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Review

Region- and Cell-Specific Vulnerability to ROS After Global Brain Ischemia-Reperfusion Injury: Molecular and Cellular Mechanisms

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Abstract

Global brain ischemia-reperfusion (I/R) injury manifests as selective neuronal vulnerability rather than uniform tissue damage. Specific neuroanatomical regions—most notably the hippocampal cornu ammonis 1 (CA1) subfield, striatum, and cerebellum—exhibit a heightened susceptibility to oxidative stress–induced degeneration. This review consolidates current understanding regarding the region- and cell-specific vulnerability to reactive oxygen species (ROS) following global I/R, highlighting the molecular and cellular determinants that drive this selectivity. We examine the spatial distribution of ROS-generating systems, including NADPH oxidases (NOX2/NOX4) and mitochondrial complexes, alongside the divergent expression of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase. Inherent neuronal properties, such as elevated synaptic activity, calcium permeability, and rigorous metabolic demands, further exacerbate oxidative injury. Additionally, we evaluate how astrocyte–neuron interactions and regional variations in blood–brain barrier (BBB) integrity modulate the severity and progression of ROS-induced damage. The roles of astrocytic endfoot polarization, aquaporin-4 (AQP4) localization, and Kir4.1-mediated potassium buffering in post-ischemic outcomes are also discussed. Furthermore, this review surveys emerging antioxidant-based therapies—ranging from edaravone and melatonin to selenium nanoparticles and miRNA-based interventions—while addressing translational hurdles like narrow therapeutic windows and inter-individual variability. By synthesizing experimental and translational evidence, this work provides a comprehensive framework for understanding oxidative stress–related vulnerability in I/R injury. Ultimately, it underscores the necessity for precision antioxidant strategies tailored to brain-region specificity, pathophysiological timing, and patient-specific factors to improve clinical outcomes.

Keywords: ischemia-reperfusion injury; reactive oxygen species; neuronal vulnerability; antioxidant enzymes; hippocampal CA1; astrocyte–neuron interaction; blood–brain barrier; targeted antioxidant therapy

1. Introduction

Ischemia-reperfusion (I/R) injury in the brain represents a fundamental pathophysiological process implicated in a variety of clinical conditions including cardiac arrest, stroke, and transient global ischemia. The restoration of cerebral blood flow following ischemia, although essential, paradoxically triggers an acute overproduction of reactive oxygen species (ROS), activation of proinflammatory cascades, mitochondrial dysfunction, and ultimately irreversible neuronal injury [1,2].

A defining feature of global cerebral I/R injury is its pronounced region- and cell-specific vulnerability. Certain neuronal populations such as hippocampal cornu ammonis 1 (CA1) pyramidal neurons, striatal medium spiny neurons, and cerebellar Purkinje cells are notably susceptible, undergoing delayed neuronal death (DND), while adjacent cells may remain viable [3,4,5,6]. Recent insights have revealed that this selective vulnerability arises from complex molecular and cellular underpinnings. These include differential expression of antioxidant enzymes such as SOD, glutathione peroxidase (GPx), and catalase; region-specific localization of ROS-generating systems including mitochondrial complexes and NADPH oxidases (e.g., NOX2 and NOX4); and intrinsic neuronal properties like heightened excitatory input, increased calcium permeability, and elevated metabolic demand [7,8,9]. In addition to neuron-intrinsic factors, astrocytic mechanisms substantially influence I/R outcomes. Astrocytes display regional heterogeneity in their ability to buffer extracellular ions, regulate cerebral blood flow, and maintain water homeostasis. Key determinants include the structural integrity of astrocytic endfeet, as well as the polarized distribution of aquaporin-4 (AQP4) and Kir4.1 channels. Ischemia-induced mislocalization of AQP4 from end-feet to neuropil impairs fluid clearance and exacerbates cerebral edema [10]. Likewise, spatial variations in Kir4.1 expression modulate potassium buffering, influencing neuronal excitability and damage severity [11,12].

These structural and regional features not only contribute to basal neuronal resilience but also modulate the brain's susceptibility to I/R-induced oxidative and inflammatory cascades. Specifically, astrocyte-neuron interactions, neuroinflammatory amplification, and oxidative injury orchestrate the post-ischemic fate of neurons. Ex-

cessive ROS generation damages deoxyribonucleic acid (DNA), proteins, and lipids while simultaneously triggering programmed cell death pathways including apoptosis, necrosis, and ferroptosis [13,14]. The delicate balance between ROS production and antioxidant defenses within the penumbra is pivotal in determining neuronal survival. Given the centrality of oxidative stress in I/R injury, antioxidant-based strategies have gained momentum. These encompass approved pharmacologic agents such as edaravone and melatonin, as well as emerging therapeutic platforms including selenium-based nanomaterials, microRNA-targeted approaches, and biomaterial-assisted drug delivery systems [15,16,17]. Additionally, lifestyle interventions emphasizing antioxidant-rich diets and functional foods combined with exercise are being explored for their synergistic potential [18,19,20].

Nonetheless, several challenges impede the clinical translation of these antioxidant therapies. Limitations include disparities between animal models and human stroke, the narrow therapeutic time window, variable blood-brain barrier (BBB) permeability, and the multifactorial complexity of redox and immune signaling networks [2,21,22]. Increasingly, precision medicine is recognized as essential, advocating for therapeutic tailoring based on individual factors such as age, comorbidities, vascular status, and inflammatory phenotype [23].

This review aims to synthesize current understanding of ROS-mediated regional vulnerability following cerebral I/R injury and evaluates state-of-the-art antioxidant therapies. We highlight (1) ROS generation and damage pathways, (2) astrocytic and neuronal region-specific responses, (3) endogenous antioxidant mechanisms, and (4) advanced therapeutic strategies including nanotechnology, gene modulation, and clinical translation. By bridging mechanistic insights and translational perspectives, we seek to inform future directions in developing effective, individualized treatments for brain I/R injury.

Methodology

To address these multifaceted issues, the present review provides a comprehensive synthesis of region- and cell-specific vulnerability to oxidative stress following global brain I/R injury. We systematically examine the spatial and temporal regulation of ROS, emphasizing the interplay between ROS generation, antioxidant defense systems, and neuronal fate in selectively vulnerable brain regions—particularly the hippocampal CA1 subfield, striatum, and cerebellar cortex. Structural and functional properties of neurons, as well as astrocyte–neuron interactions and blood–brain barrier (BBB) heterogeneity, are evaluated as contributing factors to divergent outcomes.

To construct this review, a systematic literature search was conducted using databases including PubMed, Scopus, and Web of Science, covering publications from 2000 to 2025. The search employed combinations of keywords such as ischemia-reperfusion, oxidative stress, ROS, antioxidants, neuronal vulnerability, astrocyte, CA1, BBB, and therapeutic strategies. Inclusion criteria prioritized original experimental studies and recent reviews focused on oxidative injury and regional selectivity in brain I/R models, particularly those using rodents (e.g., mouse, gerbils, rats) and translationally relevant paradigms. Articles were screened for methodological rigor, relevance to subregional mechanisms, and data richness.

By integrating evidence from well-characterized animal models, mechanistic studies, and emerging translational research, this review aims to provide an updated framework for understanding oxidative stress–mediated injury in I/R and to guide the development of region-targeted antioxidant therapies with greater clinical relevance.

2. Sources and Dynamics of ROS Generation During Global Brain Ischemia-Reperfusion

ROS act as primary mediators of cellular and tissue damage following global brain I/R. While oxygen supply is restricted during the ischemic phase, reoxygenation paradoxically triggers a massive “oxidative burst” [24,25,26]. This surge originates from multiple intracellular sources—primarily the mitochondria, NADPH oxidase (NOX), and xanthine oxidase (XO)—and follows distinct spatiotemporal patterns that contribute to the selective vulnerability of energy-demanding regions like the hippocampus and cortex [27,28,29].

Mitochondrial ROS production is particularly pivotal during reperfusion. Ischemia impairs the electron transport chain (ETC) and halts adenosine triphosphate (ATP) synthesis, leading to the accumulation of reduced carriers like NADH and $FADH_2$. Reoxygenation then drives reverse electron transport (RET) at complex I, triggering a substantial surge in superoxide production [26,30]. Confirmed in rodent models, this RET-dependent mechanism is highly pronounced in energy-demanding hippocampal CA1 neurons, directly facilitating their delayed death [31,32].

Non-mitochondrial enzymatic sources also significantly exacerbate injury. Under hypoxic conditions, xanthine dehydrogenase converts to XO, which facilitates the oxidation of hypoxanthine and xanthine to produce superoxide ($O_2^{-}\bullet$) and hydrogen peroxide (H_2O_2) [33]. This mechanism correlates strongly with oxidative damage in hippocampal and cortical tissues in gerbil models [27]. Additionally, NOX2 and NOX4 isoforms in microglia and endothelial cells are activated by post-reperfusion inflammatory stimuli and calcium influx [34,35]. NOX2-mediated ROS, in particular, promote BBB disruption and neuronal apoptosis [28].

Importantly, ROS dynamics exhibit substantial species and regional heterogeneity. Gerbil CA1 neurons show peak ROS accumulation at 6–24 hours post-reperfusion, whereas mouse models exhibit sustained mitochondrial generation for up to 48 hours [32]. Ul-

