplant models in rats and pigs, but survival after liver transplantation is reported only for a limited observation period of 2–5 days (Table 2). Four of these studies analysed the effect of hypothermic machine perfusion in DCD models. All of these reports included heparin treatment of the donor [59,70–75]. A protective *in vivo* effect for hypothermic machine perfusion compared with cold storage was concluded in the majority of these studies based on superior survival as compared with untreated cold stored control livers [59,70–72,74,75], while one study showed no clear advantage after transplantation [73] (Table 2).

In summary, current research in *ex vivo* and *in vivo* (transplant) models suggests a protective effect of hypothermic machine liver perfusion compared with cold storage. However, due to the arbitrary and varying experimental conditions of most reports in terms of the oxygenation degree, perfusion route (dual versus portal), perfusion pressure, perfusate and reperfusion settings (Tables 1 and 2) it remains yet unclear, how to define optimal conditions for continuous hypothermic liver graft perfusion. Currently, there is no evidence suggesting that hypothermic machine liver perfusion can extend preservation time.

Pre-ischaemic hypothermic liver perfusion (experimental research)

Only few studies compare pre-ischaemic condition of livers by short-term perfusion before cold storage. For example, Minor *et al.* showed that 2-h cold oxygenated perfusion prior to 16-h cold storage improved hepatocyte viability and adenosine triphosphate (ATP) recovery during isolated rat liver perfusion improved as compared with cold storage [33]. Transplant studies are yet not available.

End-ischaemic hypothermic liver perfusion (experimental research)

In contrast to pre-ischaemic machine perfusion directly after procurement, end-ischaemic machine perfusion is started after initial cold ischaemic storage and organ transport. Such hypothermic approach is clinically attractive, because of no needs to transport machine devices. Four studies investigated *ex vivo* reperfusion after end-ischaemic hypothermic oxygenated perfusion (HOPE) [64,65,67,77]. All of these studies convincingly showed cellular energy charge reloading during oxygenated perfusion in spite of different periods of cold ischaemia (5–22 h) before machine perfusion. Consistent with the results during continuous hypothermic perfusion, no oxidative stress during end-ischaemic machine perfusion was documented [64,65]. Reperfusion conditions were more adapted to physiologic conditions in two studies (reperfusion with diluted blood) [65,77], while a-sanguineous conditions were chosen in the remaining two studies [64,67] (Table 3). Of note, protection from ischaemia/reperfusion injury in rat and pig DCD transplant models was shown to be dependent on sinusoidal pressure conditions, consistent with the findings during continuous hypothermic perfusion. Severe injury occurred in pig DCD livers, if portal perfusion pressure was as high as 8 mmHg [65]. In contrast, portal perfusion with a perfusion pressures of less than 3 mmHg, that is, at 25% of physiological values, did not provoke any endothelial shear stress [65], while it was sufficient to perfuse all sinusoids in a rat model [78].

Two transplant models have been published on the HOPE technique. In a pig DCD liver transplant model with extended asystolic warm ischaemia (60 min), end-ischaemic HOPE protected significantly from hepatocyte injury, but achieved no rescue from otherwise lethal injury [79]. Shorter asystolic warm ischaemic periods (30 min asystolic warm ischaemia) were currently investigated in a rat DCD liver transplant model [68]. An end-ischaemic application of HOPE through the portal vein for 1 h was shown in this study to result in a strong protection from reperfusion injury as assessed by decreased high mobility group box-protein1 (HMGB-1) release, decreased Kupffer cell and endothelial cell activation [68]. In addition, subsequent T-cell adhesion and biliary fibrosis were prevented by 1 h HOPE within a 4-week period after transplantation [68] (Table 4), similar as in controls (no ischaemic livers). Further investigations in large animal models are needed addressing proliferation capacity of biliary epithelial cells and large bile ducts.

Of note, the effectiveness of an end-ischaemic oxygenation approach after cold storage is further highlighted by numerous studies from Minor *et al.* [52] in terms of end-ischaemic oxygen persufflation of livers and kidneys under hypothermic conditions. Likewise, normothermic oxygenated short-term perfusion provides also protection of kidneys in spite of significant cold storage periods before end-ischaemic perfusion [99,100].

Hypothermic human machine liver perfusion (clinical research)

Two companies provide commercial devices for human liver perfusion, that is, Life Port Liver Transporter® (Organ

Table 3. End-ischaemic hypothermic oxygenated perfusion (*ex vivo*).

