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REVIEW

Strategies to rescue steatotic livers before transplantation in clinical and experimental studies

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Abstract

The shortage of donor livers has led to an increased use of organs from expanded criteria donors. Included are livers with steatosis, a metabolic abnormality that increases the likelihood of graft complications post-transplantation. After a brief introduction on the etiology, pathophysiology, categories and experimental models of hepatic steatosis, we herein review the methods to rescue steatotic donor livers before transplantation applied in clinical and experimental studies. The methods span the spectrum of encouraging donor weight loss, employing drug therapy, heat shock preconditioning, ischemia preconditioning and selective anesthesia on donors, and the treatment on isolated grafts during preservation. These methods work at different stages of transplantation process, although share similar molecular mechanisms including lipid metabolism stimulation through enzymes or nuclear receptor *e.g.*, peroxisomal proliferator-activated receptor, or anti-inflammation through suppressing cytokines *e.g.*, tumor necrosis factor-α, or antioxidant therapies to alleviate oxidative stress. This similarity of molecular mechanisms implies possible future attempts to reinforce each approach by repeating the same treatment approach at several stages of procurement and preservation, as well as utilizing these alternative approaches in tandem.

Key words: Liver transplantation; Steatosis; Donor liver; Clinical; Experimental

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INTRODUCTION

Liver transplantation (LTx) is a successful therapy for end-stage liver disease, but it is severely restricted by the donor organ shortage[1-4]. In an effort to increase the size of the donor pool, livers from expanded criteria donors (ECD), including steatotic livers, are increasingly being used[3]. The current contribution of steatotic livers is marginal however, since the majority has an increased risk of ischemia-reperfusion injury (IRI) after LTx[3]. Here we review the clinical and experimental attempts to minimize this risk by modifying the quality of livers at different stages, on donors and isolated liver grafts during preservation, before LTx. We begin by briefly introducing the etiology, pathophysiology, categories and experimental models of hepatic steatosis, provide a summary of the techniques applied on donors and liver grafts, and con-

clude by providing future perspectives for these experimental approaches.

ETIOLOGY OF HEPATIC STEATOSIS

Livers are defined as steatotic or fatty when they have excessive (above 5% of wet liver weight) accumulation of lipids, mainly triglycerides. Steatosis occurs when lipid ingestion and synthesis exceed export and consumption in livers[5]. Based on the patient's alcohol consumption[5,6] fatty liver disease is classified as either alcoholic or non-alcoholic in origin (AFLD and NAFLD). Alcohol can decrease fatty acid oxidation and lipoprotein excretion, and increases the esterification of fatty acid to triglycerides *via* alpha-glycerophosphate[6]. Several factors contribute to NAFLD including dietary lipid overload; insulin resistance, which results in abnormal lipid metabolism; and ingestion of drugs or toxins such as carbon tetrachloride, which induces a decrease in apoprotein synthesis and lipid export[5-8].

PATHOPHYSIOLOGY OF HEPATIC STEATOSIS

A complicated pathophysiology has been exposed although the exact pathways have not been completely elucidated. Briefly, a "two-hit" theory is the current consensus, with the first "hit" being the initial abnormal fat accumulation and the second "hit" being the consequent inflammation ("steatohepatitis") leading to fibrosis and cirrhosis[6]. In the first "hit", excessive fat accumulates in vacuoles within hepatocytes, increasing cell volume and narrowing sinusoidal lumens[5,9]. This impairs microcirculation and decreases nutrient, oxygen and waste exchange. Excessive non-esterified fatty acids in hepatocytes inhibit β-oxidation thereby reduce acetyl-coenzyme A production[10]. Mitochondria uncoupling protein-2 is upregulated and associated with a dysfunction of adenosine triphosphate (ATP) synthesis[11-13]. Fat-induced hyperactivity of cytochrome P-450 enzymes increases the production of reactive oxygen species (ROS)[6,14]. ROS in turn lead to lipid peroxidation, phospholipid depletion and membrane dysfunction[6,14,15] as well as the release of inflammatory cytokines such as tumor necrosis factor (TNF)-α[14,16,17]. Inflammation occurs gradually and marks a significant downturn in disease progression as the second "hit"[18,19]. Alcohol can exacerbate oxidative injury and Kupffer cell activation[20], though alcoholic and non-alcoholic steatohepatitis (ASH and NASH) are thought to progress similarly[18].