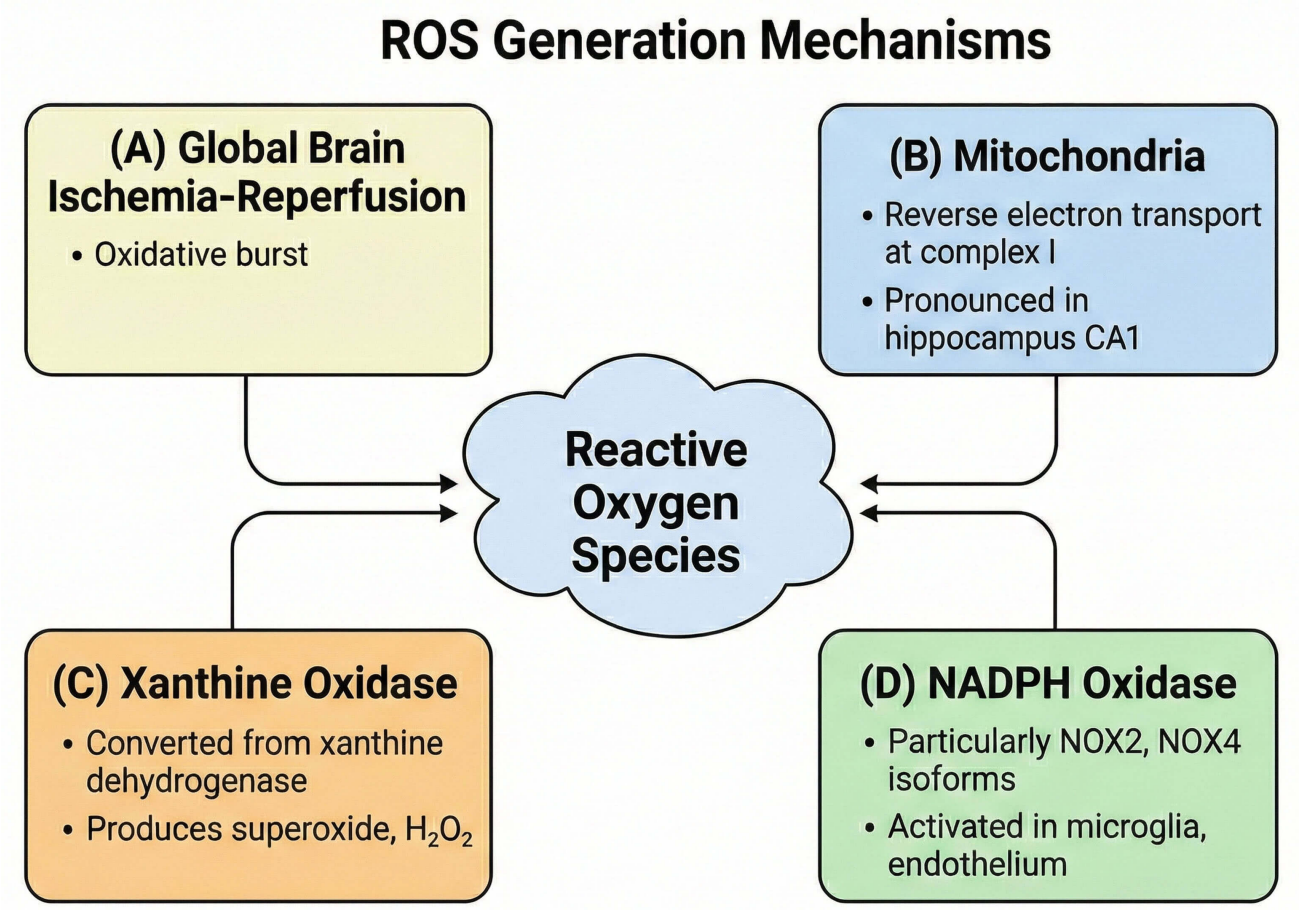

Fig. 1. Principal mechanisms of reactive oxygen species (ROS) generation during global brain ischemia–reperfusion. (A) **Global Brain Ischemia–Reperfusion**: Represents the fundamental pathophysiological trigger that initiates a sudden "oxidative burst" upon reoxygenation. (B) **Mitochondria**: Details the pivotal role of the mitochondrial electron transport chain, where impaired electron flow leads to superoxide (O_2^{-}•) production via reverse electron transport. (C) **Xanthine Oxidase**: Illustrates the conversion of xanthine dehydrogenase to xanthine oxidase under hypoxic conditions, a significant source of O_2^{-}• and H_2O_2. (D) **NADPH Oxidase**: Shows the activation of NOX2 and NOX4 isoforms in microglia and endothelial cells, which further amplifies the post-ischemic oxidative cascade. **Abbreviations**: H_2O_2, hydrogen peroxide; NOX, NADPH oxidase; ROS, reactive oxygen species; XO, xanthine oxidase; XDH, xanthine dehydrogenase; CA1, cornu ammonis 1.

timately, the interplay of these sources orchestrates the complex inflammatory and vascular responses leading to long-term cognitive dysfunction [36]. The principal mechanisms of ROS production and their spatiotemporal characteristics are illustrated in Fig. 1 and Table 1 (Ref. [26,27,28,30,31,32,33,34,35,36]).

3. Brain Region-Specific ROS Dynamics: CA1 vs CA3, Cortex vs Brainstem

I/R injury triggers ROS generation throughout the brain. However, the timing, intensity, and cellular sources of ROS exhibit substantial region-specific variation, leading to selective vulnerability or resilience among distinct neuronal populations. To elucidate these spatial dynamics, this section focuses on two well-characterized comparisons: hippocampal CA1 vs CA3 and neocortex vs brainstem.

The hippocampal CA1 subfield is extremely susceptible to oxidative stress and undergoes DND even after brief ischemia, whereas CA3 remains comparatively resistant, making this pair an ideal model for understanding intrinsic redox differences [4,37,38]. Similarly, the neocortex exhibits robust ROS-mediated damage and infarction after I/R, while the brainstem—especially nuclei critical for autonomic control—shows relative preservation [39]. Notably, the basal ROS generation rate is reportedly higher in the brainstem than in the cortex, implying distinct intrinsic oxidative resilience [39]. These two comparisons provide valuable insight into the regional redox milieu and its contribution to ischemic pathophysiology. The dominant ROS sources and their associated cell-type–specific vulnerabilities across CA1, CA3, neocortex, and brainstem are summarized schematically in Fig. 2.

Table 1. Summary of ROS sources in global brain ischemia-reperfusion injury.

Source	Key Enzyme/Pathway	Primary cell type	Peak ROS generation time	References
Mitochondria	Electron transport chain (Complex I, reverse electron transport)	Neurons (esp. hippocampal CA1)	Early reperfusion (within 1–3 h)	[26,30,32]
Purine metabolism	Xanthine oxidase (XO) converted from XDH during ischemia	Endothelial cells, neurons	Early reperfusion (~1–6 h)	[27,28,33]
NADPH oxidase	NOX2 isoform (inflammatory and Ca^{2+}-activated)	Microglia, infiltrating leukocytes	Early to mid-reperfusion (3–12 h)	[34,35,36]
Delayed ROS (secondary phase)	Mitochondrial ROS, inflammation-induced NOX reactivation	CA1 neurons, astrocytes, microglia	Delayed (1–3 days post-reperfusion)	[31,32]

Abbreviations: CA1, Cornu Ammonis 1; ETC, electron transport chain; NOX, NADPH oxidase; ROS, reactive oxygen species; XDH, xanthine dehydrogenase; XO, xanthine oxidase.

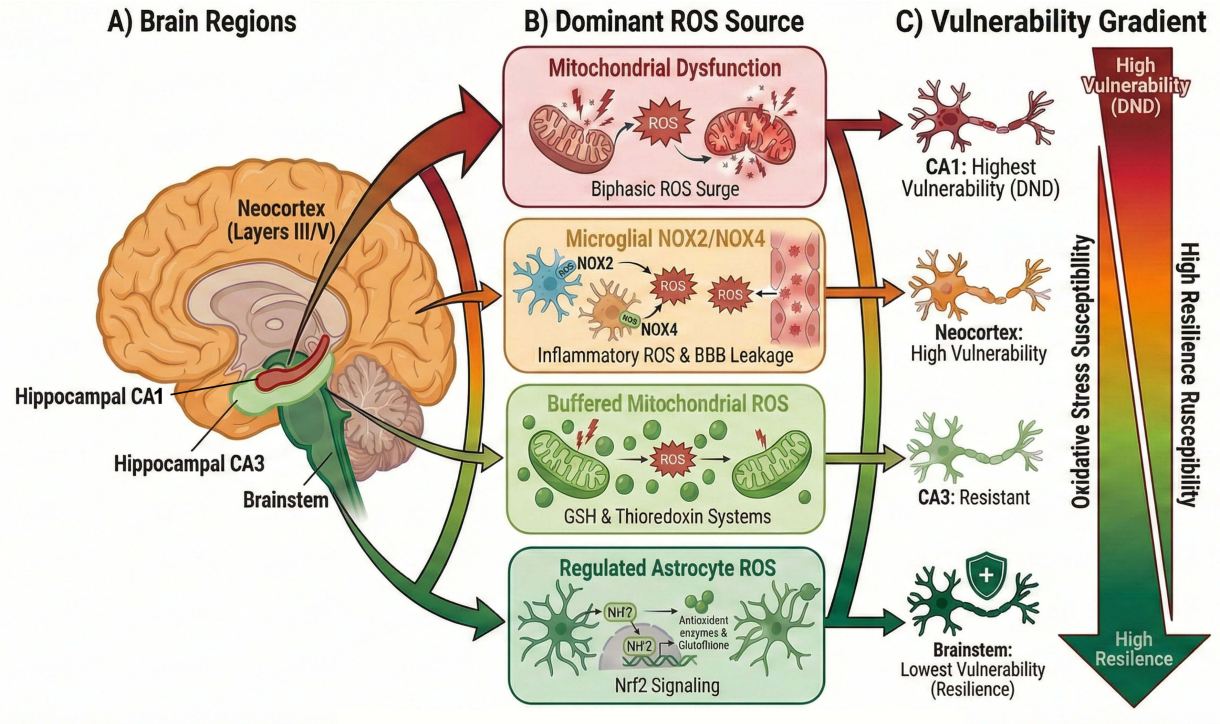

Fig. 2. Regional ROS sources and differential neuronal vulnerability after ischemia–reperfusion (I/R) injury. (A) **Brain Regions**: A schematic sagittal representation of the rodent brain highlighting specific regions with distinct susceptibility to oxidative stress, including the neocortex (Layers III/V), hippocampal subfields (CA1 and CA3), and the brainstem. (B) **Dominant ROS Source**: Distinct cellular and molecular mechanisms of reactive oxygen species (ROS) production characteristic of each region. In the hippocampal CA1, mitochondrial dysfunction drives a deleterious biphasic ROS surge. The neocortex exhibits robust inflammatory ROS generation and blood-brain barrier (BBB) leakage mediated by microglial NOX2/NOX4 activation. Conversely, the hippocampal CA3 maintains buffered mitochondrial ROS through efficient glutathione (GSH) and thioredoxin antioxidant systems. The brainstem demonstrates high resilience via regulated astrocyte-derived ROS and Nrf2 signaling pathways. (C) **Vulnerability Gradient**: A vertical scale illustrating the spectrum of oxidative stress susceptibility versus resilience across different brain regions. The gradient ranges from the highest vulnerability, characterized by delayed neuronal death (DND) in the CA1, to the highest resilience (lowest vulnerability) observed in the brainstem. Intermediate positions of the neocortex and CA3 are determined by their specific redox profiles and antioxidant capacities. **Abbreviations**: ROS, reactive oxygen species; I/R, ischemia–reperfusion; NOX, NADPH oxidase; BBB, blood–brain barrier; Nrf2, nuclear factor erythroid 2–related factor 2.

3.1 Hippocampal Subfields: CA1 vs CA3

The hippocampal CA1 subfield is most consistently associated with DND following I/R. The profound vulnerability of CA1 pyramidal neurons stems from early mitochondrial dysfunction, impaired ATP recovery, and a massive overproduction of ROS [4]. *Ex vivo* mouse models

confirm a biphasic ROS surge in this region: an initial cytosolic burst followed by a more lethal, mitochondrial-driven phase [32]. *In vivo* gerbil models further corroborate this, showing a persistent reduction in the mitochondrial redox ratio (MRR) and delayed homeostatic recovery in the CA1 [28,40].

Conversely, CA3 pyramidal neurons demonstrate remarkable resistance to ischemic insult. Despite synaptic and mitochondrial densities comparable to the CA1, the CA3 subfield maintains effective ROS buffering through superior thioredoxin and glutathione-based systems [41]. Furthermore, CA3 astrocytes retain their structural integrity longer, mitigating intracellular calcium overload and subsequent ROS amplification [42]. Transcriptomic evidence suggests that nuclear factor erythroid 2–related factor 2 (Nrf2)-dependent antioxidant genes are more robustly expressed in the CA2–CA4 subregions and dentate gyrus than in the CA1 [43]. Although the CA2 is often omitted due to its size, recent studies indicate that CA2 neurons are similarly resistant to excitotoxic and ischemic injury, likely due to specialized antioxidant and calcium-handling protein profiles [43,44].

