6 Ischemia-Reperfusion Injury and Therapeutic Strategy in Donation After Circulatory Death Liver Transplantation

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Abbreviations

cDCD	Controlled DCD
DAMPs	Damage-associated molecular patterns
DCD	Donation after circulatory death
HMBG-1	High-mobility group box 1
HMP	Hypothermic ex situ machine perfusion
IC	Ischemic cholangiopathy
IRI	Ischemia-reperfusion injury
ITBLs	Ischemic-type biliary lesions
MP	Machine perfusion
NMP	Normothermic ex situ machine perfusion
NRP	Normothermic regional machine perfusion
ROS	Reactive oxygen species
SECs	Liver sinusoidal endothelial cells
TLR	Toll-like receptor
tPA	Tissue plasminogen activator
uDCD	Uncontrolled DCD

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Introduction

Ischemia-reperfusion injury (IRI) is common during transplantation when blood flow is restored and oxygen and nutrients are returned to the liver following ischemic injury. Although donation after circulatory death (DCD) is one important strategy to expand the donor pool, it is associated with severe reperfusion injury. Liver grafts from DCD donors are exposed to the agonal phase during donation resulting in an additional warm ischemia time with insufficient blood supply.

Liver IRI is regulated by several molecular pathways. Reperfusion injury results in significant changes in hepatocytes and liver sinusoidal endothelial cells (SECs). The prolonged ischemic period results in a depletion of adenosine triphosphate (ATP) with an activation of mediators of apoptosis and necrosis in liver cells. After reperfusion, neutrophils and liver macrophages (Kupffer cells) are activated in damaged livers, which amplify IRI by secretion of paracrine and autocrine signals, such as reactive oxygen species (ROS), lipid peroxidation, and damage-associated molecular patterns (DAMPs) [1].

In this chapter, we will focus on mechanisms of IRI in hepatocytes and bile ducts and discuss therapeutic strategies targeted on molecular mechanism of IRI in liver transplantation using DCD donors.

Molecular Mechanisms of IRI: Ischemic Period

According to the revised Maastricht classification in 2013 [2], DCD transplantation was categorized into two major types: controlled DCD (cDCD) and uncontrolled DCD (uDCD).

In cDCD, during the agonal phase, the oxygen saturation and the blood pressure are decreasing following withdrawal of life-sustaining therapies (WLST), and donor death is declared 2–5 minutes after a no-touch period [3]. Following death declaration, cold flush or regional perfusion is performed, and the organs are procured. Warm ischemia time has been variably defined, but it is necessary to consider the agonal phase (from WLST to cardiac arrest) as a relative ischemia time. A retrospective study in five major liver transplant centers determined that functional DWIT with $SpO_2 \leq 60\%$ is an important predictive parameter for postoperative complications in DCD liver transplantation [4].

In uDCD, the donor underwent an unexpected cardiac arrest outside the hospital with unsuccessful cardiopulmonary resuscitation before determination of death. A prolonged time period exists between cardiac arrest and arrival at the hospital prior to death declaration. The extent of the ischemia is more uncertain in uDCD, making the post-transplant severity of IRI difficult to predict.

Prolonged warm ischemic injury of more than 30 minutes is a well-known risk factor for post-transplant liver failure [5–10]. During ischemia, the cell death is mainly caused by metabolic disturbances [11]. Depletion of oxygen causes cell hypoxia that results in an inhibition of the electron transport in the respiratory chain and a decrease in intracellular ATP levels. ATP-dependent ion channels such as Na^+/

K^+ adenosine triphosphatase (ATPase), Na^+/H^+ exchanger, and Ca channels start to fail, which induces depolarization of the cell membrane with accumulation of intracellular Na^+ and Ca^{2+} and cellular edema. This activates proteases, lipases, phospholipases, and ATPases promoting hepatic apoptosis and necrosis. At the same time, anaerobic respiration induced by insufficient oxygenation supply causes lactic acidosis that further activates intracellular proteases. The increase of Ca^{2+} influx and accumulation of adenosine diphosphate (ADP), adenosine monophosphate (AMP), and phosphate in hepatocyte lead to mitochondrial membrane permeability transition (MMPT) [12]. MMPT induces mitochondrial swelling and allows soluble molecules with a molecular weight of less than 1500 kDa to pass through the "ionic mega-channels" of the mitochondrial membrane and further enhances the liver damage [13]. Furthermore, warm ischemia decreases phospholipid cardiolipin (diphosphatidylglycerol), which is an essential predominant mitochondrial phospholipid and increases oxidized form of cardiolipin in hepatocyte [14]. These pathways cause mitochondrial dysfunction and promote cell death (Fig. 6.1).