Author	Year	Species	Asystolic donor warm ischaemia (min)	Cold storage (h)	Perfusion duration (h)	Reperfusion	Protective (y/n)
Schlegel *et al*. [65]	2013	Pig	60	7	1	3 h, diluted blood, Hct 12–14	y
Stegemann *et al*. [67]	2009	Rat	–	22	1.5	2 h, KHB, Albumin	y
Manekeller *et al*. [56]	2007	Rat	30	6, 18	1	2 h, KHB	y
Dutkowski *et al*. [77]	2006	Rat	45	5	1	3 h, diluted blood, Hct 6.4	y
Dutkowski *et al*. [64]	2006	Rat	–	10	3	40 min, KHB	y

Hct, Haematocrit; KHB, Krebs Henseleit Buffer; h, hours.

Table 4. End-ischaemic hypothermic oxygenated perfusion (Transplant studies).

Author	Year	Species	Asystolic donor warm ischaemia (min)	Perfusion duration (h)	Cold storage (h)	Survival	Protective (y/n)
Schlegel *et al*. [68]	2013	Rat	30	1	4	4 weeks	y
de Rougemont *et al*. [79]	2009	Pig	60	1	7	6 h, 25 h	y

h, hours.

Recovery Systems, Des Plaines, IL, USA) and Liver Assist®, designed by Organ Assist (Organ Assist®, Groningen, The Netherlands). Four studies have been published on discarded human livers regarding hypothermic machine perfusion (Table 5):

1. Guarrera *et al.* perfused 10 discarded human livers for 7 h through the portal vein and the hepatic artery without active oxygenation and at relatively high flow rates of 0.7 ml/g liver /min. Human livers were evaluated on the perfusion device and confirmed reliability and safety of such preservation techniques [75].

2. Monbaliu *et al.* perfused 17 discarded human livers for 24 h through the portal vein and the hepatic artery. Eleven livers were regarded as nontransplantable, and six livers were estimated retrospectively to be transplantable. Nontransplantable livers released more aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) than transplantable livers during machine liver perfusion [80].

3. Vekemans *et al.* assessed reperfusion injury after hypothermic perfusion of 13 discarded human livers during warm reperfusion with diluted red blood cells for 2 h. They reported less AST and LDH release as compared with cold stored livers [81].

4. Jomaa *et al.* tested in 16 discarded human livers single portal perfusion for 1 h after cold storage against dual perfusion through the portal vein and the hepatic artery. No difference in sinusoidal endothelial ultrastructure was seen before and after machine perfusion [57].

Only two studies present currently clinical data after implantation of machine perfused human livers (Table 5). Both studies used an end-ischaemic perfusion approach in either donation after brain death (DBD) or DCD liver grafts. The first clinical trial has been published by Guarrera *et al.* 2010 [82] including 20 patients with 3–7 h perfusion after 8- to9-h cold storage. Perfusion was performed although the portal vein and the hepatic artery with relatively high flow rates of 0.667 ml/g liver weight /min. Of note, while the perfusate was not actively oxygenated by an in line oxygenator, measurements of pO_2 levels in perfusates demonstrate the sufficient presence of oxygen during the entire hypothermic machine perfusion period (pO_2 120–160 mmHg) [82].

Perfused livers were compared with cold stored standard grafts. The results showed less early graft dysfunction and lower enzyme release as well as shorter hospital stay. Additional analysis confirmed after machine liver perfusion and transplantation significantly attenuated pro-inflammatory cytokines, including interleukin (IL)-8, tumour necrosis factor (TNF)-a, and intercellular adhesion molecule -1 (ICAM-1) in post-transplant reperfusion biopsies [83]. Meanwhile, this group reported on 40 machine perfused human liver grafts from DBD donors [76] and showed also reduced activation of adhesion molecules as well as decreased migration of leucocytes, when compared with cold stored controls [84].

The most recent results are presented by our group [78] in eight perfused DCD liver grafts compared with matched DBD liver grafts. Consistent with our previous experimental experience, we preferred perfusion only through the portal vein, with low flow and a highly oxygenated perfusate. For this purpose, we chose for human machine liver perfusion the ECOPS device, which allowed connecting the liver graft in the back table setting while keeping the liver swimming in cold perfusate (www.hope-liver.com) (Fig. 2).