3.2 Neocortex vs Brainstem

Neocortical neurons, particularly in layers III and V, are highly susceptible to ROS-mediated injury driven primarily by NADPH oxidase (NOX2/NOX4) pathways [45]. In focal ischemia models, reperfusion induces rapid NOX2 upregulation in microglia and endothelial cells, leading to extracellular superoxide accumulation that compromises the blood-brain barrier (BBB) [46,47]. Peak cortical ROS levels typically occur 6–24 hours post-reperfusion, correlating with acute neuroinflammation. This contrasts with the CA1, where injury is primarily driven by intrinsic mitochondrial dysfunction; in the neocortex, damage arises more from inflammatory ROS and microvascular failure.

In contrast, the brainstem exhibits significant resilience despite its vital autonomic role. This regional stability is attributed to enhanced baseline and reperfusion-induced activation of the Nrf2 pathway, which upregulates glutathione synthesis and detoxifying enzymes [48]. Furthermore, astrocyte activation in the brainstem is slower and less intense than in the cortex, resulting in lower production of ROS and reactive nitrogen species [49]. These features preserve mitochondrial stability and calcium homeostasis, explaining the relative sparing of brainstem structures in I/R models.

3.3 Implications for Region-Specific Therapy

Recognizing these region-specific ROS dynamics is crucial for the development of targeted neuroprotective strategies. For example: (1) In hippocampal CA1, where mitochondrial ROS dominates, mitochondria-targeted antioxidants (e.g., MitoQ, SS-31) may offer therapeutic benefit. (2) In the neocortex, where ROS arises from inflammatory NOX2/NOX4 activation, agents that inhibit NOXs or suppress microglial activation may be more effective. (3) The relative resilience of the brainstem suggests potential for redox reprogramming, such as pharmacologic Nrf2 activation in vulnerable regions like CA1 or cortex. Understanding how intrinsic (mitochondrial) and extrinsic (inflammatory/microvascular) ROS sources interact within specific neuroanatomical contexts can refine ischemia therapies and optimize outcomes based on regional vulnerability.

In summary, regional differences in ROS generation critically shape neuronal vulnerability after I/R. Hippocampal CA1 neurons display a biphasic mitochondrial ROS surge and are highly susceptible to delayed neuronal death, whereas CA3 exhibits greater antioxidant buffering and resilience. In the neocortex, NOX2/NOX4-driven ROS production and associated inflammation contribute prominently to oxidative damage, contrasting with the brainstem, where strong Nrf2 signaling and controlled astrocytic/mitochondrial ROS confer relative protection. Collectively, these findings underscore that both intrinsic mitochondrial properties and extrinsic inflammatory responses dictate the gradient of vulnerability across brain regions. A comparative overview of these region-specific dynamics and their therapeutic implications is summarized in Table 2 (Ref. [4,27,41,42,43,45,46,47,48,49]).

4. Cell-Type Vulnerability to ROS: Pyramidal Neurons, Interneurons, Astrocytes, and Oligodendrocytes

I/R injury triggers oxidative damage across diverse brain regions and cell populations. However, susceptibility to ROS is not uniform across the central nervous system (CNS). This section highlights the cell-type specific responses to ROS, focusing on pyramidal neurons, interneurons, astrocytes, and oligodendrocytes. Such insights deepen our understanding of regional pathophysiology and suggest opportunities for tailored therapeutic interventions. Notably, while the following subsections detail the vulnerability of specific cell types, the pivotal role of microglia as a major enzymatic source of ROS must be emphasized. Microglia-derived ROS, primarily generated via NOX2 and NOX4 activation, serve as a fundamental instigator of oxidative stress that dictates the fate of neighboring neurons and glia [50]. Thus, the vulnerability of the neurovascular unit is intrinsically linked to the spatiotemporal dynamics of microglial activation and its subsequent inflammatory crosstalk [28,36,45]

4.1 Pyramidal Neurons: Metabolic Burden and Mitochondrial ROS Overload

Pyramidal neurons are among the most vulnerable cell types to I/R-induced oxidative injury due to their high metabolic demand, extensive excitatory input, and limited antioxidant reserves. These neurons, particularly in the hip-

Table 2. Region-specific ROS dynamics and therapeutic implications after ischemia–reperfusion (IR) injury.

Brain Region/Subfield	ROS generation pattern	Dominant ROS source	Key cellular responses	Antioxidant capacity	Vulnerability	Potential therapeutic strategy	Representative references
Hippocampal CA1	Biphasic (early cytosolic, delayed mitochondrial)	Mitochondria	ATP depletion, mitochondrial dysfunction, delayed neuronal death	Low Nrf2, low GSH	High	Mitochondria-targeted antioxidants (e.g., SS-31, MitoQ)	[4,27]
Hippocampal CA3	Mild, delayed	Mitochondria	Astrocyte buffering, preserved Ca^{2+} homeostasis	Moderate; higher thioredoxin/GSH	Low	Endogenous antioxidant support	[41,42,43]
Neocortex	Early peak (6–24 h post-reperfusion)	NOX2/NOX4 (microglia, endothelium)	Microglial activation, BBB breakdown, superoxide burst	Moderate	High	NOX inhibition, anti-inflammatory agents	[45,46,47]
Brainstem (e.g., medulla)	High basal ROS, controlled during IR	Mitochondria + astrocytes	Stable mitochondria, slow astrocyte activation, preserved Ca^{2+}	High Nrf2, high GSH	Low	Nrf2 induction in vulnerable areas	[48,49]

Abbreviations: BBB, blood–brain barrier; Ca^{2+}, calcium; GSH, glutathione; IR, ischemia–reperfusion; NOX, NADPH oxidase; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; ATP, Adenosine Triphosphate.

pocampal CA1 and cortical layers, are early targets of ROS-mediated damage [4,27]. In *ex vivo* mouse hippocampal slice models of oxygen-glucose deprivation followed by reoxygenation, ROS generation occurs in two waves: an initial cytosolic burst and a delayed mitochondrial surge (~40 min post-reperfusion) associated with mitochondrial hyperpolarization and zinc overload [32]. In cardiomyocyte I/R models, mitochondrial-specific fluorescent sensors (mito-roGFP, MitoSOX Red) reveal post-reperfusion ROS peaks that precede mitochondrial permeability transition pore (mPTP) opening and cytochrome c release, signaling the initiation of apoptosis [51]. Additionally, in cultured rat cortical neurons, overactivation of NMDA receptors during reperfusion drives calcium overload, which enhances mitochondrial ROS production and activates calpain—a protease that degrades cytoskeletal proteins and promotes dendritic injury and delayed cell death [52].

4.2 Interneurons: Delayed Degeneration and Conditional Vulnerability

GABAergic interneurons, particularly those expressing parvalbumin (PV^+) or neuronal nitric oxide synthase (nNOS), display a unique resistance profile in the early stages of I/R injury. In transient global ischemia models (e.g., 5-min bilateral carotid occlusion in gerbils), CA1 interneurons survive up to 2 days post-reperfusion, unlike pyramidal neurons which exhibit DND during the same period [53]. However, this initial resilience wanes with time; by 5–14 days post-I/R, many interneurons undergo degeneration characterized by loss of marker expression and structural deterioration. The basis for this temporal vulnerability appears multifactorial. Certain cortical $nNOS^+$ interneurons express elevated levels of antioxidant enzymes (e.g., SOD, catalase) and anti-apoptotic proteins like Bcl-2, offering intrinsic oxidative defense [54]. Yet, their susceptibility increases in pathologic contexts. In animal models of Alzheimer's disease and transient cerebral ischemia, PV interneurons display severe morphological alterations, mitochondrial dysfunction, and synaptic disruption [53,55], indicating that stress and disease contexts override their baseline resilience. Overall, interneurons represent a dynamic cell population whose oxidative vulnerability is stage-, subtype-, and context-dependent.

4.3 Astrocytes: Guardians Turned Instigators of ROS Injury

Astrocytes serve as frontline redox regulators under homeostatic conditions by producing glutathione, removing ROS, buffering potassium, and supporting neuronal metabolism [56]. However, following I/R, astrocytes transition into a reactive phenotype marked by hypertrophy, GFAP upregulation, and ROS-producing enzyme activation.

In primary human astrocyte cultures, angiotensin II stimulation increases the translocation of NOX subunits (e.g., p47^phox, p67^phox), enhancing superoxide production and inducing oxidative stress–driven senescence [57]. *In vivo*, ischemic models such as transient middle cerebral artery occlusion (tMCAO) reveal region-specific astrocyte behavior: cortical astrocytes rapidly activate NOX2/NOX4 and contribute to BBB disruption, whereas brainstem astrocytes exhibit delayed activation, preserving redox balance [49]. Therapeutically, astrocyte-specific activation of the Nrf2 pathway holds promise. In a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's model—a neurotoxin distinct from the mitochondrial permeability transition pore (mPTP)—systemic delivery of nootkatone enhanced astrocytic Nrf2

signaling and antioxidant enzyme expression (e.g., heme oxygenase-1 [HO-1], NAD(P)H quinone dehydrogenase 1 [NQO1]), resulting in reduced oxidative stress and dopaminergic neuron loss [58]. These findings illustrate astrocytes' dualistic nature—neuroprotective under normal conditions but potentially injurious post-I/R.

4.4 Oligodendrocytes: Targets of Lipid Peroxidation and Remyelination Failure

Oligodendrocytes are especially prone to oxidative damage due to their low antioxidant capacity and high content of polyunsaturated fatty acids (PUFAs) in myelin. Lipid peroxidation impairs myelin sheaths and triggers oligodendrocyte apoptosis, contributing to white matter injury during I/R [59]. In preterm infants, elevated F_2-isoprostanes in damaged white matter reflect early oxidative injury to immature oligodendrocytes [60]. Rodent neonatal stroke models corroborate this with increased lipid peroxidation and apoptotic markers in oligodendrocyte lineage cells. Furthermore, oligodendrocytes express AMPA/kainate receptors with limited glutamate uptake ability, rendering them highly vulnerable to excitotoxic calcium influx and ROS generation [61]. Oligodendrocyte precursor cells (OPCs) also face challenges in I/R settings. *In vitro* exposure to hydrogen peroxide (H_2O_2) or 4-HNE hampers their maturation, downregulating differentiation markers like Olig2 and MBP [62]. Recent stroke models show that region-specific white matter repair is impaired due to Nrf2 dysfunction and disrupted axon–glia interactions, suggesting that redox signaling and cellular cross-talk are central to remyelination success [63].

In summary, as shown in Table 3 (Ref. [4,32,49,51,52,53,54,55,56,57,58,60,61,62,63]), the CNS exhibits profound heterogeneity in oxidative stress responses across cell types. (1) Pyramidal neurons are highly vulnerable due to their metabolic and excitatory load, making them early ROS targets. (2) Interneurons show initial resistance but deteriorate under chronic oxidative stress or disease-modified environments. (3) Astrocytes serve as both protectors and amplifiers of ROS injury, with therapeutic potential via Nrf2 modulation. (4) Oligodendrocytes and OPCs are susceptible to lipid peroxidation and glutamate toxicity, hindering white matter repair. Understanding these distinctions is essential for developing cell-type tailored interventions. Strategies may include mitochondrial-targeted antioxidants for pyramidal neurons, modulators of interneuronal redox homeostasis, Nrf2 activators in astrocytes, and glutamate buffering or remyelination-promoting therapies for oligodendrocytes. As Section 3 highlighted regional differences in ROS dynamics, integrating spatial and cellular profiles will be key to designing precision therapeutics for I/R injury.

