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Review

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Protective mechanisms and current clinical evidence of hypothermic oxygenated machine perfusion (HOPE) in preventing post-transplant cholangiopathy

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Keywords: hypothermic oxygenated perfusion; donation after circulatory death; non-anastomotic strictures; cholangiocytes; regeneration; peribiliary glands; mitochondria.

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Summary

The development of cholangiopathies after liver transplantation impacts on the quality and duration of graft and patient survival, contributing to higher costs as numerous interventions are required to treat strictures and infections at the biliary tree. Prolonged donor warm ischaemia time in combination with additional cold storage are key risk factors for the development of biliary strictures. Based on this, the clinical implementation of dynamic preservation strategies is a current hot topic in the field of donation after circulatory death (DCD) liver transplantation. Despite various retrospective studies reporting promising results, also regarding biliary complications, there are only a few randomised-controlled trials on machine perfusion. Recently, the group from Groningen has published the first randomised-controlled trial on hypothermic oxygenated perfusion (HOPE), demonstrating a significant reduction of symptomatic ischaemic cholangiopathies with the use of a short period of HOPE before DCD liver implantation. The most likely mechanism for this important effect, also shown in several experimental studies, is based on mitochondrial reprogramming under hypothermic aerobic conditions, *e.g.* exposure to oxygen in the cold, with a controlled and slow metabolism of ischaemically accumulated succinate and simultaneous ATP replenishment. This unique feature prevents mitochondrial oxidative injury and further downstream tissue inflammation. HOPE treatment therefore supports livers by protecting them from ischaemia-reperfusion injury (IRI), and thereby also prevents the development of post-transplant biliary injury. With reduced IRI-associated inflammation, recipients are also protected from activation of the innate immune system, with less acute rejections seen after HOPE.

Introduction

Complications along the biliary tree require numerous interventions, and non-anastomotic strictures (NAS) are associated with a high risk of graft loss after liver transplantation. Despite the increasing knowledge of potential risk factors and the establishment of clinical parameter thresholds, biliary complications and particularly NAS contribute to significant recipient morbidity and high costs. Among various targets for new preventive measures, modification of static cold storage (SCS) to reduce tissue hypoxia appears to be a frontrunner in attempts to reduce biliary complications. Dynamic preservation technologies are therefore a hot topic and their impact on biliary complications has been continuously explored in different experimental and clinical studies. The perfusion of donation after circulatory death (DCD) grafts under hypothermic conditions, with a highly oxygenated perfusate, was recently shown to protect recipients from the development of these most-feared biliary complications.[1]

In this review, we first describe the clinical phenomena of biliary complications in the setting of liver transplantation. Next, the risk factors and underlying pathophysiology of biliary complications, with a focus on biliary strictures, are discussed. The impact of the inevitably occurring ischaemia-reperfusion injury (IRI) is then highlighted, including the contribution of individual cell types and their crosstalk. To underline the effect of hypothermic machine liver perfusion, clinical studies reporting on biliary complications are described next, together with a discussion of the protective mechanisms involved. The different modalities used by transplant centres are described, before discussing the impact of cold perfusion on viability assessment, which is clearly linked with the aim of avoiding biliary complications and increasing the utilisation of marginal grafts. Finally, we review the future outlook, with a short excurse to potential future trials, and a discussion around the need for reliable and validated parameters to predict organ function and

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complications during hypothermic oxygenated perfusion (HOPE).

Risk factors and classification of biliary complications after liver transplantation

Biliary complications are potentially serious complications and therefore frequently referred to as the Achilles' heel of liver transplantation, being the target of various treatment strategies due to their potential severity and the associated risk of graft loss. Two main biliary entities are well known, biliary strictures and leaks. While biliary leaks occur at the site of the biliary reconstruction, biliary strictures are divided into 2 main types, anastomotic (AS) and non-anastomotic.[2] The majority of strictures at the anastomotic site can be successfully treated with endoscopic stenting. Supra-anastomotic strictures or NAS can be divided further based on the area where they are seen, for example sub-hilar or hilar (at the main duct branches) and peripheral (in the liver).[3,4] Buis *et al.* categorised the localisation of NAS into 4 different anatomical zones: hilar bifurcation and extrahepatic bile duct (zone A), ducts between first and second order branches (zone B), ducts between second and third order branches (zone C) and peripheral (zone D).[5] The vast majority of NAS occurred around the bifurcation or slightly below at the common bile duct (85%, zone A). Another criterion used to classify NAS is the time of occurrence after transplantation. In a large series of almost 500 liver transplantations, the authors described the first signs of NAS at a median of 4.1 months, with a range of 0.3-155 months.[5] Early strictures, detected within the first year are usually more central, occurring at the main biliary branches, while later strictures (>1 year after liver transplantation) occur in the liver periphery.[5] The clinical picture of NAS shows large variations and can be further divided into 3 levels of severity. The

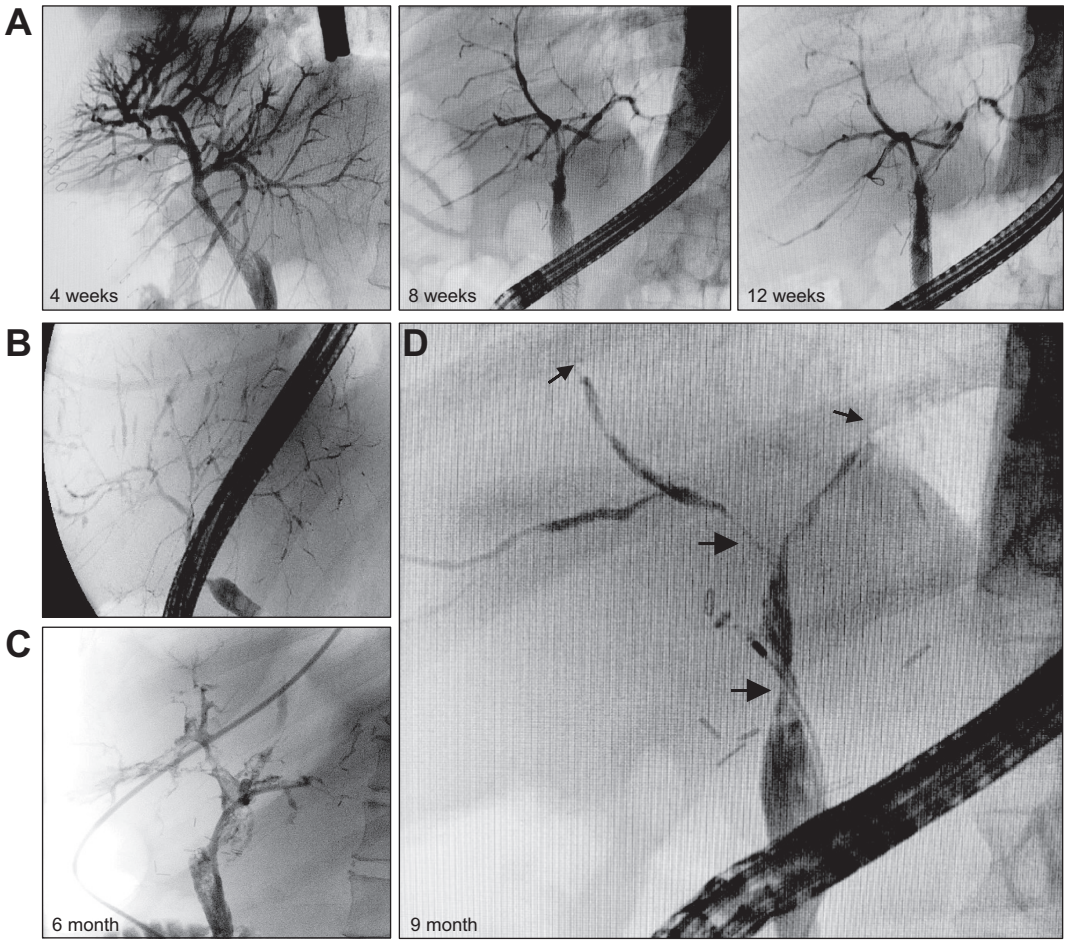

Fig. 1. Clinical example of patients with biliary complications after liver transplantation. (A) This picture series describes the biliary tree of a recipient of a standard donation after brain death liver with severe vanishing of the entire peripheral ducts within 3 months, requiring retransplantation. (B) Within 4 months the biliary tree of this donation after cardiac death liver recipient showed multiple strictures at all duct levels and ramification of small duct branches. (C-D) Vanishing of mid and small duct branches within 9 months after liver transplantation. All images were obtained during endoscopic retrograde cholangiopancreatography of the corresponding recipient.

majority appear to be mild, however 7-12% of patients present with moderate to severe NAS with rapid progression requiring multiple stenting and drains, rotating antibiotics, further medical support and retransplantation.[5,6] NAS may occur with large variations on radiological imaging, ranging from more peripheral abscesses, and individual or multiple strictures around the hilus and first biliary branches, to vanishing ducts seen along the entire biliary tree (Fig. 1). In 2021, Croome *et al.* described 4 different patterns of NAS in an American, multicentre cohort of 770 controlled DCD liver transplantations.[7] Two types were found with a more severe clinical picture, including multifocal progressive cases and patients with a pattern of diffuse necrosis. Both types required retransplantation after antibiotic cycles and repeat stenting in recipients with multifocal progressive forms. DCD liver recipients, who developed NAS predominantly around the hilar confluence were successfully managed with multiple stents, while patients with minor forms had limited need for stenting and did well without retransplantation.[7]

Key point

The risk of developing ischaemic cholangiopathy ranges from 2% to 34% after liver transplantation from donation after circulatory death donors. The hilar zone and level one duct branches are mainly affected.

