10.37021/ijper.v8i1.02

REVIEW ARTICLE

Therapeutic Approaches to Enhance Neuronal Survival in Ischemic Stroke: Insights from Experimental Models

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ABSTRACT

Ischemic stroke remains one of the leading causes of mortality and long-term disability worldwide. It occurs due to the sudden interruption of cerebral blood flow, resulting in oxygen and glucose deprivation that triggers a cascade of pathological events leading to neuronal injury and death. Key mechanisms involved in ischemic brain damage include excitotoxicity mediated by excessive glutamate release, oxidative stress caused by reactive oxygen species generation, neuroinflammation, mitochondrial dysfunction, blood–brain barrier disruption, and apoptosis. Understanding these complex pathways is essential for the development of effective neuroprotective therapies.

Experimental models such as middle cerebral artery occlusion (MCAO) in rodents and oxygen-glucose deprivation (OGD) in cultured neuronal cells have significantly contributed to the understanding of ischemic stroke pathophysiology. These models closely mimic human cerebral ischemia and provide valuable platforms for evaluating therapeutic interventions. Preclinical studies using these models have explored a wide range of neuroprotective strategies, including glutamate receptor antagonists, antioxidants, anti-inflammatory agents, anti-apoptotic compounds, stem cell-based therapies, hypothermia, ischemic conditioning, and emerging approaches such as microRNA modulation and phytochemical-based treatments.

Several pharmacological agents have demonstrated promising neuroprotective effects by reducing infarct size, attenuating oxidative stress, suppressing inflammatory responses, and improving functional recovery in experimental settings. However, despite encouraging preclinical outcomes, successful clinical translation remains limited due to factors such as species differences, variability in study design, and narrow therapeutic windows. Therefore, future research should focus on rigorous experimental methodologies and multimodal or pleiotropic therapeutic approaches to enhance translational success and improve outcomes in patients with ischemic stroke.

Keywords: Ischemic stroke; Neuroprotection; Cerebral ischemia; Oxidative stress.

How to cite this article: Rani S, Alam Q. Therapeutic Approaches to Enhance Neuronal Survival in Ischemic Stroke: Insights from Experimental Models. Int. J. Pharm. Edu. Res. 2026;8(1):9-16.

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Source of support: Nil

Conflict of interest: None

INTRODUCTION

Ischemic stroke accounts for approximately 87% of all strokes, resulting from arterial occlusion by thrombi or emboli, leading to energy failure, ionic imbalances, and subsequent neuronal injury in the ischemic core and penumbra. The penumbra, a region of hypoperfused but salvageable tissue, offers a critical therapeutic window for interventions aimed at enhancing neuronal survival. Experimental models have revealed key pathophysiological mechanisms, including glutamate-mediated excitotoxicity, reactive oxygen species (ROS) accumulation, neuroinflammation, and programmed cell death, which collectively exacerbate neuronal loss. Rodent models like MCAO simulate focal ischemia, while cellular models such as OGD in neuronal cultures mimic metabolic stress, providing platforms for testing therapies. Despite successes in models, clinical translation has been limited due to factors like model variability, lack of comorbidities in animals, and single-target approaches. This review discusses therapeutic strategies derived from these models to promote neuronal survival, focusing on multifaceted interventions.[1]

The evolution of experimental models has been pivotal in stroke research. Early global ischemia models, such as four-vessel occlusion in rats, highlighted delayed neuronal death in vulnerable regions like the hippocampus, while focal models like intraluminal suture MCAO allow for reproducible infarcts and assessment of reperfusion injury. In vitro models, including organotypic hippocampal slices subjected to OGD, enable high-throughput screening of neuroprotective agents under controlled conditions. Recent advancements, such as 3D spheroids and organoids, better replicate the neurovascular unit (NVU), incorporating cell-cell interactions and blood-brain barrier (BBB) elements, addressing limitations of traditional 2D cultures. These models underscore the importance of assessing not only infarct volume but also functional outcomes, such as sensorimotor and cognitive deficits, to predict clinical

