

PERSPECTIVE

The central paradox of ischemia–reperfusion injury: Reperfusion as the primary driver of tissue damage

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1 INTRODUCTION

Ischemia–reperfusion (IR) injury reflects secondary pathological processes arising after reperfusion of hypoperfused organs. The central paradox is that reperfusion itself drives much of the tissue damage. In the lungs, reperfusion-induced damage is associated with structural and functional impairment. This acute phase is characterized by excessive release of reactive oxygen species (ROS) and inflammatory mediators, with consequent loss of membrane integrity and dysregulation of immune responses, which can result in microvascular hyperpermeability (pulmonary edema) and acute lung injury. Mechanistically, the cathepsin C axis in alveolar macrophages is an important modulator of this process; cathepsin C has been shown to regulate p38 mitogen-activated protein kinase-mediated p47 phosphorylation for nicotinamide adenine dinucleotide phosphate oxidase activation and is associated with increased pro-inflammatory cytokines [1].

Beyond the lungs, the heart exhibits a similar paradoxical response. This cascade, driven by ROS-mediated oxidative stress, advances toward proteomic modifications which compromise membrane integrity and provoke programmed cell death (apoptosis) and contribute to myocyte loss. Importantly, IR is characterized by the selective release of lysine crotonylation-modified proteins—predominantly from the contractile apparatus—with kinetic profiles that correlate with injury

severity and suggest autonomous cross-reactivity of post-translational modifications. Despite this, there remains a significant gap in our understanding of the mechanisms at play and the exact molecular signatures that can be exploited for precision intervention.

Underlying these organ-specific manifestations is a common molecular hub: iron dysregulation. ROS generation is the initiator, with the buildup of unstable Fe(II) as a central molecular hub. The overwhelming intracellular Fe(II), in addition to directly oxidizing membrane structures, lipids, and nuclear DNA, also elicits a systemic inflammatory cascade. Interestingly, iron overload inhibits [4Fe-4S] cluster biogenesis, leading to the destabilization of lipoic acid synthase. As a result, the depletion of [4Fe-4S] inhibits protein lipoylation—a metabolic contributor to IR injury in the kidney [2]. Thus, exploring how Fe(II) interacts with the mitochondrial cofactors provides a theoretical basis to identify specific drugs against reperfusion injury in clinical practice.

2 BIOLOGICAL MECHANISMS OF REPERFUSION INJURY

2.1 The interplay of ferroptosis, apoptosis, and necrosis

Iron-overload-activated ferroptosis is an important contributor to cell death in IR injury. Ischemia produces an energetic



catastrophe, while reperfusion triggers a synergistic cascade—manifesting as bursts of ROS generation and inflammatory signaling, along with Ca^{2+} dysregulation—that acutely augments cellular demise. The ALOX15–15-HpETE axis, involving arachidonate 15-lipoxygenase and its metabolite 15-hydroperoxyeicosatetraenoic acid, is specifically involved in cardiomyocyte pathology. The pathological outcome is determined by a balance between regulated apoptosis and uncontrolled necrosis. Apoptosis is immunologically “silent”, whereas gross tissue injury tends to yield necrosis. This transition liberates damage-associated molecular patterns, generating a positive feedback loop of tertiary inflammation and tissue destruction.

2.2 Initiation and persistence of the inflammatory cascade

Overwhelming inflammation is the major pathogenetic mechanism in IR. The major immunological stimuli that recruit immune effectors to the affected organ are fluctuations in oxygen levels and metabolic substrates after reperfusion, which trigger the release of damage-associated molecular patterns and cytokines. These leukocytes release ROS and proteases, which induce a self-perpetuating inflammatory loop and promote lesion expansion. Li et al. found that NOD-like receptor family pyrin domain containing 3 inflammasome activation and astrocyte pyroptosis after cerebral IR are dependent on the lipocalin 2–24p3 receptor axis [3]. This neurodegeneration-promoting autocrine and paracrine feedback is accelerated in the final stages of programmed cell death, when functional recovery becomes unattainable.

2.3 The mitochondrial–redox nexus: From bioenergetic failure to cell death

Mitochondrial dysfunction-induced oxidative stress is the key initiator of the IR injury cascade. This pathology, instead of being a binary phenomenon, is actually a continuum between sublethal bioenergetic failure and programmed cell death execution. Ischemia modifies the intracellular milieu through accumulation of metabolic intermediates; then reoxygenation produces an explosive burst of ROS. This oxidative stress propagates through ROS-induced ROS release and converges on the mitochondrial permeability transition pore—a decisive checkpoint for cell fate. Moreover, mitochondrial collapse leads to adenosine triphosphate depletion and calcium dysregulation that drive a pathophysiologic feedback loop of dysregulated mitophagy and inflammation that exacerbates tissue damage (Figure 1).