Although the exact pathogenesis remains unclear today, early NAS (≤1 year) are more frequently related to prolonged warm and cold ischaemia, when compared to a later occurrence.[5] Later NAS are also associated with immunological risk factors and frequently detected in recipients transplanted for primary sclerosing cholangitis. Further contributing factors include Roux-en-Y reconstruction, cytomegalovirus infection, and immunological factors such as repeated episodes of rejection, a positive lymphocyte crossmatch or a poor HLA match.[8]

Because of their hypoxia-based aetiology, the terms ischaemic-type-biliary lesions or ischaemic cholangiopathy are frequently used to describe strictures at the biliary tree.[9,10] Importantly, however, such pathologies develop by definition with intact vascular supply.[1] In this review, the terminology NAS is used to summarise biliary strictures and pathologies that develop outside the anastomosis and despite intact vascular supply after liver transplantation.

The development of NAS in the context of DCD liver transplantation

Despite detailed knowledge about contributing risk factors, the ability of a specific liver to handle the accumulated injury in the donor, during procurement, preservation and implantation is difficult to estimate through a macroscopic evaluation alone. To avoid a difficult and costly post-transplant course with graft loss and recipient death, DCD livers are frequently discarded at the initial offer. The utilisation rate of DCD livers, calculated from the initial donor offer to transplantation ranges between 20-30% in the context of SCS in many countries.[11] Indeed 2.6-34% of controlled DCD liver recipients develop NAS.[6,12–20] Within the recently identified low risk benchmark DCD transplant cohort, 31.5% of patients who developed NAS lost their graft.[6] More than 10% of all DCD livers were lost within the first year after transplantation when the initial injury was advanced, with prolonged total and asystolic donor warm ischaemia time (DWIT).[21] In contrast, the development of NAS led to graft loss in only 2.2% of recipients when the DWIT was kept short.[6,21] Such recent findings are paralleled by the previous literature, where authors demonstrated the significant impact of prolonged asystolic and functional DWIT on the rate of NAS and overall complications after DCD transplantation.[20,22,23]

The type, level and duration of donor comfort therapy and the location of treatment withdrawal further impact on the duration of DWIT and subsequently on graft quality. In addition to the time required for the donor to proceed to circulatory death, the legally mandatory stand-off period, which ranges from 5 min in most countries, including the UK and Spain, to 20 min in Italy and 30 min in Russia, is a major contributor to a prolonged DWIT.[24]

Next, an elevated donor BMI and macrosteatosis are further important risk factors for the development of biliary and other complications.[25,26] Besides the metabolic status (prone to higher reperfusion injury), the time needed to perform the hepatectomy is frequently prolonged in donors with high BMI, which leads to inhomogeneous and partially delayed liver cooling, increasing warm ischaemia time and associated risks.[27] The extensive flush of the graft and the biliary tree are 2 measures performed by the procurement and transplant surgeon to reduce the risk of NAS.[21]

Of note, despite the consensus over the role of liver ischaemia, centres in Italy and Switzerland have consistently used DCD grafts, frequently declined by centres in other countries.[28] While DCD donor livers are routinely procured with the use of normothermic regional perfusion (NRP), followed by cold storage and HOPE in Italy, most DCD livers undergo super rapid retrieval and cold storage with end-ischaemic HOPE treatment in Switzerland. With the commissioning of such perfusion approaches, both countries have achieved excellent outcomes, despite high donor risk and prolonged DWIT of all types.[6,28,29]

Mechanism of injury to the biliary tree in the setting of liver transplantation

Liver cells of all types and at all levels of the biliary tree contribute to the development of cholangiopathy after liver transplantation. The identified strictures have well-known histological features, which include a loss of biliary epithelial cell integrity and their connection to the basal membrane, with concentric fibrosis and subsequent

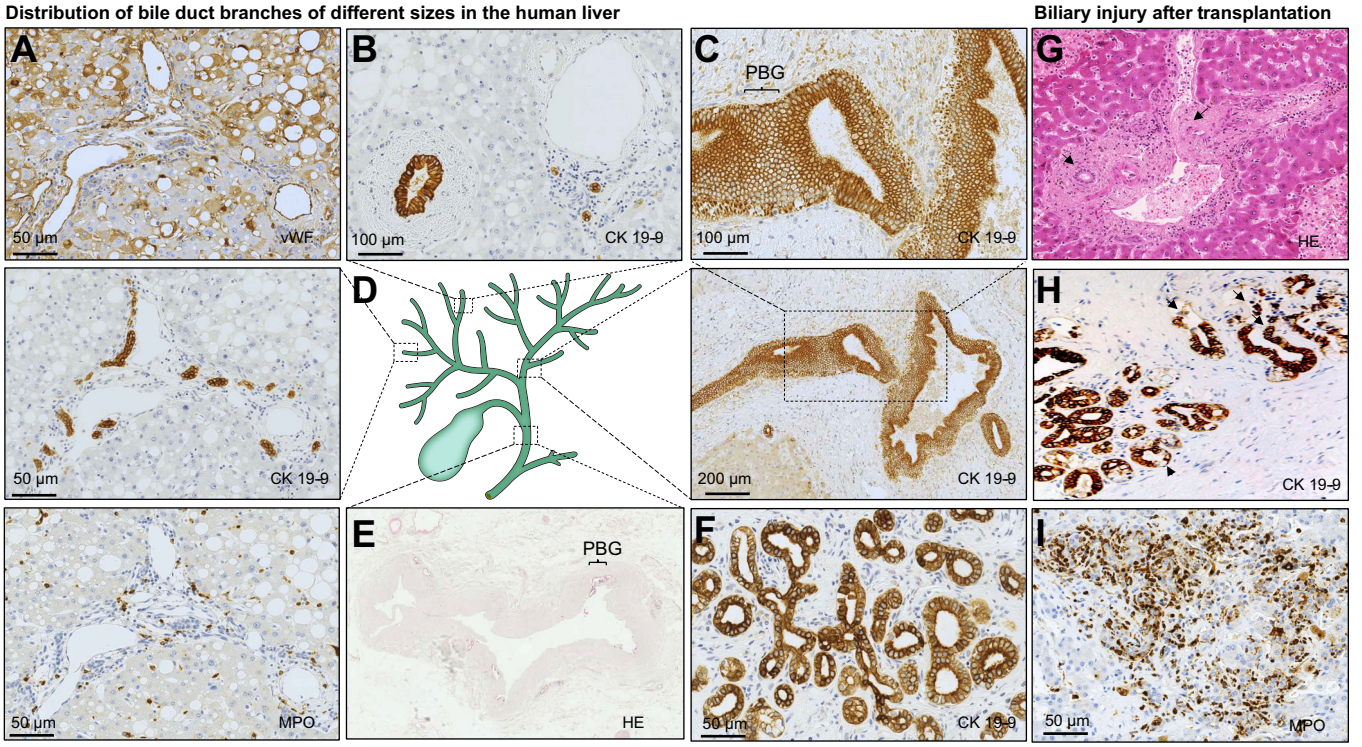

Fig. 2. Histology of healthy and injured bile ducts. (A) The 3 images under A highlight the distribution of bile ducts and vessels in the portal triad with different stainings (vessels: vWF, bile ducts: CK19-9, resident macrophages: MPO). (B-F) bile duct branches of different levels (B: level 2 branches, C: main branches level 1; D: overview biliary tree and the different levels of bile duct branches; E: common hepatic duct with peribiliary glands; F: small duct clusters); (G-I) Demonstrate the injured bile ducts and portal triads following reperfusion injury with chronic inflammation, fibre deposit and the development of biliary strictures (G: concentric fibrosis around the portal triad and explicitly surrounding portal bile duct branches, I: portal trial structure appears invisible due to significant fibrosis, MPO-positive cells proliferate and further enhance inflammation and fibrosis).

narrowing of the bile ducts at all levels of the biliary tree (from the portal triad to the hilum) (Fig. 2). The deposit of a high number of fibres is the direct consequence of ongoing chronic inflammation in the liver tissue, which is the final result of overwhelming IRI after implantation. The cascade of IRI mainly affects the larger cholangiocytes, with NAS development at larger extra- and intrahepatic bile duct branches.[30,31] On a cellular level, mitochondria are the first "domino stone" to instigate the entire cascade of inflammation.[32,33] During ischaemia, the lack of oxygen puts electron transfer through the respiratory chain complexes on hold, leading to a steady and rapid loss of ATP. While the metabolic reactions of the tricarboxylic acid cycle become blocked in a staged procedure, succinate accumulates together with other precursors of the tricarboxylic acid cycle.[33–36] Immediately following the reintroduction of oxygen into ischaemic cells, mitochondria aim to metabolise succinate and to restore the electron flow to rebuild ATP stores. This results in an acute electron overflow at complex I and subsequently to reverse electron transfer to complex I in the first seconds of oxygenated reperfusion, leading to a rapid release of reactive oxygen species (ROS) at complex I.[25,37–39] Some cells will die quickly throughout this initial phase of IRI and release further molecules, which trigger an inflammatory response in healthier surrounding cells. Various proinflammatory molecules, including danger-associated molecular patterns (DAMPs), are directly released from mitochondria through damaged cell membranes.[40,41] At transplantation, cells in the inflamed graft communicate with recipient cells entering the organ through the blood, which leads to additional cytokine release, causing ongoing and pronounced inflammation, graft dysfunction and acute complications in the recipient.[42] The amount of downstream injury and subsequent inflammation depends strictly on the level of initial damage (*e.g.*, duration of ischaemia) and the capability of the tissue to resolve it.