4.5 Bidirectional Crosstalk Between ROS and Neuroinflammation

The pathological progression of ischemic brain injury is increasingly recognized as a synergistic interplay between oxidative stress and neuroinflammatory responses. ROS act as pivotal signaling mediators that activate pro-inflammatory transcription factors, such as nuclear factor-kappa B (NF-κB), and stimulate the assembly of the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome [35,64]. These molecular events trigger the release of various cytokines and chemokines, which subsequently recruit peripheral immune cells and activate resident microglia. Crucially, these activated inflammatory cells serve as secondary sources of ROS through the upregulation of enzymes like NOX, thereby creating a self-perpetuating cycle of damage [35,36]. This bidirectional crosstalk not only accelerates BBB disruption but also intensifies regional neuronal vulnerability, effectively dictating the eventual extent of the cerebral infarction.

5. ROS-Induced Cell Death Pathways in Ischemia–Reperfusion Injury: From Apoptosis to Pyroptosis

I/R injury induces a rapid and excessive generation of ROS, which acts as a central upstream trigger for various cell death pathways. These include classical mechanisms such as apoptosis and necrosis, as well as several forms of regulated cell death (RCD) that have been increasingly recognized in neurodegenerative and ischemic conditions. Notably, as shown in Fig. 3, ferroptosis, parthanatos, necroptosis, autophagy-dependent death, and pyroptosis all exhibit ROS-sensitive checkpoints, making them highly relevant to ischemic brain injury. Each modality is defined by unique molecular markers and signaling cascades, yet they often coexist or sequentially activate within the neurovascular unit after I/R insult. Understanding the hierarchy and ROS-dependence of these mechanisms is crucial for designing effective neuroprotective strategies.

5.1 Apoptosis: Mitochondrial and Death Receptor-Mediated Pathways

Apoptosis is a hallmark mechanism of DND following cerebral I/R, particularly prominent in selectively vulnerable regions like the hippocampal CA1 subfield. It is morphologically characterized by cell shrinkage, chromatin condensation, and the formation of apoptotic bodies without plasma membrane rupture or inflammatory response (Fig. 3A). Two primary apoptotic pathways—the intrinsic mitochondrial and the extrinsic death receptor-mediated pathways—are activated following oxidative stress and calcium dysregulation. The mitochondrial pathway is initiated by ROS-induced mPTP opening, resulting in membrane depolarization and cytochrome c release. This promotes apoptosome assembly and activation of caspase-9 and caspase-

Table 3. Summary of ROS-induced vulnerability across major CNS cell types after ischemia–reperfusion injury.

Cell type	Vulnerability to ROS	Main mechanisms	IR outcomes	Therapeutic targets	References
Pyramidal neurons (CA1, cortex)	High	Mitochondrial ROS overproduction, NMDA/AMPA receptor-mediated Ca^{2+} influx, calpain activation	Delayed neuronal death, dendritic degeneration	Mitochondrial stabilizers, NMDA antagonists, antioxidants	[4,32,51,52]
GABAergic interneurons	Moderate	Lower baseline oxidative stress but susceptible in chronic stress; PV+ subtype shows redox-related protein vulnerability	Subtype-specific degeneration, inhibitory imbalance	PV-targeted proteostasis modulators	[53,54,55]
Astrocytes	Context-dependent	Reactive gliosis, NOX2/NOX4-mediated ROS generation, impaired Nrf2 activation	BBB disruption, neuroinflammation, gliotoxicity	Nrf2 activators, NOX inhibitors	[49,56,57,58]
Oligodendrocytes/ OPCs	High	Lipid peroxidation, excitotoxicity (glutamate uptake failure), impaired maturation	Myelin loss, white matter damage	Glutamate reuptake enhancers, lipid ROS scavengers	[60,61,62,63]

Abbreviations: IR, Ischemia–Reperfusion; ROS, Reactive Oxygen Species; PV, Parvalbumin; NOX, NADPH Oxidase; Nrf2, Nuclear factor erythroid 2-related factor 2; OPC, Oligodendrocyte Precursor Cell; BBB, Blood–Brain Barrier; CNS, central nervous system; NMDA, N-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

3, culminating in DNA fragmentation [65,66]. In global cerebral ischemia models, apoptotic activity is confirmed using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining and cleaved caspase-3 immunohistochemistry in CA1 neurons. These studies show that cleaved caspase-3 and TUNEL-positive cells notably increase between 24–72 hours post-reperfusion, reflecting the delayed nature of apoptosis [4,67]. The extrinsic pathway involves death ligands such as FasL and TNF-related apoptosis-inducing ligand (TRAIL) binding to their receptors (Fas/CD95 and DR4/5), which activates caspase-8 and downstream executioner caspases. In rat models of transient focal brain ischemia, blocking the Fas–FasL interaction via peptide inhibitors or gene knockdown reduces infarct volume and neuronal apoptosis [68,69]. Colocalization of CD95L and TRAIL with degenerating neurons in post-ischemic cortex and hippocampus further supports the involvement of this pathway [70].

5.2 Ferroptosis: Iron-Driven Lipid Peroxidation

Ferroptosis is a non-apoptotic form of RCD driven by iron-dependent lipid peroxidation and depletion of the antioxidant enzyme glutathione peroxidase 4 (GPX4) (Fig. 3B). Morphologically, ferroptotic cells exhibit shrunken mitochondria and outer membrane rupture without caspase activation [71]. Following I/R, free iron released from damaged ferritin and heme-containing proteins catalyzes Fenton reactions, generating hydroxyl radicals that oxidize PUFAs in cell membranes [72]. In rodent models of tMCAO, ferroptotic features are detected in the ischemic penumbra within 6–24 hours post-reperfusion, evidenced by increased iron accumulation, GPX4 depletion, and mitochondrial damage [73]. The thrombin–ACSL4 axis further sensitizes neurons to ferroptosis by promoting PUFA incorporation into membrane phospholipids [74]. Pharmacological inhibition using ferrostatin-1 or liproxstatin-1 in these models significantly reduces infarct volume and improves neurologic scores (e.g., mNSS and rotarod performance), correlating with the preservation of mitochondrial morphology. Additionally, activation of the PI3K/AKT/Nrf2 signaling axis enhances GPX4 and SLC7A11 expression, promoting ferroptosis resistance [72].

5.3 Parthanatos: PARP-1 Hyperactivation and AIF-Mediated DNA Fragmentation

Parthanatos is a caspase-independent form of RCD characterized by swollen mitochondria and large-scale, non-fragmented chromatin collapse without apoptotic body formation (Fig. 3C). It is triggered by the hyperactivation of poly(ADP-ribose) polymerase-1 (PARP-1) in response to DNA damage, leading to rapid depletion of nicotinamide adenine dinucleotide (NAD^+) and ATP and ultimately causing energy failure. Accumulated poly(ADP-ribose) (PAR) polymers signal the release of apoptosis-inducing factor (AIF) from mitochondria, which translocates to the nucleus to cause large-scale chromatin condensation and DNA fragmentation [75,76]. In rodent models of transient focal ischemia, elevated PARP-1 expression and nuclear translocation of AIF are observed in the peri-infarct cortex and striatum within 6–24 hours post-reperfusion, correlating with

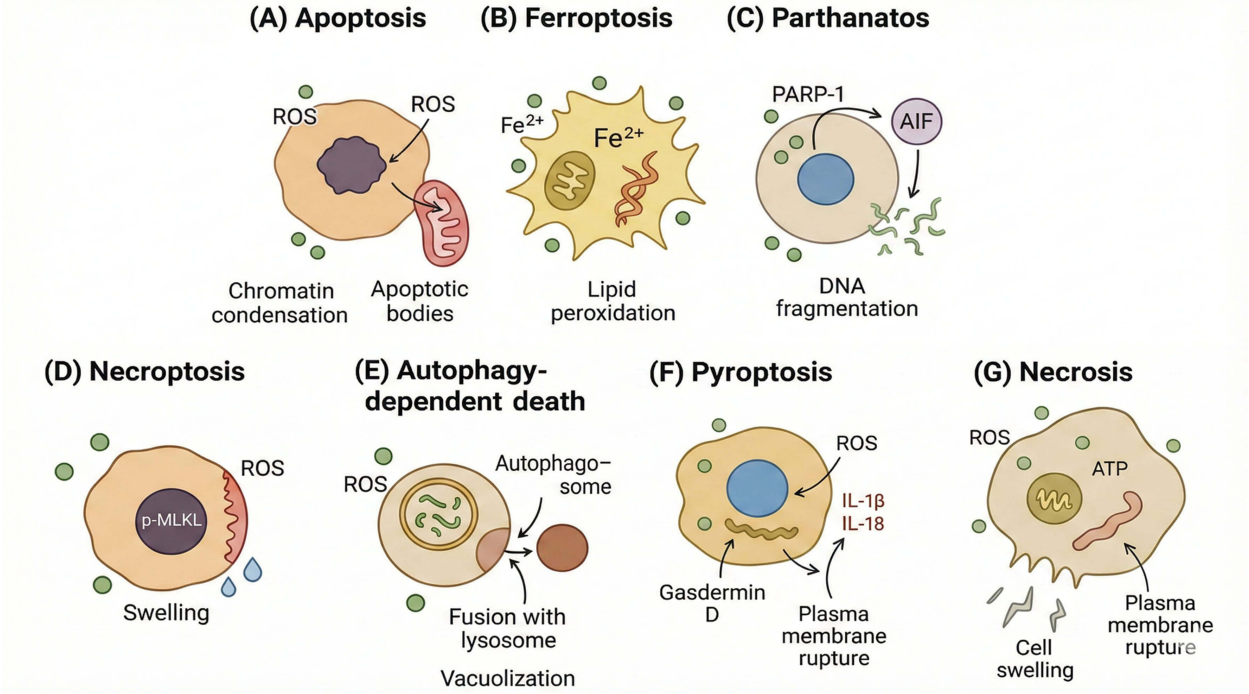

Fig. 3. Diverse ROS-mediated cell death pathways in ischemic brain injury. This figure illustrates the distinct morphological and molecular features of programmed and non-programmed cell death mechanisms triggered by reactive oxygen species (ROS) during I/R. (A) **Apoptosis**: Characterized by ROS-induced chromatin condensation and the subsequent formation of apoptotic bodies. (B) **Ferroptosis**: An iron-dependent form of regulated cell death driven by Fe^{2} accumulation and lethal lipid peroxidation. (C) **Parthanatos**: Initiated by overactivation of PARP-1, leading to the translocation of apoptosis-inducing factor (AIF) and subsequent DNA fragmentation. (D) **Necroptosis**: A form of programmed necrosis involving p-MLKL activation, resulting in significant cellular swelling. (E) **Autophagy-dependent death**: Marked by the formation of autophagosomes and their fusion with lysosomes, leading to extensive cytoplasmic vacuolization. (F) **Pyroptosis**: An inflammatory cell death pathway mediated by Gasdermin D, characterized by plasma membrane rupture and the release of pro-inflammatory cytokines, including IL-1β and IL-18. (G) **Necrosis**: A passive, unregulated process of cell death involving ATP depletion, extreme cell swelling, and catastrophic plasma membrane rupture. **Abbreviations**: AIF, apoptosis-inducing factor; ASC, apoptosis-associated speck-like protein containing a CARD; ATP, adenosine triphosphate; DAMPs, damage-associated molecular patterns; Fe^{2+}, ferrous iron; GPX4, glutathione peroxidase-4; GSDMD, gasdermin D; HMGB1, high-mobility group box-1; I/R, ischemia–reperfusion; IL-1β/IL-18, interleukin-1β/-18; MLKL, mixed lineage kinase domain-like protein; mPTP, mitochondrial permeability transition pore; NAD^{+}, nicotinamide adenine dinucleotide; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; Nrf2, nuclear factor erythroid 2-related factor 2; PAR, poly(ADP-ribose); PARP-1, poly(ADP-ribose) polymerase-1; PUFA, polyunsaturated fatty acid; RIPK, receptor-interacting protein kinase; ROS, reactive oxygen species; TLR4, Toll-like receptor-4; TRAIL, TNF-related apoptosis-inducing ligand; DNA, deoxyribonucleic acid. Figures were assisted with ChatGPT (OpenAI, GPT-4.0 version).

infarct expansion and neurological deficits [77]. Histological analysis and Western blot of nuclear fractions confirm AIF redistribution in these models. Pharmacological inhibition of PARP-1 using PJ34 or 3-aminobenzamide (3-AB) has demonstrated neuroprotective effects in both global and focal ischemia models. For instance, in a gerbil model, PJ34 significantly reduced hippocampal CA1 neuronal loss and microglial activation [78,79]. Moreover, 3-AB administration suppressed PARP-1–mediated degradation of tight junction proteins in endothelial cells, thereby attenuating BBB disruption [80]. These findings underscore parthanatos as an early and critical form of RCD in I/R injury [81].