Steatotic livers have reduced tolerance to ischemia due to low ATP stores, and are thus prone to early onset of acidosis and cellular edema during standard liver preservation method, static cold storage (SCS)[21]. Edema significantly impairs hepatic microcirculation further than the preceded impairment induced by excessive fat. Moreover, steatosis-induced inflammation is not addressed in the present liver SCS preservation solutions (Table 1).

Upon reperfusion, a complex inflammatory response involving Kupffer cells, lymphocytes, neutrophils, numerous cytokines[22] and nuclear factor kappa-B (NFκB)[23], is inevitably worse in steatotic livers compared to non-steatotic livers. Oxidative stress is also exacerbated *e.g.*, through xanthine oxidase[24,25]. The microcirculation is deteriorated further due to adherence of platelets in the sinusoids[22]. Strategies to minimize fat content and ameliorate the inflammatory and oxidative injury of steatotic livers are essential for improving these organs for transplantation.

CATEGORIES OF HEPATIC STEATOSIS

Besides AFLD and NAFLD in etiology, steatosis is also classified as "macro-" or "micro-" in histology based on the size and number of the fat vacuoles and on the location of the nucleus in the hepatocytes[5,26]. Macrosteatosis has a single fat vacuole larger than the nucleus filling the majority of the cell and pushing the nucleus to the periphery. Microsteatosis has many small fat vacuoles surrounding the nucleus in the central zone of the hepatocytes, and has more LTx success than macrosteatosis[3,27]. Steatosis can also be classified based on the proportion of hepatocytes affected, being mild (< 30%), moderate (30%-60%), or severe (> 60%), with incremental risk of graft dysfunction after LTx[5,26].

CLINICALLY APPLIED STRATEGIES

Approaches to improve steatotic livers before LTx have been tested in a handful of pilot clinical studies on living donors or donors after brain death (DBD) (Table 2). The interventions focused on reducing excessive fat (the first "hit") through limiting lipid intake and increasing lipolysis, or stimulating factors likely to be protective against inflammation and oxidative stress (the second "hit") of steatohepatitis. Living donors, though a minority in western countries[1,2], are used extensively in Asia[28] and are theoretically amenable to therapies before procurement after ethical concern is taken into account[29]. DBD livers, which comprise the majority of donor organs for LTx in western countries, could be treated between brain death declaration and organ procurement since circulation is maintained until procurement. Livers from donors after cardiac death (DCD) are seldom utilized when they have steatosis because they experience a period of warm ischemia (WI) before procurement[3] and thus were normally considered as unacceptable with two defects (steatosis and WI). Currently there are no attempts to rescue steatotic DCD livers.

Physical exercise and dietary intervention

Physical exercise and dietary restriction are general therapies for NAFLD patients, independent of whether or not they are organ donors[30,31]. But this treatment normally needs several months and might be risky to increase the mortality of recipients during the waiting time for treat-