At the biliary tree, the cascade of IRI has a few known consequences. Among all other processes, bile production is energy dependent.[43,44] Based on the lack of ATP during initial reperfusion, hepatocytes secrete less bile acids (BAs), previously absorbed from the sinusoidal blood or metabolised from cholesterol pathways. The constant supply of BAs via the enterohepatic circulation is essential to stimulate bile flow and to avoid cholestasis, which further injures hepatocytes through the accumulation of hydrophobic bile salts.[43] The additional

Key point

The duration of hypoxia and subsequent reperfusion injury impacts on the occurrence and severity of non-anastomotic strictures.

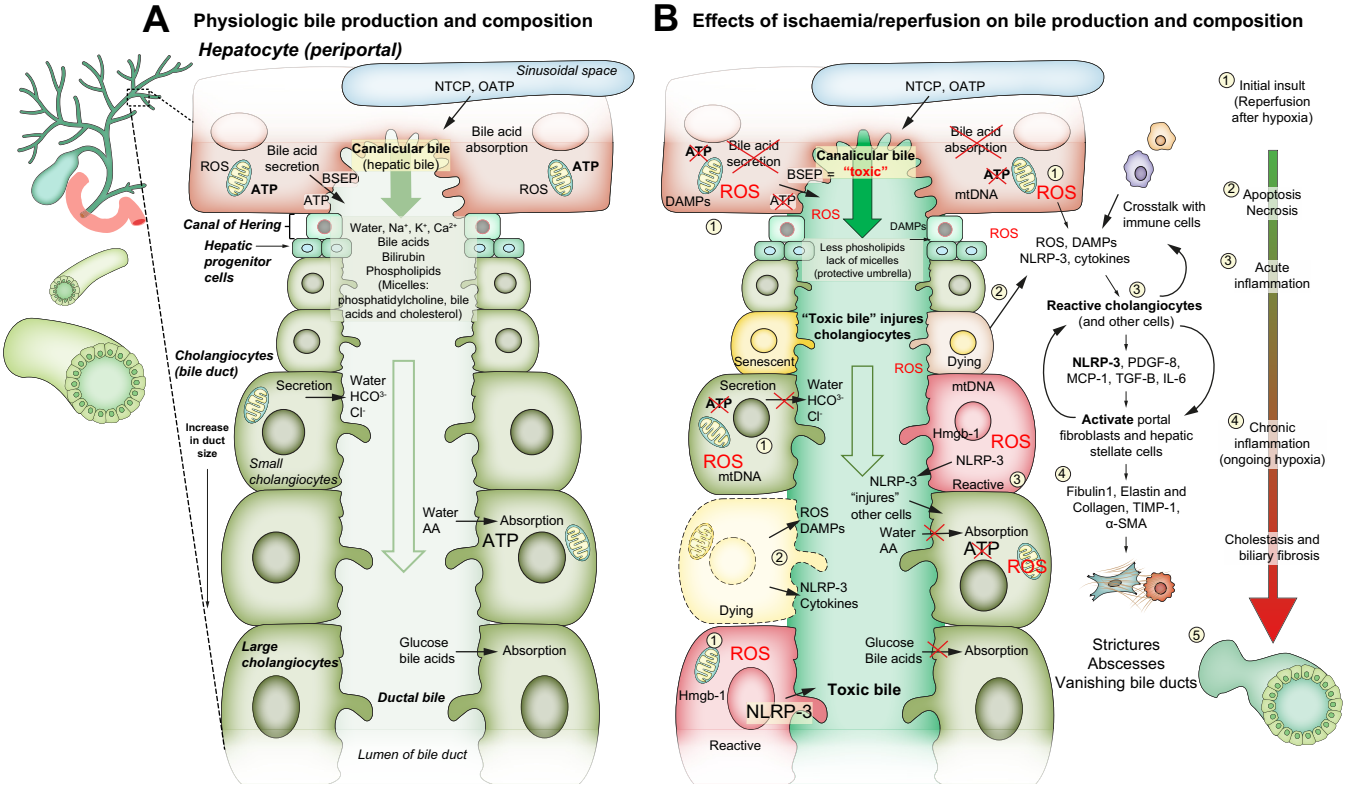

Fig. 3. Bile flow and composition in a normal liver and impairment due to ischaemia-reperfusion injury. (A) Physiologic bile production throughout the path from the initial secretion of BAs in hepatocytes to the main biliary branches and the common bile duct. BAs are the main fraction of the metabolism of cholesterol in the liver. As part of the enterohepatic circulation, BAs become absorbed at the sinusoidal side of the hepatocytes, the BAs are metabolised and actively secreted into intercellular space between hepatocytes. BA secretion though the BSEP-receptor is ATP-dependent. Hepatic bile contains water, bilirubin, bile acids, electrolytes (mainly sodium, potassium, calcium) and phospholipids, forming micelles (phosphatidylcholine, BAs & cholesterol) to protect the further downstream situated cholangiocytes. With their cilia they create a large surface and the optimal environment to secret HCO_3^- and Cl^-. Further downstream, mid-sized and large cholangiocytes re-absorb BAs, glucose, amino acids and water, thereby forming the ductal bile, which passes through the bile duct branches into the common bile duct and the duodenum. The concentrative transport of molecules in the bile creates an osmotic gradient, which leads to passive diffusion of water into bile. (B) This perfectly balanced system is however severely impaired during ischaemia and particularly reperfusion of livers. Ischaemia (or hypoxia) sets the respiratory chain and the electron flow on hold, resulting in a high level of accumulated cellular succinate and ATP depletion in all contributing cells. Such a lack of energy inhibits the entire cellular metabolism and all transport processes. However, the injury and malfunction only become visible at reperfusion. Due to the lack of ATP, BA transporters in the hepatocyte membrane are unable to secrete BAs and the hepatic bile becomes toxic to cholangiocytes. The immediate ROS release (1.) from injured (and activated or dying cells) triggers the release of other proinflammatory molecules including DAMPs, NLRP-3 and cytokines further downstream (2.). The severity of such pathophysiologic processes depends strictly on the level of initial donor injury and the duration of additional donor warm and cold injury. Of note, such an inflammatory cascade is evident in all liver cells, including hepatocytes, cholangiocytes, Kupffer cells and endothelial cells (1. +2. + 3). Reactive cholangiocytes further enhance the acute inflammation with the additional release of proinflammatory molecules and compounds, including the NLRP-3 inflammasome (3.). Periportal fibroblasts and stellate cells are subsequently activated and secrete various pro-fibrinogenic molecules (4.). The development of NAS is further characterised by chronic signs of ongoing hypoxia and inflammation (4.) and the loss of microvessels of the biliary tree. Such features lead to a reduced proliferation of progenitor cells in the peribiliary glands. This lack of stem-cell differentiation into mature cholangiocytes is key in the chronic damage and the inability to resolve injury (5.). Interrupting this cascade is the main target for novel preservation technology and machine perfusion. AA, amino acids; BAs, bile acids; DAMPs, damage-associated molecular patterns; mtDNA, mitochondrial DNA; NAS, non-anastomotic strictures; ROS, reactive oxygen species.

inability of hepatocytes to metabolise cholesterol and secrete phospholipids, and hence a lack of micelles (biliary umbrella), creates bile that is toxic to cholangiocytes, owing to the relatively high levels of BAs in the early bile (Fig. 3).[43,45,46] If the secretion of phospholipids recovers slower than BA metabolism, recipients are at higher risk of developing NAS.[43]

In addition to their own energy loss and ROS release from neighbouring cells, cholangiocytes experience a second hit through the modified initial bile they receive from hepatocytes. Additionally, their own function appears disturbed and the osmotic gradient usually seen in the bile throughout the biliary tree cannot be established. The initial bile released after liver implantation may therefore be of low quantity and "watery". In addition to ROS and DAMPs, the "inflammasome" is another characteristic molecular compound of IRI in solid organs, released by various liver cells in response to mitochondrial injury.[47,48] ROS release is crucial to activate the "inflammasome" or NLR family pyrin domain containing 3 (NLRP-3), which – in turn – appears to be key to the inflammatory response, mediating further cellular damage and death.[47,49] The stimulated NLRP-3 inflammasome

Table 1. Clinical studies assessing the impact of hypothermic machine perfusion on biliary complications after liver transplantation during the last 5 years.

