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Review Article

In Vivo Experimental Models of Cerebral Ischemia: Analysis, Translational Potential and Clinical Relevance

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Abstract

Despite all current efforts in the field of cerebrovascular disease prevention, stroke remains the leading cause of disability and death worldwide. Animal models are essential tools in stroke research to mitigate its devastating effects. The novelty of this review is the comprehensiveness of the approach and the detailed analysis of progress in the field, translational potential and clinical relevance. Recent research aimed to refine and enhance animal models to better mimic clinical scenario and improve reproducibility. This review provides extensive overview of its classification, underlining their key features, advantages, limitations, and advancements. Additionally, characterization and validation of ischemia models are discussed. Therapeutic strategies such as hypothermia, pharmacological interventions, genetic and molecular approaches, and cell-based therapies are highlighted. This review analyse the challenges and future directions, as well as translational potential and clinical relevance of ischemia models. Incorporating patient-derived cells and genetic modifications in animal models can provide a more personalized and clinically relevant approach. Bridging the gap is challenging and requires careful consideration of multiple factors, including appropriate dose translation, understanding species differences, and aligning protocols. Future studies should focus on optimizing the translational process and conducting well-designed clinical trials to assess the safety and efficacy of promising interventions.

Keywords: cerebral ischemia, animal models, therapeutic interventions, validation, translational potential, clinical relevance

Introduction

Background on cerebral ischemia

Cerebral ischemia, often referred to as a stroke, occurs when there is a disruption of blood flow to the brain, leading to the deprivation of oxygen and nutrients. This can be caused by a blockage in the arteries supplying the brain - ischemic stroke, or by bleeding into the brain tissue - haemorrhagic stroke [1, 2]. Haemorrhagic stroke is caused by leakage or burst of a blood vessel in the brain. While only 15% of strokes are of the haemorrhagic type, the majority is ischemic, the results are devastating because they are responsible for 50% of stroke related mortality [3].

The brain requires a constant supply of oxygen and glucose in order to function properly. When blood flow is interrupted, brain cells begin to die within minutes due to energy depletion and the accumulation of toxic metabolic waste. This can result in various neurological deficits, including impaired movement, speech problems, memory loss, and even death, depending on the severity and duration of the ischemic insult [2,4].

The main mechanisms underlying cerebral ischemia revealed through in vivo experimental models [1,2,5,6,] are:

Excitotoxicity - Cerebral ischemia disrupts the balance of neurotransmitters in the brain, leading to excessive release of glutamate, an excitatory neurotransmitter. This excess glutamate can cause over activation of glutamate receptors, leading to neuronal damage and cell death. This energy deficit triggers

a cascade of excitotoxicity events, which leads to an influx of calcium ions and the activation of various cell death pathways.

Inflammatory response - Additionally, the lack of blood flow to the brain causes an inflammatory response, with the release of pro-inflammatory molecules and the infiltration of immune cells such as microglia and astrocytes into the affected area. This response involves the release of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS). This inflammatory response can exacerbate tissue damage and contribute to secondary brain injury following cerebral ischemia.

Blood-brain barrier disruption - Cerebral ischemia can lead to the breakdown of the blood-brain barrier (BBB), which normally separates the brain from the bloodstream. BBB disruption allows the entry of immune cells, inflammatory mediators, and other molecules into the brain. This further amplifies the neuroinflammatory response and contributes to the development of brain injury.

Oxidative stress and mitochondrial dysfunction - Ischemic conditions lead to an increase in ROS, which can cause oxidative stress and damage to cellular structures including mitochondria. Mitochondrial dysfunction can further contribute to neuronal cell death.

Apoptosis and cell death pathways - Cerebral ischemia triggers a cascade of molecular events that ultimately lead to cell death. This can occur through several pathways, including apoptosis, a programmed cell death mechanism. Activation of apoptotic pathways can lead to widespread cell death and tissue damage after cerebral ischemia.

Need for experimental models in cerebral ischemia research

Despite all current efforts in the field of cerebrovascular disease prevention, stroke remains the leading cause of disability and one of the leading causes of death worldwide [7,8].

