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Review

Current Mechanistic Concepts in Ischemia and Reperfusion Injury

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Key Words
Ischemia-reperfusion injury • Autophagy • Mitoptosis • Necroptosis • Apoptosis

Abstract
Ischemia-reperfusion injury is associated with serious clinical manifestations, including myocardial hibernation, acute heart failure, cerebral dysfunction, gastrointestinal dysfunction, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome. Ischemia-reperfusion injury is a critical medical condition that poses an important therapeutic challenge for physicians. In this review article, we present recent advances focusing on the basic pathophysiology of ischemia-reperfusion injury, especially the involvement of reactive oxygen species and cell death pathways. The involvement of the NADPH oxidase system, nitric oxide synthase system, and xanthine oxidase system are also described. When the blood supply is re-established after prolonged ischemia, local inflammation and ROS production increase, leading to secondary injury. Cell damage induced by prolonged ischemia-reperfusion injury may lead to apoptosis, autophagy, necrosis, and necroptosis. We highlight the latest mechanistic insights into reperfusion-injury-induced cell death via these different processes. The interlinked signaling pathways of cell death could offer new targets for therapeutic approaches. Treatment approaches for ischemia-reperfusion injury are also reviewed. We believe that understanding the pathophysiology ischemia-reperfusion injury will enable the development of novel treatment interventions.

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Introduction

Ischemia-reperfusion injury is a critical condition for which physicians must control cell damage and preserve organ function. Ischemia is defined as the hypoperfusion of tissues. Several conditions, such as sepsis, acute coronary syndrome, organ transplantation, and limb injury, may cause tissue hypoperfusion. Previous studies have focused on reducing the time of hypoperfusion to preserve organ function. For sepsis, the Surviving Sepsis Campaign guidelines recommend early goal-directed therapy for timely resuscitation, including early antibiotic treatment and resuscitation with adequate fluids and vasopressors to reduce peripheral tissue hypoperfusion [1-8]. For acute coronary syndrome, the ACCF/AHA/SCAI guidelines recommend early revascularization to control myocardial injury [9]. Similar recommendations have been made for organ transplantation and limb injury. However, clinical outcomes after restoration of blood flow to ischemic tissue are not optimal. Recent studies have demonstrated that reperfusion has the potential to induce subsequent injury in ischemic tissue, a phenomenon termed ischemia-reperfusion injury. The subsequent injury induced by ischemia-reperfusion injury presents a challenge for physicians to preserve organ and neurogenic function. Despite basic and clinical research efforts, a detailed mechanism of ischemia-reperfusion injury has not been described. We believe that elucidating the pathogenesis of this condition could provide a strong foundation for the development of new therapeutic interventions. This article provides an overview of advances in ischemia-reperfusion injury research and summarizes our current understanding of related mechanisms and potential targets for potential therapeutic intervention.

Mechanism of ischemia-reperfusion injury

Several physiological mechanisms promote ischemia and lead to hypoxia and hypoperfusion, including atherosclerosis and acute myocardial infarction [10, 11]. The obstruction of arterial blood flow causes hypoxia and leads to dysfunction of the electron transport chain in mitochondria. Decreasing ATP production in mitochondria induces anaerobic metabolism, dysfunction of sodium-potassium pumps, and detachment of

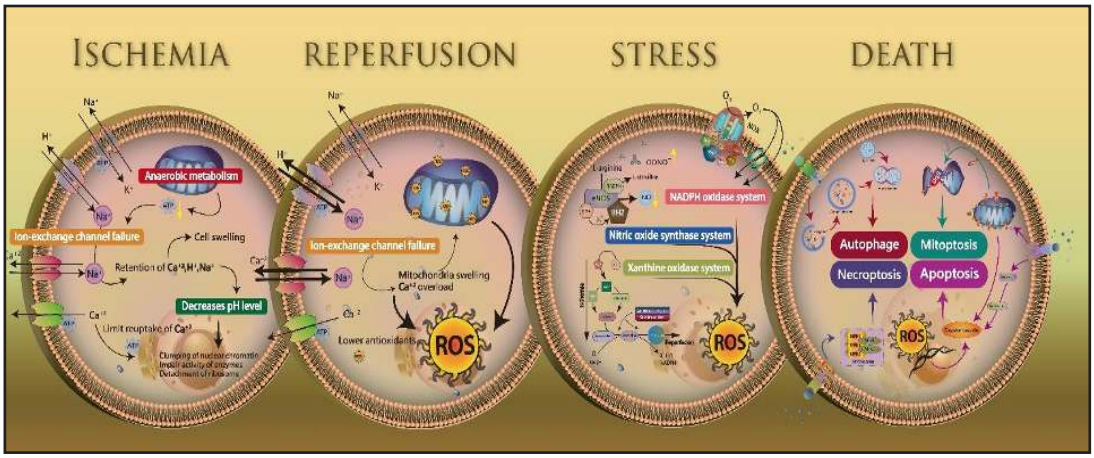

Fig. 1. Overview of the mechanism of ischemia-reperfusion injury. The ischemic state induces anaerobic metabolism, leading to a lower level of ATP production and failure of ion-exchange channels. Failure of ion-exchange channels leads to cell swelling and impaired enzymatic activity in the cytoplasm. Mitochondrial damage and electrolyte imbalance in the reperfusion state promote oxidative stress from three major systems: the NADPH oxidase system, nitric oxide synthase system, and xanthine oxidase system. ROS retention induces cell damage, leading to cell death via four pathways: autophagy, mitoptosis, necrosis and necroptosis, and apoptosis.