Authors & year	Number and type of livers	Duration of follow-up	Main findings	Anastomotic biliary complications (n/%)	Non-anastomotic biliary strictures (n/%)	Discussion
Randomised-controlled trial (level II)						
Van Rijn *et al.*, 2021, Multicentre/Europe[1]	78 DCD livers each arm (D-HOPE *vs.* CS)	6 months	Hypothermic oxygenated perfusion significantly reduces non-anastomotic biliary strictures ($p = 0.03$) and the number of required interventions	Anastomotic Strictures: CS 28.2% (n = 22/78); D-HOPE: 29.5% (n = 23/78)	CIT 17.9% (n = 14/78); 2 retransplantations, D-HOPE: 6.4% (n = 5/78), no retransplantation	Follow-up of 6 months
Czigany *et al.*, 2021, Germany (+Prague)[67]	23 DBD livers each arm (HOPE *vs.* CS)	12 months	HOPE treatment reduced the Peak ALT levels within 7 days ($p = 0.03$), the ICU ($p = 0.045$) and hospital stay ($p = 0.002$), the major complications ($p = 0.036$), the cumulative complications (CCI: $p = 0.021$) and the estimated costs ($p = 0.016$) after transplantation	Biliary complications (clinical; radiological): CS: 26% (n = 6/23); HOPE: 17% (n = 4/23), no further discrimination and specific information on NAS and other types of biliary complications provided		Study was not powered for biliary complications
Case-control cohort study (level IV)						
Rayar *et al.*, 2021, France[62]	25 extended DBD grafts, 69 unperfused controls	12 months	Less peak transaminases, less EAD, less ICU and hospital stay	Anastomotic strictures or leaks: CIT: 10.1% (n = 7/69), HOPE: 8% (n = 2/25)	CS: 1.4% (n = 1/69; ischaemic necrosis), HOPE: n = 0/25	Prospective perfusion group, retrospective control, biliary complications were not the primary endpoint
Schlegel *et al.* 2019, UK, Switzerland[61]	50 DCD (HOPE), 50 DBD (control), 50 unperfused DCD	5 years	Less PNF, HAT and ischaemic cholangiopathy result in an improved 5-year survival of HOPE-treated extended DCD liver grafts	Anastomotic strictures: CS DCD: 18% (n = 9/50), HOPE: 24% (n = 12/50), 1 biliary leak each group (2%)	CS: 22% (n = 11/50) with 10% (n = 1/69) graft loss, HOPE: 8% (n = 4/50) with 0% graft loss;	Matched cohort study
Ravaioli *et al.*, 2019, Italy[118]	Extended DBD livers (n = 10, HOPE), unperfused controls (n = 30)	12 months	No PNF and significantly lower rate of EAD, significantly lower recipient transaminases after HOPE treatment and 100% graft survival compared to the cold storage control group	Anastomotic biliary complications: CS: 10% (n = 3/30), HOPE: 10% (n = 1/10),	No specific information provided	Low case number, matched cohort study
Patrono *et al.*, 2019, Italy[63]	Extended DBD livers, macro-steatotic, n = 25 (D-HOPE), 50 unperfused extended DBD (matched)	6 months	Lower rate of post-reperfusion syndrome, acute kidney injury grade 2-3, and lower EAD due to lower recipient transaminases.	Anastomotic complications: CS: 12% (n = 6/50); D-HOPE: 16% (n = 4/25)	CS: 8% (n = 4/50), 2 symptomatic patients; D-HOPE: 8% (n = 2/25), both asymptomatic	Matched cohort study
Van Rijn *et al.*, 2017, the Netherlands[60]	DCD livers, n = 10 (D-HOPE), 20 unperfused controls	12 months	Restoration of ATP, lower transaminases and protection of the biliary tree from reperfusion injury and complications through D-HOPE	Anastomotic strictures: CS: 15% (n = 3/20); D-HOPE: 20% (n = 2/10)	CS: 45% (n = 9/20) with 2 biliary necroses, 5 retransplantations; D-HOPE: 10% (n = 1/10), no retransplantations	10 livers, matched

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Table 1. (*continued*)

Authors & year	Number and type of livers	Duration of follow-up	Main findings	Anastomotic biliary complications (n/%)	Non-anastomotic biliary strictures (n/%)	Discussion
Systematic review (level V)						
Jia *et al.*, 2020[117]	12 studies, 2 randomised, HOPE/HMP and NMP, 1 each	12 months	HMP/HOPE improved outcomes in contrast to NMP	Lower incidence of overall biliary complications in HMP-treated patients compared to CS (OR=0.45; CI: 0.25-0.80; p = 0.007), no differences between NMP and CS	Lower incidence of NAS (IC) in HMP-treated patients compared to CS (OR=0.25; CI: 0.08-0.73; p = 0.01)	Heterogenous cohort, many smaller studies
Zhang *et al.*, 2019[65]	6 studies, 144 perfused grafts, 178 unperfused controls	12 months	HOPE/HMP was associated with a better liver function, lower EAD rate, lower number of biliary complications and better survival	Lower incidence of biliary complications with HMP compared to CS (OR 0.47, CI: 0.28-0.76, p = 0.003)	No specific information provided	Heterogenous cohort, many smaller studies

CIT, cold ischaemia time; CS, cold storage; DBD, donation after brain death; DCD, donation after circulatory death; D-HOPE, dual HOPE; EAD, early allograft dysfunction; HAT, hepatic artery thrombosis; HOPE, hypothermic oxygenated perfusion; HMP, hypothermic machine perfusion; IC, ischaemic cholangiopathy; ICU, intensive care unit; NAS, non-anastomotic stricture(s); NMP, normothermic machine perfusion; OR, odds ratio; PNF, primary non function.

Key point

The level of mitochondrial injury with the amount of reactive oxygen species released during early reperfusion triggers the overall inflammatory response and impacts on the success of mechanisms to resolve biliary tree injury, including the regenerative capacity of peribiliary glands.

controls caspase-1 activation and the subsequent secretion of mature cytokines including IL-1β and IL-18, mediated through the NF-kB pathway.[47,50–52]

Such reactive cholangiocytes further contribute to the ongoing inflammation and attract recipient immune cells. Activated by the inflammasome, portal myofibroblasts and stellate cells contribute to the development of strictures and fibrosis with the release of fibulin, elastin, collagen and other molecules (Fig. 3).[53,54]

As well as cholangiocytes, peribiliary glands (PBGs) are important for bile duct integrity, as they play a central role in regenerating the injured biliary epithelial cells.[55,56]

In contrast to the more superficial biliary cell lining near the lumen of bile ducts, PBGs are pluripotent cells (crypts and glands) found at larger intra- and extrahepatic ducts, which are uniquely resistant to IRI. These cells respond to IRI by proliferating and migrating, and are able to rebuild biliary integrity.[55]

Within 24 hours after injury, such PBGs can be seen in various stages of mitosis, building small patches of new epithelial layers, which unite. On a cellular level such PBG cells communicate with surrounding microvessels and express HIF-1α (hypoxia-inducible factor 1α) and various types of vascular endothelial growth factor receptors.[30,55] With their expression of vascular endothelial growth factor receptors, PBGs not only have an activating effect on neighbouring endothelial cells, but also stimulate other PBGs.[30] When the IRI-associated inflammation overpowers repair mechanisms, the ongoing inflammation, cholestasis and fibrosis lead to a chronic hypoxia based on the loss of microvessels. The direct consequence is a reduction in PBG mass and differentiation, with an impaired regeneration of biliary epithelial cells.[30]

Clinical studies on hypothermic liver perfusion and its impact on the biliary tree

The technique of hypothermic machine perfusion (HMP) was introduced into clinical liver transplantation following the study by Guarrera *et al.*, published in 2010. The authors demonstrated the feasibility and also superiority of HMP compared to cold storage alone for marginal grafts from extended criteria brain dead donors.[57] Various retrospective studies were performed in the following years, with only a few reporting biliary complications. In 2015, the same group from the US showed that using end-ischaemic HMP for 3-7 hours (mean 3.8 hours) led to a reduction in biliary complications.[58]

Most studies within the last 10 years have been retrospective. Level I (systematic reviews of randomised-controlled trials [RCTs]) and III (prospective controlled studies – non-randomised) studies have not been performed with HMP. Only

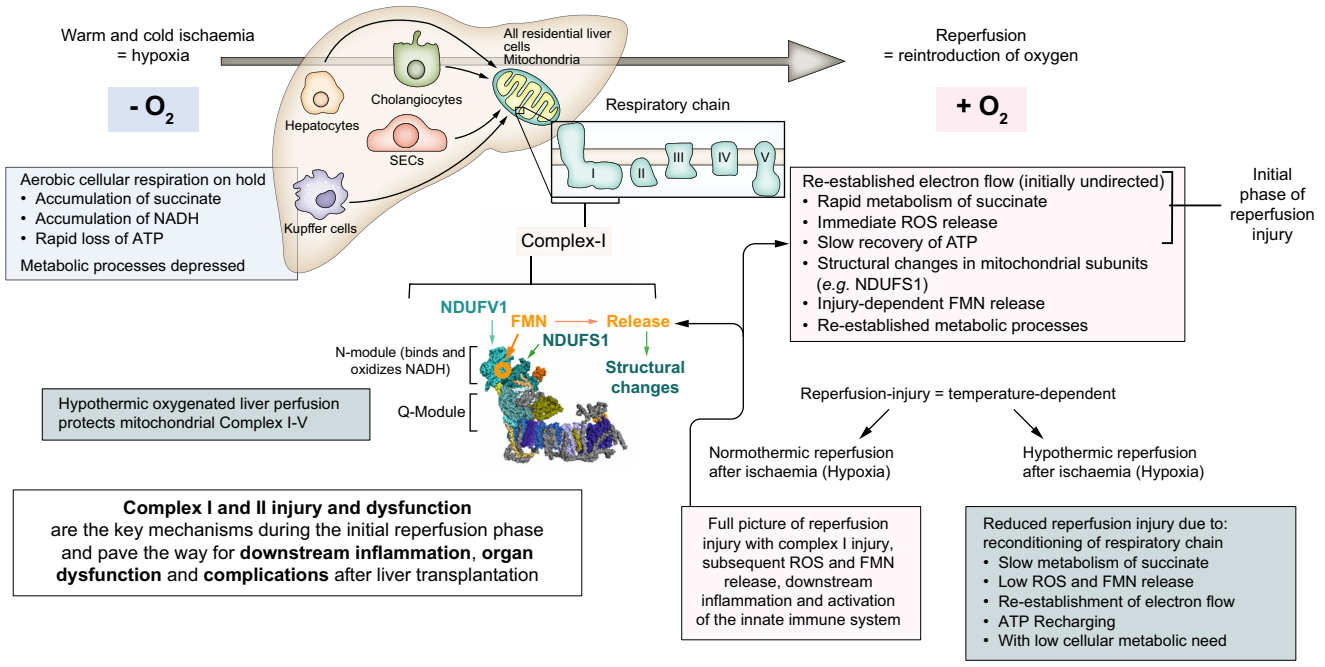

Fig. 4. Initial complex 1 injury is key during ischaemia-reperfusion injury in all cells. The mechanism of mitochondrial injury and loss of function during ischaemia and reperfusion are highlighted together with the detailed protection of complex I (to V) through hypothermic oxygenated perfusion treatment. FMN, flavin-mononucleotide; ROS, reactive oxygen species; SEC, sinusoidal endothelial cell.