The median follow-up after machine perfused DCD liver transplantation cumulates currently to 8 months without evidence for intrahepatic cholangiopathy despite extended DCD criteria. Based on these first data, hypothermic oxygenated perfusion (HOPE) of human DCD liver grafts appears well tolerated and also effective against reperfusion injury, but randomized trials are urgently needed to confirm these results [78].

Mechanisms of protection and injury during hypothermic liver perfusion

Several key steps of reperfusion injury have been discovered [85]. Accordingly liver ischaemia reperfusion injury is probably initiated by parenchymal release of danger-associated proteins (DAMPs) with later involvement of blood cells such as leucocytes and platelets and nonparenchymal liver cells, including Kupffer cells, endothelial cells, and dendritic cells [85–87]_ENREF_21. It has therefore been suggested that novel therapeutic strategies should target on DAMPs representing the most proximal instigators of the inflammatory response to reperfusion [85,86]. As DAMPs production depends on the amount of intracellular reactive oxygen species (ROS), mitochondria appear on the front as inducer of reperfusion injury [85,88]. Hypothermic oxygenated perfusion offers a unique chance to decrease ROS

Table 5. Hypothermic oxygenated perfusion of human liver grafts.

Author	Year	Donor Characteristics	*n*	Cold storage (h)	Device	Perfusion (h)	Perfusion Control	Perfusion solution	Temperature (°C)	Perfusion pressure (mmHg)	Reperfusion	Protective (y/n)
Ex Vivo (with/without reperfusion)												
Jomaa *et al*. [57]	2013	DBD	16	10.2	Life Port Kidney Transporter®	1	Pressure	KPS-1	4–8	PV: 7 HA: 30	No	Y
*Monbaliu *et al*. [80]	2012	DBD	17	3–24	Life Port Kidney Transporter®	24	Pressure	UW	4–6	PV: 7 HA: 20–30	No	Y
Vekemans *et al*. [81]	2011	DBD	13	15–17	Life Port Kidney Transporter®	4	Pressure	KPS-1	5–8	PV: 3 HA: 20	2 h, Aqix-RS-I solution + RBC, Hb 6.8)	No
*Guarrera *et al*. [75]	2005	DBD	10	Not reported	Medtronic Portable Bypass System®	5–10	Flow	Vasosol	3–6	PV: 3–5 HA: 12–15	No	No control group
Transplantation												
Dutkowski *et al*. [78]	2014	DCD (18 min asystolic WI; 38 Min total WI)	12	2.4	ECOPS® (Organ assist)	2	Pressure	KPS-1	9–11	PV: 3	OLT	Y
*Guarrera *et al*. [82]	2010	DBD	20	8–9	Medtronic analog Life Port Transporter®	3–7	Flow	Vasosol	4–8	PV: 4 HA: 6	OLT	Y

WI, warm ischaemia; DBD, donation after brain death; DCD, donation after cardiac death; HA, hepatic artery; PV, portal vein; RBC, Red blood cells; Hb, haemoglobine.
*No active oxygenation.

release by mitochondria due to changes in the mitochondrial redox state [62]. In contrast, cold storage results in hypoxic mitochondria, which are known to excessively consume oxygen during the first 10 min of reperfusion, with the release of ROS due to electron leakage within the electron transport chain [89,90]. In turn, intracellular ROS lead to DNA hydroxylation in the nucleus (8-hydroxy-2-deoxy Guanosine, 8-OHdG) and consecutive nuclear HMGB-1 release. Kupffer cells activated through Toll-like-receptor -4 (TLR-4) are primary targets of HMGB-1, as suggested in previous reports [85–88,91–93]. Endothelial cell activation and T-cell activation occur simultaneously aggravating the magnitude of the injury [65,85,91,94] including two T-cell cytokines (Interleukine-13 and Interleukine-17) [68,86,91,95] secreted by innate immune cells, which trigger additional neutrophil sequestration [86,94,96]. Further downstream activation of myofibroblasts by several pathways stimulates the development of graft fibrosis and intrahepatic cholangiocyte proliferation [95,96] (Fig. 1).