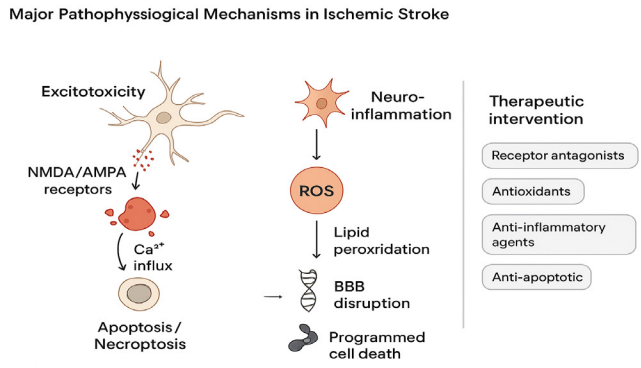

Figure 1: Pathological mechanisms of ischemic stroke involving excitotoxicity, neuroinflammation, ROS generation, BBB disruption, and programmed cell death, along with potential therapeutic interventions.

efficacy. Lessons from past failures emphasize rigorous study design, including randomization, blinding, and inclusion of aged or comorbid animals, to enhance translational potential.

Major Pathological mechanisms in Ischemic StrokeExperimental Models of Ischemic Stroke

Preclinical models of ischemic stroke are essential tools for investigating pathophysiological mechanisms and evaluating candidate neuroprotective therapies. Broadly, they are categorized into focal ischemia models, which mimic large vessel occlusion, and global ischemia models, which replicate conditions such as cardiac arrest or systemic hypoperfusion. Both in vivo and in vitro systems are employed, each with distinct advantages and limitations.[2,3]

Focal Ischemia Models

Focal ischemia is the most clinically relevant, as it reflects middle cerebral artery (MCA) occlusion in human stroke.

Intraluminal filament MCAO (middle cerebral artery occlusion)

The most widely used model, involving insertion of a nylon filament via the carotid artery to block MCA blood flow. It can be transient (with reperfusion) or permanent, allowing study of both ischemic core and penumbra. Advantages include reproducibility and clinical relevance; limitations include variability in infarct size and risk of subarachnoid hemorrhage.[1,7]

Electrocoagulation or photothrombosis MCAO

Direct coagulation of the MCA or induction of thrombus by photoactivation of a dye (e.g., Rose Bengal). These produce well-demarcated infarcts but have limited reperfusion relevance. Photothrombosis is useful for studying cortical infarcts and neuroplasticity.[4]

Endothelin-1 (ET-1)–induced focal ischemia

Local application of ET-1 causes potent vasoconstriction, producing localized ischemia. This model offers high reproducibility and is less invasive, though it does not fully mimic embolic stroke pathophysiology.[5]

Global Ischemia Models

Global ischemia replicates conditions such as cardiac arrest, severe hypotension, or systemic hypoxia, and is mainly used to study delayed neuronal death in vulnerable regions (e.g., hippocampal CA1 neurons).

Four-vessel occlusion (4VO) model in rats

Vertebral arteries are permanently occluded, followed by transient bilateral carotid occlusion, producing reproducible forebrain ischemia.[6]

Two-vessel occlusion (2VO) with hypotension

Bilateral carotid artery occlusion with systemic hypotension induces transient global ischemia. This model is technically easier but less consistent in infarct reproducibility.[6]

Embolic and Thrombotic Stroke Models

These attempt to closely replicate human thromboembolic stroke.

Autologous blood clot embolism

Injection of a preformed clot into the cerebral circulation produces occlusion, useful for evaluating thrombolytic and reperfusion therapies.

Thrombin injection model

Direct thrombin injection induces local clot formation, providing a platform for testing thrombolytics. These models reflect clinical pathophysiology more accurately but are technically challenging and less reproducible.[4]

In Vitro Models

Cell culture and organotypic slice models allow high-throughput, mechanistic investigations under controlled conditions.

Oxygen–glucose deprivation (OGD)

Mimics ischemia by depriving neuronal or glial cultures of oxygen and glucose. It provides mechanistic insights into excitotoxicity, oidative stress, and apoptosis but lacks systemic interactions and neurovascular complexity.[8]

Considerations and Limitations

Although experimental models have been invaluable in understanding ischemic pathophysiology, their predictive value for clinical translation has been limited. Key issues include the use of young, healthy animals,

whereas human patients often have comorbidities (hypertension, diabetes, aging). Reproducibility, infarct heterogeneity, and lack of standardized protocols also contribute to translational gaps. Initiatives like the Stroke Therapy Academic Industry Roundtable (STAIR) guidelines emphasize methodological rigor, including blinding, randomization, and multicenter preclinical trials, to improve translational relevance.[3,9]

