Current Pharmaceutical Research (CPR)
Vol 2, Issue 1, January-March 2026
Journal Homepage: https://cpr.org.in/

Ischemia Reperfusion Injury and Immune Modulation: New Insights into Pathogenesis and Therapy

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Keywords

Ischemia-Reperfusion Injury; Immune Modulation; Oxidative Stress; Inflammasome; Cytokines; Ischemic Conditioning; Nanomedicine; Immunotherapy; Necroptosis; Personalized Medicine

Abstract

Ischemia-reperfusion injury (IR) is a counterintuitive condition in which the reintegration of blood supply to an ischemic tissue results in an increase in cellular damage as well as an exaggeration of a harmful inflammatory response. It is an acute pathophysiological mechanism underlying morbidity and mortality of a broad range of clinical syndromes, such as myocardial infarction, stroke, organ transplantation and shock. The mechanism of IR is another complex process wherein metabolic perturbation, oxidative stress, intracellular calcium overload, and an extensive activation of innate and adaptive immune systems are intertwined. The review gives an excellent summary of the existing knowledge regarding the molecular and cellular pathways involved in the development of IR, especially the critical role of immune modulation. We explore organ-relevant phenotypes, interrogate major signal transduction mechanisms (NF-kB, NLRP3 inflammasome, and HIF), and assess the interactions among cell death pathways (apoptosis and necroptosis). Moreover, we evaluate critically the terrain of therapeutic approaches, including conventional and emerging frontiers of pharmacotherapy, cellular/gene therapy, and nanomedicine. Finally, we address more sophisticated immune-modulating approaches like monoclonal antibodies, RNA-based therapeutics, and present the guidelines in which biomarkers, personalized medicine, and merging multi-omics and artificial intelligence bridge the knowledge-to-clinical practice gap.

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Article Info
Received: 11 February 2026; Received in revised form: 29 March 2026; Accepted: 29 March 2026; Available online: 31 March 2026; Volume: 2; Issue: 1; Pages: 83-99.

ISSN: 3049-2955/The authors © 2025, under exclusive license to the Sprout Publication.
DOI: https://doi.org/10.63785/2026.2.1. 8399

1. Introduction

Ischemia reperfusion (IR) injury, a paradoxical exacerbation of tissue damage following the restoration of blood flow after ischemia, continues to pose a serious challenge across diverse clinical settings, notably myocardial infarction, stroke, acute kidney injury, transplantation, and major surgery [1]. The injurious cascade involves oxidative stress, microvascular dysfunction, calcium overload, mitochondrial failure, inflammation, and a spectrum of regulated cell death pathways, including apoptosis, necroptosis, autophagy, and ferroptosis. In transplantation, IR injury underlies primary graft dysfunction and shapes long-term outcomes; novel insights reveal how danger signals like high mobility group box 1 (HMGB1) and Toll-like receptor 4-mediated axes amplify immune responses and early graft injury [2]. In cardiovascular interventions such as percutaneous coronary procedures, real-world trials of remote ischemic conditioning (RIC) where brief limb ischemia is intentionally applied before reperfusion, have demonstrated a dramatic 62% reduction in troponin release and sustained reductions in six-year major adverse cardiovascular events. These clinical observations underscore the urgency of translating mechanistic insights into patient-centered therapies [3].

Tracing the evolution of IR research reveals a dynamic journey from early mechanistic mapping to highly targeted modern strategies. Foundational attention to oxidative injury, autophagy, and mitochondrial dynamics has laid the groundwork for decades of inquiry. More recently, the discovery and characterization of ferroptosis as an iron-dependent, lipid peroxidation-driven cell death modality has emerged as a key contributor to IR injury across multiple organs, and a promising therapeutic target [4]. Mechanistic advances continue apace: in

myocardial IR, modulation of macrophage phenotypes (M1 vs M2), influenced by chemokines, cytokines, and Toll-like receptor pathways, is now recognized as central to inflammation and healing dynamics [5]. Cutting-edge in vitro platforms, including iPSC-derived cardiomyocytes and organ-on-chip systems, are revolutionizing translational fidelity, enabling human-relevant modeling and drug testing beyond traditional animal models. Parallel strategies span epigenetic modulation (e.g., miRNAs), ischemic conditioning (pre and post), innovative perfusion techniques such as hypothermic oxygenated perfusion (HOPE) for vulnerable livers, and the development of PEG-based perfusates that preserve mitochondrial integrity during reperfusion. At the therapeutic frontier, precision tools like mitochondrial-targeted antioxidants (e.g., MitoQ, SS 31), mPTP inhibitors, anti-inflammatory agents, stem cells, exosome-based treatments, and AI enhanced multi omics design are gaining traction as viable paths toward reducing IRdamage [6].

2. Pathophysiology of Ischemia-Reperfusion Injury

2.1. Ischemic Phase

Hypoxia and Metabolic Disruption. The ischemic phase is characterized by the absence of oxygen and nutrients, which result in hypoxia and extreme metabolic interference. Ischemia leads to the inhibition of oxidative phosphorylation in mitochondria and to the rapid reabsorption of ATP [7]. This results in energy failure of the cells, which affects key functions like homeostasis of ions, protein synthesis, and cellular repair processes. In the absence of ATP ion pumps, such as the Na + /K + ATPase and the Ca2 + ATPase will no longer function well, and they will swell, acidify, and their membrane integrity will be destroyed. Along with energy failure, ischemia leads to the accumulation of metabolic wastes, e.g., lactate, that further acidifies the intracellular space [8]. The build-up of lactic acid and other metabolites further increases tissue injury, forming a cycle of viciousness that refines cell death during reperfusion. Recent reports have emphasized the exact effects of the metabolic changes that are caused by ischemia in important body systems, including the heart and brain, where ATP depletion may be essential to cell survival and performance [9].

2.2. Reperfusion Phase

Oxidative and Inflammatory Burden Reperfusion phase commences upon the restoration of blood supply to the ischemic tissue, which consists of reintroducing oxygen and nutrients. Although this is meant to ensure the recovery of tissues, reperfusion actually worsens tissues [10]. Through the instant oxygen influx during reperfusion, reactive oxygen species (ROS) are formed, which are highly reactive molecules that may cause cell damage to components such as lipids, proteins, and DNA. This is mostly evident in tissues that have high metabolic rates, like the myocardium and neurons. Research has revealed that mitochondrial dysfunction and the xanthine oxidase pathway are the major ways of ROS generation. These ROS are instrumental in the addition of further injury to the mitochondria, membrane depolarization, mitochondrial permeability transition pore (MPTP) dysfunction, and release of pro-apoptotic factors. Also, the reperfusion provokes an inflammatory reaction, inflammatory cells enter the tissue and discharge the pro-inflammatory cytokines, worsening the damage to cells. Cytokines like TNF-α, IL-6, and IL-1β also increase oxidative stress and form a cascade of immune activation that worsens tissue damage [11].

2.3. Cellular and Molecular Mechanisms

2.3.1. Mitochondrial Dysfunction

Mitochondria play a leading role in injury manifested during ischemic and reperfusion stages. In the state of ischemia, the production of ATP in mitochondria stops, which causes a deficiency of energy. During reperfusion, mitochondrial calcium overload is caused by the disruption of calcium homeostasis, which leads to the activation of proteases and phospholipases that break down cell structures. In addition, the oxidative stress of the ROS also leads to the destruction of the mitochondrial membranes and lipids by initiating the expression of a waterfall of cellular reactions contributing to necrosis and apoptosis. Research has determined that mitochondrial permeability transition pore (MPTP) is an essential occurrence during IR. Through this pore, water and solutes are moved into the mitochondria, causing the swelling and rupture of the mitochondria, as a result of which additional pro-apoptotic proteins, cytochrome c, and apoptosis-inducing factor (AIF) are released. Inhibition of MPTP opening has been suggested as a therapeutic objective to decrease mitochondrial damage and enhance the results after reperfusion [12].

2.3.2. Calcium Overload

Ca ions are at the center of the pathophysiology of IR [13]. In ischemia, calcium accumulates in the cell due to the impaired ability of ATP-dependent ion pumps. Upon reperfusion, the increased intracellular calcium (abruptly) further worsens mitochondrial performance and results in the release of such destructive enzymes as calpains, which break down cytoskeletal and contractile proteins. Pro-apoptotic proteins, such as caspases, are also aided by calcium-dependent signaling pathways and production of ROS, continuing the injury cycle [14].

