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Understudied targets of the ischemia-reperfusion injury pathogenesis in liver transplantation

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Abstract

Background. *Liver transplantation is currently the most effective method to treat diseases with end-stage liver failure. Complications are most often associated with the initially severe patient condition, imperfect*

organ preservation methods, the surgical management per se, and immune system incompetence. *The most common complications of transplantation include ischemic reperfusion injury, which occurs to some or another extent in each transplanted organ and worsens the course of the postoperative period. The process is based on complex pathophysiological mechanisms of cell damage due to ischemia and inflammation caused by reperfusion.*

Objective. *To summarize current data on the mechanisms of the ischemic reperfusion injury development in liver transplantation and to find the ways to reduce adverse effects.*

Material and methods. *The analysis of data from foreign and homeland experimental and clinical studies on the pathogenesis of ischemic reperfusion injury in liver transplantation has been performed. The search for literature data was carried out in international databases (PubMed/MedLine, ResearchGate, as well as in the scientific electronic library of Russia (eLibrary.RU) for the period from 2020-2024.*

Conclusion. *The analyzed publications have provided various algorithms for the preservation of donor organs, including those using machine perfusion.*

Keywords: ischemic reperfusion injury, liver transplantation, machine perfusion, cold ischemia, complications of ischemic reperfusion injury

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ALR/ERV1, augmenter of liver regeneration
ALT, alanine aminotransferase
AST, aspartate aminotransferase
ATP, adenosine triphosphate
BDNF, brain-derived neurotrophic factor
ECV, extracellular vesicles
ET-1, endothelin-1
GPX4, glutathione peroxidase 4
GSH, glutathione
HIF, hypoxia-induced factor
HP, hypothermic perfusion
IL, interleukin
IRI, ischemia-reperfusion injury
IT, immunosuppressive therapy
LIP, labile iron pool
MHC-AG, major histocompatibility complex antigens
mRNA, messenger ribonucleic acid
MSC, mesenchymal stem cells
NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells
OLT, orthotopic liver transplantation
PCSK9, proprotein convertase subtilisin/kexin type 9
ROS, reactive oxygen species
SPINK1, pancreatic secretory trypsin inhibitor (PSTI) also known as serine protease inhibitor Kazal-type 1 (SPINK1)
TNF-α, tumor necrosis factor-alpha
TO, transplanted organ
VEGF, vascular endothelial growth factor

Introduction

The imbalance between donor organs available for transplantation and the growing need has shifted the attention of many researchers to finding new strategies for preserving organs previously considered unsuitable for transplantation. Under these conditions, machine perfusion has been recognized as one of the most significant advances in the field of transplantation over the recent 20 years [1]. Strategies of dynamic preservation may provide the opportunity to assess organ quality before implantation or to manipulate certain functions; for example, by weakening the immune response. The most common complications of the

early period of transplantation include ischemia-reperfusion injury (IRI), which occurs to varying extent in each transplanted organ (TO) and worsens the postoperative course [2]. This process is based on complex pathophysiological mechanisms of cell damage resulting from ischemia and inflammation caused by reperfusion.

The objective of the review was to summarize current data on the mechanisms of ischemic reperfusion injury development during liver transplantation and the ways to reduce adverse consequences.

Organ preservation history

The first machine for organ perfusion was introduced in 1935 by Alexis Carrel and Charles Lindbergh. Although the prospects of developing the perfusion machines seemed clear, most of these efforts were abandoned in 1969 when Geoffrey Collins demonstrated the viability of organs after static refrigerated storage [3]. Machine perfusion (MP) is a system that provides tissues at a constant physiological level with the most important components for aerobic, and therefore cell-protective metabolism, namely oxygen and glucose. Typically, a machine perfusion system consists of a perfusate reservoir filled with a preservative solution, which is oxygenated and pumped through the organ using organ-specific parameters of temperature, pressure, and others.

Machine perfusion is a technology that both reduces cold ischemia time, and also serves as a method for assessing the graft viability [3, 4]. Currently, depending on the temperature, there are 3 types of perfusate:

- normothermic perfusion (37°C)
- hypothermic perfusion (HP) (0-4°C)
- subnormothermic perfusion (up to 20°C)

Hypo- and normothermic perfusion are the most studied ones in clinical practice. Hypothermic perfusion can be performed with or

without active oxygenation, since low temperatures allow metabolism to be slowed down in order to reduce oxygen requirements [5]. Hypothermic perfusion aims to attenuate IRI by preserving mitochondria and restoring cellular energy stores to reduce the release of reactive oxygen species after reperfusion. Hypothermic perfusion is a safe, simple method, but it does not allow for the preparation of grafts from expanded criteria donors. On the other hand, normothermic perfusion mimics normal physiological conditions by perfusing blood at a temperature of 37°C. At this temperature, oxygenation is necessary to maintain metabolic processes: the liver receives oxygenated blood, normal cellular metabolism continues, which makes it possible to assess the condition of the organ and its function.