ribosomes. Anaerobic metabolism produces a lower level of adenosine triphosphate (ATP) and antioxidative agents in cells. Moreover, the retention of lactic acid may lead to metabolic acidosis. In addition, there may be failure of sodium-potassium pumps (Na+-K+-ATPase pumps) and calcium pumps (Ca2+-ATPase pumps) on the cell surface. The failure of Na+-K+-ATPase pumps causes retention of sodium in cells and potassium out of cells. A higher level of sodium in cells decreases the activity of sodium–hydrogen exchanger pumps (Na+-H+ pumps). Calcium pumps (Ca2+-ATPase pumps) on the endoplasmic reticulum also become dysfunctional, which limits calcium reuptake. In cells, the accumulation of hydrogen, sodium and calcium ions causes hyperosmolarity, which leads to water flow into the cytoplasm and cell swelling. The retention of hydrogen decreases cellular pH, leading to impaired enzyme activity and clumping of nuclear chromatin. The detachment of ribosomes decreases protein synthesis. After the reperfusion stage, restoring blood flow to ischemic tissue provides oxygen via red blood cells. In parallel, the generation of reactive oxygen species (ROS) increases due

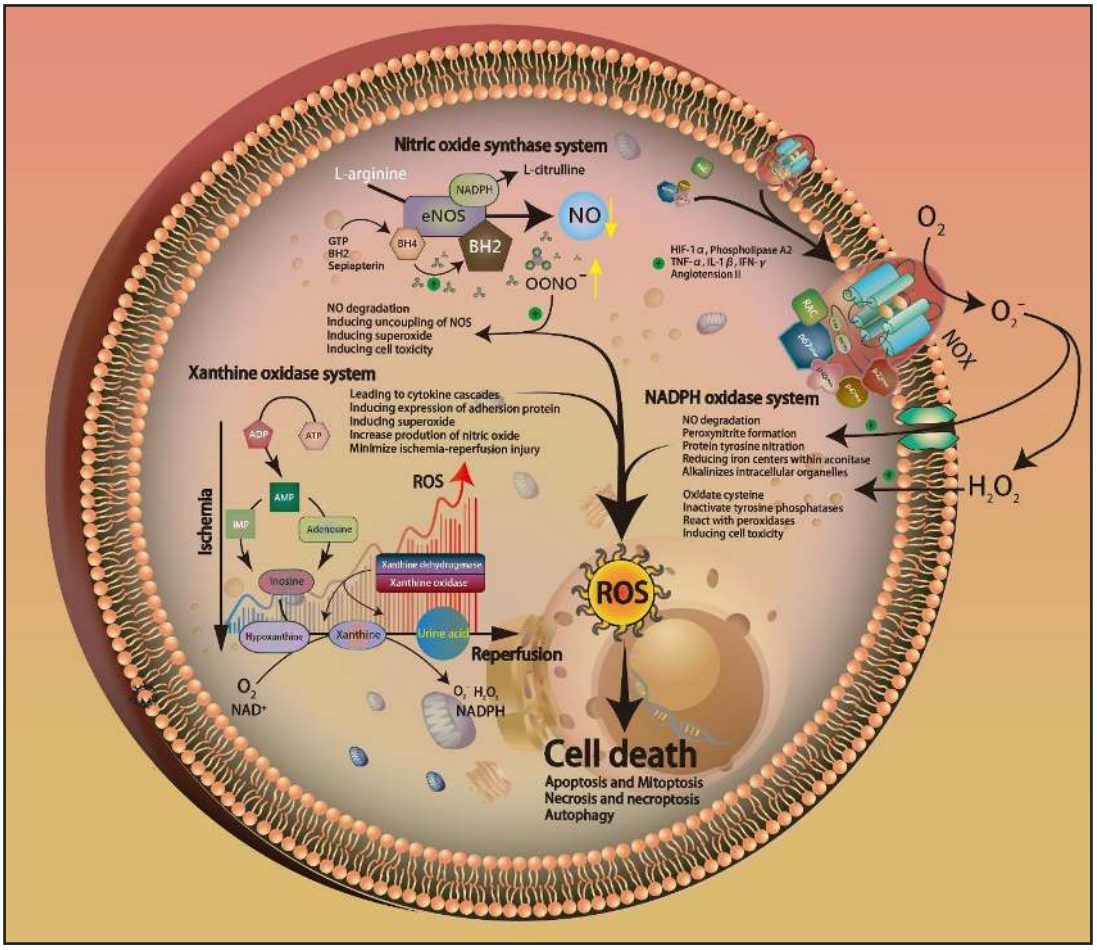

Fig. 2. Mechanism of ROS in ischemia-reperfusion injury. The xanthine oxidase system induces ROS production by oxidizing hypoxanthine to xanthine and xanthine to uric acid. Initially, ATP is converted to inosine from IMP and adenosine, then into hypoxanthine. In the ischemic state, xanthine dehydrogenase is shifted to xanthine oxidoreductase due to a lower ATP level, inducing the formation of ROS during hypoxanthine conversion to urine acid. NOX enzymes produce superoxide and hydrogen peroxide in ischemia-reperfusion injury via the activation of HIF-1α, phospholipase A2, TNF-α, IL-1β, IFN-γ, and angiotension II. Superoxides pass through the cell membrane via channels due to a negative charge. Hydrogen peroxide directly enters the cytoplasm and causes cell damage. In the hypoxic state, oxidative stress oxidizes BH4 into BH2 and induces the uncoupling of NOS. NOS uncoupling produces ROS, which induces ischemia-reperfusion injury leading to cell death.

to a lower concentration of antioxidative agents in ischemic cells. ROS cause oxidative stress that promotes endothelial dysfunction, DNA damage, and local inflammatory responses (Fig. 1). Inflammatory cascades and oxidative stress may subsequently induce a cytokine storm, resulting in cell death caused by damage to cellular structures [12]. The reperfusion stage is dynamic and may persist for several days. Understanding the detailed mechanism of ischemia-reperfusion injury may provide a strong foundation not only for novel therapeutic opportunities, but also for injury prevention.

