Dissecting Ischemic Stroke: Mechanistic Insights from in Vivo and in Vitro Models

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Abstract- Ischemic stroke remains a leading cause of morbidity and mortality worldwide, necessitating a comprehensive understanding of its underlying pathophysiological mechanisms. Central to ischemic stroke is the disruption of blood flow to the brain, resulting in a cascade of cellular and molecular events, including excitotoxicity, oxidative stress, inflammation, apoptosis, and disruption of the blood-brain barrier. Over the past decades, various in vivo and in vitro models have been developed to dissect the complexity of ischemic stroke, each offering unique mechanistic insights and translational relevance.

In vivo models, such as middle cerebral artery occlusion (MCAO), photothrombotic stroke, and embolic stroke, simulate human stroke by inducing localized cerebral ischemia. These models allow for the investigation of stroke dynamics in a physiological environment, providing valuable data on tissue damage, neuroinflammation, and post-ischemic neuroprotection. In vitro models, including oxygen-glucose deprivation (OGD) and organotypic brain slices, enable controlled study of cellular responses to ischemia at the molecular level. These simplified systems offer high-throughput opportunities to explore the neuroprotective potential of novel therapeutic agents.

Keywords: ischemic stroke; neuroinflammation; oxidative stress; neuroprotection; cellular therapy; drug repurposing

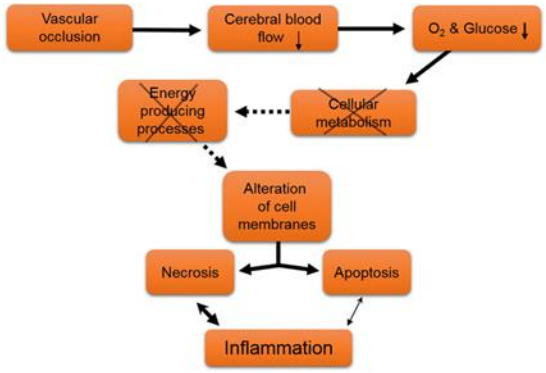
INTRODUCTION

Ischemic stroke is a leading cause of death and disability globally, characterized by the occlusion of cerebral blood vessels that leads to brain tissue damage due to oxygen and glucose deprivation. The resultant energy failure initiates a cascade of deleterious processes, including excitotoxicity, oxidative stress, inflammation, and ultimately, cell death. Despite significant advancements in our understanding of ischemic stroke pathophysiology, therapeutic strategies remain limited. Preclinical research using in vivo and in vitro models has been essential in elucidating the underlying mechanisms of ischemic stroke and providing insights for potential therapeutic interventions. The majority of approved therapies for many diseases are developed to target their underlying pathophysiology. Understanding disease pathophysiology has thus proven vital to the successful development of clinically useful medications. Stroke is generally accepted as the leading cause of adult disability globally and ischemic stroke accounts for the most common form of the two main stroke types. Despite its health and socioeconomic burden, there is still minimal availability of effective pharmacological therapies for its treatment. In this review, we take an indepth look at the etiology and pathophysiology of ischemic stroke, including molecular and cellular changes. This is followed by a highlight of drugs, cellular therapies, and complementary medicines that are approved or undergoing clinical trials for the treatment and management of ischemic stroke. We also identify unexplored potential targets in stroke pathogenesis that can be exploited to increase the pool of effective antistroke and neuroprotective agents through de novo drug development and drug repurposing.

This review discusses the pathophysiology of ischemic stroke, with a particular focus on the

mechanistic insights gained from in vivo and in vitro models.

Ischemic Stroke Pathophysiology



The pathophysiology of ischemic stroke is a complex process involving multiple phases, including:

1) Energy Failure: Occlusion of a cerebral artery leads to a dramatic reduction in blood flow, causing an immediate energy crisis in affected neurons and glial cells. ATP depletion leads to the failure of ion pumps, resulting in depolarization of neuronal membranes and the influx of sodium and calcium ions.

2) Excitotoxicity: Excessive release of excitatory neurotransmitters, primarily glutamate, triggers overstimulation of NMDA and AMPA receptors. This leads to further calcium overload within cells, activating destructive enzymatic pathways that damage cellular structures.