Both hypo- and normothermic MP are able to restore and maintain the energy reserves of the liver graft and reduce IRI after transplantation. Normothermic perfusion is a more promising trend, as it allows expanding the pool of donor organs due to the ability to assess their condition, which will help predict the success of liver transplantation from an expanded criteria donor [6].

Currently, static cold non-perfusion preservation is more often used. An HTK solution (Custodiol) is used as a preservative solution containing the electrolytes reducing the transplanted organ energy needs, the buffer systems inhibiting glycolysis and thereby slowing down the decrease in pH, mannitol reducing graft edema, and other necessary components [7]. Another feature of Custodiol is the presence of dissolved oxygen in its composition. This preservation system, despite its proven efficacy, does not fully carry out an oxygen transfer, which undoubtedly aggravates IRI, mainly in organs obtained from asystolic donors.

Process of tissue damage

Ischemic reperfusion injury is tissue damage due to restoring the blood flow to tissues after a period of ischemia. Restoration of blood flow is necessary to resolve tissue ischemia, but, paradoxically, reperfusion itself causes further damage that threatens the organ function and viability.

The ischemic organ can become involved in the IRI process either independently, or also by causing systemic damage to other organs, leading to multiple organ failure.

This is a complex multifactorial process, which results in the development of an early liver graft dysfunction [8].

In IRI pathogenesis, two phases are distinguished: ischemic and reperfusion.

The first phase is ischemic, characterized by the adenosine triphosphate (ATP) synthesis cessation, which slows down the Na/K pump, imparing the balance of fluid and ions in a cell. This leads to swelling due to the sodium accumulation inside the cell. Ischemia causes an increase in acidity, damage to cellular organelles, including lysosomes, accelerating the secretion of enzymes. Disruption of the electrochemical potential of endothelial cells and an increase in intercellular adhesion molecules promote the migration of neutrophils into the liver parenchyma. This process begins at the ischemia phase with the transmission of signaling molecules through neutrophils, slowing their movement in the bloodstream. Subsequent events include the slow movement of neutrophils along the endothelium until a complete adhesion and their passage into the liver parenchyma, where they destroy cell membranes by releasing lysosomal enzymes.

The second phase is reperfusion, which includes:

• An early phase (3–6 hours after reperfusion): the activation of Kupffer cells and sinusoidal endothelial cells may be associated with liver ischemia caused by impaired microcirculation during reperfusion. An imbalance of vasoconstrictors and vasodilators resulting from impaired synthesis of NO and endothelin-1 (ET-1) plays an important role in this process. With normal function of sinusoidal endothelial cells, the activation of hepatic stellate cells is inhibited through the synthesis of NO, which contributes to normal microcirculation and liver function. However, during liver ischemia, this balance can be disrupted, leading to the activation of Kupffer cells and sinusoidal endothelial cells, which can release proinflammatory factors and induce endothelial cell apoptosis, which can lead to further deterioration of microcirculation and liver function.

• A late phase (6–48 hours after reperfusion), which is induced by the infiltration of neutrophils and CD4+ T lymphocytes, which secrete proteases and other cytotoxic enzymes whose task is to destroy cells [9].

Tissue hypoxia during the preservation leads to a transition to anaerobic glycolysis with the production of two ATP molecules (for comparison: as a result of aerobic glycolysis, 38 ATP molecules are formed), the lactate formation, and, as a result, a decrease in pH in the cell. As a result of the described changes, the functioning of ATP-dependent ion pumps becomes impaired and the regulation of Na^+, K^+ and Ca^{2+} balance are disrupted [10]. Under ischemia conditions, the ATP breakdown predominates over its formation, which is cleaved to hypoxanthine; then, during reperfusion, xanthine oxidase is activated due to changes in pH and the accumulation of intracellular Ca^{2+}, which leads to the breakdown of hypoxanthine to uric acid and superoxide anion that disrupt the permeability of cell membranes. Reactive oxygen species stimulate endothelial cells through activation of nuclear factor kappa-bi (NF-kB), whose main function is to suppress apoptosis. Increased levels

of NF-κB cause systemic inflammation through increased expression of cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins 1, 6, 8 (IL-1, IL-6, and IL-8).

Clinically, the severity of IRI in the transplanted liver is assessed by the activity level of liver transaminases: alanine aminotransferase and aspartate aminotransferase (ALT and AST), however, there is evidence that the numerical values of these parameters do not always correlate with the severity of IRI and cannot be considered as an absolutely reliable indicator of graft dysfunction. A number of studies have shown that the level of IL-8, vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), hypoxia-induced factor (HIF-1α), and platelets in the postoperative period are more reliable markers for prognosis of the liver graft dysfunction development [11]; however, their use as the IRI markers is more labor-intensive for routine practice. Also, to diagnose the IRI severity, a non-invasive method is being considered, such as ultrasound steatofibroelastometry, which has shown greater efficacy compared to the efficacy of fine-needle biopsy [12].