The role of oxidative stress in ischemia-reperfusion injury

Oxidative stress can be produced from enzymatic sources and non-enzymatic sources. Common enzymatic sources include the xanthine oxidase system, NADPH oxidase system, mitochondrial electron transport chain, and uncoupled nitric oxide synthase (NOS) system. Non-enzymatic sources are a minor source of oxidative stress, and include hemoglobin and myoglobin, especially in extremity injury. The xanthine oxidase system, NADPH oxidase system, and mitochondrial electron transport chain are broadly implicated in oxidative stress in several organs, including the intestine, lung, heart, brain, muscle, liver, pancreas, stomach, and kidney [13]. NOS is a major oxidative stress factor in the liver, heart, and aortic endothelial cells [14-16]. These enzyme systems are discussed in detail in the following sections.

Mechanisms of xanthine oxidase induction of oxidative stress in ischemia-reperfusion injury

Xanthine oxidoreductases are complex molybdoflavoenzymes that play an important role in purine catabolism. These enzymes, which include xanthine dehydrogenase and xanthine oxidase, oxidize hypoxanthine to xanthine and xanthine to uric acid, respectively. In normal cells, purine metabolism can be initiated by two major pathyways. Initially, ATP, a purine for many energy-requiring enzymatic reactions, can be converted to inosine monophosphate by deaminase and form inosine after dephosphorylation. Purine can become adenosine by removal of a phosphate group and convert to inosine. Inosine is further converted to hypoxanthine and is then oxidized to xanthine. Xanthine is also synthesized from guanine, which is converted from guanine monophosphate. Through the activity of xanthine oxidoreductase, xanthine is then converted to uric acid (Fig. 2). In purine metabolism, there are many enzymes involved in the conversion to uric acid. Xanthine oxidases are particularly important, as their activity is accompanied by ROS production. These enzymes have two forms: xanthine dehydrogenase, which preferentially uses NAD^+ as an electron acceptor, and xanthine oxidase, which usually uses O_2 as an acceptor. During hypoxanthine oxidation to xanthine in normal cells, there is simultaneous reduction of NAD^+ or O_2, and production of NADH by xanthine dehydrogenase [17]. In an ischemic state, xanthine dehydrogenase is shifted to xanthine oxidoreductase due to a lower ATP level. In addition, xanthine dehydrogenase is shifted to xanthine oxidoreductase due to oxidation of critical cysteines and/or limited proteolysis. When blood flow is restored to ischemic tissue, xanthine oxidase reacts with O_2 to induce hypoxanthine to form xanthine and uric acid, using oxygen as the final electron acceptor. During the formation of xanthine and uric acid, superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are released, inducing more oxidative stress. ROS produced by xanthine oxidoreductase lead to cytokine cascades and pathological conditions via the expression of adhesion proteins, such as P-selectin and ICAM-1 [18].

Pharmacological inhibition of ROS production from purine metabolism has been reported in previous studies. Allopurinol and oxypurinol are commonly used to inhibit xanthine oxidase activity to decrease serum uric acid production. However, recent studies have reported inconsistent results relating to the blockade of xanthine oxidase to prevent ROS

formation [14, 17-21]. Several reasons have been proposed. For example, the required doses of allopurinol and oxypurinol are higher than the theoretical doses, and a pharmacological inhibitor may exert antioxidative effects [21]. In addition, studies have demonstrated that xanthine oxidase has several functions, including nitrate reductase activity, which increases production of nitric oxide [19, 20, 22]. The production of nitric oxide can minimize ischemia-reperfusion injury by stimulating vasodilation and inhibiting inflammatory responses. For these reasons, only inhibiting xanthine oxidase via a pharmacological inhibitor may not result in better clinical outcomes.