5.4 Necroptosis: A Programmed Form of Inflammatory Cell Death

Necroptosis is a form of regulated necrosis that morphologically resembles necrosis (e.g., cell swelling and membrane rupture) but is tightly regulated by specific molecular pathways (Fig. 3D). Driven by the receptor-interacting protein kinase 1 (RIPK1), RIPK3, and mixed lineage kinase domain-like protein (MLKL) axis, necroptosis becomes increasingly prominent in both neurons and glia under conditions of oxidative stress and ATP depletion [82,83]. In mouse MCAO models, the expression of RIPK3 and phosphorylated-MLKL (p-MLKL) peaks in the peri-infarct cortex 24 hours post-reperfusion [84]. Inhibi-

tion of necroptosis using Necrostatin-1 (Nec-1), a selective RIPK1 inhibitor, significantly reduced infarct size and improved motor outcomes in rat models, indicating its functional contribution to I/R pathology [85,86]. Notably, Nec-1 also reduced oxidative damage markers (e.g., 4-HNE and nitrotyrosine) and preserved mitochondrial structure, suggesting a feedforward loop between necroptosis and ROS. Beyond neurons, RIPK3 activation in microglia is linked to proinflammatory cytokine release (e.g., IL-1β and TNF-α) independent of NLRP3 inflammasome priming [87]. Targeting necroptosis may thus limit both neuronal injury and glial dysfunction.

5.5 Autophagy: Context-Dependent Role in ROS Homeostasis and Cell Death

Autophagy is a catabolic lysosome-mediated process that maintains cellular homeostasis by degrading dysfunctional organelles and proteins (Fig. 3E). While often cytoprotective, autophagy can contribute to cell death under sustained I/R-induced oxidative stress. Following cerebral I/R, autophagy is rapidly activated to mitigate mitochondrial dysfunction (mitophagy) [88]. In MCAO models, early activation via AMPK and JNK pathways supports ATP generation and metabolic adaptation [89,90]. However, excessive or prolonged autophagy becomes deleterious; by 24 hours post-I/R, impaired autophagosome–lysosome fusion leads to p62 accumulation and mitochondrial destabilization [91]. In global ischemia models, sustained LC3 accumulation correlates with neuronal shrinkage and cytoplasmic vacuolization, indicative of neuronal self-digestion [92,93]. *In vitro* models using PC12 and SH-SY5Y cells replicate this biphasic autophagy response [94]. Astrocytes also demonstrate context-dependent autophagic roles, where early activation suppresses NLRP3 signaling while dysregulation exacerbates dysfunction [95,96,97]. Pharmacological modulation using rapamycin has shown potential in neonatal models by enhancing autophagic flux to mitigate apoptosis [98,99].

5.6 Pyroptosis: Inflammatory Cell Death Driven by Gasdermin D

Pyroptosis is a pro-inflammatory RCD characterized by rapid cell swelling, gasdermin D (GSDMD) -mediated membrane pore formation, and release of interleukin-1β (IL-1β) and IL-18 (Fig. 3F). In I/R injury, pyroptosis is predominantly initiated through the activation of the canonical NLRP3 inflammasome in microglia and astrocytes. Recognition of damage-associated molecular patterns (DAMPs) (e.g., ATP, mitochondrial DNA, and ROS) activates NLRP3, which recruits apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase-1 to trigger IL-1β maturation and GSDMD cleavage [90,100,101]. In parallel, a non-canonical pathway involves caspase-11 in rodents or caspase-4/5 in humans, which directly sense intracellular LPS [102]. In rodent MCAO models, increased caspase-1 activity and GSDMD-N levels correlate with infarct expansion [103]. Pharmacological inhibition using VX-765, a selective caspase-1 inhibitor, ameliorates BBB disruption and improves neurological outcomes [104]. Genetic ablation of GSDMD similarly confers neuroprotection by limiting microglial pyroptosis and reducing infarct size [105]. Future directions should focus on cell-type–specific modulation to balance immune clearance with tissue preservation.

5.7 Necrosis: Energy Collapse and Oxidative Membrane Injury

Necrosis predominates in the ischemic core during the acute phase of I/R injury (Fig. 3G). It is primarily triggered by bioenergetic collapse due to rapid ATP depletion, which impairs ion transporters like Na^+/K^+-ATPase and Ca^{2+}-ATPase. This leads to cytosolic Na^+ and Ca^{2+} overload and the activation of calpains that degrade cytoskeletal and membrane proteins [52,106]. These disturbances induce cytoplasmic swelling and ultimate membrane rupture. ROS generated during mitochondrial dysfunction further exacerbate injury through lipid peroxidation [107]. Ruptured cells release DAMPs such as high-mobility group box-1 (HMGB1), which are sensed by Toll-like receptor-4 (TLR4) on microglia to initiate neuroinflammatory cascades [64]. Although traditionally regarded as irreversible, partial pharmacological modulation has been demonstrated. Inhibition of calpains using MDL28170 reduces infarct volume and preserves neuronal structure in MCAO models [108]. Moreover, disrupting the CaMKII–calpain signaling axis attenuates necrosis and improves behavioral outcomes [109].

In summary, I/R injury initiates a multifaceted cascade of cell death mechanisms extending beyond classical apoptosis and necrosis to encompass recently elucidated pathways like pyroptosis, ferroptosis, and parthanatos. As shown in Fig. 3, these modalities are driven by shared upstream triggers—primarily ROS, calcium dysregulation, and mitochondrial dysfunction—yet diverge in their specific executioner molecules and morphological features. Rather than operating in isolation, these pathways exhibit significant spatiotemporal overlap and molecular crosstalk. Understanding this networked architecture provides a framework for combinatorial neuroprotection, whereby targeting multiple death pathways in a phase- and region-specific manner may yield greater efficacy than monotherapies. Future strategies must account for the interdependence of these pathways to mitigate irreversible neuronal loss and improve post-ischemic recovery. These diverse ROS-sensitive mechanisms and their vulnerabilities are systematically summarized in Table 4 (Ref. [4,52,65,69,71,72,73,74,75,76,77,78,79,80,82,83,84,85,86,87,89,91,93,100,103,104,105,106,107,108,109]).

Table 4. ROS-dependent cell death pathways in ischemia–reperfusion (IR) injury.

Cell death type	Key mechanism/ROS role	Molecular markers	Morphological features	Timing/Localization (in IR)	Therapeutic modulation	Representative references
Apoptosis (intrinsic & extrinsic)	ROS triggers mPTP opening → cytochrome c release; death receptor (Fas, TRAIL) signaling amplifies caspase cascade	Cytochrome c, cleaved caspase-3, caspase-8, TUNEL+ nuclei	Cell shrinkage, chromatin condensation, apoptotic bodies	24–72 h post-reperfusion; CA1 pyramidal neurons, cortex	Caspase inhibitors, Fas/FasL blockade	[4,65,69]
Ferroptosis	Iron-driven lipid peroxidation; ROS (•OH) via Fenton chemistry; GPX4 depletion	GPX4 downregulation, ACSL4, lipid ROS, PUFA oxidation	Shrunken mitochondria, cristae loss, outer membrane rupture	6–24 h post-reperfusion; ischemic penumbra	Ferrostatin-1, Liproxstatin-1, Nrf2/GPX4 activation	[71,72,73,74]
Parthanatos	PARP-1 hyperactivation → NAD^+/ATP depletion; PAR → AIF nuclear translocation	PARP-1, PAR polymers, AIF nuclear translocation	Swollen mitochondria, large-scale chromatin collapse	6–24 h post-reperfusion; peri-infarct cortex, hippocampus	PARP-1 inhibitors (PJ34, 3-AB)	[75,76,77,78,79,80]
Necroptosis	ROS amplifies RIPK1/RIPK3/MLKL pathway; p-MLKL → membrane rupture	RIPK1, RIPK3, p-MLKL	Cell swelling, plasma membrane rupture, chromatin condensation	6–24 h; peri-infarct cortex, hippocampus, glia	Necrostatin-1 (RIPK1 inhibitor), ROS scavengers	[82,83,84,85,86,87]
Autophagy-dependent death	ROS-induced mitophagy; prolonged stress → autophagic collapse	LC3-II, Beclin-1, p62/SQSTM1 accumulation	Double-membrane autophagosomes; vacuolization	Early phase (protective); later (deleterious) 3–24 h post-IR	Rapamycin, autophagy modulators; context-dependent	[82,89,91,93]
Pyroptosis	ROS/DAMPs activate NLRP3 inflammasome → caspase-1 → GSDMD pore formation	NLRP3, ASC, caspase-1, cleaved GSDMD, IL-1β/IL-18	Cell swelling, membrane rupture, cytokine release	Hours–days post-IR; microglia, astrocytes, cortex	Caspase-1 inhibitors (VX-765), GSDMD knockout	[100,103,104,105]
Necrosis	ROS + ATP depletion → ionic pump failure → Ca^{2+} overload; calpain/cathepsin activation	Calpains, HMGB1, TLR4 activation	Cytoplasmic swelling, membrane rupture, organelle breakdown	Acute phase; ischemic core within hours	Calpain inhibitors (MDL28170), CaMKII–calpain blockade	[52,106,107,108,109]

Abbreviations: ACSL4, acyl-CoA synthetase long-chain family member 4; AIF, apoptosis-inducing factor; ASC, apoptosis-associated speck-like protein containing a CARD; DAMPs, damage-associated molecular patterns; FasL, Fas ligand; GSDMD, gasdermin D; GPX4, glutathione peroxidase 4; HMGB1, high mobility group box 1; IL, interleukin; IR, ischemia–reperfusion; MLKL, mixed lineage kinase domain-like protein; mPTP, mitochondrial permeability transition pore; NAD^+, nicotinamide adenine dinucleotide; NLRP3, nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3; Nrf2, nuclear factor erythroid 2–related factor 2; PAR, poly(ADP-ribose); PARP-1, poly(ADP-ribose) polymerase-1; PUFA, polyunsaturated fatty acid; RIPK, receptor-interacting protein kinase; ROS, reactive oxygen species; SQSTM1/p62, sequestosome 1; TLR, toll-like receptor; TRAIL, TNF-related apoptosis-inducing ligand; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

6. Antioxidant Defense Systems in Brain I/R Injury: Roles of SOD, GPx, and Catalase

Brain I/R injury provokes a robust surge in ROS, overwhelming endogenous antioxidant defenses and triggering oxidative stress. This oxidative burst leads to widespread damage to lipids, proteins, and nucleic acids, thereby initiating neuronal death, glial activation, and BBB disruption [24]. The brain's intrinsic antioxidant network—primarily composed of SODs, GPxs, and catalase—constitutes a multi-tiered defense system against ROS-induced cellular injury [56]. These enzymes exhibit compartment-specific localization, substrate specificity, and regulation, yet act synergistically to maintain redox homeostasis. Understanding their integrated roles provides a basis for antioxidant-based therapeutic strategies in ischemic stroke [36].