Currently, in order to reduce the consequences of IRI, improve graft survival, reduce the dosage of immunosuppressive therapy (IT) drugs, and expand the pool of donor organs, a targeted therapy is used that is targeted at well-studied links in pathogenesis. Thus, in a number of experimental studies, successful attempts were made to influence the antioxidant system by administering drugs that affect its various components [12, 13].

However, despite these results, the study of new ways to influence the IRI severity and course continues.

The most promising targets for reducing the IRI severity, according to the literature reviewed, include:

1) Liver dendritic cells, which play a role in linking innate and adaptive immunity. They are divided into plasmacytoid dendritic cells (pDCs), which produce interferon-1 (IF-1) to generate an antiviral response, and myeloid dendritic cells (mDCs) responsible for the production of interferon-3 (IF-3). The phenotype and function of liver interstitial dendritic cells are determined by the microenvironment that typically promotes their immunological tolerance. In case of host infiltration with donor myeloid dendritic cells, a change in the phenotype and functions of the cells occurs. Major histocompatibility complex antigens (MHC-AG) regulate donor T-cell responses, contributing to the suppression of immunological reactions. A preclinical study conducted in 2023 demonstrated that these cells were able to reduce the IRI severity and influence the immune response of the recipient, in particular by reducing the activity of CD8+ T cells [14, 15].

2) Proprotein convertase of subtilisin/kexin type 9 (PCSK9). The best known and clinically relevant function of PCSK9 is to promote lysosomal degradation of low-density lipoprotein receptor, to which it binds. Until recently, this protease was considered as a target in the treatment of dyslipidemia [16]; however, a recent study has shown that PCSK9 of hepatocytes suppresses the activity of pancreatic secretory inhibitor of trypsin-1(SPINK1)–mediated mitophagy leading to the activation of the stimulator of the interferon gene/Nod-receptor-like (STING/NRLP3)–induced inflammatory responses that enhance IRI [17]. Mitophagy is the process of eliminating damaged mitochondria in order to maintain mitochondrial homeostasis. Available literature data indicate that during IRI, this process slows down due to the excess need for elimination of damaged mitochondria compared to the ability of transplanted liver hepatocytes to destroy them. Slowing down the

mitophagy process is associated with mitochondrial dysfunction, oxidation-phosphorylation uncoupling, and cell death.

Based on this, the possibility of using this factor as a component of the perfusion medium to preserve the mitochondrial pool is being considered.

3) Matrix ribonucleic acid (mRNA). In one of the studies, the authors identified the dependence of the IRI severity on the expression of various types of RNA in various transplanted organs. The most important types of RNA in the regulation of the immune response in IRI during orthotopic liver transplantation (OLT) are the following: mRNA-122, mRNA-450-5b, mRNA-155, mRNA-191, mRNA-146a, mRNA-497-5p.

- mRNA-122 is a liver-specific mRNA and represents more than half of the mRNAs expressed in this organ. This species is involved in the regulation of lipid metabolism, the course of viral hepatitis, as well as IRI [18]. This mRNA is regulated by the transcription factor HIF-1α. Increasing HIF-1α expression has been shown to reduce oxidative stress, reducing the IRI severity in the myocardium in experimental models suggesting a similar effect in transplanted livers [19].

- mRNA-450-5b. It has a suppressive effect on Crystallin Alpha B (CRYAB), which plays a significant role in suppressing the immune response, as well as in regulating the synthesis of pro-inflammatory IL-1b, TNF-α, IL-6. Inhibitory therapeutic effects on this type of mRNA will allow one to control the course and severity of the immune response to IRI [20].

- mRNA-155. Its increased expression activates the proliferation of macrophages, stimulating the inflammatory response, and the activity of neutrophils. Reducing the expression of mRNA-155 inhibited the activation of Kupffer cells, resulting in a decrease in the activity of proinflammatory cytokines [21].

- mRNA-191. Enhances cell apoptosis, its expression is enhanced when exposed to HIF-1α. Suppression of the expression of this type of mRNA is a possible goal of IRI therapy.
- mRNA-497-5p. Also it increases in IRI, stimulating cell apoptosis through activation of NF-κB in an isolated population of Kupffer cells [22].
- mRNA-146a – when suppressed, the production of pro-inflammatory cytokines increases, since this type of mRNA inhibits the Toll-like receptor 4 (TLR4) pathway by directly suppressing IRAK1 (IL-1 receptor-associated kinase 1) and TRAF6 (TNF-α receptor-associated factor 6).