2.3.3. Reactive Oxygen Species (ROS) Generation

One of the most researched processes of IR is the production of ROS in reperfusion. ROS, especially the superoxide anions and hydroxyl radicals, play a part in oxidative damage by attacking lipids, proteins, and DNA. Mitochondria are a leading source of ROS, although other enzymes, including NADPH oxidases and xanthine oxidase, also play a major role. Endogenous and exogenous antioxidants have been examined and have been suggested to reduce ROS-related damage, but with variable clinical effects. There are recent studies that have discussed some of the therapeutic options that can help mitigate the production of ROS, and these are mitochondrial-

targeted antioxidants, ion channel blockers, and anti-inflammatory agents. These strategies are meant to decrease oxidative injuries and maintain mitochondrial activity during resuscitation [15].

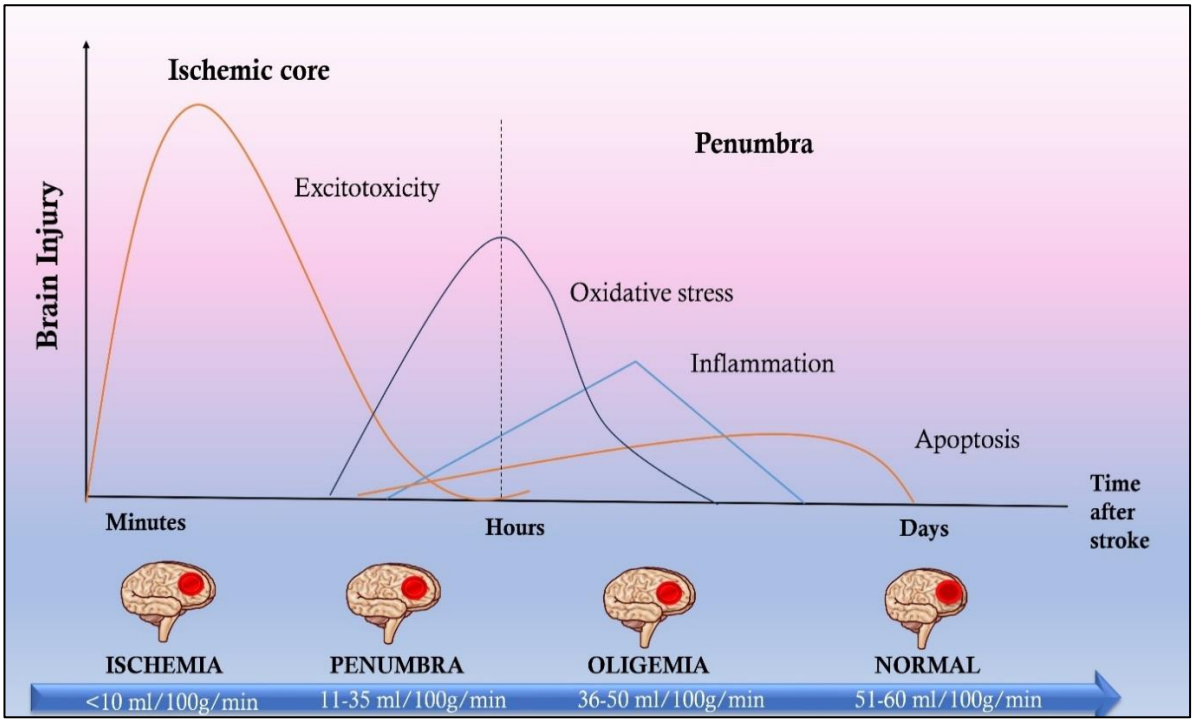

Figure 1: Temporal and spatial evolution of ischemic brain injury following stroke.

The schematic illustrates the dynamic progression of brain injury over time after ischemic stroke, distinguishing the ischemic core from the surrounding penumbra. Severe cerebral blood flow reduction in the ischemic core leads to rapid excitotoxic neuronal death within minutes. In the penumbral region, partial perfusion preserves tissue viability for several hours, during which oxidative stress and inflammatory responses progressively intensify. As reperfusion and secondary injury mechanisms evolve, delayed apoptotic cell death predominates over days. The lower panel depicts regional cerebral blood flow thresholds defining ischemia (<10 ml/100 g/min), penumbra (11–35 ml/100 g/min), oligemia (36–50 ml/100 g/min), and normal perfusion (51–60 ml/100 g/min), highlighting the therapeutic window for neuroprotection [16].

3. Immune Modulation in IR Injury

3.1. Role of Innate Immunity

The innate immune system represents the first and most powerful response to injury, through a rapid, non-specific attack that sets the stage for much of the subsequent damage. Neutrophils represent the archetypal effector cells of injury. Within minutes of reperfusion, a cascade of events leads to their massive infiltration into the affected tissue. This process, known as leukocyte trafficking, is a multistep sequence: first, activated endothelial cells express selectins (P- and E-selectin) that cause neutrophils to "roll" along the vessel wall. This is followed by firm adhesion mediated by integrins (e.g., MAC-1) on neutrophils binding to immunoglobulin-like adhesion molecules (e.g., ICAM-1) on endothelium. Finally, neutrophils transmigrate into the parenchyma. Once activated, they deploy three primary cytotoxic arsenals: (1) they release a barrage of proteolytic enzymes (e.g., matrix metalloproteinases, elastase) that degrade the extracellular matrix and directly damage cell membranes; (2) they generate a massive oxidative burst through the NADPH oxidase complex, producing copious reactive oxygen species (ROS); and (3) they form neutrophil extracellular traps (NETs), web-like structures of DNA and histones that can trap pathogens but also directly cause endothelial and parenchymal cell injury and exacerbate microvascular obstruction [17].

Macrophages represent a versatile cellular element that assumes a context-dependent dual role: Tissue-resident macrophages are among the first cells to recognize damage. They phagocytose dead cells and debris, an important clean-up task [18]. However, they also release a plethora of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and chemokines by which they amplify the inflammatory cascade. Again, the state of polarization is critical. In the early phases of the inflammatory response, it is dominated by the classically activated M1 phenotype, driven by DAMPs and IFN-γ, and highly pro-inflammatory with the production of ROS and nitric oxide [19].

Table 1. Summary of Pathophysiological Mechanisms and Therapeutic Strategies in Ischemia–Reperfusion (I/R) Injury.

S.	Pathophysiologi	Key	Consequenc	Therapeutic	Mechanism of	References

No.	cal Mechanism	Molecular/Cellular Events	es	Strategies	Action / Examples	
1.	Oxidative Stress	Overproduction of reactive oxygen species (ROS) during reperfusion, impaired antioxidant defenses	Lipid peroxidation, DNA damage, mitochondrial dysfunction	Antioxidants	Scavenging ROS (e.g., N-acetylcysteine, edaravone, vitamin E, SOD mimetics)	[20]
2.	Calcium Overload	Reperfusion-induced Ca^{2+} influx via NCX and mPTP opening	Mitochondrial swelling, enzyme activation, necrosis	Calcium Channel Blockers, mPTP inhibitors	Verapamil, cyclosporine A (blocks cyclophilin D–mediated pore opening)	[21]
3.	Mitochondrial Dysfunction	Collapse of mitochondrial membrane potential; impaired ATP generation	Energy failure, apoptosis	Mitochondria-targeted therapies	MitoQ, SS-31 peptide – preserves mitochondrial integrity and ATP production	[22]
4.	Inflammation	Activation of NF-κB, cytokine release (TNF-α, IL-1β), neutrophil infiltration	Tissue edema, endothelial injury	Anti-inflammatory agents	Corticosteroids, NSAIDs, IL-1 inhibitors (anakinra), NF-κB inhibitors	[23]
5.	Endothelial Dysfunction	Reduced NO bioavailability; adhesion molecule upregulation (ICAM-1, VCAM-1)	Microvascular obstruction, platelet aggregation	NO donors, Statins, PDE5 inhibitors	Improve vasodilation, reduce adhesion molecule expression	[24]
6.	Apoptosis and Necroptosis	Activation of caspases, Bcl-2 family imbalance; RIPK1/RIPK3 pathways	Programmed cell death	Anti-apoptotic agents, Caspase inhibitors	Z-VAD-fmk, Bcl-2 mimetics, necrostatin-1 (RIPK1 inhibitor)	[25]
7.	Autophagy Dysregulation	Excessive or insufficient autophagic flux during reperfusion	Cell death or impaired clearance	Autophagy modulators	Rapamycin (induction), 3-MA (inhibition), depending on stage and tissue	[26]
8.	Microvascular Obstruction	Capillary plugging by leukocytes, platelets, and edema	Impaired perfusion despite an open artery	Antiplatelet and antithrombotic therapy	AspIRn, P2Y12 inhibitors, tPA (tissue plasminogen activator)	[27]
9.	Immune Activation	Complement activation, DAMP release (HMGB1, ATP)	Secondary tissue damage	Complement inhibitors, Immunomodulators	Eculizumab (C5 inhibitor), anti-HMGB1 antibodies	[28]
10.	Gene and Cellular Therapy Approaches	Dysregulated repair and regeneration	Delayed recovery	RNA therapeutics, stem cells, nanoparticles	siRNA/miRNA to suppress pro-inflammatory genes; MSCs for regeneration; nano-delivery for targeted therapy	[29]

Less studied than neutrophils and macrophages, NK cells do play a role in the innate immune response. These cells could be activated by stress-induced ligands expressed by damaged cells and produce pro-inflammatory cytokines such as IFN-γ. This can further promote macrophage M1 polarization and enhance the general inflammatory milieu [30].