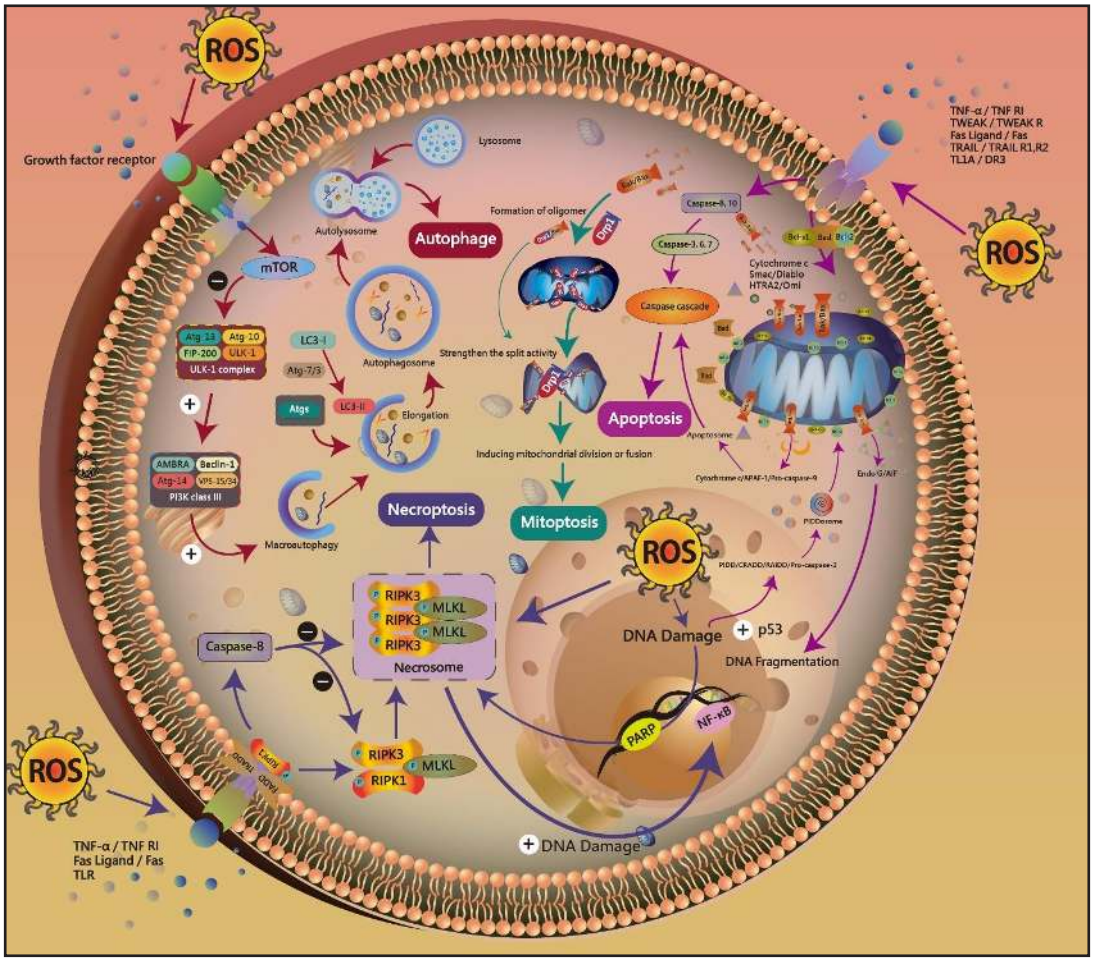

Fig. 3. Mechanism of ischemia-reperfusion injury inducing cell death. Apoptosis: Hypoxic stress from ischemia-reperfusion injury induces both the extrinsic- and intrinsic pathways. The death ligands, TNF-α, TWEAK, Fas Ligand, TRAIL, and TL1A, activate the caspase cascade to cause cell death via proteolysis. ROS also activate the pro-apoptotic Bcl-2 family to change the integrity of the mitochondrial membrane, leading to the caspase cascade. Mitoptosis: BAX/BAK regulate Drp1, leading to mitochondrial cleavage and fusion. The formation of oligomers of BAX/Drp1 strengthens the split activity and increases mitochondrial membrane potential, cytochrome c release, and cell death. Necrosis and necroptosis: Various stimuli activate necroptosis via the necrosome. The intracellular adapter molecules FADD and TRADD activate RIPK1 and promote formation of the necrosome with RIPK1 and MLKL. ROS-induced DNA damage also promotes necrosome formation by activating PARP. The overexpression of poly-(ADP-ribose) polymerase-1 (PARP-1) leads to necrotic cell death by depletion of ATP and irreversible cellular energy failure. Autophagy: The oxidative stress in ischemia-reperfusion injury inhibits the activity of mTOR, leading to formation of the ULK-1 complex and PI3K class III, which then induce autophagy. The interaction between Atg proteins and LC-1 promotes the formation of autophagosomes. Autophagosomes fuse with lysosome to induce autophagy.

Mechanisms of NADPH oxidase induction of oxidative stress in ischemia-reperfusion injury

The Nox/Duox family of NADPH oxidases, comprised of multiprotein complexes with Nox-1 to Nox-5 and dual oxidases (Duox)-1 and Duox-2, also produces ROS in ischemia-reperfusion injury [23, 24]. NOX enzymes generally consist of six transmembrane domains with two histidines at domains III and V, spanning two hemes [25-27]. The flavin adenine dinucleotide (FAD) and NADPH binding domains localize to the cytoplasmic COOH terminus of NOX enzymes [23]. NOX enzymes use oxygen as final electron acceptors via NADPH, FAD, and heme groups [28, 29]. Recent studies have reported that NOX enzymes are involved in ROS production in ischemia-reperfusion injury by their overexpression and increased activity [30-34]. NOX enzymes immediately produce $O_2^{\bullet-}$, which is transferred to H_2O_2 via enzymes. The superoxide passes through the membrane via the pores of anion channels, leading to NO degradation, peroxynitrite formation, and protein tyrosine nitration. Superoxide also reduces iron centers within aconitase and alkalinizes intracellular organelles. Hydrogen peroxide passes through the membrane to oxidize cysteine, inactivate tyrosine phosphatases and the serine-threonine phosphatase calcineurin, react with peroxidases, and cause cell toxicity [24]. Hypoxia also induces hypoxia inhibitory factor-1α (HIF-1α) to promote activation of NOX enzymes. Oxidative stess increases the production of HIF-1α to establish a positive feedback loop. Moreover, after restoring blood flow to ischemic tissue, cells release several chemical mediators to activate NADPH oxidase, such as phospholipase A2, TNF-α, IL-1β, IFN-γ, and angiotension II (Fig. 2). The release of phospholipase A2 induces production of platelet activating factor, leading to an increase in the tissue levels of thromboxane and leukotrienes, which promote local inflammation [35-38]. NOX-derived ROS in post-ischemic tissue may cause inflammatory cell accumulation, leading to reperfusion injury in a variety of organs, such as the heart, brain, intestine, stomach, lung, and skeletal muscle [39-45]. Cytokines produced by macrophages and mast cells promote overexpression of NADPH oxidase [46, 47]. Angiotensin II also stimulates local angiotensin II receptors to incease the expression of NADPH oxidase, resulting in ischemia-reperfusion injury via angiotensin converting enzyme [48-52].