Table 1 Intrinsic composition of preservation solutions in this review

		UW	HTK	Celsior	IGL-1	UW-gluconate	Kreb-Henseleit	MEM
Electrolyte	Na	25	15	100	125	125	143	143.4
(mmol/L)	K	120	9	15	30	25	5.9	5.4
	Mg	5	4	13	5	5	1.2	0.8
	Ca		0.0015	0.25		0.5	1.25	1.4
	Cl	20	32	42		1	125.2	124
	SO4	5			5		1.2	0.8
Buffer	Phosphate	25			25	15	1.2	1
(mmol/L)	Bicarbonate					25	25	26.2
	HEPES					10	20	
	Histidine		198	30				0.27
Antioxidant	Glutathione	3		3	3	3		
(mmol/L)	Allopurinol	1			1	1		
	Mannitol		38	60				
	NAC						5	
	Vit C							0.25
	Glucose					10	5	5.5
Metabolic Substrates	Adenosine	5			5	5		
(mmol/L)	Adenine					5		
	Ribose					5		
	Tryptophan		2					
	Ketoglutarate		1					
	Glutamate			20				
	Amino acid							0.7
Impermeants	Lactobionate	100		80	100			
(mmol/L)	Gluconate					95		
	Raffinose	30			30	30		
Colloid	HES	50						
(g/L)	PEG				1	50		
Other intrinsic	Insulin	100 U						
compounds	Dexamethason	8 mg						
	Penicillin	40 U						
	Phenol-red (mmol/L)							0.03
Osmolarity		340	300	363	330	360	320	310

UW: University of Wisconsin; HTK: Histidine-trypotphan-ketoglutarate; IGL-1: Institut Georges Lopez-1; MEM: Minimum essential cell culture medium; HEPES: N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid; NAC: N-acetylcysteine; HES: Hydroxyethyl starch; PEG: Polyethylene glycol.

ing donors[32]. An intensive protocol might be a solution, which was already reported to successfully reduce macrosteatosis on obese human living donors in 2-8 wk, through exercise burning 600 kcal/d, a protein-rich diet of 1000 kcal/d, and bezafibrate, an anti-hyperlipidaemia drug, at 400 mg/d[33]. However, supplementary glucose to donors a few hours before living donor liver transplantation was recommended to supply additional energy reserves, since fasting before procurement can induce glycogen depletion, decrease glycolytic ATP generation, and compromise graft transplantability[34].

The lack of omega-3 polyunsaturated fatty acids (PUFA) has been recognized in the development of NAFLD because they can activate peroxisomal proliferator-activated receptor (PPAR)-α, suppress sterol regulatory element-binding protein-1, improve microcirculation, and reduce Kupffer cell activity and inflammation[35-43]. The mechanism on microcirculation might work through reducing TXA2 synthesis after manipulating the composition of hepatic lipid (omega-3: omega-6 PUFA ratio)[43]. Based on experimental success on rodents[35-43], omega-3 PUFA was shown to be effective on NAFLD clinically after treatment at 1-2 g/d for 6-12 mo[44-46]. This has not been applied specifically on living donors but is expected to be a safe and promising approach.

Pharmacological preconditioning

Many drugs are being used clinically to treat NAFLD by decreasing lipid intake[47-49], stimulating lipid metabolism[50-53], or improving insulin sensitivity[54-57]. Ursodeoxycholic acid, a natural bile acid, was used as a non-specific hepato-protector to treat NAFLD in a pilot clinical study[58], but afterward was revealed to be controversial[59]. Pentoxifylline was used against NASH and ASH in patients[60,61] due to the effect of reducing TNF-α by inhibiting phosphodiesterase[62] and lessening oxidative stress by increasing glutathione[63]. But to date, only bezafibrate was used to treat human living donors for LTx[33]. This drug works through activating PPAR-α and β/δ to stimulate lipid metabolism and decrease fat content in livers[64,65]. While there are other candidate drugs that could potentially be taken by living donors, concerns of significant side effects are limiting their use[66,67].