Hypothermic oxygenated liver perfusion possibly interacts in this cascade as follows:

1. Mitochondrial respiration rate substantially decreases during the first hour of hypothermic oxygenated liver perfusion in rat, pig and human livers (Fig. 2b) [13,16,65]. This conclusion is based on the observation of decreased metabolism of nicotinamide adenine dinucleotide (NADH) and decreased production of CO_2 during the first hour of cold oxygenated machine perfusion despite sufficient levels of substrate (NADH, glucose, oxygen) [65]. Continuation of machine perfusion for more than 90 min causes almost complete cessation of electron transfer rates.
2. Due to reduced mitochondrial electron transfer rates during hypothermic oxygenated perfusion, any exposure to oxygen after machine liver perfusion during normothermic reperfusion leads to small amounts of mitochondrial electron leaks, and results also in minor release of reactive oxygen species and nuclear DAMPs. Further down-stream activation of Kupffer- and endothelial cells is therefore prevented (Fig. 1) [65,97].
3. In contrast, hypothermic machine perfusion in the absence of oxygen provokes the same amount of DAMPs signalling and down-stream pathways [65]. These results and others [20,27,98], support the view that oxygen supply under cold conditions is a key strategy to decrease early reperfusion injury, while complete absence of oxygen during cold machine perfusion appears disadvantageous [27,65].

It remains yet unclear, to what extend a cold storage period after hypothermic oxygenated perfusion can abrogate the protective effect and how long hypothermic oxygenated perfusion under proper pressure control can be performed safely.

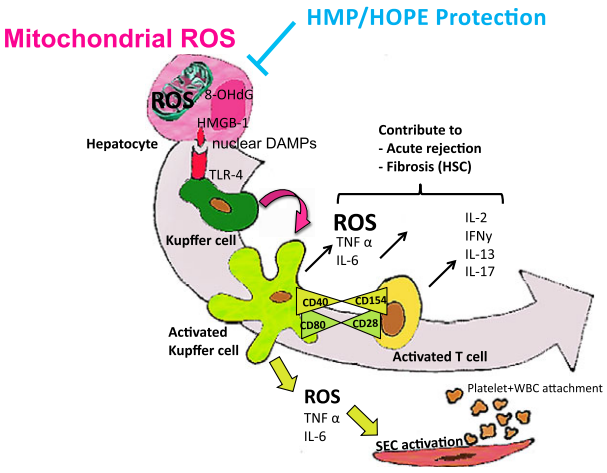

Figure 1 Proposed mechanism of protection during hypothermic oxygenated perfusion (HOPE) of livers. Untreated DCD livers release high amounts of mitochondrial reactive oxygen species (ROS) and trigger subsequent nuclear injury, detectable as release of danger-associated molecular proteins (DAMPs), which in turn activate Kupffer cells via TLR-4 receptors. Additional ROS released by Kupffer cells stimulate endothelial cells. The innate immune response is activated by T cells through cell surface interaction between T cells, Kupffer cells and endothelial cells. HOPE treatment in DCD livers protects by prevention of the initial DAMPs release by reduction of mitochondrial electron leakage.

Conclusions and future aspects

Machine liver perfusion offers the potential to improve preservation quality, particularly of marginal organs. The following key points summarize current knowledge on hypothermic machine liver perfusion:

1. Protection against initiation ischaemia reperfusion injury depends on oxygenation during hypothermic perfusion [14,27]. The optimal degree of oxygenation remains unclear. Dissolved oxygen may be sufficient at low perfusate temperatures for DBD livers.
2. Oxygenation of the perfusate during hypothermic oxygenated perfusion leads to metabolic resuscitation with resynthesis of ATP and cellular energy charge during cold perfusion [24,31,32].
3. Oxidative stress occurs at a very low degree during hypothermic oxygenated liver perfusion, even at hyperbaric conditions [27,43,65].
4. Mitochondrial respiration rates during hypothermic oxygenated perfusion decrease within the first hour of cold perfusion indicating a slow-down of mitochondrial electron transfer rates. Prolonging hypothermic oxygenated perfusion for more than 2 h has no further effect on mitochondrial electron transfer rates [13,16,65].
5. Endothelial injury by shear stress is the main risk of hypothermic machine perfusion. Hypothermic perfusion at low portal pressure conditions (<3 mmHg) can prevent this type of injury [65].