3.2. Adaptive Immune Responses

3.2.1. T Lymphocytes

Both helper CD4+ and cytotoxic CD8+ T cells invade ischemic tissue in a matter of hours to days after reperfusion. Their activation is induced by antigens

presented by antigen-presenting cells in the context differentiate into several subsets: Th1 cells, through the production of IFN-γ, promote a pro-inflammatory environment and activate macrophages; Tregs are critical for dampening the immune response and resolving inflammation, mainly through the production of IL-10 and TGF-β. The balance between Th1 versus Treg responses represents an important regulatory node. CD8+ T cells directly lyse stressed parenchymal cells (for example, cardiomyocytes, neurons) presenting antigens and thus contribute directly to cell death [31, 32].

3.2.2. B lymphocytes

B cells play a much more intricate role. These can also potentially worsen injury by the production of antibodies, which could then form immune complexes and potentially activate the complement system. They also serve as APCs themselves to activate T cells. On the other hand, subsets of B cells, especially regulatory B cells or Bregs, produce anti-inflammatory cytokines such as IL-10 and IL-35, thus reducing inflammation and promoting tolerance [33].

3.3. Complement System Activation

The complement system represents a potent, immediate humoral component of innate immunity that is potently activated following injury. DAMPs, in the form of mitochondrial fragments and mitochondrial DNA released from necrotic cells, may directly activate the complement cascade via the alternative pathway, spontaneous tick-over, as well as the lectin pathway, through the binding of mannose-binding lectin to damaged cells [34]. Key effector molecules generated include, importantly, anaphylatoxins C3a and C5a. Several of the effects of C5a, the most potent chemoattractant for neutrophils and monocytes-promoting degranulation and ROS production by these cells-are mediated directly or indirectly. The assembly of the Membrane Attack Complex, MAC (C5b-9), on the surface of endothelial and parenchymal cells can cause direct lysis or sub-lytically activate pro-inflammatory signaling pathways to further amplify the injury. The complement system thus acts to provide a critical link between the initial cell death and the full-blown inflammatory response [35].

3.4. Cytokine and Chemokine Networks in Inflammation

This network of cytokines and chemokines, which represents the chemical language of inflammation, mediates the full coordination of the entire immune response in injury [36]. The triad of cytokines, namely, Tumor Necrosis Factor-alpha, Interleukin-1 beta, and Interleukin-6, forms the core of the pro-inflammatory response [37]. TNF-α is generated early from activated macrophages and mast cells. It directly induces the activation of endothelial cells, upregulates adhesion molecules, promotes the recruitment of leukocytes, and may induce apoptosis in susceptible cells. IL-1β is usually processed and released following the activation of NLRP3. It acts in a synergistic way with TNF-α to induce other of the inflammatory milieu. CD4+ T cells cytokines, adhesion molecules, and COX-2, further amplifying the inflammatory signal [38].

IL-6 is generated by various types of cells and possesses pro- and anti-inflammatory properties. In the early phase, it enhances the recruitment and activation of neutrophils and stimulates the acute phase response. Chemokines represent chemotactic cytokines, which direct the migration of specific leukocyte subsets [39]. Key players include: IL-8 (CXCL8): Potent chemoattractant for neutrophils. MCP-1 (CCL2): Mainly attracts monocytes to the site of injury that then differentiate into macrophages. RANTES (CCL5) and MIP-1α (CCL3): These attract a wider range of leukocytes, including monocytes and T cells. Anti-inflammatory Cytokines: To balance the destructive cascade, the body simultaneously activates a resolution program. The most important anti-inflammatory cytokine is IL-10, produced by M2 macrophages, Tregs, and Bregs, which inhibits the production of pro-inflammatory cytokines by macrophages and dendritic cells. Transforming Growth Factor-beta (TGF-β) is another key player in the process that induces fibrosis and tissue repair while exerting a strong immunosuppressive effect [40, 41].

4. Organ-Specific Aspects of IR Injury

4.1. Myocardial Ischemia-Reperfusion Injury

Myocardial injury is the most extensively investigated paradigm, occurring clinically during myocardial infarction when blood supply is restored either by thrombolysis or angioplasty [42]. The main cell type involved is the cardiomyocyte, a terminally differentiated cell with extremely high metabolic demands and dependency on aerobic respiration. The ischemic phase rapidly depletes ATP, depressing the sodium-potassium pump and allowing intracellular sodium and calcium to accumulate. During reperfusion, the sudden delivery of oxygen to a compromised electron transport chain in mitochondria results in an explosive generation of ROS. This "oxidative burst", together with the normalization of pH, triggers the opening of the mitochondrial permeability transition pore (mPTP), an event critical to the necrotic death of cardiomyocytes [43].

The myocardial stunning and lethal reperfusion injury are unique and critical features in the heart. Stunning refers to a state of protracted post-ischemic contractile dysfunction of viable tissue, while lethal injury describes the death of cardiomyocytes that were salvageable at the end of an ischemic period but are killed by the events of reperfusion. The no-reflow phenomenon is also important in the heart: swollen endothelial cells, microthrombi, and trapped leukocytes may obstruct capillaries and prevent perfusion even after the epicardial coronary artery is reopened, thus extending the ischemic insult [44].

4.2. Cerebral (Stroke) Injury

Cerebral Injury occurs in ischemic stroke following thrombolytic therapy or mechanical thrombectomy.

The brain is very sensitive to ischemia due to its high negligible energy reserves. Neurons are most susceptible, followed by astrocytes and oligodendrocytes [45].

The ischemic penumbra, a region of moderately ischemic, electrically silent but potentially viable tissue surrounding the irreversibly damaged core, is a key concept in cerebral I/R. The primary goal of reperfusion therapy is to salvage the penumbra. In turn, though, reperfusion canalso worsen the injury by the development of severe edema and hemorrhage. Cerebral edema, a life-threatening complication of BBB disruption, allows the leakage of fluid and proteins into the brain parenchyma; this increases intracranial pressure and compromises the blood flow even further.

4.3. Hepatic and Renal IR Injury

IR injury commonly affects the liver and kidneys in clinical settings, such as during transplantation, resection surgery, and shock [46]. Hepatic IR injury represents a classic two-hit phenomenon. The initial ischemic insult preferentially injures the metabolically active hepatocytes most distal from the oxygenated blood supply in the centrilobular zone. Subsequent reperfusion is characterized by the activation of the liver's extensive resident macrophage population, the Kupffer cells, as a major source of ROS and pro-inflammatory cytokines, including TNF-α and IL-1. These events promote the recruitment of neutrophils, which adhere to the sinusoidal endothelium, contributing to further injury by the release of proteases and additional ROS. A characteristic of liver IR injury is the initiation of hepatocyte cell death via pathways such as necroptosis, further promoting the release of DAMPs and perpetuating the inflammatory cycle [47]. The potent regenerative capacity of the liver supports recovery from many insults, but severe injury promotes acute liver failure [48].

Renal IR injury is one of the predominant causes of AKI. Because of its high metabolic rate due to extensive reabsorption, the proximal tubular epithelial cells are particularly vulnerable to ischemia, which disrupts the cytoskeleton, leading to loss of cell polarity and shedding of the brush border, impairing reabsorptive function. This results in oxygen consumption, limited glycolytic capacity, and subsequent inflammation characterized by endothelial cell activation, infiltration of neutrophils, and a prominent role for the innate immune sensors, including the TLR4 pathway [49]. Tubular obstruction by casts of detached viable and dead cells is a hallmark of renal IR that increases intratubular pressure and lowers the glomerular filtration rate (GFR) further. Kidney dysfunction resulting in systemic consequences includes fluid and electrolyte imbalances and the retention of waste products [50].