3 CLINICAL CONTEXTS OF REPERFUSION INJURY

3.1 Myocardial IR injury: Clinical insights and therapeutic innovations

Herbal mixtures have long served as a source of pharmacological agents against IR injury after acute myocardial infarction

(AMI), and are increasingly used as adjuvants in the clinical management of myocardial ischemia–reperfusion injury (MIRI). Emerging evidence indicates that the Yiqi Liangxue Shengji decoction improves cardiac function and attenuates MIRI, thereby relieving clinical symptoms of AMI and reducing the incidence of major adverse cardiovascular (CV) events [4]. Progress in the prevention of MIRI offers transformative approaches for improving AMI outcomes and advancing integrated CV care models. In addition, in-depth mechanistic studies and novel interventional approaches have the potential to attenuate or even reverse myocardial injury, providing a robust basis for early diagnosis and precision medicine in CV care.

3.2 Neurological sequelae of reperfusion injury

The neurological sequelae of reperfusion injury appear as a secondary event after restoration of blood flow to the brain. This process is characterized by the rapid disruption of cellular homeostasis; although flow is restored, neurons often irreversibly transition into a state of metabolic and structural dysfunction mediated by an intense inflammatory response. One important contributor to this neuroinflammatory cascade is the HDAC3–p65–cGAS–STING signaling pathway, which can potentiate innate immunity and contribute to focal neurological deficits [5]. In addition, disrupted neurotransmitter release in the damaged areas prevents synaptic stability, resulting in a loss of function. Finally, cerebral reperfusion injury not only amplifies the primary ischemic insult but also triggers sustained—and in many cases permanent—neurological dysfunction, which constitutes a critical barrier to clinical recovery.

3.3 Challenges of reperfusion injury in organ transplantation

IR injury is a nearly unavoidable pathological phenomenon in organ transplantation that profoundly damages early graft function and long-term graft survival, and further narrows the available donor pool [6]. In the presence of reperfusion, reflow and a sudden increase in oxygen level induce a massive spike of ROS overproduction, provoking oxidative damage to cellular membranes, proteins, and DNA. At the same time, this response activates damage-associated innate immune signaling and amplifies inflammatory cascades, both of which contribute to tissue injury and dysfunction. Such multistep insults impair graft viability and increase susceptibility to transplant rejection. This rejection remains a significant clinical barrier to successful transplantation outcomes.

4 THERAPEUTIC LANDSCAPES AND PREVENTIVE STRATEGIES

4.1 Preconditioning: Priming the endogenous defense

Ischemic preconditioning is a classical cytoprotective strategy against reperfusion injury that involves exposing cells to brief