3) Oxidative Stress: Mitochondrial dysfunction and the production of reactive oxygen species (ROS) exacerbate cellular injury. ROS causes damage to lipids, proteins, and DNA, leading to cell death via apoptosis or necrosis.

4) Inflammation: The ischemic injury activates resident microglia and recruits peripheral immune cells, including neutrophils and macrophages. These cells release cytokines and chemokines, exacerbating tissue damage.

5) BloodBrain Barrier (BBB) Breakdown: The integrity of the BBB is compromised, allowing the infiltration of immune cells and the leakage of proteins and other harmful molecules into the brain parenchyma.

6) Cell Death: Depending on the extent and duration of ischemia, neurons undergo apoptosis, necrosis, or autophagic cell death.

METHODS

➢ IN VIVO MODELS OF ISCHEMIC STROKE

In vivo models are indispensable for studying ischemic stroke because they simulate the complex interactions between brain cells, the vascular system, and the immune system under pathophysiological conditions. The most widely used in vivo models include:

1) Middle Cerebral Artery Occlusion (MCAO): The MCAO model is the most commonly used model for mimicking ischemic stroke in rodents. It involves the occlusion of the middle cerebral artery, leading to focal ischemia that closely resembles human ischemic stroke. Both transient and permanent occlusions can be used, allowing researchers to study different aspects of stroke pathophysiology.

Pathophysiology:
MCAO results in focal ischemia, where the supply of oxygen and glucose is interrupted in brain tissue.

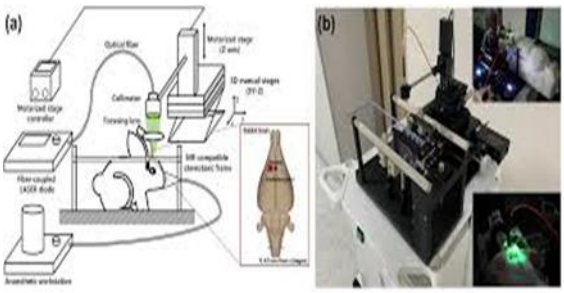
This leads to neuronal damage through excitotoxicity, oxidative stress, inflammation, and the formation of free radicals.
Symptoms in Humans:
Hemiparesis (weakness on one side of the body)
Aphasia (language impairment)
Sensory deficits
Visual field disturbances
Animal Models: Applications in Research:
Stroke Mechanisms: MCAO models help in understanding the molecular and cellular mechanisms underlying ischemic damage.

Neuroprotection: Studying potential therapeutic agents, such as neuroprotective drugs, to limit brain damage.

Recovery and Rehabilitation: Investigating poststroke recovery and the role of rehabilitation techniques.

Reperfusion: After temporary occlusion, reperfusion (restoring blood flow) can lead to additional injury due

to oxidative stress and inflammation (reperfusion injury).



Photothrombotic Stroke Model: This model involves the use of light to induce localized clot formation within the cerebral vasculature, creating a highly reproducible focal ischemic stroke. It is particularly useful for studying the spatial dynamics of ischemic lesions. The Photothrombotic Stroke Model is another widely used experimental model to study ischemic stroke, primarily in rodents. This model involves inducing focal cerebral ischemia through light activation of a photosensitive dye, which leads to the formation of a thrombus (blood clot) and subsequent occlusion of blood vessels in a specific brain region.

Key Features:

1. Mechanism:

The model is based on the administration of a photosensitive dye, such as Rose Bengal, which circulates in the bloodstream. A specific area of the brain is exposed to focused light (usually through the skull), typically using a laser or halogen light source. The light activates the dye, causing the generation of free radicals that damage the endothelium of blood vessels. This leads to platelet aggregation and thrombus formation, occluding the vessels and causing ischemia in the targeted region.

2. Advantages:

Highly localized stroke: The photothrombotic model allows precise control over the size and location of the infarct, making it ideal for studying specific brain regions. Reproducibility: It offers high reproducibility in terms of infarct size and location. Noninvasive: It does not require invasive surgery to directly occlude vessels, which reduces variability and mortality in animal models.

3. Applications in Research:

Cerebral Ischemia: The model is used to study the effects of ischemia on brain function, neuronal damage, and death. Neuroprotection and Therapeutics: It is commonly employed to test the efficacy of neuroprotective drugs and interventions aimed at reducing brain damage after a stroke. Poststroke recovery: Researchers use this model to study the mechanisms of recovery and the effects of rehabilitative therapies following a stroke.