4.4 Intestinal and Pulmonary IR Injury

Although less commonly the initiating event, IR in the gut and lungs frequently serves as a driver of systemic injury and multiorgan failure. Intestinal IR injury is particularly perilous, since the gut serves as a "motor" for systemic inflammation. The intestinal mucosa has rapid cell turnover and sits adjacent to a vast reservoir of commensal bacteria [51]. Ischemia disrupts the tight junctions between epithelial cells, damaging the critical gut-vascular barrier. With reperfusion, this breach enables the translocation of bacteria and their endotoxins (e.g., Lipopolysaccharide - LPS) into the portal circulation and mesenteric lymphatics [52]. This sets off a powerful systemic inflammatory response syndrome (SIRS), activating neutrophils and cytokine storms capable of causing remote damage to other organs, especially the lungs and liver. Thus, intestinal IR injury is often not an isolated event but rather an instigator of multi-organ dysfunction. While lung transplantation is the most common association with pulmonary IR injury, the unique vulnerability of the lung has its basis in its direct exposure to both the circulation and the alveolar space [53]. The main targets are the pulmonary endothelial cells and alveolar epithelial cells. A characteristic hallmark of the IR injury here is the profound inflammatory response with massive neutrophil sequestration within the pulmonary capillaries. These neutrophils become activated, transmigrate into the alveolar space, and release ROS and proteases such as elastase, destroying the delicate gas-exchange interface. This causes increased vascular permeability, non-cardiogenic pulmonary edema, impaired oxygenation, and the clinical picture of primary graft dysfunction in transplant recipients [54].

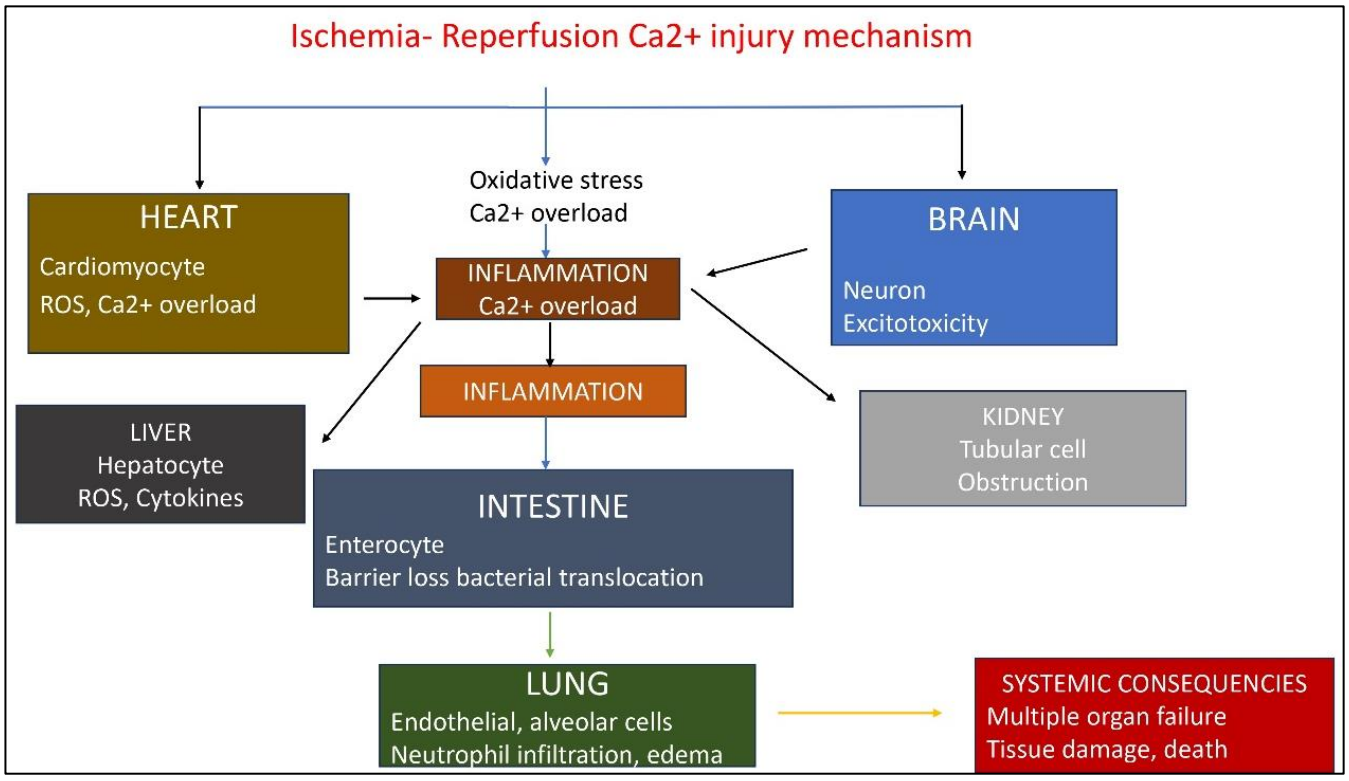

Figure 2: Ischemia–reperfusion–induced Ca^{2+} injury and multiorgan dysfunction.
Ischemia–reperfusion triggers oxidative stress and intracellular Ca^{2+} overload, leading to inflammatory activation and cellular injury. In the heart, cardiomyocytes exhibit reactive oxygen species (ROS) generation and Ca^{2+} overload. In the brain, neuronal excitotoxicity predominates, while renal tubular obstruction characterizes kidney injury. Hepatic hepatocytes release ROS and pro-inflammatory cytokines, and intestinal enterocytes undergo barrier disruption, promoting bacterial translocation. Secondary lung involvement includes endothelial and alveolar cell injury with neutrophil infiltration and edema. The cumulative inflammatory and Ca^{2}-mediated responses result in systemic consequences, ultimately leading to multiple organ failure, tissue damage, and death.

5. Molecular Signaling Pathways in IR Injury

5.1. NF-κB, NLRP3 Inflammasome, and MAPK Signaling: The Pro-Inflammatory Triad

This triplet constitutes the central inflammatory machinery in IR injury, able to amplify the inciting insult into a full-bore sterile inflammatory response. Nuclear Factor-Kappa B (NF-κB) is generally recognized as a master transcriptional regulator of inflammation [55]. Cellular stress of ischemia initiates its activation, although this process is fully unleashed upon reperfusion. The influx of oxygen and calcium results in the massive production of Reactive Oxygen Species (ROS), which act as potent second messengers. Other DAMPs and ROS activate upstream kinases-IKK complex-which phosphorylate the inhibitory protein IκBα and target it for degradation. This releases the NF-κB dimer-usually p50-p65-which then translocates to the nucleus. Here, it binds to DNA and initiates the transcription of a whole host of pro-inflammatory genes that include cytokines such as TNF-α, IL-1β, and IL-6; chemokines, an example of which is MCP-1; and adhesion molecules like ICAM-1 and VCAM-1. This "priming" signal is important because it allows neutrophils and other immune cells to be recruited to the site of injury, but its dysregulation leads to excessive tissue damage [56].

The NLRP3 Inflammasome acts as a molecular platform that translates these initial NF-κB-derived signals into a powerful, localized inflammatory burst. Transcription of pro-IL-1β and NLRP3 itself is first "primed" via NF-κB. The actual "activation" of the inflammasome occurs during reperfusion and is induced by a confluence of danger signals: mtROS, potassium efflux, and calcium overload. Upon activation, multiple NLRP3 proteins oligomerize to recruit the adapter protein ASC, which in turn recruits procaspase-1. This complex enables the autocleavage of procaspase-1 into its active form, caspase-1. Active caspase-1 subsequently cleaves pro-IL-1β and pro-IL-18 into their mature, highly active forms, which are then secreted to drive a potent inflammatory response. This pathway is a critical link between initial cellular damage through mtROS and the execution of inflammatory cell death [57].

Mitogen-Activated Protein Kinase (MAPK) pathways such as JNK, p38, and ERK constitute parallel stress-sensing routes, which synergize with NF-κB. They are serine/threonine kinases that rapidly phosphorylate and become activated under ROS, osmotic stress, and cytokine signaling during I/R. Among them, p38 and JNK pathways are associated with promoting inflammation and apoptosis. Both can enhance NF-κB activity and directly phosphorylate transcription factors involved in stress responses. On the other hand, the ERK pathway can sometimes promote cell survival but at other times contributes to hypercontracture and damage in cardiomyocytes. The

cross-talk between MAPKs, NF-κB, and the NLRP3 inflammasome results in a potent self-reinforcing inflammatory network that is a major target for therapeutic suppression [58].