2 RCTs have been published, 2 are completed and under review, while 4 are currently recruiting.[59] Most clinical studies are retrospective, with a matched control group of cold-stored livers. HOPE is more frequently applied in DCD cohorts, mainly reported by centres in Italy, the Netherlands or Switzerland.[60,61] Colleagues from France and Italy have also described the impact of HOPE or dual HOPE (D-HOPE) on marginal livers from brain dead donors.[62–64] Table 1 provides an overview of available clinical studies within the past 5 years, where authors report the impact of HMP on biliary complications and include a control group. Despite the retrospective design, HOPE treatment was found to protect from the development of NAS. Based on donor risk, a NAS rate of 1.4–45% was reported for cold-stored DCD livers, while this rate was reduced to 0–10% with the addition of hypothermic perfusion. Importantly, no grafts were lost in the hypothermic perfusion group, in contrast to the cold storage group, where up to 55.6% of recipients with NAS required retransplantation (Table 1).[60]

Most of those case-control cohort studies were also summarised in 2 systematic reviews (level V). Of note, both reports demonstrated not only the superiority of HMP compared to cold storage alone regarding biliary complications, but also remarkably showed the lack of a clear and uniform definition of biliary complications and particularly ischaemic biliary lesions in currently available clinical studies.[65] In the meta-analysis by Zhang *et al.*, which analysed 6 retrospective studies, including 144 human livers transplanted with HMP, the authors reported that all manuscripts either lack a definition for NAS in general or do not present clear and clinically relevant criteria.[65] Another 6 single-arm cohort studies have been presented within the last 5 years, however, they did not include a matched cold storage control group.

The study presented by the Groningen group in February 2021 therefore appears to be highly relevant. With their multicentre, European RCT, the authors demonstrate a significant reduction of symptomatic NAS in DCD livers allocated to a simple end-ischaemic D-HOPE of 2 hours.[1] NAS occurred in 17.9% (n = 14/78) of recipients of cold-stored DCD livers, with 2 patients requiring retransplantation. In contrast, only 6.4% (n = 5/78) of recipients of D-HOPE-treated grafts experienced symptomatic NAS and no recipient required retransplantation within a follow-up of 6 months (Table 1).[1] In addition to a reduction in the number of cholangiopathies, D-HOPE treatment also reduced their severity. This was demonstrated by the D-HOPE group requiring 4-fold fewer interventions at the biliary tree compared to the SCS group. Further secondary endpoints were of interest, with a lower rate of early allograft dysfunction (EAD; 26% *vs.* 40%) and acute rejections (11.5% *vs.* 20.5%) in the D-HOPE group than the SCS group, respectively.[1] Consecutively, the protective effect seen in DCD livers with D-HOPE should also result

in graft survival differences over longer follow-up. Another important aspect is that the discard rate in both study arms (control *vs.* HOPE treatment) was equal in the D-HOPE trial, which is in sharp contrast to the recent RCT presented by Nasralla *et al.* on normothermic machine perfusion (NMP). The authors of this trial reported a high drop-out rate in the cold storage control group, with more control livers discarded after randomisation, despite not appearing to be higher risk than grafts in the D-HOPE trial; this would thus appear to be more of a study design failure than a result.[66]

Of note, however, the current RCT from Groningen demonstrates an equally high rate of anastomotic strictures in both study arms (D-HOPE: 29.5% *vs.* cold storage: 28.2%).[1] Such findings parallel previous retrospective studies within the past 5 years, where the frequency of anastomotic strictures ranged from 8% to 24% following hypothermic perfusion (Table 1).[60–63]

In contrast, another recently published RCT was presented by the group from Berlin. Although the primary endpoint was peak liver transaminases within 1 week after transplantation, which were significantly lower in the HOPE group, the authors also reported a lower rate of biliary complications, which occurred in 17% of recipients in the HOPE arm compared to 26% in the cold storage group, though such findings were not statistically significant.[67] No further details regarding type, location and timing of the described biliary complications are provided in their paper. However, based on the included extended criteria donor livers donated after brain death (DBD), one could assume that the majority of the biliary complications were of anastomotic origin. Recipients of HOPE-treated livers were also found with significantly lower plasma bilirubin levels at 6 months after transplantation compared to the unperfused controls.

In contrast, the effect of NMP on the development of biliary complications remains controversial. In their RCT, Nasralla *et al.* included a mixed cohort of standard DBD and DCD livers. The authors did not observe a significant impact of upfront NMP on the rate of NAS in DCD livers, with the limitation that the study was not powered for this endpoint. In the NMP group, 11.1% (3/27) of DCD liver recipients were found with signs of NAS on MRI imaging, compared to 26.3% (5/19) in the unperfused cold storage control arm.[66] Only 1 recipient in each study group required retransplantation due to clinically relevant NAS.

In addition, 3 retrospective studies from the UK have explored the impact of end-ischaemic NMP. The group of Chris Watson reported that NAS occurred in 27% (n = 3/11) and 18.2% (n = 4/22) of recipients after end-ischaemic NMP in 2 series from Cambridge.[68,69] Such findings were paralleled by the recent prospective trial from Birmingham (UK), wherein the authors described the need for regrafting due to ischaemic biliary strictures in 18.2% of marginal livers from DCD and DBD donors, which underwent end-ischaemic NMP after cold storage.[70] These results are contrary to published reports from Innsbruck, where the authors demonstrated a protective effect of end-ischaemic NMP against NAS, though this cohort included predominantly DBD livers with standard risk.[71] However, the results of this study are questionable because of the fairly high rate of NAS in unperfused controls, *e.g.* 14% NAS in mainly DBD liver recipients (55 DBD/59 overall), although we may assume that most are not of clinical relevance. In this context, currently ongoing RCTs in Germany and Italy, comparing end-ischaemic HOPE to NMP, are awaited to provide further insights.[72]

Key point

The combination of hepatocyte and cholangiocyte reperfusion injury triggers the production of toxic bile. Together with a crosstalk between various liver-related and recipient-derived cells, a milieu of chronic inflammation with deposition of fibres is established leading to the development of strictures.

Protection of the biliary tree through HOPE

Since the first studies on hypothermic liver and kidney perfusion more than 50 years ago, the ATP replenishment of tissues has been frequently described. Of note, high perfusate oxygen partial pressures are required to fully recover ATP levels.[73] Despite the lack of a complete mechanistic understanding, an effect on mitochondria was considered most likely. With the development of novel technologies, the impact of HOPE on subcellular compounds is much better understood today. Of note, the modification of mitochondrial metabolism appears similar in various solid organs.[36,74–76]

Exposure of mammalian cells to high levels of oxygen under cold conditions seems to trigger a slow but steady electron flow throughout mitochondrial complexes, following previous complete respiratory chain “standstill” due to ongoing hypoxia. Energy recovery and succinate metabolism during HOPE are the main metabolic events that prevent the initial burst of ROS within the first few minutes of subsequent warm reperfusion (Fig. 4).[36] The group of Mike Murphy has recently described 2 key targets to protect mammalian tissue from IRI and inflammation. First, the prevention of succinate accumulation during ischaemia, and second, the metabolism of succinate before normothermic reoxygenation.[32] The lower tissue succinate at the end of HOPE compared to SCS alone was confirmed in experimental studies.[25,36,75] Based on a functional recovery of complex I to V, HOPE protects against severe inflammation during later normothermic reperfusion.[36,77] The Zurich group has quantified the initial ROS release during normothermic reperfusion of macrosteatotic livers. HOPE treatment led to significantly lower ROS concentrations in perfusates, compared to the cold storage control group.[25] The entire cascade of downstream inflammation and functional impairment was reverted accordingly.[25] Such findings were in clear contrast to the immediate reintroduction of oxygen