4. Infarct Formation:

The ischemic lesion created is highly localized and consistent in size. The core of the lesion typically involves necrotic cell death, while surrounding areas (the penumbra) can show varying degrees of damage and potential recovery.

5. Limitations:

Lack of reperfusion: In contrast to other stroke models like MCAO, the photothrombotic stroke model does not involve reperfusion, which means it doesn't mimic the full pathophysiology of strok where blood flow is restored after an initial occlusion. Penumbra dynamics: The penumbral region in the photothrombotic model is less prominent compared to other stroke models, limiting its utility for studying salvageable brain tissue.

Protocol Overview:

1. Dye Injection: A photosensitive dye (e.g., Rose Bengal) is injected intravenously into the animal.

2. Light Exposure: A focused light source is directed at a specific brain region through the intact skull for a set duration.

3. Thrombus Formation: The dye is activated by the light, causing endothelial damage and thrombus formation, leading to ischemia in the target area.

4. Stroke Assessment: After the stroke is induced, the neurological deficits and infarct size can be evaluated using behavioral tests and histological analysis.

Advantages for Research:

The model is especially useful for creating cortical strokes, which are often implicated in motor and

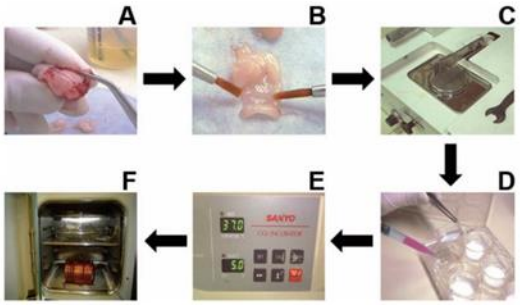
sensory deficits. It's often favored for studying mechanisms of ischemic injury in specific brain regions and assessing potential treatments that could protect neurons or promote recovery. Would you like more details on experimental setups, or are you interested in comparing this model with other stroke models?

Embolic Stroke Model: This model uses the injection of preformed emboli into the bloodstream to mimic embolic stroke, where a blood clot travels to the brain and causes vessel occlusion. This model closely mimics clinical stroke caused by thromboembolic events.

Global Cerebral Ischemia Models: These models induce global ischemia by occluding both common carotid arteries, simulating conditions of cardiac arrest or severe hypotension. They are valuable for understanding the global impact of ischemia on the brain. While in vivo models offer valuable mechanistic insights, they also present limitations such as variability in infarct size, difficulty in controlling confounding variables, and ethical considerations regarding animal use.

- IN VITRO MODELS OF ISCHEMIC STROKE

In vitro models offer a more controlled environment to investigate specific molecular and cellular responses to ischemic conditions. These models are essential for highthroughput drug screening and for understanding the fundamental processes of ischemic injury. Common in vitro models include:

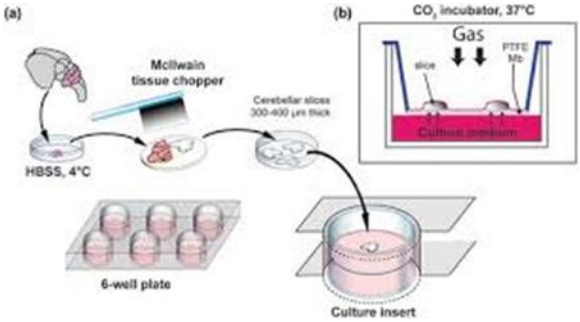


1) OxygenGlucose Deprivation (OGD): OGD is the most widely used in vitro model for simulating ischemic conditions. Cultured neurons, astrocytes, or mixed cultures are subjected to oxygen and glucose deprivation, mimicking the energy failure seen during ischemia. This model allows for the study of cellautonomous responses to ischemia and the screening of neuroprotective agents.

OxygenGlucose Deprivation (OGD) is an in vitro model used to simulate the effects of ischemia, such as what occurs in stroke, on brain cells or tissues. By depriving cultured neurons, glial cells, or organotypic brain slices of oxygen and glucose, researchers mimic the pathophysiological conditions that occur during a stroke. This model is widely used to study the mechanisms of neuronal injury, cell death, and potential therapeutic interventions.