Despite the proven correlation between the IRI severity and the level of expression of various mRNA types in experimental animal models, there are a number of limitations associated with the inability to simulate cold ischemia conditions. There are also certain difficulties in assessing the expression of various types of mRNA associated with the need to administer anticoagulants, storage conditions and processing of biomaterial. In this regard, there is a need to develop protocols regulating the collection, storage, and processing of material to obtain reliable results of the expression of certain mRNA types. Therefore, to consider mRNA as a biomarker of the severity of IRI and other processes, it is necessary to take into account many factors, which makes it difficult to draw up unified protocols.

4) Factor-2 associated with erythroid nuclear factor (NRF2) is in an inhibited state in the absence of homeostasis disruption, it is activated under oxidative stress, promoting the activation of the antioxidant system [23]. Experimental studies on mouse models demonstrated the ability of NRF2 to improve the course of IRI. A population of Cd4+ T cells expressing NRF2 reduced hepatocyte damage during prolonged cold ischemia; and

perioperative increases in NRF2 expression in human liver grafts reduced the severity of hepatocyte damage [24].

Based on the data obtained, we can conclude that the ex vivo preservation using normothermic machine perfusion may in turn enhance NRF2 expression, which improves graft survival. Presence of macrovesicular steatosis did not mean the unsuitability of the graft, which can be improved after normothermic machine perfusion. Further exploration of the NRF2 use in marginal allografts being more susceptible to IRI is needed. In the future, working with this factor will expand the pool of donor organs.

- Extracellular vesicles (ECVs) are nanoscopic vesicles of various sizes, formed in two ways. The first type (microparticles) are formed by budding and splitting the plasma membrane of the cell. The second type (exosomes) are formed inside multivesicular endosomal bodies. Apoptotic bodies are the third type of extracellular vesicles. Considering that these vesicles are involved in the intercellular interaction, regulation of the immune response, and are also capable of transporting various types of proteins, their role as drug “deliverers” in patients with rejection was considered [25]. In parallel with graft-derived ECVs, other types of vesicles have been studied, and among them, mesenchymal stem cell-derived ECVs (MSC-ECVs) have been shown to be capable of transferring specific molecules to the recipient's immune cells. Thus, MSC-ECVs can transfer specific microRNA (22-3p) into Kupffer cells inhibiting the expression of interferon regulator factor-8 (IRF8), which promotes the proliferation of progenitor cells into monocytes [26]. This mechanism leads to the suppression of local immunity and prevention of liver rejection. T cells exposed to ECVs derived from CD80+ dendritic cells decreased the expression of NLRP3 (cliopyrin involved in the formation of the active form of IL-18, 1β) and showed a decrease in

proliferation and adhesion, which indicates the possibility of induced tolerance [27]. Unlike stem cell therapy, ECVs have a number of advantages, such as low immunogenicity, no risk of transformation into tumor cells, ease of storage and high clinical safety. In the liver, the ECV protective mechanisms against IRI are mainly aimed at modulating the immune response, regulating autophagy [28] and activating regeneration pathways [29]. In the liver of rats exposed to IRI, the intravenous administration of MSC-ECV reduced the infiltration of neutrophils and macrophages, as well as the level of oxidative stress markers [30]. Similarly, MSC-ECVs were found to reduce liver IRI by reducing the secretion of pro-inflammatory cytokines such as TNF-α, IL-1α, IL-1β, IL-6, IL-12 [31]. The therapeutic efficacy of ECVs can be improved by adding a targeting molecule (antibody, aptamer – peptide molecules that specifically bind to target cells, etc.) to their surface, by loading the nanoparticle with a specific biologically active agent, or by increasing the time taken by ECVs in circulation through chemical modifications, creating the so-called “hybrid ECV” [32]. Overall, ECVs clearly have great potential for widespread application in disease therapy, either in their parent form, which has anti-inflammatory effects, or as drug delivery vehicles. However, one possible disadvantage is the fact that unmodified ECVs undergo rapid clearance from the circulation and exhibit relatively low accumulation in target tissues [33]. They are also incapable of replication, which reduces the risk of tumor formation after their delivery, and their inherent targeting mechanisms reduce the likelihood of side effects. These factors make them a promising drug delivery system for organ transplantation, ensuring long-term graft function. The donor liver is an ideal organ for studying the action of vesicles, since most of them are able to accumulate in the liver [34].

Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor that plays a key role in mediating adaptive responses to hypoxia. This factor consists of the oxygen-sensing subunit HIF-α and the subunit HIF-β, also known as nuclear translocator aryl hydrocarbon receptor (ARNT). In mammals, there are three isoforms of the HIF-α subunit (HIF-1α, HIF-2α, and HIF-3α). HIF-1α is a key factor in the oxygen sensing pathway discovered by Greg Semenza in 1991, and is especially important for maintaining oxygen homeostasis in mammalian cells. In cancer, coronary heart disease, or chronic obstructive pulmonary disease, the partial pressure of oxygen in tissues decreases, resulting in activation of HIF-1α. Under hypoxic conditions, the HIF-1α protein does not subject to degradation by oxygen-dependent ubiquitin proteasome system and is stably expressed. HIF-1α accumulates in the cytoplasm, translocates to the nucleus, and subsequently forms a dimer with ARNT to regulate target gene transcription. Activation of HIF-1α improves cell survival in hypoxic environments by altering energy metabolism, proliferation, angiogenesis, and vascular remodeling. HIF-1α is required for cardioprotection against IRI [35]. Since the discovery of the HIF-1α (erythropoietin) target gene, multiple downstream targets have been discovered, highlighting the complexity and importance of the HIF-1α signaling pathway. In IRI, HIF-1α can regulate and participate in various signaling pathways. Thus, pre-exposure to sevoflurane can increase VEGF expression through activation of the AKT/HIF-1α/VEGF signaling pathway. VEGF is closely associated with the process of angiogenesis. Enhancing angiogenesis in IRI can effectively reduce hypoxia in the lesions. In addition, iNOS, heme oxygenase-1 (HO-1), adiponectin, insulin-like growth factor-2, GLUT are also involved in the protective effect of HIF-1α against IRI [36].

During hypoxia, HIF-1α regulates various signaling pathways, including mitochondrial function and autophagy, which may have protective effects on myocardial cells following the ischemic response. However, prolonged autophagy can be harmful. HIF-1α also plays an important role in the control of oxidative stress and inflammation, which helps reduce the severity of the ischemic response. HIF-1α can affect mitochondrial function reducing mitochondrial damage and mitigating the severity of the ischemic response. It is also capable of inducing mitochondrial autophagy through various signaling pathways, which helps myocardial cell survival after an ischemic response. In the initial stage of ischemia, the activation of HIF-1α may be a protective mechanism, but prolonged autophagy can lead to cardiomyocyte death. Additionally, in the context of oxidative stress, HIF-1α activates Nrf2 to protect cells through increasing intrinsic autophagy clearance. Activation of HIF-1α can reduce tissue inflammation and the severity of the ischemic response by inhibiting NF-κB, which leads to a decreased production of proinflammatory cytokines [37]. In addition, HIF-1α regulates various signaling pathways, such as GSK3β/mitochondrial PTP, β-catenin, ERK1/2, Bcl-2, PI3K/AKT and mTOR, which influences a variety of cellular functions and helps reduce the severity of the ischemic response.

HIF-1α is a complex protein heterodimer consisting of α and β subunits that binds to the hypoxia response element (HRE) in the promoter of genes, regulating their expression. Some metal ions, such as Ni(II), Co(II), V(V), and Mn(II), contribute to the stabilization of HIF-1α and the activation of HIF-1α-dependent genes. These metals also affect ferritin levels and induce transcription of genes regulated by HIF-1α. There are also factors that interact with HIF-1α mRNA that may control its synthesis, including iron-regulating proteins. The effect of inhibition of HIF-1α activity during early stages of brain injury in rats on infarct

volume and survival by reducing apoptosis has been studied, but it is unclear whether a similar effect is possible during ischemic reperfusion injury. The mechanisms of the protective effect of HIF-1α on ischemic reperfusion injury, including the role of genes associated with glycolysis, mitochondrial function, cell survival, apoptosis and oxidative stress, remain poorly understood. HIF-1α regulates multiple target genes and therefore may have different functions at different stages of ischemic reperfusion injury through a variety of mechanisms. Therefore, further studies on the role of HIF-1α in the pathophysiology of IRI and its underlying mechanisms are needed [38].

Another pathway of regulated cell death in IRI is the relatively recently described and poorly understood ferroptosis. Ferroptosis is a recently described form of programmed cell death, which is realized by the interaction of iron ions and reactive oxygen species (ROS). Research on IRI has been conducted in many organs, including the heart, brain, kidney, and liver. Inhibition of ferroptosis may be an effective strategy for the treatment of such related organ diseases as well as IRI [39].

Ferroptosis is characterized by iron-dependent accumulation of lipid peroxides and is caused by loss of lipid-reducing enzyme activity and inhibition of antioxidant glutathione (GSH) synthesis, leading to iron-dependent cell death. During ferroptosis, several biological regulation pathways operate simultaneously. GSH deficiency or inactivation of glutathione peroxidase 4 (GPX4) causes ferroptosis. In addition, ferroptosis is characterized by excessive iron-mediated peroxidation of polyunsaturated fatty acids. Excessive accumulation of iron-dependent lipid hydroperoxides leads to ferroptosis [40].