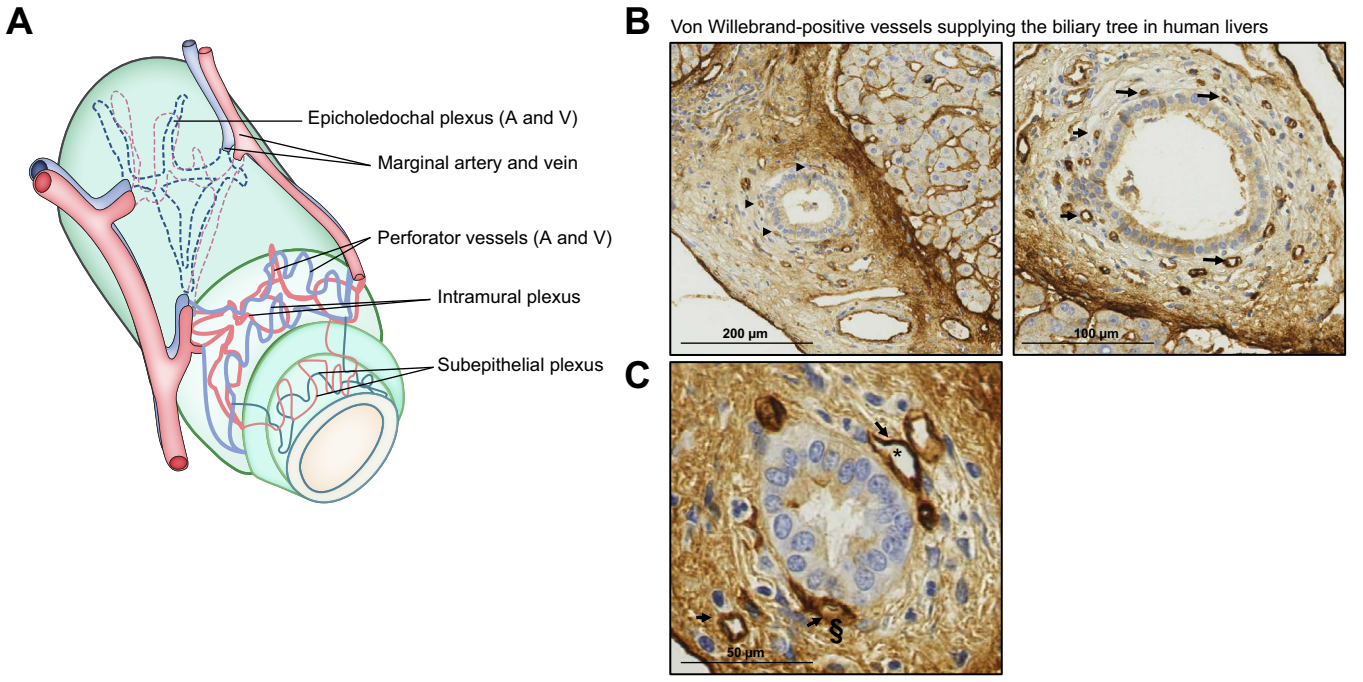

Fig. 5. Arterial and venous blood supply to biliary tree (schematic and histology). (A) Vascular supply to the biliary tree: The arrangement of the arterial and venous plexus to the biliary tree are similar. The epicholedochal plexus is fed by the marginal arteries and veins and lies superficial on the surface of the common bile duct. Within the next deeper layer, the intramural (intrinsic) plexus is found and is connected through perforator vessels to the epicholedochal and subepithelial plexus. (B) Von Willebrand positivity in vessels supplying larger and smaller bile ducts within the liver. Arterial and venous branches are seen. (C) Vascular supply of a small ductulus with venous (*) and arterial (§) subepithelial branches. The destruction and loss of such microvasculature (arterial and venous branches) after liver reperfusion in combination with a mechanical hit through the surgical anastomosis of the common bile duct contributes significantly to the persistent hypoxia of the biliary tree and the subsequent development of biliary strictures. High perfusate oxygen concentration during hypothermic oxygenated perfusion achieves full mitochondrial reprogramming in all cells, including cholangiocytes, despite perfusion through the portal vein only.

under normothermic conditions, which led to the full picture of IRI – depending on the level of initial liver injury and donor warm and cold ischaemia time.[78]

Improved liver function and reduced inflammation were also seen when risky livers underwent HOPE treatment prior to normothermic reperfusion.[79,80] The protection from mitochondrial injury appears to be key to improved hepatocyte function and reduced injury.

Regarding the biliary tree, HOPE of human livers provides more ATP and improves metabolic function, including cholesterol metabolism, BA secretion and cellular regeneration, at subsequent normothermic reperfusion.[43] This leads to a less toxic initial hepatic bile and reduced intracellular bile salt accumulation and cholestasis.[43] Equally, cholangiocytes experience less ROS release and inflammation within a healthier milieu. The overall reduced acute proinflammatory reaction within the entire organ ultimately protects from chronic inflammation, and subsequent cholestasis and fibrosis, thereby preventing the development of biliary strictures.[81]

The effect of HOPE on graft function

In correlation to a reduction of inflammation and liver injury, HOPE treatment has also been shown to improve initial graft function after transplantation (Fig. 4). A retrospective cohort study compared outcomes after transplantation of HOPE-treated DCD livers and unperfused, cold-stored controls. A lower rate of primary non function was seen in the HOPE group together with a significantly better immediate coagulative function, as quantified by a lower recipient international normalised ratio on day 1.[29] Such clinical outcomes were paralleled by results from experimental studies. Authors from Zurich have demonstrated higher Quick (corresponding to a lower international normalised ratio) and factor V values after rodent DCD liver transplantation using HOPE treatment.[77]

Further surrogate markers support the functional improvement of livers by HOPE. Lower recipient potassium levels were identified after reperfusion of HOPE-treated livers in clinical practice.[82] This finding can be explained by the better functioning of the sodium-potassium pump

Key point

During hypothermic oxygenated perfusion (HOPE), mitochondrial complexes experience a reprogramming, with metabolism of accumulated succinate and ATP reloading. HOPE therefore provides enough energy to fuel metabolic and secretory processes required immediately during normothermic reperfusion to produce bile of good quality and protect cholangiocytes.

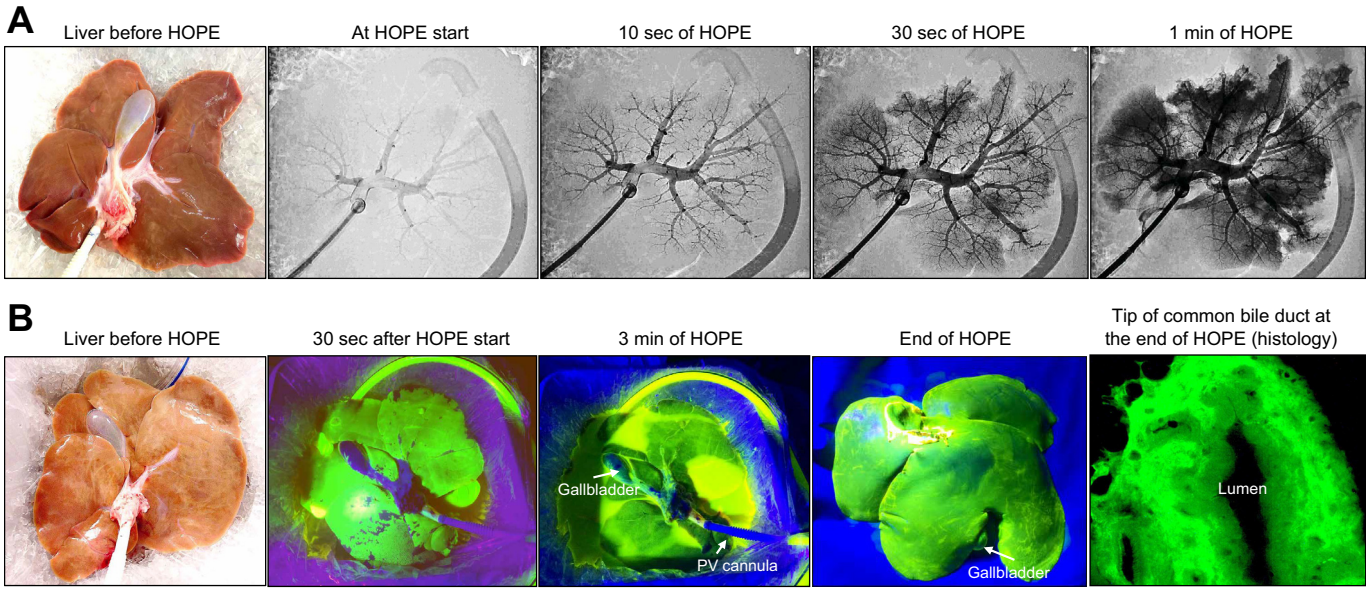

Fig. 6. Perfusion quality of DCD pig livers during HOPE through the portal vein. In a pig model with 30 minutes asystolic donor warm ischaemia time and 5 hours cold storage, the perfusion quality during HOPE was assessed. Pig livers were flushed and retrieved with the standard super rapid technique and underwent cold storage. The portal vein was cannulated prior to HOPE and contrast medium (A) or fluoresceine (B) was added to the standard Belzer machine perfusion solution. Perfusion parameters included the perfusion temperature at 9-10°C, pressure control with a maximum of 3 mmHg and a subsequent perfusion flow, ranging between 140-270 ml/min. The complete liver perfusion was confirmed within the first few minutes of HOPE through the portal vein in both experimental settings. Of note, the extrahepatic biliary tree was macroscopically stained with fluoresceine within a few minutes after the start of HOPE and all layers of the common hepatic duct were entirely perfused and demonstrated the intense green colour of fluoresceine as confirmed with histology. DCD, donation after circulatory death; HOPE, hypothermic oxygenated perfusion.

in hepatocytes, triggered by the higher cellular ATP levels achieved during HOPE,[82] and are paralleled by lower rates of post-reperfusion syndrome and recipient hypotension. Likewise, in 2019, Patrono *et al.* from Turin described a reduction of post-reperfusion syndrome from 20% in recipients of cold-stored extended criteria DBD livers to 4% when HOPE was applied.[63]

Consistent with the functional liver graft improvement, most retrospective studies and RCTs on HOPE and D-HOPE demonstrate their impact on EAD.[83] For example, in an RCT from Germany, the rate of EAD was reduced from 35% in the cold storage arm to 17% in the HOPE group.[67] The RCT from Groningen paralleled these results in their controlled DCD population, with a reduced EAD rate of 26% after D-HOPE compared to 40% for cold-stored controls.[1] However, these results should be interpreted with cautions, as significant hepatocellular enzyme loss may occur during machine perfusion, regardless of the technique, which is often not reported.