Ischemic preconditioning

Though extended ischemia is deleterious to organs, it has been recognized since the 1980s that a short period of ischemia with subsequent reperfusion triggers natural de-

Table 2 Overview of the clinical and experimental strategies in this review

	Clinically applied	Experimentally applied
Donors	Dietary	Pharmacological
	Pharmacological	Heat shock
	Ischemic (except remote ischemic)	
	Anesthetic	
Liver grafts		SCS preservation
		New solution
		Pharmacological additives
		Additional oxygen (VSOP)
		MP preservation
		MP solely
		MP + pharmacological additives
		Flushing
		Pharmacological additives

SCS: Simple cold storage; MP: Machine perfusion; VSOP: Venous systemic oxygen persufflation.

fense mechanisms against future ischemic insults and protects the organ against IRI[68]. Ischemic preconditioning (IP) was first observed in kidneys and hearts[69,70], and then employed for clinical liver resections and transplantation[68]. It can be applied intermittently[71], or as a single short period (5-10 min) of ischemia followed by 10-15 min reperfusion before cold flush during liver procurement[72,73]. Franchello *et al*[73] have used the technique clinically on marginal DBD livers including steatotic livers, and observed a reduction of hepatocyte swelling and enzyme release in recipients after LTx.

IP is protective because ATP consumption during the short ischemic period increases endogenous adenosine and nitric oxide[74]. Adenosine protects sinusoidal endothelial cells through adenosine A2 receptor[75]. Cyclic adenosine monophosphate (cAMP) worked as the second messenger, but whether increasing or blocking cAMP would be beneficial was still controversial[75,76]. Nitric oxide is a vasodilator, and further it attenuates the release of TNF-α, decreases the injurious interleukin (IL)-1β and increases the anti-inflammatory IL-10[77]. Another effect of the intermittent ATP consumption is to increase the level of adenosine monophosphate (AMP), which stimulates AMP-activated protein kinase (AMPK). AMPK can regulate an energy-conserving state, decrease inflammation through inhibiting NFκB, and induce the synthesis of nitric oxide as well[78-80]. Overall, the advantages of IP were an improved microcirculation[81,82], mitochondrial permeability transition and mitochondrial function[83], cytochrome oxidase C activity and tissue oxygenation[82,84], and the reduction of oxidative stress such as the xanthine accumulation and xanthine oxidase activity[85,86].

Interestingly, IP can work remotely, *e.g.*, liver IP decreased lung IRI[25,87] and limb IP decreased liver IRI[88-90]. This possibly works through some protective agents, *e.g.*, heme oxygenase-1, endothelial nitric oxide synthase, and nuclear protein High Mobility Group-Box 1[88-90]. But foreseeable ethical concerns exist with the logistics of implementing this technique in human donors.

Anesthesia selection

During clinical liver resection, Beck-Schimmer *et al*[91] observed the superiority of volatile anesthesia using sevoflurane in the prevention of hepatic injury after reperfusion compared to intravenous anesthesia with propofol. The mechanism was suggested to be the increased synthesis of nitric oxide with sevoflurane, which alleviates some of the effects of IRI as discussed above. Moreover they observed that patients with steatosis did benefit more[91]. This method could be easily applied during the procurement of steatotic livers from both DBD and living donors.

EXPERIMENTAL STRATEGIES

Induction of steatosis in animal models

Steatosis in animal models are established with or without alcohol and classified also as micro- *vs* macro- or as mild, moderate, *vs* severe. The timeline to develop the different classifications largely depends on strain and method used (Table 3).