Pharmacological inhibition of ROS production from NOX enzymes by apocynin or diphenyliodonium has been reported [53-57]. Apocynin inhibits NOX enzymes by decreasing membrane translocation of p47phox and p67phox. Diphenyliodonium is a flavoprotein inhibitor that decreases the electron transport function of NOX enzymes. In endothelial cells, inhibiton of NOX activation decreases NADPH oxidase expression and superoxide production. It also reduces the expresion of adhesion proteins, including E-selectin and ICAM [58]. Similar results were reported in cardiac cells, lung cells, and brain cells [59-61]. However, curcumin, apocynin and diphenyliodonium have antioxidant functions that inhibit nitric oxide synthesis, xanthine oxidase, cytochrome P450 reductase, and mitochondrial enzymes. For these reasons, definitively attributing the neutralization of ischemia-reperfusion injury by apocynin and diphenyliodonium to NOX enzyme inhibition is difficult [62-67].

Mechanisms of NOS induction of oxidative stress in ischemia-reperfusion injury

The three recognized types of NOS are neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) [68, 69]. All types of NOS produce nitric oxide (NO) by the conversion of L-arginine to L-citrulline. NO also plays a protective role via its antioxidative and anti-inflammatory functions [69-72]. However, in a hypoxic state, NOS is converted to NOS uncoupling and produces ROS to induce ischemia-reperfusion injury. The structure of NOS consists of an oxygenase, which contains heme and tetrahydrobiopterin (BH_4) and binds arginine, and a reductase domain, which contains flavins (FAD and FMN) and binds NADPH. Tetrahydrobiopterin (BH_4) is a cofactor, synthesized from guanosine 5□-triphosphate (GTP) via GTP cyclohydrolase I, quinonoid dihydrobiopterin (BH_2), and sepiapterin [73-77]. The

BH_4 concentration plays a critical role in NO production by regulating eNOS activity. However, in ischemia-reperfusion injury, oxidative stress oxidizes BH_4, causing the tissue level of BH_4 to decrease. Decreases in the BH_4/NOS ratio induces the uncoupling of NOS and superoxide, resulting in cell death (Fig. 2). The mechanisms of ROS production in ischemia-reperfusion injury are multifactorial and complex. In the ischemic stage, ROS produced from the xanthine oxidase system, NADPH oxidase system, mitochondrial electron transport chain, and uncoupled NOS system may accumulate in cells and reduce the effects of an antioxidative agent. After the blood supply is restored to ischemic tissue, oxidative stress may lead to cell damage and cell death if the severity is sufficient. The mechanism of ischemia-reperfusion-injury-induced cell death is discussed in the following section.

Cell damage in ischemia-reperfusion injury

The total tissue injury induced by ischemia-reperfusion injury is divided into two parts: ischemia injury and reperfusion injury. Ischemic injury may initially cause hypoxia and hyponutrition. After prolonged ischemia, the metabolic products from cells are retained and cause metabolic acidosis. When the blood supply is re-established, local inflammation and ROS production increase, leading to secondary injury. The cell response is dependent on the severity of total tissue injury [78]. Cell damage induced by prolonged ischemia-reperfusion injury may lead to apoptosis, autophagy, necrosis, and necroptosis [79-81]. Moderate ischemia-reperfusion injury may cause cell dysfunction by autophagy and activate recovery systems for survival. If damage is severe, cell death may be induced via apoptotic or necrotic pathways [82]. A shorter duration of ischemia-reperfusion injury may activate cell survival programs to control ROS generation and cell damage [83]. Different mechanisms of cell death may be induced based on different pathogenesis pathways of ischemia-reperfusion injury. Four types of cell death mechanisms induced in ischemia-reperfusion injury are discussed in the next section.