The cystine glutamate transporter is composed of heterodimers of solute transporter family 3 member 2 (SLC3A2) and solute transporter family 7 member 11 catalytic subunit (SLC7A11). The system promotes

the exchange process of cysteine and glutamate across the plasma membrane. Cysteine is an amino acid essential for cell survival, especially in large quantities outside the cell. Its presence is important for the process in which the exchange of glutamate and GSH (glutathione) occurs. This helps maintain the redox balance in the cell. GPX4 is an important antioxidant enzyme involved in the fight against free radicals, especially phospholipid peroxides. Glutathione and selenium are required for its functioning. Glutathione also plays a role in antioxidant defense and helps restore GPX4. Several proteins involved in glutathione metabolism are also associated with ferroptosis [41]. GPX4 can be directly or indirectly affected through a process called ferroptosis, which reduces its activity. RSL3, which causes ferroptosis, inhibits GPX4 by reacting chemically with selenium contained in the amino acid selenocysteine, causing the accumulation of lipid peroxides and cell death. This inhibition of GPX4 leads to the accumulation of lipid peroxides, which form toxic lipid radicals that are harmful to the cell [42]. Erastin, another ferroptosis inducer, reduces glutathione (GSH) levels, which disrupts GPX4 indirectly and reduces the cell's antioxidant capacity by promoting the formation of reactive oxygen species (ROS). The different ways in which RSL3 and Erastin regulate redox processes and cell death involve different mechanisms: RSL3 induces ferroptosis depending on the presence of iron and lipid peroxides, while Erastin induces cell death independent of the presence of iron, but still via peroxide lipids. Erastin is a typical inducer [42]. Recent studies show that FSP1 (ferroptosis suppression protein 1) in conjunction with CoQ10 (ubiquinone) through the NADP(P)H pathway synergistically suppresses the proliferation of lipid peroxides and the ferroptosis process through its effect on GPX4 (glutathione peroxidase 4) and GSH (reduced glutathione) both independent system. FSP1 works as an oxidoreductor,

reducing CoQ10 through NADP(P)H and producing a lipophilic free radical scavenger (RTA) to prevent the formation of lipid peroxides. There is evidence that a ferroptosis-inducing agent (e.g., FIN56) induces ferroptosis by blocking the process. The P53 protein compound, which is a tumor suppressor, plays a role in a variety of cell death processes such as apoptosis, cellular autodestruction and necrosis. P53 is able to suppress the SLC7A11 gene, thereby affecting the activity of GPX4, which leads to the accumulation of lipid oxidation. SAT1 acts as a transcriptional target of P53 and its activation can induce ROS-induced lipid peroxidation and ferroptosis.

Polyunsaturated fatty acids are oxidized to form lipid peroxides. Important factors causing ferroptosis include the accumulation of lipid peroxides. Oxidized phosphatidylethanolamine containing arachidonic acid (AA-PE) acts as a signal for ferroptosis [43]. A recent study showed that AA-OOH-PE, instead of other types of phospholipids-OOH, is the key phospholipid inducing ferroptosis. The creation of AA-OOH-PE requires three enzymes: arachidonate lipoxygenase (ALOX), long-chain acyl-CoA synthetase 4 (ACSL4), and lysophosphatidylcholine acyltransferase 3 (LPCAT3). ACSL4 converts PUFA, especially AA, into the CoA form, while LPCAT3 regulates the conversion of AA-CoA to AA-PE. Under the influence of lipoxygenase (LOX), AA-PE can be converted into AA-OOH-PE, which leads to oxidative processes. When AA-OOH-PE levels are excessive, ferroptosis occurs, requiring iron as a catalyst. In addition to lipid peroxidation, ferroptosis requires a redox-active metal-iron. Iron released from the labile iron pool (LIP) can contribute to the accumulation of ROS through the Fenton reaction.

Recent studies indicate that the ferroprotein FSP1 in the compound with the coenzyme CoQ10 through the NADP(P)H mechanism synergistically reduces the growth of lipid peroxides and ferroptosis by

influencing GPX4 and reduced glutathione GSH as an autonomous system. This ferroprotein functions as an oxidoreductase and reduces CoQ10 via NADP(P)H and creates the lipophilic antioxidant RTA to prevent the formation of lipid peroxides. Evidence demonstrates that ferroptosis inducers (such as the agent FIN56) cause ferroptosis by blocking this process. The P53 tumor suppressor protein is involved in numerous cell death processes, including apoptosis, cell autolysis, and necrosis. This protein can suppress the expression of the SLC7A11 gene and thus affect the function of GPX4.