6.1 SODs

SODs catalyze the dismutation of superoxide anions (O_2^{-}•) into H_2O_2, serving as the first line of antioxidant defense during I/R injury. The three isoforms—SOD1 (cytosolic Cu/Zn-SOD), SOD2 (mitochondrial Mn-SOD), and SOD3 (extracellular SOD)—are differentially expressed in the brain, with SOD2 playing a particularly vital role due to its mitochondrial localization and proximity to the primary ROS production sites [24]. Experimental evidence strongly supports the neuroprotective role of SOD2. In a SOD2+/– heterozygous knockout mouse model subjected to middle cerebral artery occlusion (MCAO), infarct size and lipid peroxidation were significantly increased, along with elevated peroxynitrite levels [110]. Conversely, transgenic mice overexpressing SOD2 exhibited reduced oxidative damage, preserved mitochondrial cristae, and attenuated apoptotic cell death following transient focal cerebral ischemia [111]. Furthermore, pharmacological interventions that enhance mitochondrial redox buffering have shown promise. In rat MCAO models, Dengzhan Xixin injection—a flavonoid-rich extract from *Erigeron breviscapus*—promoted mitochondrial fusion and suppressed ferroptosis via upregulation of SOD2 and PINK1-mediated mitophagy [112]. Similarly, selenium-enriched diets increased SOD2 expression and reduced mitochondrial ROS in ischemic hippocampal tissue, suggesting therapeutic synergy between trace elements and endogenous antioxidants [113]. Collectively, these findings highlight the central role of SOD2 in maintaining mitochondrial redox homeostasis and limiting I/R-induced neuronal injury through structural and signaling preservation of mitochondrial function.

6.2 Glutathione Peroxidases (GPxs)

GPxs are a family of selenium-dependent enzymes that catalyze the reduction of H_2O_2 and lipid hydroperoxides to water and lipid alcohols, using reduced glutathione (GSH) as a cofactor. Among them, GPx1 is highly expressed in both neurons and astrocytes and serves as a key cytosolic enzyme that protects cellular and mitochondrial components from oxidative injury during I/R stress [114]. The neuroprotective function of GPx1 has been demonstrated in multiple rodent models of cerebral ischemia. In GPx1 knockout mice subjected to transient focal ischemia, infarct volumes were significantly larger, with marked increases in lipid peroxidation and apoptotic neuronal loss, compared to wild-type controls [115]. Conversely, transgenic mice overexpressing GPx1 exhibited smaller infarct areas, delayed hippocampal neuronal death, and better preservation of mitochondrial membrane potential following global ischemia [116]. These protective effects were mechanistically linked to reduced cytochrome c release and inhibition of downstream caspase activation, indicating that GPx1 plays a pivotal role in suppressing mitochondria-mediated apoptosis. Pharmacological strategies to enhance GPx activity or mimic its function have also shown translational potential. In rat MCAO models, administration of N-acetylcysteine (NAC), a glutathione precursor, led to increased GSH levels, reduced pro-inflammatory cytokines, and attenuated infarct volume and neuronal damage [117]. Additionally, ebselen, a synthetic seleno-organic compound with GPx-like activity, decreased ROS accumulation and neuronal injury in both mouse and rat ischemic models [118]. These findings collectively support the therapeutic relevance of targeting the GPx-GSH axis to mitigate I/R-induced oxidative stress and preserve neuronal viability.

6.3 Catalase

Catalase is a peroxisomal antioxidant enzyme that decomposes H_2O_2 into water and oxygen, thereby preventing the Fenton reaction-mediated generation of highly reactive hydroxyl radicals [119]. While its baseline expression in the brain is lower than in peripheral tissues, ischemic stress triggers peroxisomal biogenesis and catalase upregulation in both neurons and astrocytes—particularly within the cortex and hippocampus—suggesting an adaptive compensatory response [120,121]. Paradoxically, catalase activity is frequently downregulated following reperfusion, resulting in H_2O_2 accumulation, lipid peroxidation, and the acceleration of neuronal death [122]. Experimental efforts to bolster this defense have yielded significant neuroprotective results. Selective overexpression of catalase in astrocytes significantly reduces infarct volume, preserves BBB integrity, and suppresses apoptotic markers such as cleaved caspase-3 and TUNEL-positive neurons [123]. Furthermore, therapeutic approaches including gene delivery via catalase-expressing plasmids and the administration of synthetic mimetics like EUK-134 and EUK-8 have demonstrated robust efficacy in reducing oxidative burden and sensorimotor deficits in various stroke models [124,125]. Collectively, these multimodal interventions underscore the translational potential of catalase-centered strategies to mitigate post-ischemic H_2O_2 toxicity.

Table 5. Major antioxidant enzymes in brain ischemia–reperfusion (I/R) injury: roles, mechanisms, and therapeutic implications.

Enzyme	Localization/Isoform	Primary function	Experimental findings in I/R models	Therapeutic Strategy/Modulation	Representative references
Superoxide dismutase (SOD)	SOD1 (cytosol), SOD2 (mitochondria), SOD3 (extracellular)	Catalyzes dismutation of superoxide (O_2^-•) to H_2O_2; first-line antioxidant defense	$SOD2^{+/-}$ mice show larger infarcts and lipid peroxidation; SOD2-overexpressing mice exhibit preserved mitochondrial morphology and reduced apoptosis after transient ischemia	Mitochondrial redox enhancement via SOD2 upregulation; flavonoid extract (Erigeron breviscapus) increases SOD2 and mitophagy; selenium-enriched diets augment SOD2 expression	[110,111,112,113]
Glutathione peroxidase (GPx)	GPx1 (cytosol, mitochondria), GPx4 (membrane-associated)	Reduces H_2O_2 and lipid hydroperoxides to water and lipid alcohols using GSH	GPx1 knockout mice show increased infarct size and apoptosis; GPx1-overexpressing mice exhibit smaller infarcts, preserved mitochondrial potential, and reduced caspase activation	N-acetylcysteine (GSH precursor) increases GPx activity, reduces inflammation; ebselen (selenium compound) mimics GPx function, decreases infarct volume and ROS	[115,116,117,118]
Catalase	Peroxisomes (neurons & astrocytes, especially in cortex and hippocampus)	Decomposes $H_2O_2 \rightarrow H_2O + O_2$, limiting hydroxyl radical (•OH) formation	Ischemia decreases catalase activity; overexpression in astrocytes reduces infarct volume and apoptosis; catalase mimetics improve BBB integrity and motor outcomes	Gene therapy (adenoviral catalase), EUK-134 and EUK-8 mimetics enhance H_2O_2 detoxification and neuroprotection	[119,120,121,122,123,125]
Integrated enzyme network (SOD + GPx + Catalase)	Cytosolic, mitochondrial, and peroxisomal compartments	Sequential detoxification of ROS: O_2^-• → H_2O_2 → H_2O; maintains redox homeostasis	Coordinated loss of enzyme activity exacerbates oxidative injury; mitochondrial SOD2/GPx4 most critical for redox balance; catalase buffers peroxisomal and vascular H_2O_2	Multi-target Nrf2 activation enhances SOD, GPx, and catalase expression; synergistic protection in stroke models	[29,126,127]

Abbreviations: BBB, blood–brain barrier; GSH, glutathione; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; I/R, ischemia–reperfusion; Nrf2, nuclear factor erythroid 2–related factor 2; ROS, reactive oxygen species; SOD, superoxide dismutase.

6.4 Comparative Insights: Coordinated Antioxidant Enzyme Responses in Brain I/R Injury

The brain's antioxidant defense operates as a tiered and compartmentalized network in which SODs, GPxs, and catalase function in concert to neutralize ROS at successive stages of oxidative metabolism. SODs catalyze the dismutation of $O_2^{-}\bullet$ into H_2O_2, which is subsequently detoxified by GPxs and catalase through distinct yet complementary mechanisms [29,126]. Among these, mitochondrial isoforms such as SOD2 and GPx4 play pivotal roles because mitochondria represent the predominant ROS source during reperfusion [126]. Catalase, by contrast, acts mainly at the neurovascular and peroxisomal interfaces, limiting H_2O_2 accumulation and protecting endothelial and glial compartments [29]. This relay-like orchestration ensures timely detoxification of ROS intermediates and prevents propagation of oxidative chain reactions. Dysregulation in any component—particularly deficiency or inactivation of GPx or catalase—amplifies neuronal oxidative stress and structural damage. Conversely, simultaneous enhancement of multiple antioxidant enzymes, such as through Nrf2 pathway activation, has shown synergistic neuroprotection in experimental stroke models [127]. In summary, endogenous antioxidant enzymes form a sequential and spatially integrated defense system against I/R-induced oxidative stress. SODs convert $O_2^{-}\bullet$ to H_2O_2, which GPxs and catalase subsequently detoxify within cytosolic, mitochondrial, and peroxisomal domains. Experimental models, including MCAO in rodents, demonstrate that reductions in SOD2, GPx1, or catalase activity exacerbate neuronal injury, especially in mitochondria-rich regions. In contrast, pharmacological agents such as NAC, ebselen, and EUK-134 enhance enzymatic activity, reduce infarct volume, and preserve mitochondrial function. Together, these enzymes operate as an interlinked redox network whose compartment-specific synergy is indispensable for neuronal survival, emphasizing the value of multi-target antioxidant strategies in mitigating I/R-induced neurodegeneration (Table 5, Ref. [29,110,111,112,113,115,116,117,118,119,120,121,122,123,125,126,127]).

7. Therapeutic Implications: Targeting ROS in I/R Injury

ROS are central mediators in the pathophysiology of I/R injury, contributing to neuronal death, mitochondrial dysfunction, BBB breakdown, and inflammatory responses [24,35]. Therapeutic strategies focused on ROS modulation—including antioxidant enzyme mimetics, gene therapy to enhance endogenous defenses (e.g., SOD2, GPx1, catalase), and pharmacological upregulation via redox-sensitive pathways like Nrf2—show promise in preclinical stroke models [35,48]. This section reviews translationally relevant interventions aimed at restoring redox balance and mitigating I/R-induced brain injury.