Primary Neuronal Cultures: Neurons cultured from rodent brain regions such as the hippocampus or cortex are commonly used to study OGD effects on specific neuronal populations.
Organotypic Brain Slices: These are slices of brain tissue that retain much of their original cellular architecture, allowing for more accurate modeling of in vivo brain ischemia.
Cell Lines: Immortalized cell lines, such as PC12 or SHSY5Y cells, are sometimes used due to their ease of handling.

2) Organotypic Brain Slices: Organotypic slice cultures retain the threedimensional architecture of brain tissue, providing a more physiologically relevant environment compared to dissociated cell cultures. These slices can be subjected to OGD, allowing the study of neurovascular and neuroglial interactions during ischemic injury.
Neurodevelopment: Studying how brain circuits develop and how various genetic or environmental factors influence brain formation.

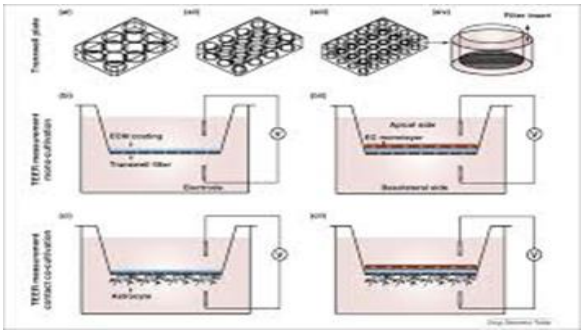


Neurophysiology: Researchers can measure synaptic transmission, neuronal firing, and network activity using electrophysiological techniques like patchclamp recordings.

Neurodegenerative Diseases: Organotypic slices are often used to model neurodegenerative conditions, such as Alzheimer's disease, Parkinson's disease, or epilepsy, allowing scientists to observe disease progression and screen potential treatments.

Ischemia/Stroke Research: Oxygenglucose deprivation (OGD) can be applied to slices to simulate ischemic conditions (as discussed earlier), making organotypic slices valuable for stroke research.

Neuroprotection: This model allows for testing the neuroprotective effects of drugs or therapies aimed at preventing neuronal injury, especially in the context of neurodegenerative diseases, ischemia, or traumatic brain injury.



3) BloodBrain Barrier Models: In vitro models of the BBB, such as cocultures of endothelial cells and astrocytes, are used to study how ischemia affects BBB integrity and permeability. These models are useful for investigating potential therapeutic strategies to protect or restore the BBB during ischemic stroke.

In vitro models are widely used to study the function of the BBB, mechanisms of transport, and drug permeability. They are simpler, costeffective, and scalable compared to in vivo models.

a) Static Models:

Endothelial Monolayers: The simplest BBB models involve culturing brain endothelial cells (often derived from humans, rodents, or immortalized lines) on permeable inserts to form a monolayer. This simulates the endothelial layer of the BBB, and researchers can measure the passage of substances across the monolayer.

Primary Endothelial Cells: More physiologically relevant, but challenging to maintain in culture.

Immortalized Endothelial Cell Lines: Easier to culture but less representative of in vivo conditions.

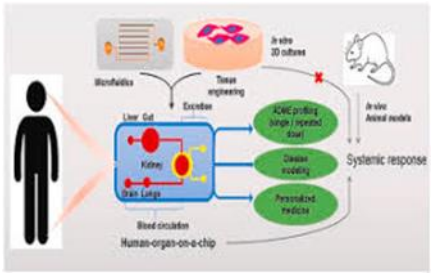
Coculture Models: These models improve physiological relevance by incorporating other cell types present in the BBB, such as astrocytes, pericytes, and neurons. Triple Coculture Models: Endothelial cells are cultured with astrocytes and pericytes, providing a more complete representation of the BBB's complexity.The astrocytes and pericytes help tighten the junctions between endothelial cells, improving the barrier properties and allowing for a better simulation of in vivo conditions.

b) Dynamic Models:

Microfluidic Models (BBBonaChip): These models use microfluidic platforms to simulate the dynamic flow of blood and shear stress that endothelial cells experience in vivo. By incorporating flow, these models more accurately replicate the physiological conditions of the BBB and allow for realtime monitoring of molecular transport.Microfluidic models allow for precise control of fluid flow, chemical gradients, and the cellular environment, improving the accuracy of BBB simulation.