A common rodent NAFLD model induces cerebral deficiency of the leptin receptor through a genetic mutation that causes the animals to become obese through overeating[92-98]. The methods inducing lipid overload with high fat or cholesterol diets are also quite common. A high fat diet (50% dextrose, 18% casein, 25% lipids, 4% minerals, 1% cholesterol, 0.5% sodium cholate, 0.2% choline, and 1% vitamins) for 7 d induces severe steatosis in rats[99]. A high-cholesterol (2%) diet for 12 wk in rats[82] and 8 wk in rabbits results in moderate steatosis[84]. A cafeteria diet (65% of fat) for 4-15 wk was also used to create NAFLD on rats[100,101]. Recent studies suggested it reflects human metabolic syndrome better than high-fat diet[100,101]. A choline/methionine-deficient diet (CMDD) was another rather common method to develop steatosis, for 4-6 wk or short as 7 d in rodents[5,27,102,103]. Choline is essential for the formation of phosphatidyl-choline and very low density lipoprotein needed for lipid export, while methionine is a good source of methyl groups for the endogenous synthesis of choline. This model could be criticized for not being clinically accurate because NAFLD patients due to CMDD are rather unrealistic. The most rapid induction of NAFLD in rats was with a high-starch, fat-free diet [saccharose (40%), starch (40%), casein (16%), and a mineral and vitamin mix (4%)] administered for 2 d after fasting for 2 d, which can lead to mild to moderate steatosis[104-106]. While none of these models are unanimously agreed to be ideal in replicating clinical NAFLD, the high fat or cholesterol diet model was the most widely used to mimic steatosis in humans.

There are fewer large animal NAFLD models, which usually combine more than one method described above to achieve steatosis. Takahashi *et al*[107] established a dog model using a diet rich in fat and deficient in choline, which produced moderate to severe macrosteatosis after 8-12 wk feeding. Lee *et al*[108] used a high fat and high cholesterol diet (20% kcal from fructose, 46% kcal from fat,

Table 3 Animal models of hepatic steatosis for liver transplantation

Disease	Approaches	Description	Animals	Treatment time
NAFLD	Genetic	Cerebral leptin receptor deficiency	Rodent	
	Dietary	High fat	Rodent	7 d
		High cholesterol	Rodent, rabbits	8-12 wk
		Cafeteria diet	Rodent	4-15 wk
		Choline/methionine-deficient	Rodent	7 d-6 wk
		High starch and fat free after fasting	Rodent	4 d
		High fat and choline deficiency	Dog	8-12 wk
		High fat and high cholesterol, plus choline deficiency or not	Miniature pig	24 wk
		High fat and carbohydrate with streptozotocin for a diabetic state	Landrace pig	5 wk
AFLD	Dietary	Ethanol in liquid diet, intragastric infusion or gavage	Rodent	20 h-9 wk
		Ethanol and high fat diet	Rodent	6 wk
		Ethanol and deficient folate diet	Micropig	12 wk

NAFLD: Non-alcoholic fatty liver disease; AFLD: Alcoholic fatty liver disease.

2% cholesterol and 0.7% cholate by weight) for 24 wk to develop microsteatosis on miniature pigs. When using lowered choline content simultaneously, severe steatosis with fibrosis was observed with increased TNF-α and oxidative stress. A recent study on Landrace pigs used a diet rich in fat (20% in volume) for 5 wk together with intravenous streptozotocin (125 mg/kg) to induce a diabetic state in the last 2 wk; but this treatment led only to mild steatosis[109].

In rodent AFLD models, ethanol was provided in a liquid diet[110-114] or *via* intragastric infusion[115,116]. Different degrees of alcohol exposure have been reported: 5%-8% in the concentration of liquid diet; 35%-40% of total energy consumption; 8-16 g/kg per day; or 150-300 mg/dL of blood ethanol[110-116]. Normally several days are needed for the animals to adapt to the alcoholic diet, and an additional 4-9 wk to observe steatosis. Acute responses to ethanol have also been reported as early as 20 h after feeding 6 g/kg ethanol by gavage on rats[117]. Combined ethanol with high-fat diet to develop steatosis on rats was also reported[118]. Large animal AFLD models are uncommon though micropigs have been fed a diet with ethanol and a deficiency of folate, as a substrate for methionine synthase, with some efficacy[119,120].