After procurement with an organ preservation solution, the liver is stored on ice (at 4 degrees Celsius). This second ischemic phase is called cold ischemia time (CIT) and is associated with cold ischemic injury until liver is successfully reperfused in the recipient. During this time, liver metabolism is reduced, and ATP stores within cells are depleted less rapidly [15]. SEC is sensitive against cold storage [16, 17]. ATP depletion during the ischemic phase in SEC induces not only mitochondrial dysfunction but also actin-fiber disassembly [18] and the release of matrix metalloproteinases (MMP-2, MMP-9) [19]. This results in an expression of von Willebrand factor (vWF) and P-selectin on the endothelial cell surface, which promotes thrombosis after reperfusion [20].

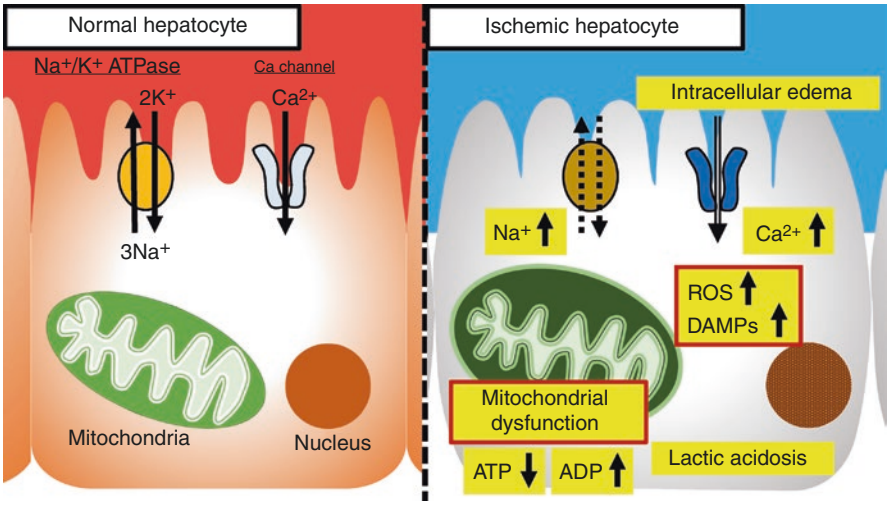

Fig. 6.1 Mechanism of cell damage in ischemic period. *Abbreviation*: ROS reactive oxygen species, DAMPs damage-associated molecular patterns, ATP adenosine triphosphate, ADP adenosine diphosphate

Molecular Mechanism of IRI: Reperfusion Period

While the warm and cold ischemic phases condition the liver cells to preservation injury, it is the reperfusion phase when the apoptotic and necrotic pathways are executed, and the cell death occurs. Reperfusion increases the intracellular Ca^{2+} concentration and the production of reactive oxygen species (ROS) by several pathways such as neutrophil migration and inflammatory cytokines and chemokines such as TNF-α, IL-1β, IFN-γ, and IL-12 (Fig. 6.2). This leads to irreversible cellular and mitochondrial changes and cell death.

Reperfusion injury involves numerous parenchymal cells as well as non-parenchymal cells that interact in a network of simultaneous events prompting pro-inflammatory change and cell injury.

Hepatocytes develop cell swelling, lactic acidosis, and mitochondrial dysfunction induced by ATP depletion and hypoxia during the ischemic phase. After reperfusion, the surplus oxygen is not used in the respiratory chain which results in the generation of oxygen free radicals that lead to cell death [21]. In addition, damaged hepatocytes secrete DAMPs such as HMGB-1, histone/DNA, and ATP to activate Kupffer cells and neutrophils as a sterile inflammation [22]. These productions of ROS and DAMPs promote more severe damage of hepatocytes.