Based on the reduction of IRI-associated inflammation and cytokine release through HOPE, various authors have also described less acute kidney complications after liver transplantation. The rate of acute kidney injury (grade 2 and 3) was reduced from 42% in cold storage controls to 16% by end-ischaemic D-HOPE after transplantation of marginal DBD livers – *i.e.*, from elderly donors (>80 years) or with relevant steatosis (>40% mixed) and extended cold ischaemia time (>10 hours).[63]

The effect of HOPE on the immune system and acute rejection

The proinflammatory status of severely injured cells during IRI promotes the expression of cellular surface markers, recognised by recipient immune cells, thereby activating the innate immune system.[84,85] Correlating with its direct effect on mitochondrial metabolism and IRI-associated inflammation, HOPE was also shown to reduce the response of the innate immune system after transplantation (Fig. 4).[86] Accordingly, recipients of allogeneic HOPE-treated rodent livers were protected from T cell-mediated rejection, demonstrated by a reduced number of infiltrating T cells and cytokine levels in the HOPE group, despite the absence of immunosuppression.[86] Interestingly, the end-ischaemic application of the HOPE concept in DCD kidneys led to similar findings in a model of allogeneic kidney transplantation.[87]

These results from experimental studies are further supported by clinical trials. In 2019, a significant reduction of acute rejections from 28% in unperfused controls to 4% after HOPE was described in DCD livers ($p = 0.0019$).[61] A recent RCT also demonstrated a reduced number of acute

rejections after D-HOPE. Of recipients of unperfused cold-stored DCD livers, 20.5% developed acute rejection compared to 11.5% after D-HOPE.[1]

Technical differences and implications on the protective effect of HOPE

Based on the recent discovery of the detailed underlying mechanisms of HMP in various organs, the importance of perfusate oxygen has been widely accepted.[36,74–76,88,89] All cell types in the liver require treatment, while both hepatocyte and cholangiocyte injury contributes to the development of NAS in an inflammatory crosstalk with various cells.[33,36,48,49,90–92] In addition to different oxygen levels in hypothermic perfusates, the perfusion route (*e.g.* the use of a single or both inflow vessels) has been debated.[59] Since the introduction of HMP into clinical liver transplantation by Guarrera *et al.*, 2 main perfusion strategies have been used. First, perfusion through the portal vein only ("classic HOPE") advocated by the Zurich group, and secondly, the additional perfusion through the hepatic artery (dual or D-HOPE).[1,57,63,93] In context of the need for oxygen supply to the biliary tree, the group from Groningen has promoted the D-HOPE technique, based on the known higher oxygen content in the hepatic artery when compared to portal vein branches.[94] To achieve this and to reduce the risk of injuring the hepatic artery, the additional procurement of donor aortic vessels to be used for arterial cannulation during D-HOPE was suggested.[1,60]

Anatomically, the biliary tree is fed with both arterial and venous blood through cranial and caudal branches from the hepatic artery and portal, or supra-mesenteric vein (right hepatic artery, portal vein and retro-pancreatic artery and vein). Together these vessels build a mesh around the ducts in the biliary tree (Fig. 5).[59,95,96] Additionally, such vessels communicate at all levels, from the sinusoids to the common bile duct.[97] Clinically, this is seen during liver implantation, when venous back bleeding from the common bile duct stump occurs routinely prior to the connection of the donor and recipient hepatic artery. With the high oxygen content in HOPE perfusates of 60-80 kPa, all liver cells including the cholangiocytes of the entire biliary tree receive the required oxygen and undergo the necessary mitochondrial reprogramming, despite perfusion through the portal vein only.[45,93,96] Experimental studies using contrast medium- and fluoresceine-loaded perfusates, during HOPE through the portal vein, have confirmed the rapid and homogenous oxygen distribution to the entire organ (Fig. 6).[96,98] The high oxygen level in the portal vein during HOPE is clearly different from physiological conditions. The reduced oxygen level in the portal vein in situ appears insufficient for the biliary tree; thus, arterial blood supply is absolutely required, particularly after ischaemia and for the recovery of the bile ducts from hypoxia and inflammation after transplantation. In the context of high portal vein oxygenation, experimental studies did not identify differences in liver preservation when comparing HOPE through the portal vein only to the dual vessel approach.[99] Of note, a recent clinical study from Italy did not demonstrate any differences in outcomes after liver transplantation between these procedures.[100] However, robust comparative studies with a focus on biliary complications after transplantation of high-risk livers are lacking and will be required to fully confirm the equality of both techniques.[59] Although multiple other factors besides donor ischaemia contribute to the development of anastomotic strictures, the equally high rate of strictures at the anastomotic site, seen with single portal vein-HOPE (24%) and D-HOPE (29.5%), appears to be additional evidence of their equivalence.[1,61]

In addition to the different vascular entry for HOPE and D-HOPE, discussions on the best timing and the need for device transport are ongoing.[59] Despite the naturally expected benefit of an early HOPE, *i.e.* performed immediately at or after liver procurement, a continuous perfusion throughout liver transport may not necessarily be required, because the HOPE technique reprogrammes mitochondria within 60-120 min and maintains this effect thereafter.[77] Such results should always be interpreted in the context of the organ type and quality. For example, very short HOPE perfusions of 1 hour were not long enough for severely macrosteatotic livers.[25] Continuous perfusion entails various challenges and is currently not supported by the literature. In the 2 available RCTs and most currently awaited trials, HOPE procedures were routinely performed after cold storage. And the larger body of retrospective studies shows similar perfusion settings with a cold storage time of 4.4-14.5 hours prior to HOPE.[59] Although data on the metabolic effect of using HOPE at different timepoints, *e.g.* before or after cold storage, are scarce, we have learned from the literature that prolonged oxygenation in the cold beyond 2 hours does not seem to increase ATP levels further. The group from Essen has explored this in a model of cold oxygen persufflation and has described similar tissue ATP concentrations after 2 and 3 hours of treatment.[101] Equally the protective HOPE effect was maintained in a prolonged perfusion model.[102] Parameters of injury and inflammation remained equally low or even lower after 20 hours of D-HOPE compared to the first 6 hours of perfusion.

Mike Murphy's group has shown that most ATP molecules are lost within the first 4 hours of warm and cold ischaemia in hearts.[103] An initial HOPE, started within this period, could therefore be of interest to revert this metabolic feature. A comparative study of HOPE before and after cold

Key point

Due to mitochondrial protection, HOPE reduces ischaemia-reperfusion injury and acute and chronic inflammation, resulting in less cholestasis and fibrosis.

Key point

This simple cold liver perfusion with high oxygen levels, therefore, protects recipients of high-risk livers from the development of ischaemic cholangiopathy.

storage is required, particularly in the context of the current discussion around whether to implement "organ perfusion hubs", where livers could be treated and assessed prior to additional cold storage and transport to the recipient centre.

Viability assessment of the biliary tree

To ultimately improve the poor DCD liver utilisation rates, the optimal perfusion approach should also provide an assessment of viability and give surgeons confidence to use risky grafts based on a combination of parameter thresholds measured from perfusates, which are linked with post-transplant outcomes.[104] For example bile flow and composition are both frequently used to explore biliary viability during NMP.[43,105] Biliary pH, absolute perfusate bicarbonate and glucose levels or perfusate/bile ratios are some key measures, which have recently been suggested for the assessment of cholangiocyte viability. Such parameters were used in smaller cohort studies to explore liver viability. Grafts that exceeded specific parameter thresholds in bile or perfusate were declined.[105,106] While bile appears a useful compound to assess cholangiocyte viability, the metabolic profile of hepatocytes with more than 500 metabolic processes is insufficiently captured.[104] Despite the potential quantification of specific BAs, these molecules represent only a very small proportion of hepatocyte function, and bile alone does not represent the greater metabolic cluster of liver tissues.

Under hypothermic conditions, the fluid released throughout the biliary tree appears rather unphysiological due to reduced metabolic and secretory processes. Other parameters are therefore of higher interest. In the context of the underlying mechanism of IRI and protection conveyed through HOPE, both cell types, hepatocytes and cholangiocytes, and mitochondria, should be considered.[36,37,104] When mammalian tissues receive oxygen after a period of hypoxia, in addition to ROS, further molecules are released from mitochondrial complex I. Flavin-mononucleotide (FMN), also known as Vitamin B_2 derivate, represents one known group of such molecules.[107,108] The release of FMN from mitochondria was first described in the 60s[107] and has recently garnered renewed interest. With recirculating perfusate, liver quality and injury can be assessed in real-time due to the autofluoresecent characteristics of FMN.[109] The concentration of FMN in HOPE perfusates was recently shown to correlate with liver function, complications and graft survival after DCD liver transplantation. While perfusate FMN concentrations beyond suggested cut-offs led to primary liver non-function or NAS in the vast majority of DCD recipients, livers where such thresholds were respected were associated with excellent graft and recipient survival rates and low complications, despite advanced donor risk profiles.[36,109]

In Switzerland, DCD donor livers undergo standard super rapid flush and retrieval, with subsequent transport during cold storage. At arrival in the recipient centre, such livers are routinely assessed and connected to the HOPE device. During HOPE, the perfusate is sampled and FMN concentrations are measured using laboratory fluoroscopy. When FMN perfusate levels are far below the established threshold at 30 min of HOPE, the recipient surgery is started and the graft is transplanted. Livers with intermediate range FMN concentrations (suboptimal but acceptable), might be re-allocated to a different recipient, provided the initial candidate carried too high a risk, *e.g.* retransplantation or high lab model for end-stage liver disease score. Livers were discarded if perfusate FMN levels went beyond the accepted threshold, irrespective of the previously assessed clinical risk factors of the donor. About 10-15% of human livers show a fairly steep increase of perfusate FMN at 15 and 30 min of HOPE. Such livers undergo repeat FMN measurement after 45 and 60 min, sometimes even after 90 min, with a decision according to previously described and aforementioned perfusate FMN values.[36,104] Despite the validation of FMN in different cohorts and other solid organs, the establishment of reliable thresholds, accepted and used by many centres, requires higher caseloads and further prospective studies, which are currently being performed.[74,110,111]