Stroke is second leading cause of death worldwide, affecting 15 million people annually, it is also a source of enormous disability in survivors [9]. Stroke-related costs in the EU came to nearly 60€ billion/year [9] and in the US $56.5 billion/year [8]. Stroke care accounted for approximately 3% of total health care expenditures in EU [8]. Patients who have suffered an ischaemic stroke are at high risk for recurrence of a cerebrovascular event as well as subsequent dementia, myocardial infarction, and vascular death [8].

Given the devastating consequences of cerebral ischemia, extensive research is conducted to understand its underlying mechanisms and develop effective strategies for neuroprotection and recovery. Experimental models play a crucial role in this research as they allow us to simulate and study the different aspects of cerebral ischemia in controlled laboratory settings.

Experimental models of cerebral ischemia can be broadly classified into in vitro and in vivo models. In vitro models involve culturing brain cells or tissues in a controlled environment, allowing us to investigate various cellular and molecular mechanisms. These models provide valuable insights into the molecular pathways involved in ischemic injury and help identify potential therapeutic targets.

In contrast, in vivo models more closely mimic the complex physiological and pathological processes observed in human stroke. These models provide a more realistic representation of the complex pathophysiology and responses to ischemia compared to in vitro models. Animal models, such as rodents (rats, mice, gerbils, rabbits), are commonly used for in vivo studies due to their physiological and genetic similarities to humans, and due to similar neuroanatomy and relatively short lifespan, allowing for long-term monitoring and analysis. These models help us understand the dynamics of ischemic injury, characterize the temporal progression of cellular and molecular events, and evaluate potential neuroprotective strategies.

Moreover, cerebral ischemia animal models enable us to assess the efficacy and safety of potential therapeutic interventions, including pharmacological agents, stem cell transplantation, and neurostimulation techniques. They can also be used to investigate the effects of genetic manipulation, environmental factors, and comorbidities on stroke outcomes.

Experimental models of cerebral ischemia are essential tools in stroke research to mitigate its devastating effects. Recent research has aimed to refine and enhance these models to better mimic the clinical scenario and improve reproducibility. However, it is important to note that no single model can fully replicate the complexity of human stroke, and therefore a combination of different models is often used to provide a comprehensive understanding. Additionally, various modifications and refinements of the technique exist depending on the specific requirements of the research study and the animal model being used.

This review is more focused on in vivo animal models of ischemic stroke, since it represent majority of 85% of cerebral ischemia insults. We applied historical time-line approach, starting from the first introduction of in vivo models in the field in 60-70's year of the last century up to the most recent refinements. We also tried to have balance in the number of references, in order not to overload the manuscript.

Classification of in vivo experimental models of cerebral ischemia

In order to improve our understanding of the mechanisms underlying cerebral ischemia, it has been developed various in vivo experimental models. There are several different classification

systems used to categorize in vivo experimental models of cerebral ischemia [6,10].

Global vs. Focal Ischemia - This classification is based on the extent of the ischemic insult. Global ischemia models involve a systemic reduction in blood flow to the brain, resulting in widespread damage. Focal ischemia models, on the other hand, involve a localized reduction in blood flow to a specific region of the brain, typically induced by occlusion of a major cerebral artery.

Transient vs. Permanent Ischemia - The classification is based on the duration of the ischemic insult. Transient ischemia models involve a temporary reduction in blood flow to the brain, followed by reperfusion (restoration of blood flow), whereas permanent ischemia models involve a sustained reduction in blood flow without reperfusion.

Surgical vs. Non-Surgical Models - This classification is based on how the ischemic insult is induced. Surgical models involve direct manipulation of the cerebral vasculature, such as occluding a major cerebral artery, whereas non-surgical models use other methods to induce ischemia, such as chemical agents or genetic manipulations.

Species - The classification is based on the species of animal used in the model. Commonly used species include rodents (e.g., rats, mice, gerbils), rabbits, cats, and non-human primates.

Age - This classification is based on the age of the animals used in the model. Age can influence the susceptibility and response to cerebral ischemia, so different age groups may be used to study different aspects of the condition.