Experimental strategies applied to donors

Pharmacological preconditioning: Reduction of oxidative and inflammatory activity with heme oxygenase-1, a microsomal enzyme[121-123], was used intravenously or intraperitoneally on AFLD and NAFLD rats 24 h before liver procurement. It decreased macrophage infiltration, improved portal venous blood flow, bile production, and survival rate after LTx[121-123]. Bortezomib, a NFκB inhibitor, was used intravenously on obese donor rats and reduced IRI after LTx[23]. N-acetylcysteine, a precursor of glutathione, was injected through the mesenteric vein of CMDD rats 15 min before liver procurement and showed a protective effect on IRI in an isolated reperfusion system[124]. The subcutaneous injection of IL-6 for 10 d was observed to be protective against IRI after *in situ* partial ischemia-reperfusion on NAFLD and ALFD mice[92]. The mechanism might be the prevention of cell death and the reduction of TNF-α[125], in addition to stimulating PPAR-α, β-oxidation of fatty acids, and the export of triglycerides and cholesterol[92,125]. Theaflavin, a polyphenol substance extracted from black tea, was tested on CMDD mice and observed to have antioxidant, anti-inflammatory, and anti-apoptotic effect[126]. A multi-drug approach was reported by von Heesen *et al*[127] including N-acetylcysteine as an antioxidant, pentoxifylline for anti-inflammation, glycine to stabilize Kupffer cells, deferoxamine as an iron chelator to reduce ROS, and erythropoietin, melatonin and simvastatin to protect against IRI. In the treated rats they observed no inflammatory response with significantly reduced parenchymal dysfunction and injury compared to the untreated rats.

Heat shock preconditioning: An intriguing experimental method to improve the quality of steatotic donor livers has been to induce protective heat shock proteins (HSPs) endogenously by exposure to heat. Termed "heat shock preconditioning" and applied at 3-48 h before organ procurement by exposing anesthetized donor animals to warm (42 ℃) bath water for 10-15 min[128-132], obese and CMDD rats showed an increased expression of HSP-32 (heme oxygenase-1), -72 and -90[128,129]. These HSPs can decrease TNF-α production[129], improve microcirculation through producing carbon monoxide, and inhibit platelet aggregation[62,64]. Our group has also reported the inhibition of CD4+ T lymphocytes in CMDD rats after LTx with heat shock preconditioning[130]. Other factors might be involved in the treatment since studies on normal and WI rat livers showed IL-6, inter-cellular adhesion molecule-1, and some neutrophil chemo-attractants were also impacted[131,132].

Strategies applied on liver grafts during ex vivo preservation

Obviously, strategies to improve steatotic liver quality during preservation are more desirable than those on donors, as they have no effect on the donor's other organs, and are practical when it's not possible to work on the donor. The clinical standard for liver graft preservation has been SCS with University of Wisconsin (UW)

solution for more than 20 years[3,21]. In the past decade, histidine-trypotphan-ketoglutarate (HTK) solution[133-135] and Celsior solution[136,137] were recognized as having similar efficacy and safety as UW solution; Institut Georges Lopez-1 (IGL-1) was reported to be comparable to UW for healthy human livers[138], and better for steatotic rat livers[139]. Besides the arising new solutions, many adaptations have been suggested through enriching the intrinsic composition of the solutions with additives, or replacing SCS by machine perfusion (MP) preservation in experimental studies to rescue steatotic livers.

Pharmacological additives during SCS preservation: Liver preservation solutions normally comprise electrolytes, pH buffers, antioxidants, metabolic substrates, impermeants with or without colloid, insulin, dexamethasone, and antibiotics (Table 1)[139-143]. The additives for improving steatotic liver preservation were intended to ameliorate metabolism or suppress oxidative injury and inflammation. They were reported to be effective during SCS, despite the reduced metabolic rate of liver grafts during hypothermic preservation.

Addition of IL-6 into UW solution for donor liver flushing and SCS was tested by Sun *et al*[144], leading to improvement in microcirculation and reduced IRI after LTx of NAFLD and AFLD rats. Arnault *et al*[99] added pentoxifylline into UW solution and also observed a benefit to the microcirculation, but the exact mechanism is yet to be identified.