Sinusoidal endothelial cells (SECs) play a key role to control sinusoidal blood flow, oxygen supply, and delivery of nutrients for liver tissue by regulating vascular tone [23]. SEC injury gives rise to cell swelling as well as detachment. Mitochondrial injury results in decreased NO (nitric oxide) production and depletion of NO stores.

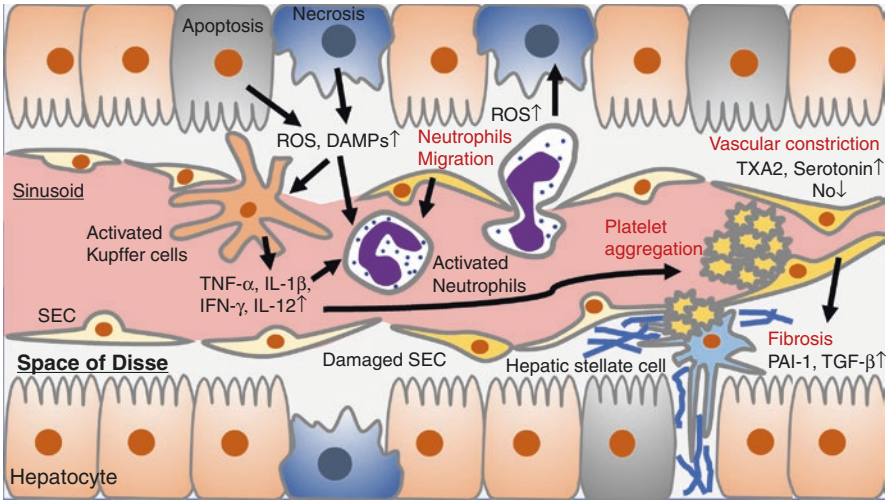

Fig. 6.2 Main mechanism of ischemia-reperfusion injury. *Abbreviation*: SECs Liver sinusoidal endothelial cells, ROS reactive oxygen species, DAMPs damage-associated molecular patterns, TNF-α tumor necrosis factor-α, IL-1β interleukin-1β, IFN-γ interferon-γ, IL-12 interleukin-12, TXA2 thromboxane A2, NO nitric oxide, PAI-1 plasminogen activator inhibitor-1, TGF-β transforming growth factor-β

The balance between the vasorelaxation effect of NO and the vasoconstrictor effects of TXA2 (thromboxane A2) from platelet becomes disturbed, which leads to an increase of the vascular tone and decrease of the hepatic blood flow [24, 25]. In addition, activated SECs express P-selectin which enhances platelet adhesion and activation. Adhesion of platelet further promotes cell death and decreases sinusoidal microcirculation by inducing congestion and reducing flow [26, 27].

Kupffer cells play a central role in the pro-inflammatory cascade after reperfusion. Under normal circumstances in the absence of preservation injury, Kupffer cells present circulating antigens from the blood to T cells and induce tolerogenic T cells to produce anti-inflammatory cytokines (IL-10) [28]. In contrast, during IRI, Kupffer cells recognize DAMPs from hepatocytes and SEC through Toll-like receptors 3, 4, and 9 and secrete pro-inflammatory cytokines such as TNF-α, IL-1β, IFN-γ, and IL-12. These mediators induce neutrophil migration to the liver and the release of ROS from neutrophils and promote platelet adhesion on SEC [29–31].