7.1 Enzyme Mimetics and Genetic Augmentation

Therapeutic strategies aiming to reestablish redox balance following I/R injury have increasingly focused on enhancing the activity of endogenous antioxidant enzymes—particularly SOD, GPx, and catalase—through the use of enzyme mimetics or gene delivery systems. Among the most promising pharmacological candidates are manganese (Mn) porphyrin-based SOD mimetics, including MnTE-2-PyP^{5+} and MnTnHex-2-PyP^{5+}, collectively referred to as BMX-001. These compounds mimic mitochondrial SOD activity and are capable of scavenging both superoxide and peroxynitrite. In preclinical rodent models of global and focal cerebral ischemia, administration of MnTnHex-2-PyP^{5+} has been shown to reduce infarct volume, attenuate NF-κB–mediated inflammatory responses, activate Nrf2-driven antioxidant signaling, and improve neurological outcomes—even when administered after reperfusion onset [128,129,130]. In contrast, MnTBAP, another widely used Mn-porphyrin compound, lacks genuine SOD activity in its pure form. Its observed *in vivo* efficacy appears to derive primarily from peroxynitrite scavenging, rather than from direct superoxide dismutation [131].

Genetic augmentation of antioxidant defenses has also shown neuroprotective efficacy. Although studies specifically targeting astrocytic expression are limited, overexpression of SOD1 in transgenic rodents has been reported to significantly reduce hippocampal and cortical neuronal loss after global cerebral ischemia, likely through the suppression of oxidative stress–induced apoptosis [132]. Similarly, catalase overexpression via viral vectors, when administered prior to MCAO, protected striatal neurons against I/R-induced oxidative injury in rats [123]. In another study, gene delivery of GPx1 suppressed cytochrome c release, preserved mitochondrial membrane potential, and reduced apoptotic signaling after experimental stroke in mice [116]. An innovative advancement in this domain involves the use of chimeric fusion enzymes. For example, PEP-1-catalase, a cell-permeable fusion protein, was successfully delivered intranasally in a murine model of ischemia and was found to reduce infarct volume, inhibit oxidative stress markers, and improve neurological outcomes [133].

Collectively, these findings demonstrate the therapeutic potential of both mimetic compounds and genetic enhancement of antioxidant enzymes in attenuating neuronal death after I/R injury. However, translational success will depend heavily on delivery method, timing of intervention, and cell-type specificity—particularly in selectively vulnerable brain regions.

7.2 Upregulation of Endogenous Antioxidants (Nrf2-Independent or Mixed)

Beyond direct antioxidant enzyme mimetics and gene delivery approaches, several natural and pharmacological compounds have demonstrated the capacity to enhance endogenous antioxidant defenses through Nrf2-independent

or mixed mechanisms. These interventions contribute to mitochondrial redox stabilization, suppression of ROS overproduction, and attenuation of I/R-induced neuronal injury.

Melatonin is one of the most widely studied endogenous compounds with antioxidant properties. It enhances the activity of key antioxidant enzymes—including SOD, GPx, and catalase—through both receptor-mediated signaling and direct mitochondrial interactions. In a rat model of global cerebral ischemia, melatonin administration significantly increased SOD and GPx activities, reduced lipid peroxidation, and mitigated DND in the hippocampus [134]. A recent systematic review confirmed the therapeutic potential of melatonin in rodent models of cerebral I/R injury, highlighting consistent reductions in infarct volume, preservation of BBB integrity, and improved neurologic outcomes, which were largely attributed to enhanced antioxidant enzyme activity [135]. Furthermore, co-administration of melatonin with NAC in a rat MCAO model resulted in additive restoration of SOD activity and suppression of oxidative stress biomarkers [136]. Notably, melatonin pretreatment was shown to preserve mitochondrial membrane potential and prevent cytochrome c release, correlating with upregulated catalase activity and reduced oxidative injury [136].

Edaravone, a clinically approved free radical scavenger for ischemic stroke in Japan and China—including its newer formulation in combination with dexborneol—exerts neuroprotection primarily through efficient elimination of hydroxyl radicals and lipid peroxides. In animal models of cerebral I/R, edaravone treatment prevented mitochondrial swelling, preserved mitochondrial membrane potential, and reduced neuronal apoptosis [137,138]. These mitochondrial protective effects align with decreased oxidative product accumulation and dampened neuroinflammatory responses. While its principal mechanism is direct ROS neutralization, emerging studies report that edaravone–dexborneol can activate the Nrf2/HO-1 antioxidant pathway in ischemic brain tissue, thus supporting its role in modulating endogenous antioxidant defenses [138,139].

Curcumin, a polyphenolic compound derived from turmeric, also demonstrates multifaceted antioxidant effects in cerebral I/R models. In rats subjected to transient MCAO, curcumin significantly reduced infarct volume and lipid peroxidation while elevating SOD and GPx activity [140]. Mechanistically, curcumin's neuroprotective actions involve activation of the PI3K/Akt signaling cascade, which subsequently promotes Nrf2 nuclear translocation and upregulation of downstream antioxidant genes such as NAD(P)H quinone dehydrogenase 1 (NQO1), suggesting both Nrf2-dependent and independent contributions [140].

Resveratrol, another well-characterized polyphenol found in grapes, enhances antioxidant capacity through both Nrf2-mediated transcriptional activation and SIRT1-dependent modulation of mitochondrial function. In models of ischemic brain injury, resveratrol elevated catalase and SOD2 expression, suppressed hippocampal oxidative stress, and preserved neuronal viability [141]. These effects are partly attributed to resveratrol's ability to maintain mitochondrial redox balance and inhibit ROS-driven apoptotic pathways.

Expanding on this pharmacological approach, an essential neuroprotective perspective involves utilizing agents that curb endogenous ROS production at its source. Statins, traditionally used for lipid management, provide a prime example of this strategy due to their pleiotropic antioxidant properties [53]. In addition to their cholesterol-lowering effects, statins have been shown to mitigate oxidative damage in the ischemic brain by inhibiting NOX activation and enhancing endothelial nitric oxide synthase (eNOS) activity [53,142]. These mechanisms contribute to the maintenance of vascular integrity and the reduction of reperfusion-induced radical formation. Recent clinical evidence further underscores the efficacy of statins in providing robust neuroprotection through the modulation of these oxidative pathways [142].

Collectively, these compounds underscore the therapeutic promise of enhancing endogenous antioxidant systems via diverse mechanisms. Their pleiotropic effects on mitochondria, enzymatic defenses, and signaling cascades broaden the neuroprotective landscape beyond canonical Nrf2 activation. However, challenges such as poor bioavailability, variability in dosing regimens, and timing of administration remain significant translational hurdles for clinical application.

7.3 Nrf2 Pathway Activation

The nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway plays a central role in cellular defense against oxidative stress by regulating the transcription of antioxidant and cytoprotective genes. Upon activation, Nrf2 translocates to the nucleus and binds to antioxidant response elements (AREs), inducing the expression of enzymes such as heme oxygenase-1 (HO-1), NQO1, glutamate cysteine ligase (GCL), and thioredoxin reductase [50]. These enzymes orchestrate a coordinated cellular response that mitigates ROS-induced injury and restores redox homeostasis.

In the context of cerebral I/R injury, several Nrf2 activators have demonstrated neurovascular protection through antioxidant, anti-inflammatory, and mitochondrial-preserving mechanisms.

Sulforaphane, a natural isothiocyanate derived from cruciferous vegetables, is one of the most extensively studied Nrf2 inducers. In a rat model of global cerebral ischemia, sulforaphane pretreatment enhanced Nrf2 nuclear translocation and upregulated downstream targets such as HO-1 and NQO1, leading to reduced lipid peroxidation, smaller infarct volume, and improved neuronal survival in the hippocampus [143]. In a mouse model of tMCAO, sul-

foraphane preserved mitochondrial membrane potential and reduced ROS accumulation, further supporting its neuroprotective efficacy [144]. Notably, sulforaphane also increased Akt phosphorylation, implicating PI3K/Akt signaling in facilitating Nrf2 activation in ischemic brain tissue [144].

Dimethyl fumarate (DMF), an FDA-approved immunomodulatory drug for multiple sclerosis, also activates Nrf2 by modifying critical cysteine residues on Keap1—the cytosolic inhibitor of Nrf2—thereby promoting its nuclear stabilization [145]. In a focal cerebral I/R model, both DMF and its metabolite monomethyl fumarate (MMF) significantly decreased infarct size, brain edema, and glial activation while enhancing Nrf2-dependent gene expression such as HO-1 and NQO1 [146]. These findings underscore DMF's dual immunoregulatory and antioxidant actions in the post-ischemic brain.

Tert-butylhydroquinone (tBHQ), a synthetic phenolic antioxidant, exerts neuroprotective effects through similar mechanisms. By covalently modifying Keap1 cysteines, tBHQ facilitates Nrf2 nuclear accumulation and subsequent transcription of ARE-regulated genes. In rodent models of cerebral ischemia and subarachnoid hemorrhage, intracerebroventricular administration of tBHQ attenuated brain injury, improved sensorimotor outcomes, and elevated antioxidant gene expression levels [147,148].

Bardoxolone methyl (CDDO-Me), a synthetic triterpenoid and potent Nrf2 inducer, has demonstrated promising antioxidant properties in ocular ischemic models. Although direct evidence in cerebral I/R is lacking, studies using a rat model of nonarteritic anterior ischemic optic neuropathy (rAION) revealed that intravitreal CDDO-Me administration promoted Nrf2 nuclear translocation and upregulated HO-1 and NQO1, while suppressing oxidative DNA damage and proinflammatory cytokine expression. These molecular effects were associated with enhanced retinal ganglion cell survival and reduced ischemic damage [149]. Given the embryologic and physiological similarities between the retina and central nervous system, these findings suggest a potential translational application of Bardoxolone methyl in cerebral I/R injury, pending further validation in brain-targeted models.

Importantly, many of these Nrf2 modulators also intersect with upstream kinase signaling, including ERK1/2, PI3K/Akt, and p38 MAPK, which further influence Nrf2 stabilization and transcriptional efficacy. For instance, in sulforaphane-treated ischemic cortex, Akt phosphorylation was essential for optimal Nrf2-mediated neuroprotection [144].

Taken together, these studies establish Nrf2 as a master regulator of the antioxidant defense system in cerebral I/R injury. Pharmacological activation of Nrf2 represents a promising therapeutic avenue for limiting oxidative damage and enhancing neuronal survival. However, the clinical translation of Nrf2-targeting compounds warrants careful optimization of timing, dosing, and tissue specificity. Furthermore, the potential for off-target effects or disruption of physiological redox signaling must be considered in the development of long-term Nrf2-based therapies.

7.4 Advanced Antioxidant Strategies: From Synergistic Therapies to Clinical Translation

Recent advances in antioxidant-based therapeutic strategies for I/R injury have focused on the development of synergistic combination regimens, novel nanocarriers, and gene-targeted therapies to improve clinical translatability. These strategies aim to overcome the limitations of single-agent antioxidants by enhancing BBB penetration, modulating multiple molecular targets, and increasing neuroprotective efficacy in both experimental and clinical settings.