Tolba *et al*[106] added L-carnitine into HTK solution for SCS preservation of steatotic rat livers and observed a reduction of IRI in an isolated reperfusion system. L-carnitine is a nonessential amino acid but is essential for transporting fatty acids through the inner mitochondrial membrane and for β-oxidation[106]. It has also been reported to function as an antioxidant and to stabilize the membrane fluidity and stability *in vitro* and *in vivo*[145].

Ben Mosbah *et al*[146] added carvedilol, a cardiologic drug to block α- and β-adrenergic receptor, into UW solution for SCS of obese rat livers, and reduced oxidative stress and mitochondrial damage after isolated reperfusion. The mechanism might be an enhanced release of nitric oxide that facilitates vasodilatation and ROS scavenging[147]. AMPK activators, trimetazidine and aminoimidazole-4-carboxamide ribonucleoside (AICAR) were also tested as UW additives on obese rat livers by this group. Increased bile production, decreased enzyme release and vascular resistance, and reduced oxidative stress after isolated reperfusion were observed. It was noted that combination of trimetazidine and AICAR was not necessary[95].

Zaouali *et al*[148] tested the use of epidermal growth factor and insulin-like growth factor-I as UW additives and observed that each additive resulted in the improvement of fatty rat liver function after LTx. The mechanisms are suggested to be upregulation of Akt, a cytoprotector[149], and the subsequent over-expression of PPAR-γ. They also tested melatonin as additive in IGL solution and reported its protective role through generating nitric oxide and decreasing oxidative stress and inflammation[150].

Venous systemic oxygen persufflation during SCS preservation: In 1990s, Minor *et al*[151] developed a new method, called venous systemic oxygen persufflation (VSOP) to supply gaseous oxygen to livers during SCS preservation. The oxygen was introduced into hepatic vasculature *via* the suprahepatic vena cava and allowed to exit *via* several small pin pricks on the liver capsule made using an acupuncture needle. This technique was employed on steatotic rat livers for 24 h, and resulted in improved preservation of mitochondria and sinusoidal endothelial linings, less Kupffer cell activation and reduced hepatocellular enzyme release compared to SCS preservation[105]. Recently, by assessing the enzyme release, energy storage, bile production, and cell death during isolated reperfusion, it was demonstrated that application of VSOP for 90 min may rescue steatotic livers after extended (18 h) SCS preservation[152].

MP preservation: MP is an alternative preservation method to SCS[153], which can be further categorized based on the temperature employed[154]. Hypothermic (4 ℃) machine perfusion (HMP) preservation has proven to be superior to SCS for human kidneys[155], and feasible for normal human livers[156]. Normothermic (32 ℃-37 ℃) and sub-normothermic (20 ℃-30 ℃) machine perfusion (NMP and subNMP) preservation have been reported in experimental studies on livers, but mostly on their advantages for DCD models[143,157-162]. MP preservation of steatotic livers is limited, but also reported to be beneficial on preserving energy content and liver function experimentally[109,143,163,164]. The advantages of MP preservation result from continuously supplying nutrients, removing waste products, and maintaining microcirculation[154]. Because MP, especially NMP, provides a physiologically-relevant environment to the isolated donor organ, the quality of liver grafts can be manipulated more efficiently than those simply stored in an ice-box during SCS. Another advantage of MP is the considerable convenience for non-invasively evaluating liver viability, a key issue when ECD livers are used[153].

Bessems *et al*[163] employed HMP preservation with UW-gluconate solution on steatotic rat livers for 24 h and alleviated IRI compared to SCS. Vairetti *et al*[143] preserved steatotic rat livers by subNMP (20 ℃) with Kreb-Henseleit solution for 6 h and obtained similar results. The longest preservation of steatotic livers was the NMP preservation for 48 h in a pig model by Jamieson *et al*[109], who employed blood containing additional insulin and vasodilators as perfusate, and observed a mild reduction of steatosis from 28% to 15%. This NMP setting provided the most physiological environment to liver grafts and lead to an activated function of the isolated organs with sufficient oxygen and nutritional support. This is expected to be the best preservation method in spite of the

highest logistic restriction.