Apoptosis and mitoptosis

Cell death is important in the pathogenesis of several diseases. Apoptosis is a process of programmed cell death that is activated under hypoxic stress in ischemic injury and during the production of ROS in reperfusion injury [84, 85]. Apoptotic mechanisms are divided into two major pathways: the extrinsic- and intrinsic pathways. Both pathways can crosstalk and influence the other. The extrinsic pathway, also known as the death receptor pathway, is activated by death ligands and receptors, including TNF-α, TWEAK, Fas ligand, TRAIL, and TL1A. This death-signaling complex activates a protease, caspase-8, to cleave caspase-3, which then induces cell death via proteolysis in damaged cells. The intrinsic pathway, also known as the mitochondrial pathway, is activated by hypoxia, radiation, or cellular toxins, and changes the integrity of the mitochondrial membrane, leading to activation of the pro-apoptotic Bcl-2 family. In ischemia-reperfusion injury, cytoplasmic Bad increases and binds to Bcl-2 and Bcl-XL. In parallel, Bax and Bak are processed and inserted into the mitochondrial membrane to release downstream pro-apoptotic proteins such as cytochrome c, Smac/Diablo, HTRA2/Omi, apoptosis-inducing factor, and endonuclease G. Cytochrome c binds apoptotic protease activating factor 1 (APAF-1) to activate pro-caspase-9 to form the apoptosome. The apoptosome induces a caspase cascade to promote apoptosis via activation of caspase-9 [86]. Endo G also interacts with AIF to cause DNA fragmentation. After ROS-induced DNA damage, p53 induces the formation of the PIDDosome by the expression of PIDD and interaction of CRADD/RAIDD and pro-caspase-2. The complex activates caspase-2 and cleaves downstream caspases to drive apoptosis (Fig. 3). Ischemia-reperfusion injury-induced apoptosis is not as common as necrosis [87]. However, apoptosis is not as frequently accompanied by a local inflammatory response.

Mitochondria become fragmented to achieve programmed cell death in a process termed mitoptosis [88]. However, during mitoptosis, BAX and BAK regulate mitochondrial cleavage and can be linked to the mitochondrial outer membrane in conjunction with Drp1 rather than through the regulation of other cleavage proteins such as Fis1 (fission 1) and Mdv1 (mitochondrial division 1). Regulation of mitochondrial cleavage and fusion of any protein cause inactivation of the protein, and leads to intracellular mitochondrial division or fusion abnormalities [89]. The K38A Drp1 (dynamin-related protein 1) pathway inhibits Drp1 function, leading to mitosis, is inhibited, and fusion is not affected, mitochondrial morphology showed long strip, than the normal cell mitochondrial length increased significantly in cardiac ischemia-reperfusion injury [90]. Excessive expression of Drp1 resulted in overexpression of mitochondria, a significant decrease in the number of mitochondria, increased mitochondrial membrane potential, cytochrome c release, and cell death [91]. Which BAX/Drp1 cannot only co-split, damage to the heavier cells will also occur BAX/Drp1 formation of oligomer to strengthen the split activity [92]. BAX is a pro-apoptotic protein that assists mitochondrial division. In addition, BAX can change the distribution of mitochondria to promote mitochondrial fusion through the activation of mitochondrial fusion protein Mfn2 (mitofusion 2) synthesis (Fig. 3). Cristae in the mitochondrial inner membrane structure also play a key role in the regulation of cytochrome c during apoptosis. An important protein molecule in cristae is OPA1 (optic atrophy 1). Abnormal OPA1 function may cause damage to the endometrial structure, promoting mitochondrial fragmentation and apoptosis [93]. The regulatory mechanism is through Bid to distract cristae wrinkles to destroy the OPA1 formation of oligomer to facilitate the connection and maintain the form of cristae. In addition, the release of cytochrome c promotes the release of additional cytochrome c. In contrast, overexpression of OPA1 delays structural changes in the mitochondrial inner membrane and reduces the damage caused by Bid in heart ischemia injury [94].

Necrosis and necroptosis

Necrosis, like apoptosis, is considered a form of the execution phase of programmed cell death, although the consequences of necrotic and apoptotic cell death are quite different for a whole organism. Necrosis is a form of cell death characterized by early plasma membrane permeation and organelle swelling. Necrosis occurs when cells are subjected to excessive external stress [95]. Necrosis is considered passive and unregulated. Although necrosis is widely found in human pathology, there have been few studies on the mechanism of necrosis regulation in ischemia-reperfusion injury [96]. Necrosis is caused by a dramatic change in the external environment caused by physical, chemical, or biological damage. Cell disintegration, organ swelling and loss of mitochondrial function are the main features of necrosis, and the process induces a large number of local inflammatory responses in ischemic tissue [97]. Although necrosis is observed in a variety of pathophysiological processes, traditional necrosis is considered unregulated. However, recent studies have confirmed the characteristics of necrosis, known as necroptosis in mouse models of inflammation and ischemia-reperfusion injury [98]. Necroptosis is a form of programmed cell death that is controlled by death signals and displays a death pattern like that of necrosis [98]. Local ischemia is caused by obstruction of blood flow to tissue, resulting in a limited supply of oxygen and nutrients, which if prolonged, can impair energy metabolism and cell death [99]. The recovery of blood loss (reperfusion) leads to the reintroduction of oxygen and the production of ROS, leading to cell death associated with inactivation [100, 101].

The formation of a necrosome key protein in the RIP1, RIP3 is phosphorylation, not only the structural changes, because the two protein active domains have high degree of similarity. Promoting TNFR complex II more stable, and at the same time activation of RIP3 downstream regulatory signal path. RIP1 interacts with RIP3 and forms necrosome-induced necroptosis with dimerization, while cell death is still performed with conventional apoptosis or necrosis in the absence of ischemia-reperfusion injury in rat retina [96]. However, in the

case of RIP3 overexpression, not only was a high concentration of ROS detected, but also enhanced NF-κB protein regulation, this regulation ability was significantly lower than that of RIP1, so many diseases are in this way necroptosis way to control. These findings suggest that RIP3 may be associated with the activity of RIP3 in disease formation and that RIP3 can control the transformation of cells *in vivo* to necroptosis [102]. In addition to relying on the activity of RIP1 and RIP3, necroptosis, including RIP1 activity, can be directly or indirectly involved in the phosphorylation of RIP3 at Ser161 phosphate sites. Studies have shown that viral infection in mouse cells is mainly regulated by RIP3 rather than RIP1-regulated necroptosis [103]. However, increasing evidence has clarified that certain types of necrosis are mediated by the intrinsic cellular procedures that are strictly controlled and are now called necroptosis (Fig. 3) [95]. Because of the association between necroptosis and inflammation in the pathogenesis of human diseases, understanding the molecular mechanisms underlying necrosis and the pathophysiological importance of necroptosis remains an important goal of ischemia-reperfusion injury research.