3D BBB Models: Recent advances allow for the creation of threedimensional cultures, often involving hydrogels or scaffolds to support the formation of a 3D structure that better mimics the in vivo BBB environment.Organoids or spheroids can be created from BBB cell types, providing a more physiologically accurate model for longterm studies.

4) Microfluidic Models: These models use microfluidic devices to recreate ischemic stroke conditions in a highly controlled microenvironment. They allow precise manipulation of oxygen and glucose levels, as well as the study of fluid dynamics during ischemia. Microfluidic models are increasingly being used for highthroughput screening and mechanistic studies.



Comparison of In Vivo and In Vitro Models In vivo and in vitro models each offer unique advantages and limitations in the study of ischemic stroke. In vivo models provide a holistic view of the ischemic process, including the interaction between brain cells and systemic factors such as immune responses and vascular changes. However, they are timeconsuming, expensive, and subject to ethical considerations.

Feature	In Vivo BBB Models	In Vitro BBB Models
Definition	Wholeanimal models that mimic the BBB in its natural context.	Cellbased or tissuebased systems that simulate the BBB in a controlled laboratory environment.
Physiological Complexity	High: Fully captures the complexity of BBB, including interactions with the circulatory system, immune responses, and all brain cell types.	Moderate to Low: Simulates specific cellular interactions (e.g., endothelial cells, astrocytes) but lacks fullbody integration.
Cellular Components	Includes endothelial cells, astrocytes, pericytes, neurons, and blood flow, along with systemic influences like immune responses.	Typically includes only endothelial cells or coculture with astrocytes/pericytes, but lacks systemic influences.
Tight Junctions & BBB Integrity	More physiologically accurate with dynamic tight junctions and intact barrier function.	Variable: Can mimic tight junctions, but often less stringent and may vary between models.
Blood Flow & Shear Stress	Naturally includes blood flow, shear stress, and systemic pressure, which are important for BBB function.	Absent in static models; present in microfluidic models (BBBonachip) that simulate shear stress.
Time Frame of Experimentation	Longterm: Can be used for chronic experiments over weeks to months.	Shortterm to mediumterm: Limited to hours, days, or a few weeks, depending on the model used.
Relevance to Human BBB	Closer to human physiology, especially in genetically modified or disease models.	May differ from human physiology depending on the cell source (human vs. rodent cells) and culture conditions.
Ethical Considerations	High: Involves the use of animals (rodents, transgenic models), which raises ethical concerns and requires regulatory approval.	Lower: No use of live animals. Ethical concerns are limited to the source of primary cells or humanderived materials.
Cost and Scalability	Expensive: High costs due to animal care, genetic modifications, and longer study durations.	Costeffective: Lower costs, especially with immortalized cell lines, and scalable for highthroughput screening.
Drug Permeability Testing	More accurate representation of drug delivery, transport mechanisms, and BBB penetration in a living system.	Allows for highthroughput screening of drug permeability, but with lower physiological relevance than in vivo models.
Disease Modeling	Can replicate human disease states like stroke, neurodegenerative diseases, and BBB disruption in conditions like multiple sclerosis.	Limited disease modeling capabilities; however, some in vitro systems can model aspects of neuroinflammation or ischemic conditions (e.g., oxygenglucose deprivation (OGD)).
Transport Mechanisms	Naturally incorporates complex active and passive transport mechanisms, including receptormediated transcytosis and efflux pumps.	Can replicate these mechanisms to a degree, but less accurately than in vivo models; some models include Pglycoprotein and other transporters.

CONCLUSION

In vivo and in vitro models are indispensable tools for studying the pathophysiology of ischemic stroke and for exploring potential therapeutic interventions. In vivo models such as MCAO and photothrombosis closely replicate the clinical features of stroke, while in vitro models like OGD and microfluidic platforms allow for detailed mechanistic studies in a controlled environment. Together, these models provide a comprehensive framework for studying ischemic stroke and hold promise for the development of novel treatments that could reduce the burden of this devastating disease.

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