NEUROPROTECTIVE STRATEGIES TARGETING EXCITOTOXICITY

Pathophysiological Background

Excitotoxicity is a central mechanism of neuronal injury in ischemic stroke, triggered by excessive release of glutamate and overactivation of ionotropic glutamate receptors such as N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. This leads to massive Ca^{2+} influx, activation of proteases, lipases, and endonucleases, mitochondrial dysfunction, and eventual neuronal death. The pivotal role of excitotoxicity in ischemic brain injury has made it an attractive therapeutic target.[11,12]

NMDA Receptor Antagonists

Competitive antagonists (e.g., APV, selfotel) and non-competitive antagonists (e.g., MK-801, ketamine) have shown potent neuroprotection in animal models.

However, clinical trials were disappointing due to adverse effects such as hallucinations, psychotomimetic symptoms, and hemodynamic instability.[13]

Recent approaches focus on low-affinity blockers (e.g., memantine) or subunit-selective antagonists (e.g., NR2B-selective ifenprodil) to minimize side effects.[14]

AMPA and Kainate Receptor Antagonists

AMPA receptor antagonists (e.g., NBQX, perampanel) reduce excitotoxicity and have shown neuroprotective effects in rodent ischemia models.[15]

Translation is limited by sedation and cognitive impairment, but selective targeting of calcium-permeable AMPA receptors remains a promising strategy.

Modulation of Glutamate Release and Uptake

Glutamate release inhibitors (e.g., riluzole) reduce synaptic glutamate overflow and demonstrated neuroprotection in preclinical stroke models.[16]

Enhancing glutamate transporter (EAAT2/GLT-1) activity promotes clearance of extracellular glutamate. Agents such as ceftriaxone upregulate GLT-1 expression and have shown beneficial effects in rodent ischemia models.[17]

Ion Channel Modulators

Calcium channel blockers (e.g., nimodipine) were evaluated for their potential to reduce Ca^{2+} overload, but clinical benefit in ischemic stroke has been limited.[18]

Sodium channel blockers (e.g., lamotrigine, fosphenytoin) reduce glutamate release and downstream excitotoxic injury; preclinical studies show efficacy, but clinical translation remains incomplete.[19]

Targeting Downstream Signaling Pathways

Excitotoxic Ca^{2+} entry activates calpains, caspases, and nitric oxide synthase (nNOS).

Neuroprotective strategies include calpain inhibitors, nNOS inhibitors, and poly(ADP-ribose) polymerase (PARP) inhibitors, which aim to block excitotoxic downstream cascades.[12,20]

Combination and Multi-Target Approaches

Given the complexity of excitotoxic pathways, combination therapies (e.g., glutamate antagonists plus antioxidants or anti-inflammatory drugs) may provide superior neuroprotection.

Recent studies suggest that hypothermia enhances the efficacy of anti-excitotoxic agents by reducing metabolic demand and glutamate release.[21]

ANTIOXIDANTS AND MITIGATION OF OXIDATIVE STRESS

Endogenous Antioxidant Systems

The brain is particularly vulnerable to oxidative stress due to high oxygen consumption, abundant polyunsaturated fatty acids, and relatively low antioxidant capacity. Major endogenous antioxidants include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione (GSH). Enhancing these defense mechanisms through pharmacological or gene therapy approaches has shown neuroprotection in animal models.[24]

Exogenous Antioxidants

Free Radical Scavengers

Edaravone (MCI-186): A small-molecule free radical scavenger approved in Japan for acute ischemic stroke. It neutralizes hydroxyl and peroxyl radicals, reduces lipid peroxidation, and protects the BBB. Clinical studies suggest modest improvements in neurological outcomes when administered early.[25,26]

Tirilazad: A lipid peroxidation inhibitor tested in clinical trials, but efficacy was not demonstrated, possibly due to inadequate trial design or patient selection.[27]

Vitamins and Nutraceuticals

Vitamin E (α-tocopherol): Lipid-soluble antioxidant that interrupts lipid peroxidation chains. Preclinical studies suggest neuroprotection, but clinical trials in stroke prevention show inconsistent results.[28]