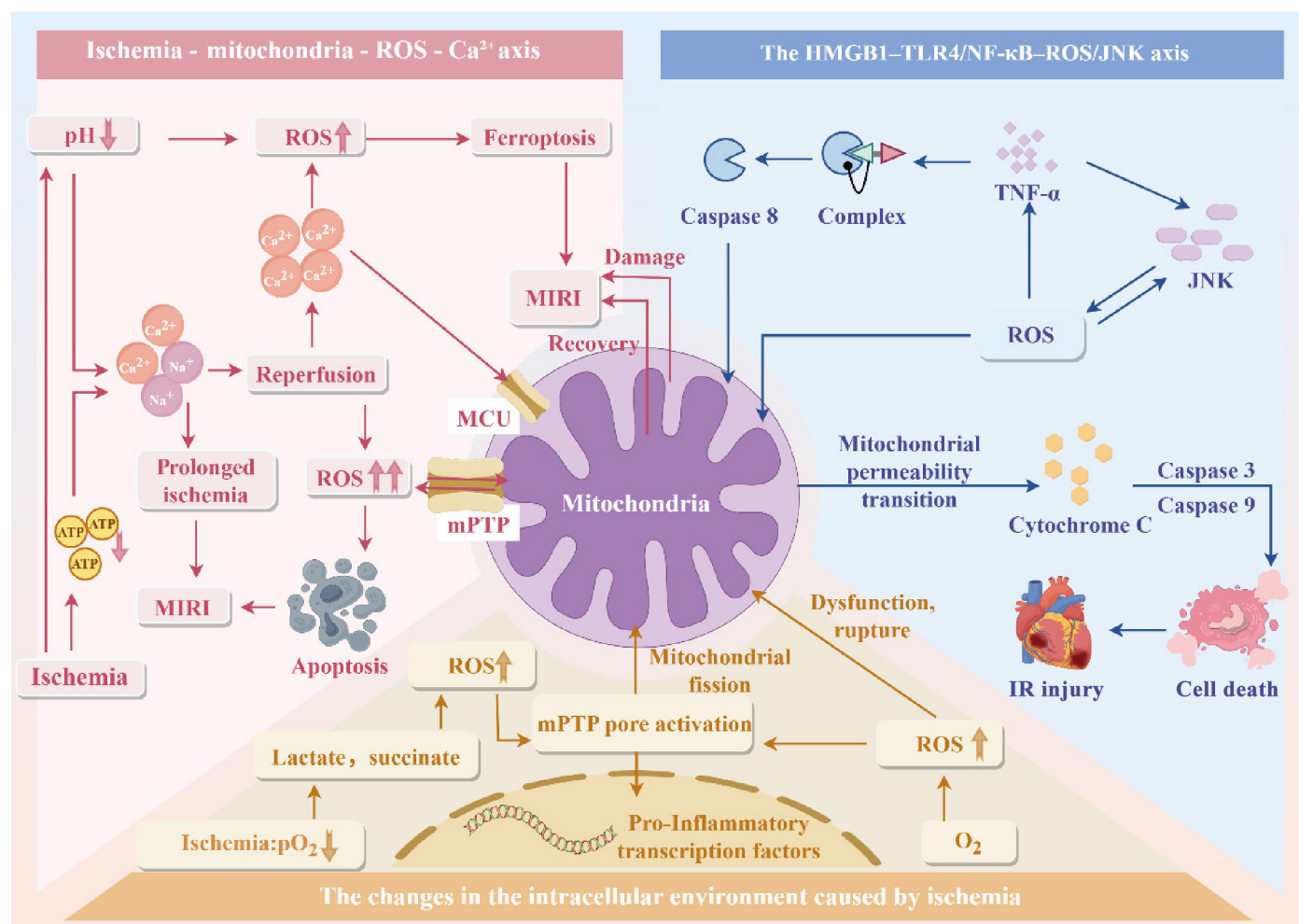


Figure 1. Schematic overview of key signaling pathways in IR injury. The left panel illustrates the mitochondrial–ROS–Ca²⁺ axis, depicting the transition from ischemic metabolic derangement to reperfusion-induced oxidative stress and mPTP opening, leading to apoptotic and ferroptotic cell death. The right panel depicts the HMGB1–TLR4/NF-κB–ROS/JNK inflammatory axis and its interaction with pro-apoptotic mediators (caspase-8, TNF-α), culminating in mitochondrial dysfunction and tissue injury. Created with BioRender. HMGB1, high mobility group box 1; TLR4, Toll-like receptor 4; NF-κB, nuclear factor kappa-B; JNK, c-Jun N-terminal kinase; TNF-α, tumor necrosis factor-alpha; mPTP, mitochondrial permeability transition pore; MIRI, myocardial ischemia–reperfusion injury; ROS, reactive oxygen species; MCU, mitochondrial calcium uniporter; pO₂, partial pressure of oxygen; IR, ischemia–reperfusion; ATP, adenosine triphosphate.

sublethal hypoxia, thereby equipping cardiomyocytes with an intrinsic protective mechanism against subsequent stressors. Recent studies demonstrate that stannocalcin 1 is induced by ischemic preconditioning and contributes to cellular defense against metabolic and reperfusion-induced oxidative stress, reducing necrosis and apoptosis [7]. This protective effect is primarily mediated by the upregulation of antioxidant defense systems, repression of pro-inflammatory signaling, and down-regulation of stress-responsive pathways. Ultimately, these adaptive responses allow for better functional maintenance and protection of the heart in response to the imposed IR insult.

4.2 Pharmacological modulation of reperfusion injury

Pharmacological approaches to reperfusion injury focus mainly on oxidative stress, inflammatory pathways, and pro-

grammed cell death. Ligustilide (LIG) is an emerging candidate with potent therapeutic efficacy by virtue of its dual antioxidant and anti-inflammatory properties. LIG exerts cytoprotective effects by inhibiting ROS bursts, limiting inflammatory responses, and preventing microvascular perfusion loss. In cerebral IR models, LIG protects mitochondrial function and protects against both apoptosis and necrosis. Mechanistically, these effects are mediated by crosstalk with the PTEN-induced kinase 1 axis, which both prevents functional deterioration and promotes recovery. As such, a stratified phase-based intervention paradigm is proposed, targeting tissue resilience during ischemia, ROS scavenging and mitochondrial stabilization during reperfusion, and structural remodeling modulation during prolonged recovery (Figure 2).