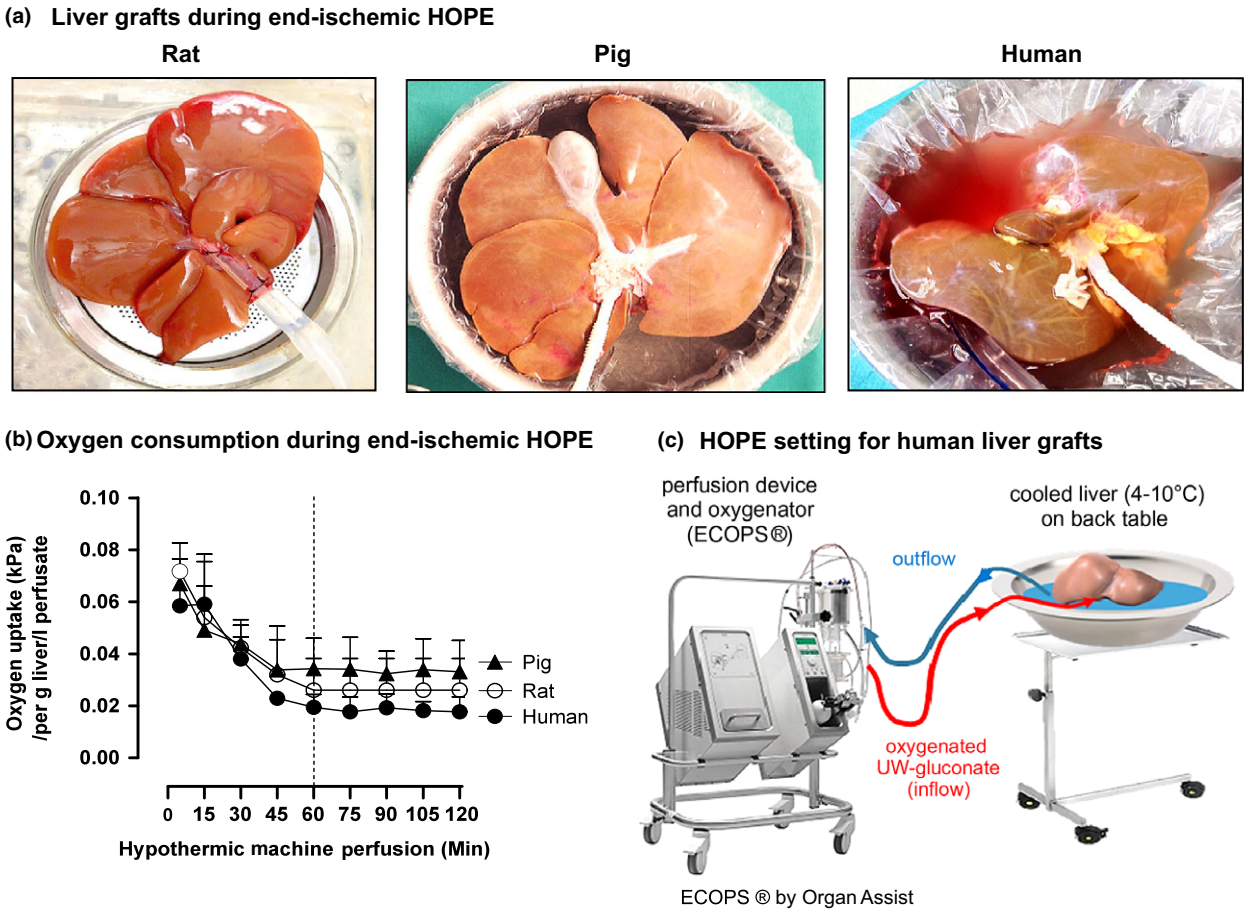

Figure 2 Hypothermic oxygenated perfusion (HOPE) in different species. (a) HOPE through the portal vein with low pressure perfusion (<3 mmHg) and oxygenated UW gluconate (KPS-1) was applied to rat, pig and human livers donated after cardiac arrest with different periods of asystolic warm ischaemia, that is, 30 min, 60 min and 18 min, respectively. (b) Oxygen consumption during the first hour of HOPE decreased in all livers to the same degree despite different warm ischaemic intervals before machine perfusion. This point to a unique slow-down in mitochondrial respiration independently from previous injury by warm ischaemia. (c) HOPE in human livers can be performed easily after back table by connection of the oxygenated pump device (ECOPS, Organ Assist®) to the portal vein.

6. Hypothermic oxygenated perfusion is well tolerated after cold storage periods [78].
7. Currently no evidence exists supporting that hypothermic oxygenated perfusion can extend the preservation time of livers.
8. Randomized trials are needed to further evaluate hypothermic machine perfusion in human. Prospective studies should target first comparison of end-ischaemic hypothermic oxygenated perfusion (HOPE) against cold storage in DBD livers (including ECD grafts). Furthermore, the HOPE approach should be compared with normothermic or subnormothermic perfusion techniques.

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