Neutrophils are main actors during IRI. After reperfusion, the complement system is activated and enhances production of complement protein 3a (C3a), complement protein 5a (C5a), and the membrane attack complex (MAC). This complement activation leads to the recruitment of pro-inflammatory cells including neutrophils to the damaged liver and promotes in cell death [32, 33]. In the liver, neutrophils detect chemokines such as CXCL1 and CXCL2 secreted by activated Kupffer cells, which guides them into the sinusoids [34]. Chemokines also bind to glycosoaminoglycans on the vascular surface of SEC. When neutrophils reach the SEC, chemokine-chemokine receptor interactions activate the integrins. Neutrophils bind SEC through integrin CD11b/CD18a (Mac-1) on the neutrophils and intracellular adhesion molecule-1 (ICAM-1) on SEC [35]. Neutrophils also respond to inflammatory signals (DAMPs) in the liver, such as high-mobility group box 1 (HMBG-1) and DNA fragment released from injured hepatocytes. These substances enhance the production of ROS from neutrophils through DAMP receptors including Toll-like receptor (TLR) [36]. DNA fragments activate TLR9 on neutrophils, which plays a significant role in neutrophil migration, activation, and production of ROS [37]. Damaged hepatocytes release the HMGB-1, which activates TLR4 and amplifies hepatic injury [38]. This cascade causes further migration of inflammatory cells, and liver tissue damage creates a positive feedback loop [39].

Platelets have been recognized as important players within the hepatic reperfusion injury cascade. Activated Kupffer cells by DAMPs from hepatocytes and SEC release TNF-α. TNF-α induces the P-selectin on SEC and promotes platelet adhesion and activation [40]. This leads to microthrombosis in sinusoid and induces apoptosis of SEC [27]. On the other hand, SECs express CD39 (ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1)) on the luminal side, which is a regulator of ATP and ADP in platelets. When SECs are injured, CD39 activity decreases and ADP increases in the extracellular environment. ADP is a key inducer of platelet aggregation, and platelets are activated [41]. Furthermore, damaged SECs can result in endothelial fenestrations allowing platelets to enter the space of Disse. Platelets attach to the collagen type III in space of Disse and aggregate, which is called “extravasated platelet aggregation” [42]. Activated platelets release negative mediators, such as thromboxane A2 (TXA2) [24],

serotonin [27], plasminogen activator inhibitor-1 (PAI-1) [43], and TGF-β [44]. TXA2 and secretin can induce portal hypertension, while PAI-1 and TGF-β promote hepatic fibrosis and suppression of liver regeneration.

Therapeutic Strategies

Minimizing Ischemia Times

It is important to realize that minimizing ischemia is a low-cost and highly effective way to reduce preservation injury in liver grafts from DCD donors. Warm ischemia has severe effects in DCD grafts, but the length of warm ischemia does not linearly correlate with the severity of injury [45]. Prolonged CIT of more than 8 hours is a risk factor for graft failure and ischemic cholangiopathy [6–9, 46–48]. To shorten the cold ischemia time, some institutes start the recipient hepatectomy prior to the procurement team's return when using grafts from DCD donors [7, 49]. Other possible strategies to minimize warm ischemic injury are ante-mortem procedures such as donor anticoagulation, administration of vasodilators, and femoral cannulation for regional perfusion. Limitations include legal and cultural restrictions as well as the limited scientific evidence of the beneficial effects of antemortem strategies on postoperative outcomes [50].

Thrombolytic Therapy

Biliary complications are common after DCD liver transplantation. Ischemic-type biliary lesions (ITBLs) and ischemic cholangiopathy (IC) occur in 12% to 50% of DCD transplantations resulting frequently in graft loss [7, 10, 51, 52]. These biliary complications are thought to be caused by insufficient arterial blood supply of the intra- and extrahepatic bile ducts. While the liver parenchyma receives the dual blood flow from the hepatic artery and the portal vein, the blood supply for bile duct comes only from hepatic artery via peribiliary vascular plexus [53]. Dries et al. analyzed biliary injury of 128 liver transplants including 29 from DCD donors. The authors demonstrated that 92% of the bile epithelium was injured at the end of cold storage with a luminal epithelium loss >50%. In addition, the peribiliary glands which promote biliary regeneration were damaged in 57% of the superficial periluminal side and 18% in deep bile duct wall. Furthermore, the mural stroma necrosis, vascular injury, intramural bleeding, and inflammation worsened after reperfusion [54].