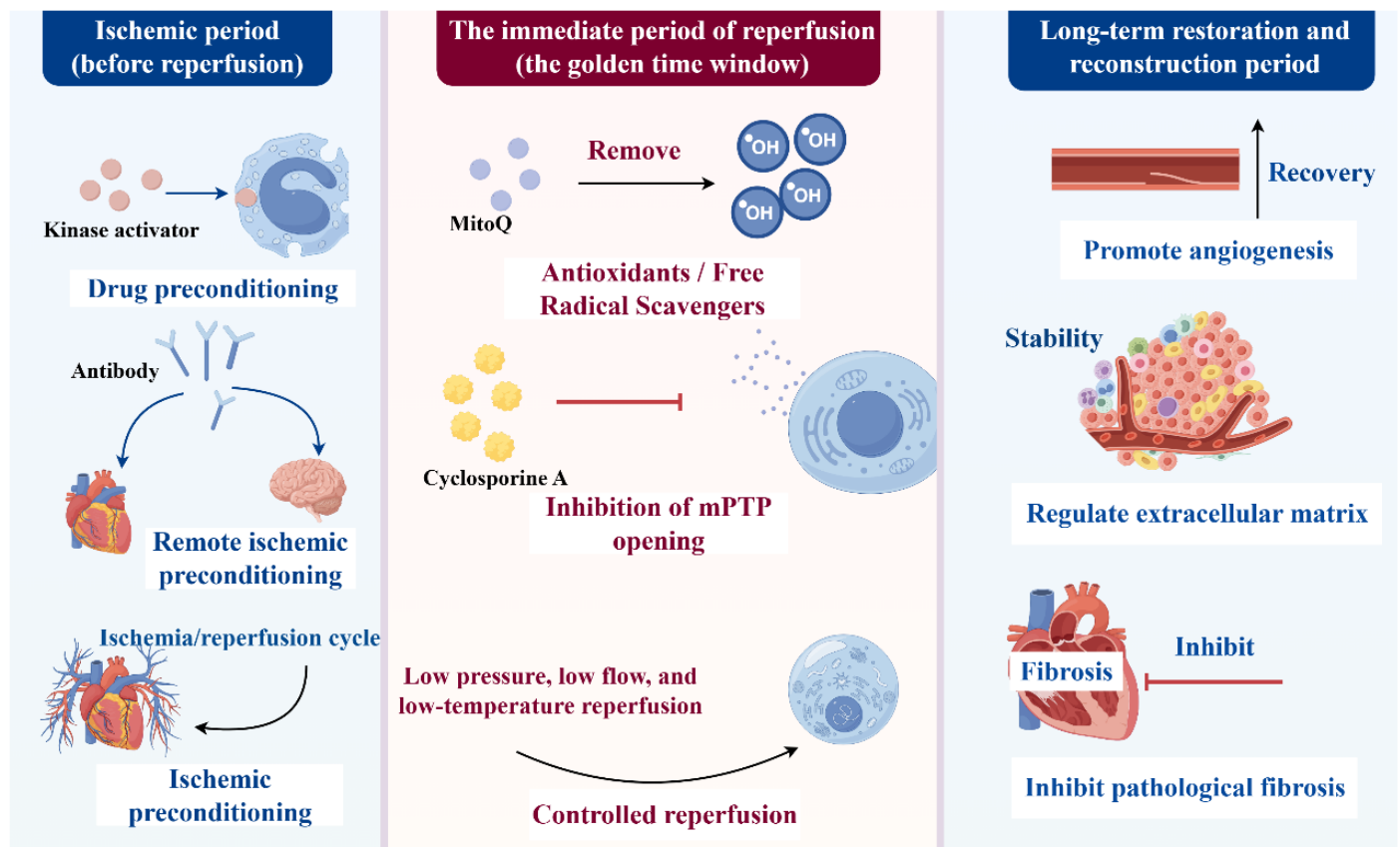


Figure 2. Stratified therapeutic interventions across the IR continuum. This schematic outlines phase-specific therapeutic strategies applicable to different stages of the injury timeline. In the ischemic (pre-reperfusion) phase, interventions include prophylactic pathway priming through kinase activation, pharmacological preconditioning, RIPC, and antibody-based approaches. The early reperfusion period represents a “golden time window” for acute injury rescue via controlled reperfusion, antioxidant therapy, and mPTP inhibition. The later phase involves structural healing through restoration of tissue homeostasis, modulation of extracellular matrix dynamics, and attenuation of pathological fibrosis. Created with BioRender. mPTP, mitochondrial permeability transition pore; RIPC, remote ischemic preconditioning; IR, ischemia–reperfusion.