Polyunsaturated fatty acids undergo oxidation to form lipid peroxides. An important factor provoking ferroptosis is the accumulation of lipid peroxides. Oxidized phosphatidylethanolamine enriched with arachidonic acid (AA-PE) acts as a signal for ferroptosis. Recent study has shown that AA-OOH-PE (instead of other types of oxidized phospholipids) is the main phospholipid component causing ferroptosis. To create AA-UN-PE, three enzymes are required: arachidonate lipoxygenase (ALOX), long-chain Acyl-CoA synthase4 (ACSL4), lysophosphatidylcholine acyltransferase3 (LPCAT3). ACSL4 transforms PUFA (especially AA) into the CoA form. LPCAT3 controls the conversion of AA- CoA to AA-PE. Lipoxygenase (LOX) transforms AA-PE into AA-UN-PE, causing an oxidative process. At the same time, nuclear ferritin receptor coactivator 4 (NCOA4) leads to autophagy and ferritin accumulation. Oxidative stress induces HO-1 expression by activating the p62-Keap1-NRF2 pathway, counteracting ferroptosis. Nrf2 activates the expression of various target genes required for the regulation of ferroptosis, regulating the metabolism of GSH, lipid, iron and the mitochondrial function [44, 45].

In the recent few years, the effect of ferroptosis on the development of ischemic reperfusion injury of the kidneys has been actively studied. Researchers suggest that ferroptosis may play a role in the development

of acute kidney failure. Experiments in mice have shown that pre-administration of the enzyme ferrostatin at various periods of time before the onset of ischemia reduces the level of kidney tissue damage, creatinine and urea levels 48 hours after the onset of ischemia. This indicates the important role of ferroptosis in the mechanism of development of acute renal failure. In mouse models of acute renal failure by ischemic reperfusion injury, ferroptosis was directly associated with synchronous renal tubular death.

S.J. Dixon et al. demonstrated that I/R induces the activation of miR-182-5p and miR-378a-3p and induces ferroptosis in renal injury through downregulation of GPX4 and SLC7A11 [46]. Recent studies have shown that ferroptosis plays an important role in renal ischemia-reperfusion injury; however, the exact mechanism of ferroptosis requires further study.

Pannexin1 (PANX1) is an ATP release pathway family protein that may promote apoptosis in renal injury. A previous study showed that deletion of PANX1 prevents renal IRI by attenuating the ferroptosis activated by mitogen-activated protein kinase/extracellular signaling kinase signal transmitting. Macrophage migration inhibitory factor (MIF) is able to limit necrosis, restore intracellular glutathione (GSH) levels and reduce lipid oxidation to reduce oxidative stress. This cytokine plays an important role in the protection of renal tubular epithelial cells and provides kidney protection under experimental ischemia-reperfusion conditions. Pachymic acid found in coconut pores is a lanostane triterpenoid. According to studies, with pachymic acid treatment, kidney function in mice with IRI can be improved, and kidney damage can be alleviated [47]. The protective effect of pachymic acid may be due to the inhibition of renal ferroptosis through direct or indirect activation of Nrf2 and upregulation of the downstream GPX4 system and HO-1.

Additionally, XJB-5-131 is a mitochondria-targeted nitroxide with high affinity for tubular epithelial cells [48]. XJB-5-131 inhibits lipid peroxidation after IRI and inhibits ferroptosis in tubular epithelial cells, thereby ameliorating ischemic renal injury. Irisin is a type of exercise-inducible hormone that can improve mitochondrial function and reduce ROS production. Irisin treatment in mice can reduce acute kidney injury by upregulating GPX4, a master regulator of ferroptosis. These results indicate that the essential endogenous antioxidant GPX4 and its cofactor GSH are important targets for protection against kidney injury [49].

Major factors promoting ferroptosis, including the generation of ROS and increased lipid peroxidation, are associated with intestinal IRI. Researchers have found that capsiat, a gut microflora metabolite, can increase GPX4 expression by activating the TRPV1 receptor and suppressing ferroptosis. The latter is caused by ischemia-reperfusion syndrome in the intestines.

It is also known that intestinal ischemia-reperfusion injury can lead to ferroptosis and acute lung injury. Previous studies have already noted the presence of ferroptosis in this pathology.

Scientists believe that the Nrf2 protein is able to suppress ferroptosis and stimulate the expression of SLC7A11 and HO-1 proteins. It plays an important role in protection against acute lung injury [50].

In addition, researchers have found that iASPP (p53 protein inhibitor) can block the apoptosis-stimulating protein p53, thereby preventing the development of pathology [51].

A recent study showed that in Nrf2−/− mice with acute lung injury induced by intestinal I/R, iASPP could effectively alleviate acute lung injury and inhibit ferroptosis through the Nrf2/HIF-1α/TF signaling pathway [52].