Vitamin C (ascorbic acid): Water-soluble antioxidant that regenerates vitamin E and scavenges ROS; supportive in animal models but lacking strong clinical validation.[29]

Polyphenols (resveratrol, curcumin, EGCG): Exhibit antioxidant, anti-inflammatory, and anti-apoptotic properties. While effective in rodent ischemia models, their bioavailability in humans remains a limitation.[30]

Enzyme Modulators and Mimetics

SOD mimetics (e.g., tempol) and catalase mimetics reduce infarct volume and oxidative injury in animal models.[31]

Nrf2 activators (e.g., sulforaphane, bardoxolone) upregulate antioxidant response elements (AREs), enhancing the expression of cytoprotective genes such as HO-1 and NQO1.[32]

Mitochondria-Targeted Antioxidants

Mitochondria are central to ROS generation and apoptotic signaling. Targeted antioxidants like MitoQ and SkQ1 have shown promise in preclinical models by localizing scavenging activity within mitochondria and preserving energy metabolism.[33]

Combination and Adjunctive Approaches

Combining antioxidants with reperfusion therapies (e.g., thrombolysis, thrombectomy) may reduce reperfusion injury.

Multi-target strategies (e.g., edaravone combined with dexborneol, an anti-inflammatory agent) have demonstrated synergistic benefits in clinical trials.[26]

ANTI-INFLAMMATORY THERAPIES

Neuroinflammation, mediated by microglia and cytokines, amplifies ischemic damage. In MCAO models, M1 microglia release proinflammatory IL-1β and TNF-α, while M2 phenotypes promote repair. Therapies targeting TLR4/NF-κB, like ApTOLL, reduce infarct volume in rodents and show safety in phase I/II trials. Fingolimod, an S1P modulator, decreases lymphocyte infiltration and inflammation in clinical studies, enhancing neuronal survival.

Inhibition of chemokines like CCL2/CCR2 or cytokines with IL-1Ra mitigates BBB disruption in models. Complement inhibitors and IVIG reduce inflammation and apoptosis in experimental stroke, with pleiotropic effects on multiple pathways. Anti-inflammatory phytochemicals, such as ginsenosides, suppress NF-κB in vitro and in vivo, offering natural alternatives for neuronal protection.

Cytokine Modulation

IL-1 Receptor Antagonist (IL-1Ra)

Blocks IL-1 signaling and reduces infarct size in preclinical stroke models. Clinical trials (e.g., SCIL-STROKE) indicate safety and potential functional benefit.[36,37]

TNF-α inhibitors

Agents such as etanercept and infliximab reduce post-ischemic inflammation in experimental models, but their systemic immunosuppressive effects limit clinical translation.[38]

Microglial Activation Inhibitors

Minocycline

A tetracycline derivative that inhibits microglial activation, reduces matrix metalloproteinase (MMP) activity, and attenuates apoptosis. Clinical trials suggest improved neurological outcomes when administered early after stroke.[39,40]

Colony-Stimulating Factor 1 Receptor (CSF1R) inhibitors

Target microglial proliferation and activation; preclinical studies demonstrate neuroprotection but require further evaluation for clinical safety.[41]

Adhesion Molecule and Leukocyte Infiltration Modulation

Anti-ICAM-1 and Anti-selectin therapies

Inhibit leukocyte adhesion and transmigration, reducing inflammatory infiltration. Although effective in animal models, clinical trials like Enlimomab Acute Stroke Trial failed due to immune reactions and adverse effects.[42]

Strategies targeting integrins (e.g., anti-CD11/CD18) show preclinical promise but have not yet translated into safe clinical therapies.[43]]

Inflammasome and Intracellular Signaling Modulation

NLRP3 inflammasome inhibitors

Reduce caspase-1 activation and IL-1β release, thereby limiting post-ischemic inflammation. Small molecules such as MCC950 demonstrate significant neuroprotection in rodent models.[44]

NF-κB pathway inhibitors

NF-κB controls transcription of multiple pro-inflammatory genes. Agents targeting IκB kinase (IKK)