Autophagy

Autophagy is a biological process in which biological macromolecules and damaged organelles in the cytoplasm are degraded in membrane vesicles. Autophagic common inclusions mainly contain cytoplasmic components and some damaged organelles such as granules. According to the source of the autophagosomes, the inclusion is transported to the lysosome in different ways, and the autophagy process is roughly divided into three main types: 1. macroautophagy: the main source of the membrane originates from the endoplasmic reticulum and Golgi apparatus. The monolayer film of the net forms a double-layer separation film with a cup-like structure, and completely contains the object to be degraded to form an autophagosome, which is then fused with the lysosomal membrane to form an autophagosome. The degradation products are degraded by lysosomal enzymes. 2. microautophagy: the lysosomal membrane is directly wrapped to be degraded and digested in the lysosome. 3. chaperone-mediated autophagy (CMA): the chaperone is first bound to the protein to be degraded to guide the transport of the substance to lysosomes, digested and decomposed by enzymatic action, and the CMA pathway is a soluble protein molecule. Therefore, the CMA pathway has a clear selectivity, at this point with the first two ways there is a huge difference [104].

During ischemia-reperfusion injury, cells induce cell autophagy [105]. Autophagy is regulated by autophagy-related proteins, which are encoded by autophagy-related genes (ATG). Autophagy is mainly mediated by hormones. The ULK1-Atg13-RB1CC1-Atg10 complex formed by Atg13 and RB1CC1/Atg17 interacts with mTORC1 and participates in the initial phase of autophagy. In the absence of nutrients and energy, cells display inhibited mTOR activity, while ULK1 is activated, and then control Atg13 and RB1CC, autophagy membrane began to form [106]. Autophagic vesicle extension requires the participation of the Atg12-Atg5-Atg16 complex and Atg8/LC3, two ubiquitination binding systems. The initial free LC3-I in the cytoplasm binds to PE and is lipidated to form LC3-II, which is localized to the phagocytic membrane. The presence of specific markers of LC3-II, the formation of autophagosomes, can be used as autophagy-induced subject matter, autophagosomes in the lysosomal hydrolase was degraded [107]. Regulatory autophagy pathways include mTOR, PI3K-Akt, p53, CA-AMPK and endoplasmic reticulum pressure (Fig. 3) [108, 109]. mTOR is located downstream of PI3K-Akt signaling, regulates cell growth, and inhibits the initial process of autophagy [110, 111]. The mTOR inhibitor rapamycin can induce autophagy. At the beginning of reperfusion, mitochondria generate ATP, which induces autophagy. In parallel, reperfusion can induce calcium and accumulation in mitochondria. When increased autophagy did not completely neutralize the reperfusion pressure, liver cell damage occurred [112]. In previous studies, the use of chloroquine and GFP-LC3 to observe the autophagic image confirmed that the initial stage of reperfusion promotes autophagy. However,

autophagy did not clear all dysfunctional mitochondria during the intracellular perfusion stage, as there was mitochondrial calcium overload and excessive ROS, which exceeded the autophagic clearance ability, leading to mitochondrial permeability transition (MPT) [112]. MPT leads to irreversible oxidative phosphorylation de-coupling, ATP formation is insufficient, and there is energy failure, eventually leading to hepatocellular death [113]. An understanding of the mechanisms that regulate autophagy to reduce the ischemia-reperfusion injury has a guiding significance.

Therapeutic approaches in ischemia-reperfusion injury

The clinical manifestations of ischemia-reperfusion injury have been previously described, and include myocardial hibernation, acute heart failure, cerebral dysfunction, gastrointestinal dysfunction, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome. Therapeutic approaches are different according to the injured organ. For sepsis, timely resuscitation with adequate fluids and vasopressors is recommended [1-7]. In acute myocardial infarction, reperfusion arrhythmias are common complications in patients undergoing revascularization. To control malignant arrhythmias, staged gradual reflow or transient acid reperfusion are useful management approaches to reduce ischemia-reperfusion injury [114, 115]. In acute ischemic extremity injuries, the first step is shortening ischemic time, correcting metabolic acidosis, and preventing acute renal injury using metabolic techniques or anti-inflammatory treatments [116-118]. In this review, we focus on the therapeutic approaches of cell biology in ischemia-reperfusion injury.