5.2. Hypoxia-Inducible Factor (HIF) and Reoxygenation Pathways: The Oxygen-Sensing Switch

The body's principal molecular sensor for oxygen deprivation, the Hypoxia-Inducible Factor pathway, has a complex and context-dependent role in IR injury. Under normal oxygen conditions, the HIF-1α subunit is constitutively produced but quickly degraded. This is through the action of PHD enzymes, utilizing oxygen as substrate to hydroxylate HIF-1α, thereby marking it for recognition by the VHL protein and subsequent proteasomal degradation [59].

In ischemia, low oxygen suppresses PHD activity, allowing the stabilization and accumulation of HIF-1α. The latter dimerizes with a constitutive partner, HIF-1β, translocates to the nucleus, and stimulates the transcription of more than 100 genes whose actions promote survival in an environment deprived of adequate oxygen. These include genes encoding glycolytic enzymes to aid energy production in the absence of oxygen, angiogenic factors such as VEGF to stimulate the growth of new blood vessels, and erythropoietin to increase oxygen-carrying capacity. Generally, HIF activation in this phase is protective and adaptive [60].

The paradox occurs at the time of reperfusion. The sudden readmission of oxygen rapidly reactivates the PHD enzymes, causing the swift degradation of HIF-1α. This abrupt shutdown of the adaptive HIF response is dangerous. But even worse, the reoxygenation phase can evoke HIF-1α-independent, harmful signaling [61]. The rapid reintroduction of oxygen, especially through mitochondrial dysfunction, has been shown to produce an explosive generation of ROS, which can damage DNA, proteins, and lipids. This reoxygenation stress activates the pathways described in section 5.1, effectively overriding the adaptive HIF signaling and pivoting the cell towards inflammation and death. Therapeutic strategies are exploring the pharmacological stabilization of HIF during ischemia or the inhibition of its destructive downstream effects upon reperfusion [62].

5.3. Autophagy, Apoptosis, and Necroptosis

The Cell Death Trilemma outcome of IR injury is determined by the balance between pro-survival and pro-death pathways, with cells facing a fate decided between three principal mechanisms. Autophagy is a catabolic process for the degradation and recycling of damaged organelles and proteins through lysosomes. During ischemia, autophagy is generally upregulated as a pro-survival mechanism, creating essential nutrients and clearing dysfunctional mitochondria (mitophagy), thus preventing apoptosis. However, during reperfusion, the role of autophagy is highly contextual. Controlled autophagy may continue to be protective by removing proteins and organelles damaged by ROS. On the other hand, excessive or dysregulated autophagy itself can be a cell death mechanism, sometimes referred to as autophagic cell death [63].The switch between its pro-survival and pro-death roles depends on the severity and duration of the stress and the cell's ability to manage the process. Apoptosis or programmed cell death is a highly regulated, energy-dependent process that is characterized by shrinkage of the cell, fragmentation of the nucleus, and formation of apoptotic bodies, which are neatly phagocytosed without causing inflammation. During IRnjury, it is mainly induced through the intrinsic, mitochondrial pathway. Mitochondrial outer membrane permeabilization (MOMP) is induced by cellular stressors, such as ROS and DNA damage, and is controlled by the Bcl-2 family of proteins [64]. The critical event consists of the release of cytochrome c into the cytosol, where it forms the "apoptosome" with Apaf-1, activating caspase-9. Caspase-9, in turn, activates the executioner caspases-3 and -7, which systematically degrade the cell. While apoptosis is a "clean" form of death, its significant contribution to the overall infarct size makes it a major therapeutic target. Necroptosis is a highly inflammatory form of programmed necrosis. Cells default to this alternate death pathway when apoptosis is inhibited, which commonly occurs in IR injury [65]. It is induced by death receptors, such as TNFR1, and depends on the receptor-interacting protein kinases RIPK1 and RIPK3. These interact via a complex called the "necrosome," which phosphorylates the mixed lineage kinase domain-like pseudokinase (MLKL). Phosphorylated MLKL oligomerizes and translocates to the plasma membrane, where it forms pores that disrupt ionic homeostasis and cause cell swelling and lysis. The release of intracellular contents upon rupture acts as DAMPs, strongly driving the inflammatory response described in section 5.1. Necroptosis is increasingly recognized as important in the heart, brain, and kidneys following IR injury [66].

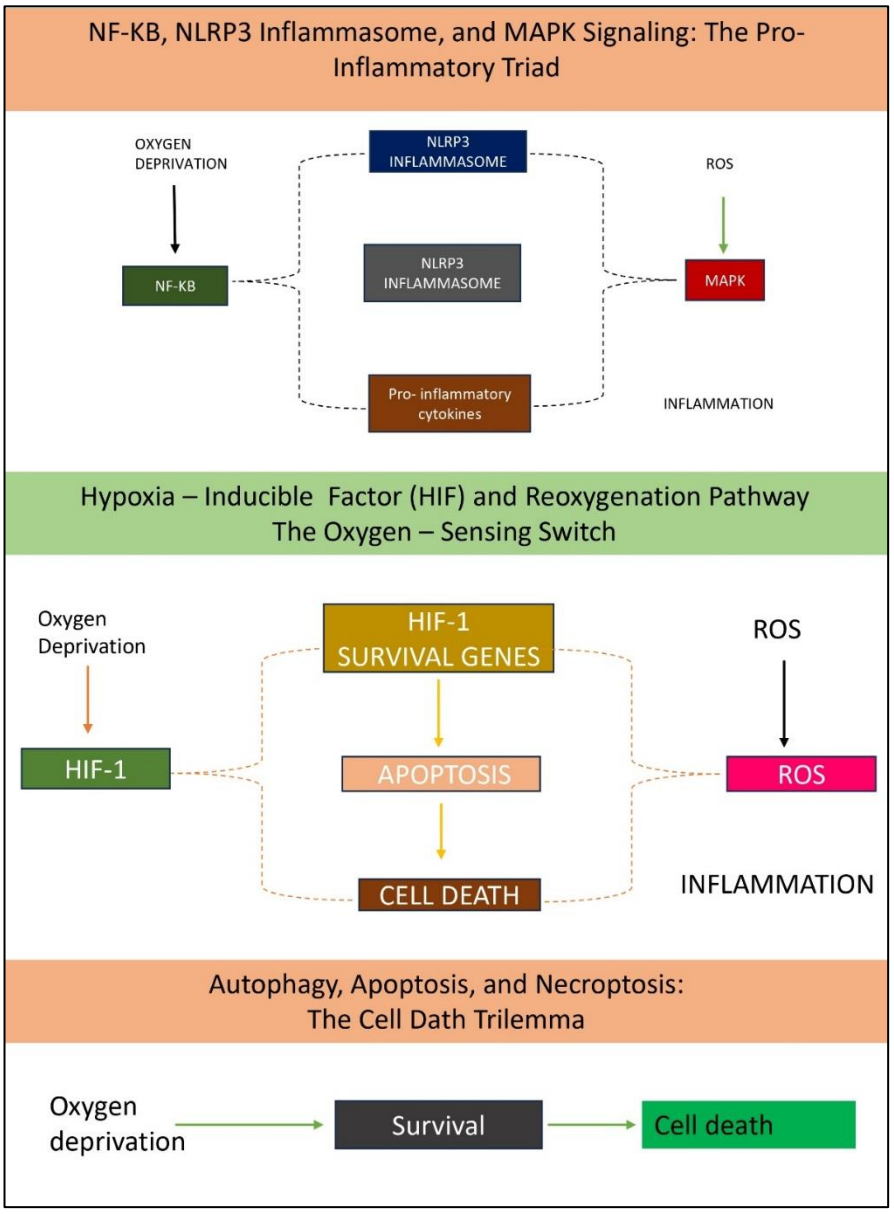

Figure 3: Molecular signaling pathways involved in ischemia–reperfusion (IR) injury. Ischemia–reperfusion initiates a complex network of oxygen deprivation– and reoxygenation-dependent signaling cascades that collectively drive inflammation and cell death. Oxygen deprivation activates NF-κB signaling, which primes the NLRP3 inflammasome, while reoxygenation-associated reactive oxygen species (ROS) activate MAPK pathways, together forming a pro-inflammatory triad that amplifies cytokine release. Concurrently, hypoxia stabilizes hypoxia-inducible factor-1 (HIF-1), promoting survival gene expression during ischemia; however, excessive ROS generation during reperfusion shifts signaling toward apoptosis and inflammatory cell injury. The balance between adaptive responses and oxidative stress ultimately determines cell fate, engaging interconnected processes of autophagy, apoptosis, and necroptosis, and culminating in either cellular survival or programmed cell death.