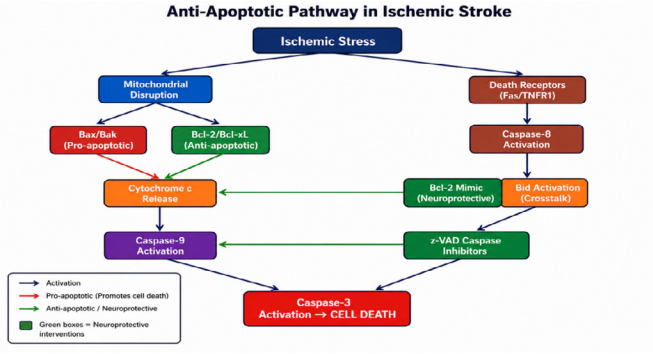

Figure 2: Anti-Apoptotic Pathway in Ischemic Stroke. The intrinsic (mitochondrial) and extrinsic (death receptor) pathways converge on caspase-3 activation leading to neuronal death. Green boxes indicate key therapeutic intervention points.

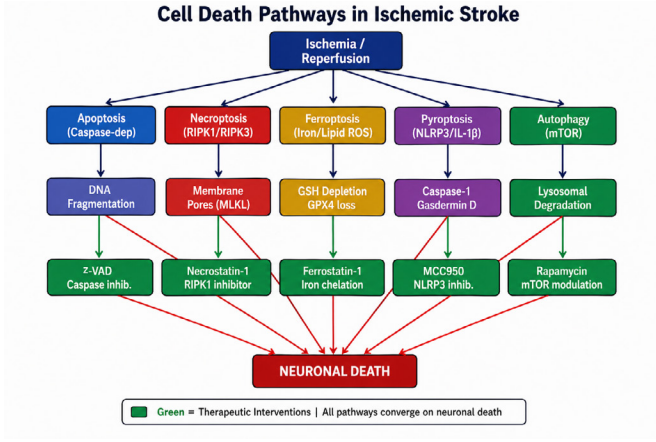

Figure 3: Cell Death Pathways in Ischemic Stroke. Five major regulated cell death modalities—apoptosis, necroptosis, ferroptosis, pyroptosis, and autophagy—converge on neuronal death. Corresponding therapeutic interventions (green) targeting each pathway are highlighted.

or NF-κB translocation attenuate infarct size and improve functional outcomes in preclinical studies.[45]

Combination Anti-inflammatory Approaches

Multi-target strategies combining anti-inflammatory therapy with antioxidants, anti-excitotoxic drugs, or reperfusion therapies have shown synergistic effects in animal models.[46]

Early intervention is critical, as delayed inhibition of inflammation may impair tissue repair and post-stroke recovery

ANTI-APOPTOTIC AND CELL DEATH PATHWAYS

Apoptosis involves intrinsic (mitochondrial) and extrinsic (death receptor) pathways, with caspase activation central in ischemic models. Caspase inhibitors like z-VAD reduce neuronal death in OGD and MCAO. p53-mediated apoptosis is targeted by inhibitors, while necroptosis blockers like necrostatin-1 (RIPK1 inhibitor) decrease infarct size in rodents. Ferroptosis inhibition via iron chelators and pyroptosis blockade with MCC950 limit cell death in experimental stroke.

miRNA modulation, such as antagomirs against miR-155-5p, enhances survival in MCAO models by regulating NF-κB and leukocyte pathways. Activated Protein C (3K3A-APC) reduces apoptosis and hemorrhage in comorbid rodent models, advancing to phase II trials. These strategies emphasize targeting multiple death modalities for comprehensive neuroprotection.

Mitochondrial (Intrinsic) Apoptotic Pathway

Ischemic stress triggers mitochondrial outer membrane permeabilization (MOMP), releasing cytochrome c, which binds Apaf-1 and activates caspase-9, leading to downstream caspase-3 activation and DNA fragmentation .[49]
Anti-apoptotic strategies aim to upregulate Bcl-2, Bcl-xL or inhibit pro-apoptotic proteins such as Bax and Bak. Preclinical studies using Bcl-2 mimetics or gene therapy have shown reduced infarct size.[50]

Death Receptor (Extrinsic) Pathway

Activation of TNF receptor 1 (TNFR1) or Fas/CD95 receptors triggers caspase-8, which can directly activate caspase-3 or amplify apoptosis via Bid-mediated mitochondrial crosstalk [51].