Nitric oxide corresponding protection strategy

NO is synthesized by vascular endothelial cells to prevent the reduction of antioxidants in the body to reduce oxidative stress injury. In ischemia and reperfusion injury, NO reduces the harmful effects of endothelin and improves microcirculation. Using ischemia-reperfusion injury animal experiments, Peralta et al. [119] found that NO significantly improved liver function by improving liver blood flow. Adenosine plays a critical role in stimulating the production of NO in ischemia-reperfusion injury. In a study by Lang et al. [120], using NO inhalants after liver transplantation protected hepatocytes, promoted liver function recovery, and shortened the duration of hospital stay. In a meta-analysis by Bice et al. [121], NO interventions also limited infarct size during the early reperfusion period. In myocardial injury, inhaled gaseous NO inhibited cardiomyocyte apoptosis and reduced infarct size [122-125]. The concentration of creatine kinase also decreased [122-124, 126, 127]. Increasing NO mediated respiratory complexes, leading to improved myocardial oxygen consumption and reduced production of superoxide. Administration of NO for ischemia-reperfusion injury may be useful to control ROS formation and cell death.

Nuclear transcription factors

NF-κB is a dimer redox-sensitive transcription factor consisting of p50 and p65 with an important role in ischemia-reperfusion injury involving a rapid response to oxidative stress. The activation of NF-kB regulates cell survival, apoptosis and inflammation, via effectors such as MnSOD, Bcl-2, TNF-α, ICAM and P-selectin. In normal Kupffer cells, NF-κB is inactive, and its complexes inhibit protein I-κB formation. In ischemia-reperfusion injury, NF-κB is activated in a variety of ways: the classical pathway of NF-κB activation, in which IκB is phosphorylated by its kinase complex, followed by degradation; without the IKK complex (IκB kinase) pathway, IκBα tyrosine residues are phosphorylated and NF-κB is activated. Activated NF-κB is transferred to the nucleus and activates the target gene for transcription,

thereby participating in ischemia-reperfusion injury. NF-κB exerts its effects by regulating NOS, cytokines (TNF-α, IL-1), chemokines (ENA78), and ICAM-1. Matsui et al. [128] found that activation of NF-κB was associated with ischemia-reperfusion injury in animal experiments, and administration of NF-κB inhibitors reduced ischemia-reperfusion injury.

Reduced ischemia-reperfusion injury caused by apoptosis

Cells release ATP through calcium as a distress signal to attract phagocytic cells and inhibiting renal ischemia-reperfusion injury cell by apoptosis is a treatment strategy for ischemia-reperfusion injury. Studies have shown that damaged proximal tubular cells produce matricellular thrombospondin 1 (THBS1) and High Mobility Group Box 1 (HMGB1), which can induce apoptosis; administration of THBS1 and HMGB1 inhibitors protected mice from kidney ischemia-reperfusion injury [129-131]. Previous studies have also suggested that an increase in platelet-derived factors can lead to an increase in GSK-3βSer9 phosphorylation levels and that Ser9 phosphorylation can inhibit apoptosis. Hotchkis et al. [132] found that the immunosuppressive agent cyclosporine inhibited the mitochondrial permeability of renal tubular cells through mPTP open inhibitor, is an important inhibitor of tubular death in the preparation [133].

Calcium overload and corresponding protection measures

In the process of ischemia-reperfusion injury, ischemia and hypoxia disturb the physiological balance of cells, causing an insufficient cell energy supply. A lower ATP level decreases the activity of energy-dependent Na^+-K^+-ATPase, leading to an increase in intracellular Na^+. Na^+-Ca^{+2} ion exchange compensates to reduce the concentration of intracellular Na^+. To maintain the membrane potential, the compensation of ion exchange results in a higher concentration of intracellular Ca^{+2}; Ca^{+2}-ATP/Ca^{+2}-Mg^{+2}-ATPase dysfunction worsens this condition. Calcium overload injures cells from multiple aspects, by increasing ROS, activating inflammatory cells, inducing cell apoptosis, destroying cell membranes, and interfering with mitochondrial function. Pretreatment with verapamil, a voltage-dependent calcium channel blocker, prior to animal ischemia-reperfusion resulted in significant inhibition of Ca^{+2} accumulation in hepatocytes [134]. Mitochondrial ATD-sensitive potassium channels (mitoKATP) and mPTP differences altered mitochondrial Ca^{+2} balance, induced mitoKATP opening to mitochondrial membrane partial depolarization and inhibition of mPTP can reduce Ca^{+2} influx, thereby reducing calcium overload [135]. A Na^+-Ca^{+2} exchange inhibitor, KB-R7943, inhibited apoptosis, activated K^+-ATP channels, promoted K^+ and Ca^{+2} exchange, and inhibited calcium overload [136].

Conclusion

The clinical manifestations of ischemia-reperfusion injury are diverse. Ischemia-reperfusion injury is a critical challenge for physicians from a treatment standpoint. In this review, we presented current research findings on the basic pathophysiology of ischemia-reperfusion injury, especially the involvement of ROS and cell death pathways. Several principles described here are worth reiterating. First, hypoxia and ischemia induce anaerobic metabolism and dysfunction of the electron transport chain in mitochondria. Decreased ATP production causes dysfunction of ion-exchange channels, leading to retention of sodium, hydrogen and calcium, which results in cell swelling and impaired enzyme activity in the cytoplasm. Second, mitochondrial damage and electrolyte imbalance in the reperfusion state promotes the production of oxidative stress via the NADPH oxidase system, NOS system, and xanthine oxidase system. Finally, ROS retention induces cell damage and leads to cell death

via autophagy, mitoptosis, necrosis and necroptosis, and apoptosis. Although the detailed mechanisms associated with ischemia-reperfusion injury remain to be fully elucidated, we believe an understanding of the pathophysiology has the potential to provide a strong foundation for the exploration of new therapeutic avenues.

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Disclosure Statement

No conflict of interests exists.

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