6. Therapeutic Strategies Targeting Ischemia/Reperfusion Injury

6.1. Ischemic Preconditioning and Postconditioning Approaches

Ischemic conditioning protocols are among the most powerful endogenous protective strategies that serve anticipated extended ischemic event [67]. Such a maneuver results in a complex signaling cascade that renders the tissues resistant to further injury. There are two phases: an early phase, which occurs within hours due to post-translational modifications, and a late phase, occurring after 24-72 hours, with de novo protein synthesis giving a second window of protection. The major mediators include adenosine, bradykinin, and opioids, which activate prosurvival pathways, including the RISK and SAFE pathways, to ultimately preserve mitochondrial integrity and inhibit the opening of mPTP, a critical event in cell death [68].

to "prep" the organ to withstand a subsequent, more severe ischemic insult. The process of IPC involves the application of one or several brief, non-lethal cycles of ischemia and reperfusion to the target organ before the

A more clinically feasible modality is Ischemic Postconditioning (IPost), applied at the moment of

reperfusion. It involves administering short, intermittent cycles of reperfusion and re-occlusion immediately after the restoration of blood flow. This "stuttering" reperfusion seems to moderate the sudden surge of oxygen and calcium, consequently damping the explosive generation of ROS and the associated inflammatory response typical of uncontrolled reperfusion. IPost has indeed demonstrated significant promise in cardiac catheterization laboratories during percutaneous coronary intervention for heart attacks as a clinically practical means of myocardial protection at the most critical juncture [69].

6.2. Pharmacological Interventions

6.2.1. Antioxidants

The "oxidative burst" during reperfusion is a major driver of IRnjury. Antioxidants work by neutralizing the excess ROS. While simple antioxidants, such as SOD mimetics or N-acetylcysteine, have been effective in a number of preclinical studies, clinical translation has been limited, often due to poor bioavailability or timing of administration. Attention is now being directed at more sophisticated agents that not only scavenge ROS but also enhance the endogenous antioxidant defense system; examples include agents targeting the Nrf2 pathway [70].

6.2.2. Anti-inflammatory Agents

The sterile inflammatory response is one of the hallmarks of IRnjury. Damaged cells release DAMPs, which, through the activation of innate immune receptors such as TLRs, result in the production of cytokines and chemokines responsible for the recruitment of neutrophils and other inflammatory cells. Therapeutic approaches include monoclonal antibodies against adhesion molecules (e.g., ICAM-1), antagonists of pro-inflammatory cytokines (e.g., IL-1β), and inhibitors of the complement cascade. However, the pleiotropic nature of inflammation renders targeted intervention without compromising host defense very challenging [71].

6.2.3. Immune Checkpoint Modulators

A more recent and revolutionary insight is the role of immune checkpoints in IRnjury. These are inhibitory administration of PD-L1, or otherwise activating the PD-1/PD-L1 axis, during the reperfusion window reduces infarct size, lowers proinflammatory cytokines, attenuates edema, and improves functional recovery in models of stroke and myocardial infarction. Soluble PD-L1 was shown in a laboratory study to be able to reprogram circulating monocytes, reduce cerebral edema, and decrease neurologic injury after stroke. These effects appear to be mediated by suppressing effector T cells, increasing regulatory T cells, and skewing macrophages toward less inflammatory phenotypes. Other checkpoints include CTLA-4 agonism and the manipulation of TIM-3/Galectin-9, which have shown beneficial immunomodulatory effects in preclinical models of IRby reducing T-cell costimulation or promoting M2 macrophage polarization, thus being helpful for tissue repair [74].

pathways that usually maintain self-tolerance and control the duration of immune responses. It has been learned that ligands such as PD-L1 are upregulated on ischemic tissue, and signaling through the PD-1/PD-L1 axis acts as a natural brake on the destructive immune response. Agonists of these checkpoints, or administration of recombinant PD-L1, have shown remarkable efficacy in animal models of stroke and myocardial infarction through suppression of T-cell and macrophage-driven inflammation, thus opening a new avenue for immunotherapy in a non-oncological context. In recent years, the understanding of the immune system's dual role in ischemia–reperfusion (I/R) injury has led to the identification of immune checkpoints as potential therapeutic targets. Normally, immune checkpoints are regulatory mechanisms that maintain self-tolerance and prevent excessive or prolonged immune activation, ensuring tissue integrity during immune responses. However, in the setting of IR injury, where reperfusion triggers a sterile inflammatory cascade, these checkpoints become pivotal determinants of tissue damage or repair [72].

One of the most studied pathways is the Programmed Death-1 (PD-1)/Programmed Death Ligand-1 (PD-L1) axis. During ischemic stress, PD-L1 expression is upregulated on endothelial and parenchymal cells within the affected tissue. Activation of PD-1 on infiltrating T lymphocytes and macrophages attenuates their pro-inflammatory activity, thereby limiting secondary tissue injury. Experimental studies have demonstrated that agonists of PD-1 or PD-L1, or recombinant PD-L1 fusion proteins, can significantly reduce infarct size and improve functional recovery in animal models of myocardial infarction, cerebral ischemia, and renal injury. The mechanism involves suppression of effector T-cell proliferation, reduction in proinflammatory cytokine release (TNF-α, IFN-γ, IL-6), and promotion of regulatory T-cell (Treg) expansion, which collectively restore immune homeostasis [73].

PD-L1 / PD-1 agonism or recombinant PD-L1 - Several animal studies have shown that

6.3 Cellular and Gene Therapies

6.3.1. Cellular Therapy

Cellular therapy for injury represents a paradigm shift from symptomatic treatment to regenerative intervention, focusing on restoring the structural and functional integrity of damaged tissues. The most extensively studied cell types include mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), endothelial progenitor cells (EPCs), and cardiac progenitor cells (CPCs). Mesenchymal Stem Cells (MSCs), derived from bone marrow, adipose tissue, or umbilical cord, exert multifaceted therapeutic effects not primarily through direct differentiation but via paracrine signaling. Preclinical models of myocardial, cerebral, and hepatic injury have shown functional recovery, reduction in infarct size, and improved microvascular integrity following MSC transplantation. Clinical trials, particularly in cardiac ischemia, have yielded

encouraging but variable outcomes, limited by issues such as poor cell survival, immune rejection, and low retention at the target site. Current innovations include genetically engineered MSCs, exosome-based therapy, and biomaterial scaffolds or hydrogels designed to improve cell delivery and retention. Other promising cellular strategies involve iPSC-derived cardiomyocytes and neurons, which offer the advantage of patient-specific therapy, reducing immune incompatibility. However, concerns regarding tumorigenicity and genetic instability warrant cautious optimization before widespread clinical application [75].