To dissolve the microthrombi and to obtain sufficient blood flow in biliary microcirculation, some transplant programs used thrombolytic therapy during back-table preparation or implantation of the liver graft. Hashimoto et al. reported their experience with the tissue plasminogen activator (tPA) flush on the back table for 22 DCD liver grafts [55]. Several other groups reported the use of tPA during DCD liver transplantation, and a systematic review indicated that thrombolytic therapies in

DCD liver transplantation statistically decreased ITBLs and retransplantation rate and improved 1-year graft survival without the risk of increasing blood transfusion [53]. However, the efficacy of thrombolytic therapy is still controversial in the absence of randomized controlled trials and the differences within the tPA injection protocols. In addition, there is a significant variation in functional DWIT and CIT between studies, which makes the comparison of the different trials difficult [56]. This topic is covered in more depth in Chap. 8, "tPA and Thrombolytic Therapy."

Machine Perfusion

Machine perfusion (MP) is a novel strategy for preservation of DCD grafts. Machine perfusion can be performed as in situ and ex situ machine perfusion. Ex situ machine perfusion has been performed at physiologic temperatures (warm perfusion) with oxygen and nutrition, while cold (4 °C degrees) ex situ machine perfusion has been developed with or without oxygen.

Ex situ MP is currently categorized into three groups: post-static cold storage (SCS) MP, replacing cold storage with MP, and ischemia-free liver transplantation without warm or cold ischemic preservation [57]. In post-SCS MP, liver graft is perfused after cold storage and transport of the liver graft to the recipient hospital. With preservation MP, the perfusion starts at the donor hospital after cold flush and continues until transplantation. Ischemia-free liver transplantation is a novel method to connect the perfusion machine to the donor vessels and continue perfusion until reperfusion without any ischemia. In clinical settings, perfusate temperature and perfusate type differ between each perfusion strategy. Two different temperature settings have been used during machine perfusion: hypothermic MP (HMP, 0–12 degrees) and normothermic MP (NMP, 35–38 degrees). Although each perfusion has its own protective effects against IRI, the basic merits of MP in both settings are decreased preservation injury, graft assessment, and graft reconditioning, compared with SCS.

Hypothermic Oxygenated Ex Situ Machine Perfusion

Oxygenated HMP increases ATP and attenuates the inflammatory IRI cascade compared with static cold storage. Oxygenated HMP reduces mitochondrial injury [58] and improves ATP storage during preservation [15]. Furthermore, compared with SCS group, HMGB-1, which is one of DAMPs and representative of nuclear damage, was lower, Kupffer cell activation was suppressed, and expression of vWF on LSECs was significantly decreased in HMP group [58]. In a matched control clinical trial, Schlegel et al. demonstrated that liver grafts from DCD donors with oxygenated HMP had significantly lower graft loss at 5 years after transplantation (HMP-DCD 8% vs SCS-DCD 32%) [59]. Oxygenated HMP in DCD liver grafts also decreased biliary injury after transplantation by reducing biliary fibrosis with less activated myofibroblasts compared with SCS-preserved grafts [60]. In a clinical trial, Rijn et al. demonstrated that oxygenated HMP-DCD liver transplantation reduced IRI of the bile duct when compared with DCD-SCS controls, with less

mural stroma necrosis and better preservation of periluminal peribiliary glands after reperfusion [61, 62]. This was associated with a significantly lower rate of graft loss by ischemic cholangiopathy (HMP-DCD 0% vs SCS-DCD 10%) [59] (Fig. 6.3a).

Normothermic Ex Situ Machine Perfusion (NMP)

The liver is metabolically active during normothermic ex situ perfusion, which offers the opportunity to assess the hepatocyte and cholangiocyte viability. Aminotransferase levels in the perfusate can be determined as hepatocyte injury maker. As hepatocellular functional parameters, lactate clearance, bile volume and quality (bile pH, bicarbonate and glucose levels), and glucose consumption can be measured [63]. Several markers during normothermic ex situ perfusion were reported to be associated with post-transplant primary non-function liver. Mergental et al. defined viability criteria during NMP. These viability criteria consisted of lactate clearance, pH maintenance, bile production, vascular flow patterns, and liver macroscopic appearance based on data of human discarded livers [64].