4.3 Frontiers in targeted precision therapy

Targeted interventions have also emerged as a focus of IR research. One such agent is complement receptor 2–complement receptor 1-related protein y (CR2–Crry), a molecular species well-positioned for targeted intervention. CR2–Crry is a high-affinity, domain-specific inhibitor of the complement cascade that constrains pathogenic immune activation and thereby prevents tissue injury. CR2–Crry provides strong local protection with limited systemic coverage when administered prophylactically, a beneficial feature in the context of brain death-induced IR injury potentiation [8]. Indeed, the significant free radical scavenging activity, Hedgehog signaling inhibition, and anti-inflammatory properties of this compound suggest broad applicability across a spectrum of CV and cerebrovascular disorders. In fact, CR2–Crry is a prototype of the vectorial specificity needed to modulate reperfusion injuries that can best be applied using a site-directed mechanism of action and where expectations for a high-efficacy, low-toxicity paradigm can be realized.

5 FUTURE PERSPECTIVES AND CLINICAL CHALLENGES

Current biomarkers for IR injury have notable limitations, prompting the search for more sensitive and early indicators. The most widely used circulating biomarkers include lactate dehydrogenase and creatine kinase-MB, which are commonly applied to support the clinical assessment of IR injury. In this context, experimental modeling—including studies of epigenetic and metabolic remodeling—demonstrates systemic alterations in the levels of these enzymes, reflecting membrane disruption and leakage of cytosolic proteins, thereby contributing to their diagnostic relevance in cardiac injury assessment [9]. There is a shift away from traditional diagnostic markers toward mechanism-based biomarkers and next-generation multi-parameter panels. Therefore, future studies should explore ultra-early injury biomarkers with high sensitivity, such as microRNAs, selective post-translational modifications, or changes in metabolic intermediates that allow quantification of therapeutic effects.

Another promising direction is research on solute carrier family 26 member 6 (Slc26a6), which highlights its importance in modulating the IR environment. This mechanism has two components: modulation of acid-base balance in the myocardium via the $\text{Cl}^-/\text{HCO}_3^-$ exchanger, and modulation of ischemia-induced acidosis. It has been demonstrated that ablation of Slc26a6 protects against ischemic metabolic acidosis through elevation of myocardial pH. Importantly, a key role of Slc26a6 has been described in the regulation of transmembrane ionic balance, which is a major determinant of myocardial susceptibility to IR injury [10]. Elucidation of these complex Slc26a6 functions should augment our understanding of cardiovascular pathophysiology with potential relevance to novel therapeutic frontiers and interdisciplinary science.

Finally, inadequate blinding in trial designs represents a significant barrier to the translation of IR-based interventions. Trials lacking double-blinding—where either the experimenter or the subjects remain unblinded—are susceptible to experimenter bias and subject expectancy effects. These confounders act to cloud therapeutic assessment and mask the actual effect of therapies directed toward reperfusion. Thus, future studies should be double-blind randomized controlled trials with rigorous methodological quality and greater clinical relevance. Here, we emphasize the importance of a strict blinding process to ensure objectivity and accurate assessment of treatment effects, thereby facilitating the bench-to-bedside translation of IR therapies.

ABBREVIATIONS

AMI, Acute myocardial infarction; CV, Cardiovascular; HDAC3, Histone deacetylase 3; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; IR, Ischemia-reperfusion; LIG, Ligustilide; MIRI, Myocardial ischemia-reperfusion injury; RIPC, Remote ischemic preconditioning; ROS, Reactive oxygen species; CR2–Crry, complement receptor 2–complement receptor 1-related protein y; Slc26a6, solute carrier family 26 member 6.

DECLARATIONS

Author contributions

Haotian Wu contributed to manuscript writing and figure preparation; Huan Zhang designed the work; Siyi Yan supervised the work. All authors have read and approved the final manuscript.

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Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study. All information is derived from publicly available articles and datasets.

Ethics approval and consent to participate

Not applicable. This manuscript does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable. This manuscript does not include details, images, or videos relating to an individual person.

Competing interests

The authors declare that they have no competing interests.

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