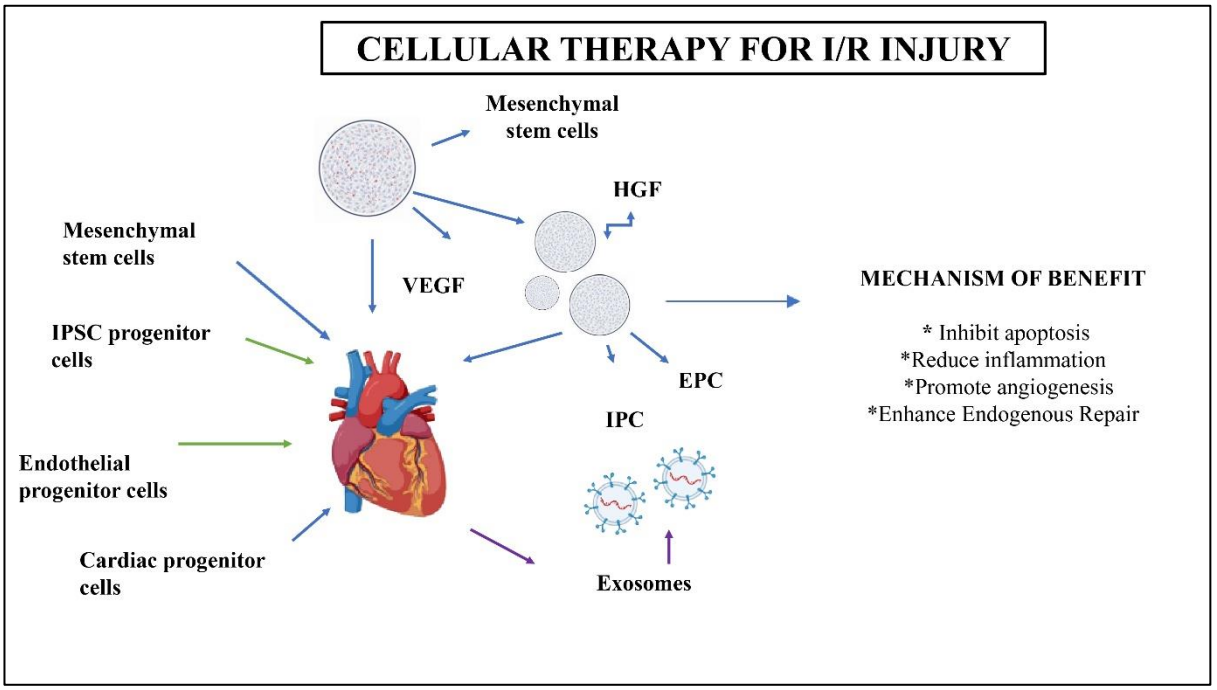

Figure 4. Cellular therapy strategies for ischemia–reperfusion (I/R) injury.
Cell-based therapeutic approaches for I/R injury involve the administration of mesenchymal stem cells (MSCs), induced pluripotent stem cell (iPSC)–derived progenitor cells, endothelial progenitor cells, and cardiac progenitor cells to restore damaged myocardium. MSCs exert paracrine effects through the secretion of angiogenic and cytoprotective factors, including vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), and by releasing extracellular vesicles/exosomes. These mediators promote endothelial repair, neovascularization, and tissue regeneration while inhibiting apoptosis and inflammatory signaling. Collectively, cellular therapies enhance endogenous repair mechanisms, improve cardiac function, and reduce ischemia–reperfusion–induced tissue injury.

MSCs and cell-derived exosomes consistently reduce infarct size and improve functional endpoints in rodent and large-animal models of myocardial and cerebral I/R. The advantages of exosome preparations, known as MSC-EVs, include better tissue penetration and lower immunogenicity, with easier standardization [76].

MSC trials in acute myocardial infarction/heart failure: dozens of early-phase trials and several meta-analyses report modest but statistically significant improvements in LVEF and safety signals overall, but results are heterogeneous across studies (different cell source(s), doses, timing, routes). Larger, more standardized trials continue to be planned or reported within 2024–2025. Clinical translation is accelerating as manufacturing and regulatory frameworks are improving. Several review articles and 2023-2025 analyses argue that exosomes may become the next-generation therapeutic platform for stroke and cardiac repair, but well-controlled clinical efficacy trials remain limited. Cell survival/retention: Low engraftment is a recurring limitation; proposed solutions include biomaterial scaffolds, hydrogel carriers, and cell preconditioning/genetic modification. Variability between donors, culture methods, and EV-isolation techniques hampers interpretation and regulatory approval. Safety concerns, such as arrhythmogenic risk for cardiomyocyte grafts, tumorigenicity for iPSC derivatives, and immune reactions, need to be overcome. Many of these risks are diminished in exosome-based approaches [77].

6.3.2. Gene Therapy

Gene therapy offers a means to precisely reprogram cellular responses to injury by delivering protective or restorative genetic material. Using viral vectors (such as adenoviral or adeno-associated viruses) or non-viral delivery systems (liposomes, nanoparticles, or plasmids), therapeutic genes can be introduced to modify oxidative stress pathways, apoptosis regulation, angiogenesis, and calcium handling. Antioxidant enzymes: *Heme oxygenase-1 (HO-1)*, *superoxide dismutase (SOD)*, and *catalase* to enhance cellular resilience against reactive oxygen species. Anti-apoptotic proteins: *Bcl-2*, *Akt*, and *HSP70*, which prevent programmed cell death during reperfusion. Pro-angiogenic and vasodilatory factors: *VEGF*, *eNOS*, and *FGF2*, which restore blood flow

and promote tissue recovery. Cytokine and chemokine regulators: to modulate immune cell recruitment and polarization. Emerging approaches combine CRISPR/Cas9-based genome editing with nanocarrier systems for precise, transient, and tissue-specific modulation of target genes. Despite remarkable preclinical success, translation to clinical use faces challenges such as vector immunogenicity, control of gene expression duration, and off-target effects. Hybrid strategies, combining gene therapy with stem cell delivery (gene-enhanced cell therapy), are being explored to synergistically improve therapeutic efficacy [78].

Gene therapy seeks to durably enhance cytoprotective pathways in vulnerable tissue or to provide transient high expression of protective proteins at the time of reperfusion. Common targets tested in IRmodels include: Free radical scavenging enzymes: HO-1, SOD, catalase. Anti-apoptotic factors include Bcl-2, Akt, and HSP70. Pro-angiogenic / vasodilatory factors: VEGF, eNOS, FGF2. Cytoprotective cytokines or modulators that adjust the recruitment and polarization of immune cells [79].

HO-1 gene transfer (rAAV/HO-1): Long-term AAV-mediated cardiac HO-1 expression has been shown to reduce infarct size and confer durable cardioprotection in mouse models and has demonstrated efficacy in preclinical porcine IRstudies. Both cytoprotective and anti-inflammatory effects are seen with sustained expression of the delivered gene. Infarct sparing with preserved function has been demonstrated several months after delivery in animals. eNOS and VEGF gene delivery: Numerous preclinical reports show that local eNOS or VEGF gene delivery improves perfusion and reduces IR injury; a range of non-viral and viral delivery vehicles have been utilized [80].

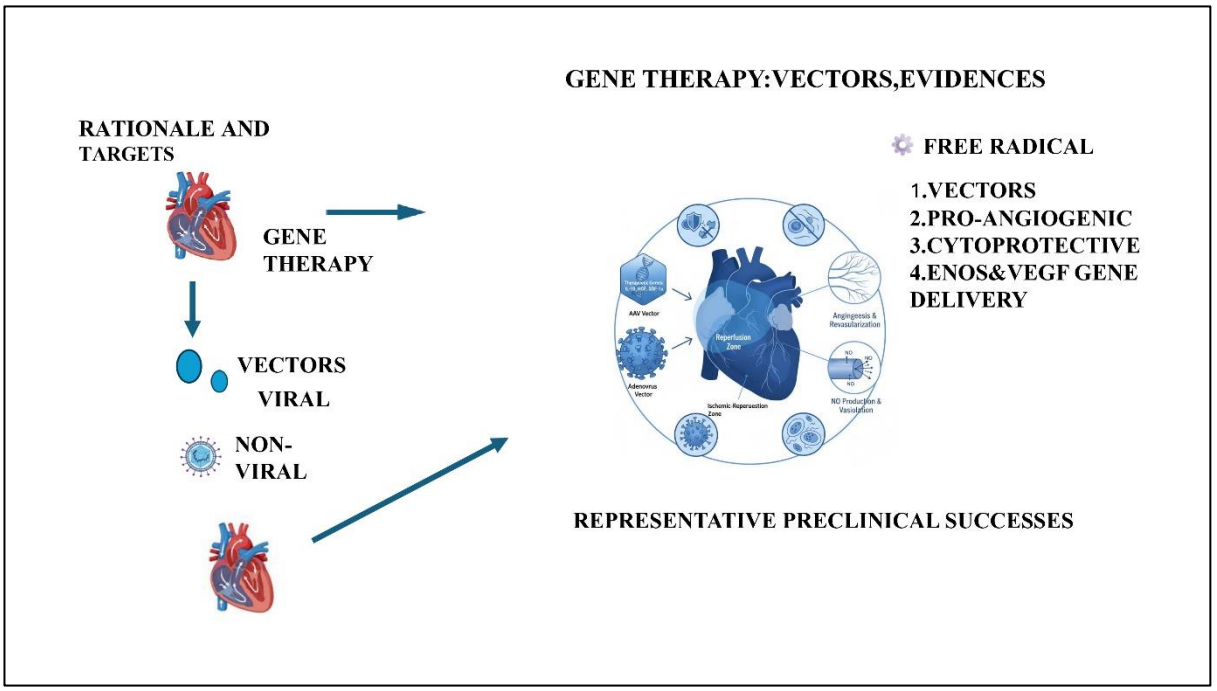

Figure 5. Gene therapy approaches for ischemia–reperfusion (I/R) injury: vectors, targets, and preclinical evidence. Gene therapy for I/R injury is designed to modulate oxidative stress, inflammation, and tissue repair pathways in the ischemic myocardium. Both viral (adenoviral, adeno-associated viral) and non-viral vectors are employed to deliver therapeutic genes to target cardiac tissues. These strategies focus on pro-angiogenic, cytoprotective, and antioxidant gene delivery, including endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF), to enhance neovascularization, reduce free radical–mediated damage, and promote cardiomyocyte survival. Representative preclinical studies demonstrate improved myocardial perfusion, attenuation of ischemia–reperfusion injury, and functional recovery, supporting gene therapy as a promising adjunct for cardioprotection.