NMP has been demonstrated to replenish ATP levels in hepatocyte [65–68], significantly lower aminotransferase after transplantation, and result in better survivals in pig DCD liver transplant models [69]. Recently, Jassem demonstrated that NMP leads to an upregulation of gene expression of tissue regeneration and platelet function and a reduced expression of immune-related genes. NMP induces regulatory T cells and reduces the proportion of CD4-positive T cells producing IL-2, IL-4, IFN-γ, and IL-17 and CD8-positive T cells producing IFN-γ. This results in a suppression of neutrophil infiltration and reduction of parenchymal cell death compared with SCS [70]. Nasralla et al. reported the first randomized trial of NMP with 220 human livers including 53 DCD livers. They demonstrated lower level of graft injury (peak AST NMP 488.1 vs SCS 964.9 IU/L), lower discarded rate (NMP 11.7% vs SCS 24.1%), and lower rate of early allograft dysfunction (NMP 10.2% vs SCS 29.9%) [71].

NMP also decreases biliary IRI and promotes bile regeneration in DCD liver grafts. NMP-DCD livers showed mild epithelial injury, while SCS-DCD showed diffuse epithelial injury in extrahepatic duct and the peribiliary gland. Furthermore, Ki-67 staining revealed active cholangiocyte regeneration in NMP-DCD livers in the bile duct lumen and superficial and deep peribiliary gland, whereas Ki-67 staining was absent in SCS-DCD [72].

As a new type of perfusion, Boteon et al. demonstrated that a combined perfusion of HMP and NMP (2-hour HMP and 4-hour NMP) had 1.77 times higher ATP levels and lower tissue expression markers of oxidative injury (4-hydroxynonenal) and inflammation (CD11b, vascular cell adhesion molecule) compared with 6-hour NMP in ten human discarded livers (DCD 70%) [73] (Fig. 6.3b).

Normothermic In Situ Regional Perfusion (NRP)

Normothermic in situ regional perfusion was developed to assess the organ function in cDCD and uDCD prior to organ excision in the donor. NRP restarts blood flow to the abdominal organs after death declaration via extracorporeal membrane oxygenation (ECMO) prior to the graft cooling. Watson et al. compared NRP-DCD ($n = 43$)

a

Author	Year	Donor type, N	Preservation	Perfusion	Perfusion duration (min)	WIT (min)	CIT (min)	peak AST/ALT (U/L)	PNF	Ischemic cholangiopathy	Graft survival
Dutkowski et al.	2015	DCD 25	HMP	PV only, open circuit	118 (101-149)	18 (17-21)	188 (141-264)	1808 (AST) / 1239 (ALT)	0%	0%	90% (1 year)
		DCD 50	SCS	–	–	17.5 (16-20)	395 (349-447)	2848 (AST) / 2065 (ALT)	6%	22%	69% (1 year)
		DBD 50	SCS	–	–	–	386 (286-425)	1473 (AST) / 1124 (ALT)	0%	4%	96% (1 year)
Rijn et al.	2016	DCD 10	HMP	HA, PV, open circuit	126 (12-135)	15 (13-17)	NA	966 (ALT)	0%	10%	100% (1 year)
		DCD 20	SCS	–	–	16 (14-18)	503 (476-526)	1658 (ALT)	0%	4.5%	67% (1 year)
Schlegel et al.	2019	DCD 50	HMP	PV only, open circuit	120 (96-144)	36 (31-40)	264 (210-312)	1226 (ALT)	0%	8%	94% (5 year)
		DCD 50	SCS	–	–	25.5 (21-31)	282 (258-318)	1331 (ALT)	4%	22%	78% (5 year)
		DBD 50	SCS	–	–	–	300 (240-300)	1425 (ALT)	2%	2%	Showed in Figure