Until such results are available, and to further explore the field, an end-ischaemic combined perfusion approach with initial HOPE, followed by controlled oxygenated rewarming and NMP (to assess viability), is being applied by different groups.[112] Despite the lack of validation studies and the evolution of parameter thresholds based on advancing knowledge, many clinicians use NMP to assess liver viability. Perfusate lactate, pH and enzyme release are leading parameters, which are frequently combined with markers obtained from bile produced during NMP. There can be difficulties adapting such strategies to less-experienced centres, as, for example, 4 different thresholds are used in practice for perfusate lactate levels.[70,104,106,113]

The Impact of HOPE on costs related to transplantation

Next to the required RCTs showing the benefit of a specific treatment, studies which provide more details on the impact on costs, ideally showing a cost benefit, are ultimately required for the full commissioning of HOPE. The currently available literature specifically tailored to costs is very scarce in the entire field; in fact, it is not only the costs required for machine perfusion, but also for liver donation and transplantation surgery that have been incompletely assessed.[114,115] The lack of large

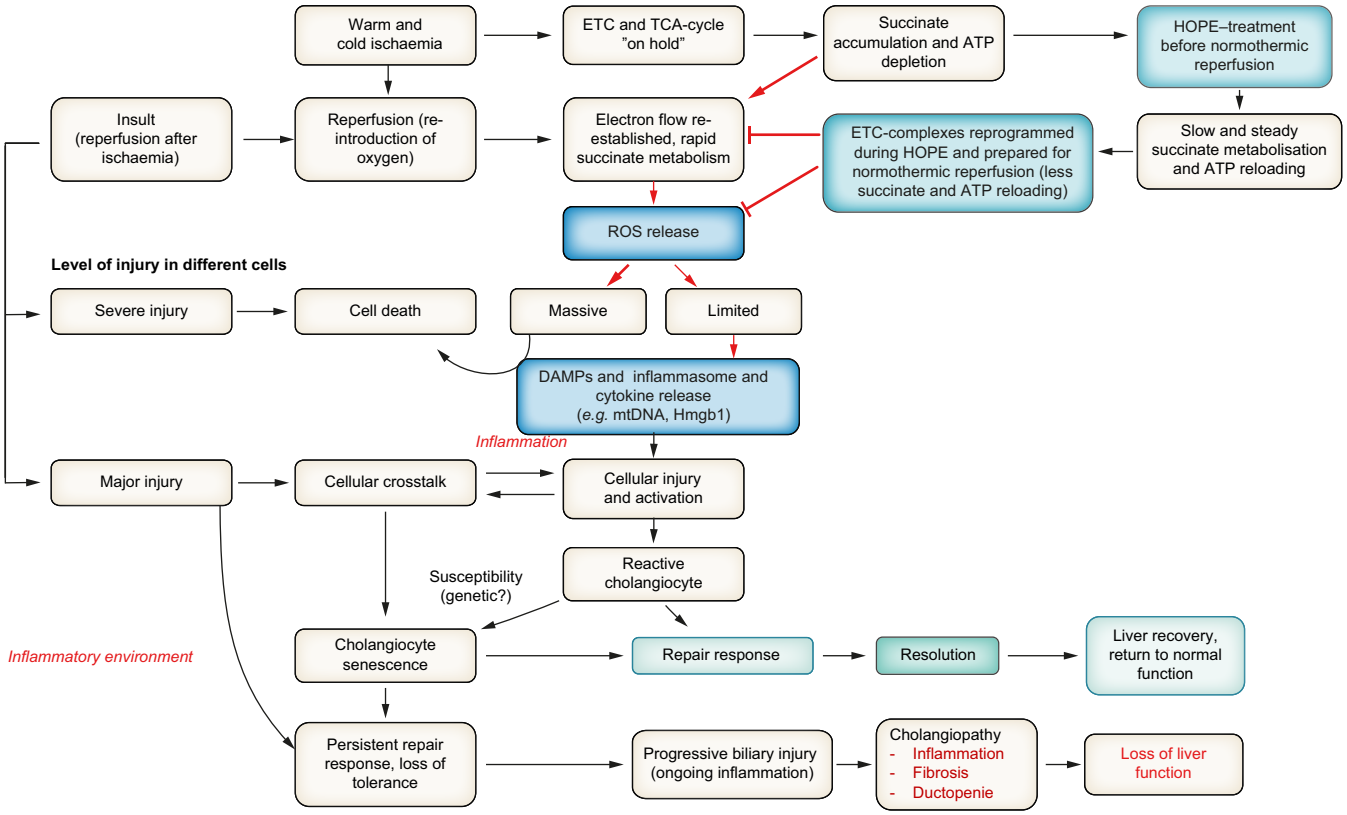

Fig. 7. Pathway of injury and protection of the biliary tree through hypothermic oxygenated liver perfusion. The pathway of liver IRI is summarised together with the impact on the structures and function of the biliary tree and downstream consequences. The initial mitochondrial reprogramming during HOPE treatment protects all liver structures from ongoing inflammation, leads to resolution of injury and functional recovers. This contrasts with overwhelming IRI with high levels of ROS, DAMPs and cytokine release and subsequent chronic inflammation, cholestasis and the development of cholangiopathy and related complications. DAMPs, damage-associated molecular patterns; ETC, electron transport chain; HOPE, hypothermic oxygenated perfusion; IRI, ischaemia-reperfusion injury; ROS, reactive oxygen species.

and well-designed cohort studies appears to be one reason for the limited information regarding the impact of machine perfusion on costs.

Cost analyses in liver transplantation should include the procurement surgery, organ transport, transplantation surgery, as well as the post-operative course with hospital stay and post-transplant complications. Further cost contributors are device depreciation and hourly use, staff required to operate the machine, consumables and perfusion solutions. The use of operation theatres might incur additional costs, which are frequently not considered.[114] Four studies provide up-to-date information regarding the financial impact of MP. Importantly, 3 were not based on large registry data or randomised cohorts, and only 1 described the cost benefit in a randomised albeit small cohort.[62,114,115] Normothermic approaches in general appear much more cost intensive due to the required equipment, including disposables, which is currently 3–4-fold more expensive for each single transplantation when compared to the HOPE technique.[62,114,115] Rayar *et al.* list the costs for a single HOPE treatment in France as 5,298 Euro.[62] Two major companies provide the disposables for HOPE at a current cost range of 3,500.00 to 6,000.00 Euro. Based on the involved distributors and specific contracts, such costs can spiral to values of up to 12,000 Euro. In their study, Rayar *et al.* demonstrate that additional costs for the HOPE approach are amortised through the shorter hospital stay and the reduction of post-transplant complications. However, this study did not consider liver donation costs.[62] With a focus on post-transplant major complications as the main driver of costs, the team from Germany has demonstrated a cost benefit when using the HOPE approach. Cold perfusion reduced the median costs from 59,000 Euro to 39,000 Euro per transplant case, mainly achieved through a shorter hospital stay and less major complications.[67] The impact of HMP on costs appears much better analysed in the context of kidney transplantation, where the cold perfusion approach appears cost effective, through a lower number of post-transplant complications such as delayed kidney function.[116] Further studies

are required to provide robust evidence to convince commissioners in various countries.

Future directions

Despite the great progress in the field of machine perfusion, and particular with hypothermic approaches, a few key questions will define future studies. Most cold perfusions are currently performed after SCS in the recipient centre, while the impact of HOPE initially at the donor centre appears unclear and is currently being explored. Next, the technical differences with perfusion through different liver inflow vessels or maximal perfusion pressures will be debated in the future. To further improve liver grafts, which are insufficiently reconditioned by HOPE treatment and are identified through a high perfusate FMN release beyond currently applied thresholds, the addition of specific molecules into perfusates to better shield mitochondrial complex I and II may be considered.

Conclusions

As shown in a recent RCT, DCD liver transplantation can be performed at a new level of safety with end-ischaemic HOPE – a simple preservation approach which is clearly protective against ischaemic cholangiopathies. The protection is conveyed through mitochondrial reprogramming and preparation of cells for the normothermic reperfusion during implantation. With succinate metabolism and ATP recovery during HOPE, the level of mitochondrial injury is reduced and subsequent inflammatory processes, including ROS release, are limited during subsequent normothermic reperfusion. Protection from acute and chronic inflammation is the initial key to prevent later cholestasis and tissue fibrosis – 2 main features of NAS – seen along the biliary tree (Fig. 7).

The recent RCTs on the effect of HOPE have confirmed previous experimental studies and underlying mechanisms, and provide the first insights into the financial benefits of this technique.[1,67,81] Therefore, both the HOPE and D-HOPE approaches are being implemented as the routine clinical preservation techniques for higher risk grafts in various countries.

Abbreviations

AS, anastomotic strictures; ATP, adenosine-triphosphate, CS, cold storage; DBD, donation after brain death; DCD, donation after circulatory death; DWIT, donor warm ischaemia time; EAD, early allograft dysfunction; FMN, flavin-mononucleotide; HMP, hypothermic machine perfusion; HOPE, hypothermic oxygenated perfusion; IC, ischemic cholangiopathy; IRI, ischaemia-reperfusion injury; ITBL, ischemic-type-biliary-lesion; LT, liver transplantation; NAS, non-anastomotic stricture(s); NMP, normothermic machine perfusion; PBGs, peribiliary glands; RCT, randomised-controlled trial; SCS, static cold storage.

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Authors' contributions

Design and layout of article: all co-authors. Design of figures and completion of table: table & figure 1, 6: all co-authors; figure 2-5, 7: AS. Manuscript draft: all co-authors. Revision and approval of manuscript: all co-authors.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.01.024.

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