Clinical trials in cardiovascular gene therapy have focused more on chronic ischemia/angiogenesis (VEGF, FGF) than on acute IRcytoprotection. Translation to human IRs is limited but evolving with the improvement of vector safety and targeted delivery. Reviews through 2021-2024 summarize a mixed history: strong preclinical efficacy but translational hurdles concerning delivery, immune responses against vectors, and controlling the transgene expression duration. Vector and safety improvements include the development of AAV vectors with improved serotypes, tissue-specific promoters, and nanoparticle/nucleic-acid carriers to reduce off-target expression and immunogenicity. CRISPR/Cas approaches enable transient, targeted edits in preclinical work but remain experimental clinically for acute I/R [81].

6.4. Nanomedicine and Targeted Drug Delivery Systems

Unfortunately, most promising therapeutics have

failed in clinical trials due to their inability to effectively reach the target site in sufficient concentrations without systemic side effects. Nanomedicine offers a sophisticated solution to this delivery problem. Nanoparticles are artificial structures in the range of 1-100 nanometers that can be engineered to encapsulate drugs, genes, and even antioxidants, thereby protecting them from degradation and renal clearance. Their surface can be functionalized with specific ligands, for instance, antibodies or peptides capable of recognizing and binding to molecules uniquely expressed on the surface of ischemic or inflamed endothelial cells, such as selectins or VCAM-1. This active targeting ensures delivery of the therapeutic payload with high precision to the jeopardized tissue, hence assuring high efficacy with minimal off-target effects. Nanoparticles can also be designed as responsive or "smart" systems that discharge their cargo only in the specific microenvironment of the IRzone, for example, in response to low pH, elevated ROS levels, or overexpressed enzymes such as matrix metalloproteinases. This site-specific release further enhances therapeutic precision. Applications are being explored for targeted delivery of anti-inflammatory drugs, siRNA to silence pro-death genes, and therapeutic gases like hydrogen sulfide. Nanomedicine thus represents a unifying platform that can enhance the efficacy of all the aforementioned pharmacological, cellular, and genetic strategies,heralding a new era of targeted, personalized therapy for injury [82].

7. Recent Advances in Immune Modulation Therapies

The understanding of injury has dramatically shifted from a purely metabolic and oxidative stress model to one in which the immune system is recognized as the main orchestrator of tissue damage. While initially protective, the sterile inflammatory response often becomes self-amplifying, resulting in a vicious cycle of cell death and immune-mediated destruction. For this reason, the cutting edge of IRtherapeutics is now dominated by sophisticated immune modulation strategies aimed at precisely interrupting this destructive dialogue. Recent advances can be categorized into targeted biologics, nucleic acid-based interventions, and novel biological vector therapies [83].

Table 2: Recent Advances in Immune Modulation, Cellular Therapy, and Nanomedicine for IRnjury

S. No.	Therapeutic Category	Strategy / Approach	Mechanistic Target	Key Outcomes / Effects	Examples / Advances	References
1.	**Immune Modulation**	d. RNA-based Immune Regulation	Gene silencing of pro-inflammatory mediators	Targeted suppression of NF-κB, NLRP3, and Caspase-1	siRNA (NLRP3, Casp1), miR-146a mimics, CRISPR/Cas9 inhibition of miR-155	[84]
2.	**Cellular Therapy**	a. Mesenchymal Stem Cells (MSCs)	Paracrine secretion of anti-inflammatory and pro-angiogenic factors	Reduced apoptosis and inflammation, enhanced repair	MSCs from bone marrow, adipose, or umbilical cord; exosome-mediated signaling	[85]
		b. Endothelial Progenitor Cells (EPCs)	Vascular repair and neovascularization	Restoration of endothelial integrity and microcirculation	EPC transplantation, EPC-derived exosomes	[86]
		c. Induced Pluripotent Stem Cells (iPSCs)	Differentiation into cardiac or neuronal lineages	Replacement of damaged cells, mitochondrial transfer	iPSC-derived cardiomyocytes or neurons	[87]
		d. Immune Cell-based Therapies	Macrophage polarization (M2 phenotype), DC modulation	Promotes resolution of inflammation	M2 macrophage transfer, tolerogenic dendritic cell therapy	[88]
3.	**Nanomedicine Approaches**	a. Targeted Nanocarriers	Delivery of antioxidants and anti-inflammatory drugs to ischemic tissue	Enhanced bioavailability, site-specific action	Liposomes, polymeric nanoparticles, and solid lipid nanoparticles	[89]
		b. Stimuli-responsive Nanoparticles	ROS-, pH-, or enzyme-sensitive release	Controlled drug delivery in oxidative microenvironment	ROS-responsive PEGylated nanoparticles, pH-sensitive micelles	[90]

		c. Biomimetic Nanoparticles	Camouflaged with cell membranes (RBCs, platelets, macrophages)	Immune evasion and targeted adhesion to inflamed endothelium	Macrophage-membrane-coated NPs, platelet-derived vesicles	[91]
		d. Exosome-based Nanocarriers	Natural vesicles carrying proteins, miRNAs	Intercellular communication, repair signaling	MSC-derived exosomes delivering miR-21, miR-24, or anti-apoptotic agents	[92]

8. Future Projections and Gaps of Knowledge

The most important challenge is the translation of the encouraging pre-clinical outcomes into useful clinical therapies. There is a major gap in the understanding of the exact spatiotemporal dynamics of immune response in humans in various organs. The unsuccessful experience of numerous anti-inflammatory trials points to the complexity of immune modulation, in which timing, situation, and patient-specific variables play a decisive role. Future studies need to go beyond one-target studies. Multi-omics technologies (genomics, transcriptomics, proteomics, and metabolomics) will be integrated to give a systems-level picture of IR. Such massive datasets will demand artificial intelligence and machine learning to detect new predictive biomarkers, patient endotypes (subgroups with unique biological mechanisms), and the best therapeutic targets. This opens the door to personalized medicine: the possibility to anticipate the risk of severe IR of an individual and the possibility to choose the appropriate immunomodulatory therapy and the appropriate time. The main areas of interest will be the verification of non-invasive biomarkers to diagnose early and the design of targeted delivery systems to ensure the product is most effective and the least effects are on non-targets, thereby bridging the sorrowful translational gap of IR research [93].

Conclusion

Ischemia-reperfusion injury is a multifaceted pathology as the original metabolic disaster is conducted by a dysadaptive innate and adaptive immune reaction. The paradigm has turned around the thinking of IR being merely a chemical imbalance, to that of a sterile inflammatory disease. Such a fine sense has revealed a new panoply of new therapeutic targets, especially in the field of immune modulation. Although translation obstacles remain, the combination of sophisticated biologics, high-order conditioning methods, cell-free exosome therapies, and state-of-the-art nanomedicine presents a promising prospect. The future of IR therapy is a tailored system medicine, which can utilize AI-powered information on multi-omics data to finally control the complexities of reperfusion damage to improve the outcomes of millions of patients across the globe.

Acknowledgment

The authors sincerely acknowledge their respective institutions for providing the necessary facilities and academic support to carry out this review work. The authors are also grateful to colleagues and peers for their constructive suggestions during the preparation of the manuscript.

Author Contribution

HK: Conceptualization; **MNK:** Supervision; **PG:** Literature review; **SA:** Writing – original draft; **AB:** Data curation; **AA:** Editing; **FA:** Visualization; **SP:** Proofreading.

Conflict of Interest

The authors declare no conflict of interest.

Source of Funding

There is no funding available to conduct this study.

AI Declarations

The authors declare that they used AI language tools (ChatGPT and Grammarly Premium) to enhance this manuscript's linguistic clarity and readability. They carefully reviewed and edited all generated text to ensure accuracy and alignment with the research's intended meaning.

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