Recently our group has combined NMP and pharmacological preconditioning for decreasing steatosis[96]. A "defatting cocktail" was developed with 6 compounds to activate nuclear receptors such as PPARs, pregnane X receptor, and constitutive androstane receptor, to exert an insulin mimetic effect and to stimulate intracellular cAMP. This cocktail was added into Minimum Essential cell culture medium as a perfusate to stimulate lipid metabolism of obese rat liver grafts preserved at NMP. A significant decrease (50%) in steatosis was observed after 3 h NMP[96].

Other experimental approaches

The solution used for SCS preservation needs to be flushed out of the donor organ prior to implantation to remove possible air bubbles, and preservative components such as high potassium, which are deleterious to the recipient. This provides an opportunity for treatment of ischemic injury, although it has been very little explored. In one study, polyphenols, an antioxidant extracted from Camellia sinensis (green tea), was added to the flushing solution and improved the hepatic injury and survival rate after LTx in a steatotic rat model[117].

Another option is applying preconditioning at several phases. While it appears little explored, in one study Ye *et al*[118] injected glutathione intraperitoneally in rats with hepatic steatosis 2 d before procurement and preserved the livers by SCS with VSOP and additional glutathione. Remarkable improvement on survival rate, liver function, and oxidative stress in liver tissues after transplantation was observed.

SUMMARY AND FUTURE PERSPECTIVES

Based on the understanding on pathophysiology, current strategies to rescue steatotic donor livers work through ameliorating the abnormal lipid metabolism (the first "hit"), and the oxidative stress and inflammation (the second "hit"). Each approach employs various methods at different phases of organ recovery and preservation but generally targets similar molecular mechanisms. Notably, it may be possible to attack the same mechanism but reinforce the effects by applying the treatment at multiple points of organ recovery and preservation process, thereby producing a stacking effect. The use of glutathione on donors and in SCS solution by Ye *et al*[118] is a good example in this direction that demonstrates the therapeutic effects may stack. Stacking other medications, *e.g.*, IL-6 and pentoxifylline could also be promising as these could be given to the donors prior to recovery or readily added into preservation solutions to treat liver grafts. Whether the stacking approach works for different targets in lipid metabolism, oxidative stress or inflammation remain to be elucidated.

Similar to stacking the same/similar pharmacological agents at different stages, it is a reasonable idea to attack the two "hits" simultaneously by stacking medications targeting different pathways. Development of such combinations is usually shunned because it exponentially increases the complexity of development and clinical testing, but given that the disease itself is a very complex phenomenon spanning multiple pathways, it may be unavoidable, and a single silver bullet to treat steatosis simply nonexistent.

An intriguing alternative in development is the efforts to use MP for liver preservation. Especially in near normothermic conditions, MP provides a combined opportunity to improve energy storage, maintain microcirculation, and support pharmacological approaches to decrease fat content and treat IRI. While machine perfusion by definition is more complex than simple storage on ice, it is a very promising approach available in the near future and could be the ultimate solution to rescue steatotic as well as ischemic livers.

Both steatotic livers and DCD livers are highly susceptible to IRI. Therefore, potentially, a method to rescue DCD livers could be also applicable to steatotic livers even with the fat content intact. For instance, MP preservation was able to rescue DCD livers and steatotic livers[143,157-164]. Similarly, perfluorocarbon as an artificial oxygen carrier to improve SCS preservation of DCD livers[165] could also be tested on steatotic livers. If successful, we would then secure both DCD and fatty livers for transplantation, which would boost the organ availability dramatically and resolve donor liver shortage for a decade or more.

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