7.4.1 Synergistic and Combination Therapies

Among the most promising clinical advances is the combination therapy of edaravone and dexborneol (EDB), which synergistically targets oxidative stress and inflammatory cascades. Edaravone, a free radical scavenger approved for acute ischemic stroke (AIS), is complemented by dexborneol, a bicyclic monoterpene that enhances cerebral microcirculation and suppresses inflammation. In a multicenter, randomized, double-blind Phase III trial, EDB treatment resulted in significantly better functional outcomes (*modified Rankin Scale* $\leq$1 at 90 days) compared to edaravone alone (67.18% vs 58.97%) [150]. A recent meta-analysis further confirmed the efficacy and cost-effectiveness of EDB in AIS patients, reporting improvements in NIHSS scores, reduced mortality, and favorable pharmacoeconomic profiles across several randomized controlled trials [139]. Preclinical data also support the value of antioxidant combination therapy. Co-administration of antioxidants with anti-inflammatory agents (e.g., minocycline) or therapeutic hypothermia has been shown to reduce infarct volume, preserve mitochondrial function, and attenuate apoptosis in rat models of tMCAO, with treatment administered intraperitoneally within 1 hour of reperfusion [17]. Natural compound pairings—such as curcumin with resveratrol or ginsenosides with flavonoids—have demonstrated enhanced efficacy in mouse tMCAO models, where combination therapy reduced oxidative stress markers (e.g., MDA, 4-HNE), infarct size, and behavioral deficits (e.g., Morris water maze latency and target crossings). Mechanistically, these combinations concurrently activated Nrf2 signaling, inhibited NF-κB, and stabilized mitochondrial dynamics, thus outperforming monotherapies [151].

7.4.2 Nanocarriers and Gene/miRNA-Based Interventions

The application of nanotechnology has opened new avenues for targeted antioxidant delivery in cerebral I/R injury. Selenium nanoparticles (SeNPs)—engineered nanocarriers composed of the essential trace element sele-

nium and synthesized via redox templating with polysaccharide coatings—exhibit potent ROS-scavenging, anti-inflammatory, and cytoprotective properties. In rat models of cerebral and myocardial I/R, intravenous administration of SeNPs significantly attenuated histological damage (HE staining and TUNEL assay), suppressed STAT1-mediated oxidative signaling, and improved neurological scores and spatial learning in the Morris water maze [16,113]. In parallel, novel synthetic small-molecule antioxidants have shown promise. Z3, a newly developed imide-based compound structurally optimized for BBB permeability, was administered intravenously in Sprague–Dawley rats subjected to tMCAO. Z3 treatment activated the Nrf2/HO-1 axis, significantly reduced infarct volume (via TTC staining), lowered ROS levels (DCF fluorescence assay), and decreased inflammatory cytokines (TNF-α, IL-1β, IL-6 measured by ELISA), indicating its translational potential as a cerebroprotective agent [152]. Gene therapy approaches targeting key antioxidant enzymes have further broadened therapeutic possibilities. In rat tMCAO models, adeno-associated virus vectors encoding SOD2 or HO-1 were stereotaxically injected into the cortex and hippocampus 7 days prior to ischemia induction. Overexpression of these enzymes led to smaller infarcts (MRI and histology), reduced neuronal apoptosis (cleaved caspase-3 IHC), and preserved BBB integrity—as assessed by Evans blue extravasation and ZO-1/occludin immunostaining [17]. Additionally, miRNA-based interventions targeting oxidative stress pathways have shown translational relevance. In rat models of tMCAO, intracerebroventricular injection of miRNA mimics (e.g., miR-124) or antagomirs (e.g., miR-210 inhibitors) 1 hour after reperfusion modulated Nrf2 and NF-κB signaling, resulting in improved sensorimotor function (rotarod test, neurological severity score) and spatial memory (Y-maze and Barnes maze) [17].

7.4.3 Clinical Translation and Limitations

Despite substantial experimental progress, the clinical translation of antioxidant-based strategies remains challenged by several factors: (1) BBB permeability: Many antioxidants, particularly those with high molecular weight or hydrophilic properties, fail to accumulate in therapeutic concentrations within the brain parenchyma. (2) Therapeutic time window: Antioxidant efficacy is highly time-sensitive, with optimal effects observed when administered within hours of reperfusion onset. (3) Safety and pharmacokinetics: Emerging compounds, including nanoparticles and gene therapies, require extensive evaluation for long-term toxicity, off-target effects, and metabolic stability. (4) Patient variability: Factors such as age, comorbidities (e.g., diabetes, hypertension), and genetic background influence both oxidative stress profiles and therapeutic responses. Nevertheless, ongoing clinical trials—such as NCT07091994 evaluating multi-targeted antioxidant therapies—and the integration of artificial intelligence (AI), transcriptomics, and precision medicine are expected to refine patient stratification and improve the success of future interventions [151]. In this context, the pronounced heterogeneity in cell-type vulnerability, as detailed in Section 4, provides a compelling biological rationale for the development of cell-type targeting therapies. Rather than relying on global ROS scavenging, precision neuroprotection can be pursued through functionalized nanocarriers, such as selenium-based platforms [16], which can be engineered for cell-specific accumulation. Furthermore, gene-targeted interventions utilizing adeno-associated virus vectors equipped with cell-specific promoters allow for the selective upregulation of endogenous antioxidant enzymes—such as SOD2 or HO-1—within vulnerable populations like hippocampal pyramidal neurons or reactive astrocytes [17]. Such cell-specific approaches could strategically bolster the resilience of highly susceptible neurovascular components without disrupting essential redox signaling in more resilient brain regions.

Collectively, the therapeutic landscape of antioxidant strategies for I/R injury is rapidly evolving toward integrated, multi-modal approaches. Clinically validated combinations like EDB have demonstrated efficacy, while novel platforms such as SeNPs, the synthetic antioxidant Z3, and gene/miRNA-targeted interventions offer new directions for translational neuroscience. Future research must continue to optimize delivery systems, expand preclinical testing across age and comorbidity models, and leverage personalized medicine tools to bring these strategies into clinical use.

7.5 Limitations, Safety Concerns, and Future Perspectives

Despite substantial progress in developing antioxidant strategies for I/R injury, significant barriers to clinical translation persist. A primary concern is the predominant use of young, healthy rodent models in preclinical studies, which fail to replicate the physiological complexity of the aged or comorbid populations—such as those with hypertension or diabetes—that represent the majority of stroke patients. Research indicates that aged animals exhibit larger infarct volumes, impaired neurovascular remodeling, and altered inflammatory responses compared to younger subjects [2,153,154]. Thus, utilizing age-related and comorbidity-matched models is vital for translational success. Additionally, the therapeutic window remains a major hurdle. Preclinical administration is often immediate, yet clinical delays are inevitable due to symptom recognition and transport. Given that delayed antioxidant treatment significantly reduces efficacy in animal models, testing realistic post-ischemic windows is essential [17,21].

Safety considerations also restrict the clinical adoption of potent antioxidant therapies. Although ROS are drivers of oxidative damage, they are also indispensable for intracellular signaling, vascular regulation, and immune defense; thus, over-suppression may paradoxically

disrupt homeostatic balance [155]. Furthermore, many widely studied natural antioxidants, such as resveratrol and curcumin, suffer from poor oral bioavailability and rapid metabolism in humans, complicating dose standardization and therapeutic consistency [156,157]. Similarly, emerging nanocarrier-based formulations, including SeNPs, require rigorous evaluation of their long-term toxicity, biodistribution, and immune compatibility prior to clinical implementation [16].

The biological heterogeneity of ischemic injury—varying across brain regions, time points, and individual patient profiles—further complicates trial outcomes. Previous failures in human trials have often been attributed to a lack of accounting for stroke subtypes and specific patient comorbidities [2,22]. Addressing this variability necessitates a shift toward personalized neurotherapeutics. Emerging technologies, such as AI-guided drug discovery and multi-omics screening platforms (incorporating genomic, transcriptomic, and proteomic data), offer innovative pathways to optimize synergistic regimens and identify individualized redox vulnerabilities. These multi-targeted and adaptive approaches represent a move toward precision medicine, aligning treatment strategies with patient-specific pathophysiological signatures [151].

In conclusion, while antioxidant therapies hold substantial promise for mitigating I/R-induced neurodegeneration, future research must prioritize clinically relevant animal models, realistic treatment windows, and long-term safety evaluations. Incorporating systems biology, AI, and precision medicine frameworks will be essential to bridging the translational gap and advancing these therapies toward successful clinical application.

8. Conclusions and Future Perspectives

Cerebral I/R injury continues to be a leading cause of acute and long-term neurological disability, with oxidative stress acting as a central pathogenic driver. Over the past few decades, significant progress has been made in characterizing the mechanisms by which ROS trigger neuronal damage, BBB disruption, and synaptic dysfunction. This review has underscored the dual nature of ROS—as both deleterious agents and vital physiological modulators—while highlighting the inherent complexity of developing antioxidant therapies. Despite the promising results observed in various preclinical models—ranging from classical enzymatic defenses like SOD and GPx to innovative mitochondria-targeted therapies—translation into clinical practice has remained largely unsuccessful.

Key obstacles include narrow therapeutic windows, limited BBB penetration, and the biological heterogeneity of human stroke patients, which is often not reflected in standard rodent models. To overcome these hurdles, future research must prioritize the development of multi-targeted and precision-based therapies. Emerging platforms, such as AI-guided drug discovery, multi-omics, and personalized nanomedicine, offer exciting opportunities to adapt antioxidant strategies to individual pathophysiological profiles. Furthermore, combining redox modulation with neurorestorative agents may provide essential synergistic benefits. Moving forward, the field must adopt clinically relevant models and stratified trial designs to bridge the gap between bench and bedside. By embracing personalized medicine, antioxidant therapies may finally evolve from experimental adjuncts into cornerstone interventions for ischemic brain injury.

Abbreviations

AIF, Apoptosis-Inducing Factor; AIS, Acute Ischemic Stroke; ATP, Adenosine Triphosphate; BBB, Blood–Brain Barrier; CA, Cornu Ammonis; DAMPs, Damage-Associated Molecular Patterns; DND, Delayed Neuronal Death; DNA, Deoxyribonucleic Acid; EDB, Edaravone and Dexborneol; GPx, Glutathione Peroxidase; GPX4, Glutathione Peroxidase 4; GSDMD, Gasdermin D; GSH, Glutathione; HO-1, Heme Oxygenase-1; I/R, Ischemia-Reperfusion; IL-1β, Interleukin-1β; MCAO, Middle Cerebral Artery Occlusion; MLKL, Mixed Lineage Kinase Domain-Like Protein; mPTP, Mitochondrial Permeability Transition Pore; NF-κB, Nuclear factor-kappa B; NLRP3, Nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3; NOX, NADPH Oxidase; NQO1, NAD(P)H Quinone Dehydrogenase 1; Nrf2, Nuclear Factor Erythroid 2–Related Factor 2; PARP-1, Poly(ADP-ribose) Polymerase-1; PUFA, Polyunsaturated Fatty Acid; RCD, Regulated Cell Death.

Author Contributions

KYY conceptualized the research strategy and supervised the overall review process. BHJ performed the comprehensive literature search and data curation. Both authors took the lead in synthesizing the literature, conducting the formal analysis, and drafting the initial manuscript, including the preparation of all figures and tables. Furthermore, both authors contributed to editorial revisions, read and approved the final manuscript, and agreed to be accountable for all aspects of the work.

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Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT to assist in generating the initial visual elements for Figs. 1,2,3. The authors subsequently oversaw, reviewed, and edited the generated images to ensure their scientific accuracy and coherence with the manuscript's content. The authors take full responsibility for the content of the final published figures.

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