Targeting death receptor signaling using soluble receptor decoys, Fas-Fc, or small molecule inhibitors has demonstrated neuroprotection in animal models.[52]

Caspase Inhibition

Broad-spectrum caspase inhibitors (e.g., z-VAD-fmk) reduce apoptosis and infarct volume in experimental stroke.

Specific inhibitors of caspase-3, -8, or -9 have also shown preclinical efficacy, though clinical translation remains limited by pharmacokinetics and timing of administration.[53,54]

Necroptosis and Programmed Necrosis

Necroptosis is a regulated form of necrosis mediated by RIPK1, RIPK3, and MLKL. Inhibition of RIPK1 (e.g., necrostatin-1) protects neurons in preclinical ischemia models [55].

Targeting necroptotic pathways may complement anti-apoptotic strategies to protect the ischemic penumbra.

Autophagy Modulation

Autophagy is a cellular degradation pathway that can be protective or deleterious depending on context. Controlled activation of autophagy (e.g., via mTOR inhibition) can reduce ischemic injury, while excessive autophagy contributes to cell death.[56,57]

Combination Therapies

Multi-target approaches combining anti-apoptotic agents with antioxidants, anti-inflammatory drugs, or anti-excitotoxic therapies have shown synergistic neuroprotection in preclinical studies.[58]

Timing and targeting are critical, as intervention must occur before irreversible cell death pathways dominate.

Cell-Based Therapies

Stem cell therapies promote neuronal survival through trophic support rather than direct replacement. In MCAO rats, bone marrow-derived mesenchymal stem cells (MSCs) improve functional outcomes via anti-inflammatory and angiogenic effects, tracked by MRI. Adipose-derived stem cells (ASCs) offer autologous potential, migrating to infarcts and upregulating neurotrophins. Neural stem cells (NSCs) co-transplanted with astrocytes enhance differentiation and survival in murine models.

Exogenous delivery methods include intravenous or intra-arterial routes, with challenges like tumorigenicity addressed in long-term studies. Endogenous mobilization with growth factors like EGF boosts NSC migration to penumbras, though benefits are debated. These model insights support ongoing clinical trials for cell therapies in stroke recovery.

Mechanisms of Action

- Cell-based therapies mediate recovery through multiple pathways
- Replacement of lost cells via differentiation and integration.
- Secretion of neurotrophic factors promoting survival and plasticity.
- Immunomodulation reducing post-ischemic inflammation.
- Angiogenesis and vascular remodeling, enhancing blood flow to the penumbra.
- Stimulation of endogenous repair mechanisms, including neural progenitor proliferation.[59-61]

CONCLUSION

Ischemic stroke triggers a cascade of interconnected pathophysiological events—including excitotoxicity, oxidative stress, neuroinflammation, and activation of apoptotic and necroptotic pathways—that culminate in neuronal death. Experimental models have been invaluable in elucidating these mechanisms and in guiding the development of diverse therapeutic strategies aimed at enhancing neuronal survival.

Neuroprotective approaches targeting excitotoxicity, such as NMDA and AMPA receptor modulators, can mitigate calcium overload and downstream cell injury. Antioxidant therapies, including free radical scavengers, Nrf2 activators, and mitochondria-targeted agents, have demonstrated efficacy in reducing oxidative damage and infarct volume. Anti-inflammatory strategies—including cytokine modulation, microglial inhibition, and inflammasome targeting—attenuate secondary injury and preserve neuronal function. Furthermore, modulation of apoptotic and necroptotic pathways through caspase inhibition, Bcl-2 family protein regulation, and necroptosis blockade protects the penumbral neurons. Finally, cell-based therapies, including neural stem/progenitor cells, mesenchymal stem cells, induced pluripotent stem cell-derived neurons, and umbilical cord blood cells, offer regenerative potential by promoting neurogenesis, angiogenesis, immunomodulation, and synaptic repair .

The integration of multi-targeted approaches that combine neuroprotection, anti-inflammation, antioxidation, anti-apoptotic modulation, and regenerative therapies holds the greatest promise for enhancing neuronal survival and functional recovery. Future research should focus on optimizing therapeutic windows, routes of administration, combination strategies, and translational studies in well-characterized experimental models. A mechanistic understanding from experimental models continues to provide a roadmap for the development of effective therapies aimed at reducing neuronal loss and improving outcomes in ischemic stroke patients..

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