b

Author	Year	Donor type, N	Preservation	Perfusion	Perfusion duration (min)	WIT (min)	CIT (min)	peak AST/ALT (U/L)	PNF	Ischemic cholangiopathy	Graft survival
Selzner et al.	2016	10 (DCD 2, DBD 8)	NMP	HA, PV, closed circuit	480 (340-580)	DCD: NA (28-29)	NA	619 (ALT)	0%	0%	100% (3 months)
		30 (DCD 6, DBD 24)	SCS	–	–	DCD: NA (28-29)	534 (523-783)	949 (ALT)	0%	0%	100% (3 months)
Ravikumar et al.	2016	20 (DCD 4, DBD 16)	NMP	HA, PV, closed circuit	5580 (210-1110)	DCD: 21 (14-31)	NA	417 (AST)	0%	NA	NA
		40 (DCD 8, DBD 32)	SCS	–	–	DCD: 15 (9-23)	534 (252-684)	902 (AST)	0%	NA	NA
Bral et al.	2017	10 (DCD 4, DBD 6)	NMP*1	HA, PV, closed circuit	690 (198-1350)	21 (16-26)	167 (95-293)	1252 (AST)	0%	0%	NA
		30 (DCD 8, DBD 22)	SCS	–	–	NA	233 (64-890)	839 (AST)	0%	NA	NA
Nasralla et al.	2018	121 (DCD 34, DBD 87)	NMP	HA, PV, closed circuit	547.5 (372.5-710.5)	DCD: 21 (17-25)	126 (106.5-143.0)	488.1 (AST)	0.8%	DBD 7.4% DCD 11.1%	95% (1 year)
		101 (DCD 21, DBD 80)	SCS	–	–	DCD: 16 (10-20)	465 (375-575)	964.9 (AST)	0%	DBD 5.4% DCD 26.3%	96% (1 year)

c

Author	Year	Donor type, N	Preservation	Perfusion	Perfusion duration (min)	WIT (min)	CIT (min)	peak AST/ALT (U/L)	PNF	Ischemic cholangiopathy	Graft survival
Watson et al.	2018	DCD 43	NRP	NRP	123 (103-130)	30 (26-36)	382 (303-502)	633 (ALT)	0%	0%	97.7% (90 days)
		DCD 187	SCS	–	–	27 (22-32)	444 (395-493)	1154 (ALT)	7%	27%	89.8% (90 days)
Hessheimer et al.	2019	DCD 95	NRP	NRP	120 (79-136)	18 (13-23)	315 (265-365)	N	2%	2%	88% (3 years)
		DCD 117	SCS	–	–	22 (19-26)	340 (285-383)	N	3%	13%	76% (3 years)

Fig. 6.3 Clinical evidence of ex vivo machine perfusion for DCD liver graft. (**a**) Hypothermic ex situ perfusion. (**b**) Normothermic ex situ perfusion. ∗1 1 liver was not perfused because of portal vein torsion. (**c**) Normothermic regional perfusion. *Abbreviation*: WIT warm ischemia time, CIT cold ischemia time, PNF primary non-function, DCD donation after cardiocirculatory death, DBD donor after brainstem death, HMP hypothermic machine perfusion, NMP normothermic machine perfusion, NRP normothermic regional perfusion, HA hepatic artery, PV portal vein, NA not available

with non-NRP-DCD (n = 187) liver transplantation. The NRP-DCD group had decreased liver injury (peak ALT; 633 vs 1154 IU/L), lower early allograft dysfunction rate (3.5% vs 5.0%), and lower IC (0% vs 27%) [74]. Hessheimer et al. reported that NRP group showed significantly lower ITBLs (2% vs 13%) and lower graft loss (12% vs 24%) compared with super-rapid recovery group [75] (Fig. 6.3c).

Conclusion

IRI in liver transplantation is induced by a simultaneous activation of parenchymal and non-parenchymal cells within the liver. In liver grafts from DCD donors, the prolonged ischemia times are a crucial factor for postoperative liver function and bile duct injury. To reduce graft injury and improve post-transplant graft function, minimizing WIT and CIT is critical. In addition, novel preservation methods, such as cold and warm ex situ perfusion, as well as in situ regional perfusion, are promising approaches to improve reperfusion injury in DCD grafts. Currently, several organ perfusion settings demonstrated feasibility and improved results in transplantation with DCD grafts. It is expected that future research will result in the development of new targeted drugs for more effective protection against IRI and reconditioning of grafts from DCD donors in the future.

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