Edaravone (Radicava), an antioxidant protective agent, has effects against cerebral ischemic reperfusion injury (CIRI), but its mechanism of action is unclear [53]. W. Li et al. results [54] showed that cerebral infarct volume and rates of neurological impairment were increased in rats with cerebral ischemia-reperfusion injury and impaired sensorimotor ability; in addition, the content of glutathione (GSH) in brain tissues decreased, the content of Fe2+, malonic dialdehyde (MDA), and lipid peroxide (LPO), and also the expression level of glutathione peroxidase 4 (GPX4), a key protein of ferroptosis, decreased. Moreover, the Nrf2 expression level increased, and the FPN expression level of decreased after cerebral ischemia-reperfusion, while the levels of interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, and myeloperoxidase decreased. However, in relevant tests, edaravone demonstrated protective effects on cerebral infarction, as well as on neurological and sensorimotor functions. Edaravone inhibits ferroptosis, attenuating CIRI, likely through activation of the Nrf2/FPN pathway [55, 56].

Discussion

In the reviewed literature, IRI after liver transplantation is described as a sequence of processes with different mechanisms of action. Preventing IRI therefore requires drugs that target specific links in this chain of damage reactions (figure).

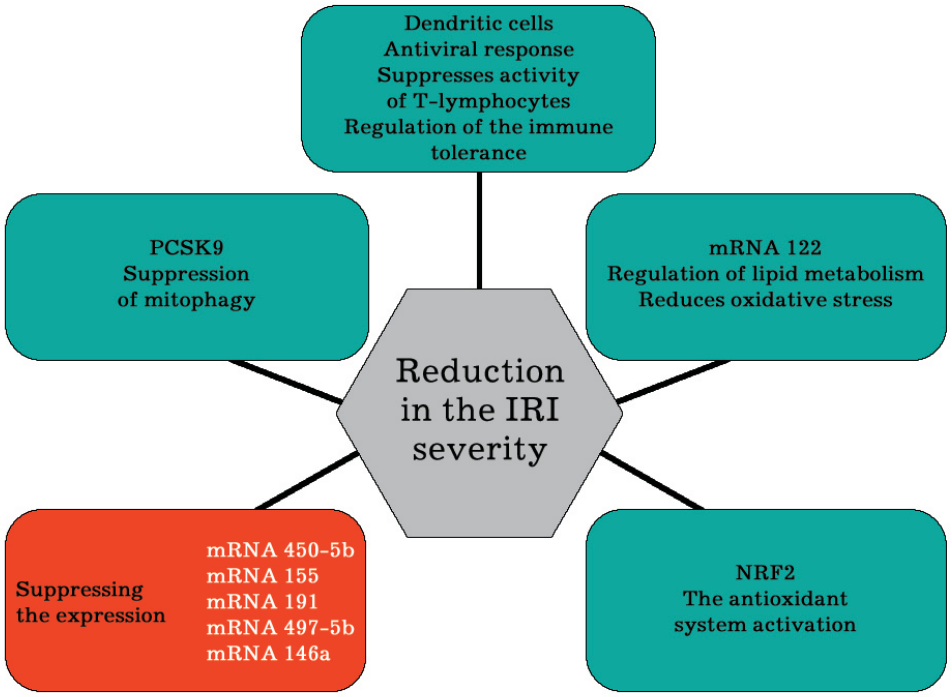

Figure. Schematic representation of the main literature-described mechanisms of reducing the ischemia-reperfusion injury severity

In recent years, significant progress has been made in studying the mechanisms of ischemia-reperfusion injury. Initial studies were mainly conducted at the animal cellular level, and the effectiveness of ferroptosis in clinical practice remains poorly understood. Additional studies are needed to determine the specific effects of ferroptosis on ischemia-reperfusion injury in various organs, as well as to develop targeted therapies. More in-depth research in this area may help suppress ferroptosis and develop effective treatment strategies for diseases associated with organ ischemia-reperfusion injury. It is expected that the creation of ferroptosis modulators will expand the possibilities in the treatment of ischemia-reperfusion injuries and related diseases [57]. In this regard, conducting research to develop innovative treatments that can provide protection against ischemia-reperfusion injury is extremely important.

Conclusion

The most promising mechanisms and targets for influencing the severity of ischemic reperfusion injury, according to the literature studied, are: a) the regulation of the expression of certain types of matrix ribonucleic acid, affecting ischemic reperfusion injury in the transplanted liver; b) an impact on mitophagy activity; c) the inhibition of ferroptosis, and d) the use of perfusion technologies in the regulation of intercellular interaction and the immune response. In the future, the development of effective therapeutic methods for influencing these targets can help both reduce the severity of ischemia-reperfusion injuries, and also reduce the dosage of immunosuppressive drugs, as well as expand the pool of donor organs.

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