Global cerebral ischemia models

Global cerebral ischemia is a severe medical condition characterized by a reduced blood flow to the entire brain, leading to impaired neuronal function and, in severe cases, neuronal death. Global cerebral ischemia occurs as a result of a sudden, severe decrease in systemic blood flow, leading to compromised oxygen and nutrient supply to the entire brain [1,2]. This condition can arise from cardiac arrest, hypoxic events, or systemic hypotension and can have devastating consequences [1,2]. Rapid reperfusion is crucial to prevent irreversible damage; however, this can paradoxically result in additional injury known as reperfusion injury. Experimental models resembling global cerebral ischemia and reperfusion play a pivotal role in understanding the underlying mechanisms and exploring novel therapeutic interventions.

Rationale for bilateral carotid artery occlusion models

Bilateral common carotid artery occlusion (BCCAO) in patients is a condition in which both common carotid arteries, which are major blood vessels in the neck supplying blood to the brain, become blocked or obstructed. This can result in a reduction or complete cessation of blood flow to the brain, leading to a variety of neurological symptoms [11,12].

BCCAO is often caused by atherosclerosis, a condition characterized by the build-up of plaque in the arteries. Plaque consists of cholesterol, fat, calcium, and other substances that can narrow the arterial lumen and impede blood flow [13,14].

The prognosis of BCCAO depends on various factors, including the severity of the occlusion, the presence of collateral blood vessels, and the promptness of medical intervention. Early diagnosis and treatment are crucial to prevent further neurological complications and improve outcomes.

BCAO model is one of the most commonly employed stroke models in animals, first introduce by Levine and Payan in 1966 [15]. This model involves the occlusion of both carotid arteries, which leads to global cerebral ischemia and subsequent brain damage BCAO models closely mimic the pathophysiological conditions seen in human stroke. This model mainly affects the forebrain regions and is widely used to study global cerebral ischemia. By inducing global brain ischemia, we can study the effects of reduced blood supply to the entire brain, which is relevant to conditions like cardiac arrest or severe hypotension, where the entire brain is affected [12,16]. The brain's ability to regulate blood flow is also compromised, resulting in further hemodynamic alterations [16].

The reduction in blood flow triggers a series of pathological events known as the ischemic cascade. This includes energy failure, excitotoxicity, oxidative stress, and inflammation. These processes contribute to further brain damage, to cell death and tissue damage [2].

Prolonged BCAO results in neurodegeneration, characterized by the loss of neurons and their connections. This leads to cognitive impairment and neurological deficits [6]. Additionally, BCAO models also exhibit white matter injury, which involves damage to the myelin sheath surrounding nerve fibers. Impaired white matter integrity further contributes to cognitive dysfunction [6].

The BCAO model enables us to evaluate the efficacy of potential neuroprotective strategies [2]. By comparing outcomes in animals subjected to BCAO with and without interventions (e.g., drugs, stem cell therapies, or gene therapies), it can be assess the effectiveness of various neuroprotective approaches in reducing brain damage, improving functional recovery, and enhancing survival rates.

BCAO models provide opportunities to identify and evaluate clinically relevant endpoints and biomarkers for stroke. These models allow us to assess various parameters, such as neurological deficits, cognitive function, brain tissue damage, BBB integrity, and changes in gene expression or protein levels, to identify and validate potential biomarkers for stroke diagnosis, prognosis, and treatment response.

Permanent vs. transient models

Permanent BCAO models and transient BCAO models are both used in research to study ischemic stroke. However, these models differ in their methods of inducing the occlusion and their physiological effects on the brain [17,18]. Each of these models has its advantages and limitations [6,17].

Permanent BCAO models involve the complete occlusion of the common carotid arteries, leading to sustained ischemia in the brain. In this model, the common carotid arteries are permanently occluded, resulting in a complete and permanent disruption of blood flow to the brain. This is typically achieved by surgically ligating or clamping the common carotid arteries. As a result, there is a rapid and sustained reduction in blood flow to the brain, leading to cerebral ischemia.

Advantages of permanent BCAO - It provides a consistent and severe model of cerebral ischemia, mimicking a complete blockage of blood flow that occurs in some stroke patients. It allows for long-term studies of the effects of chronic cerebral ischemia, as the occlusion is permanent.

Limitations of permanent BCAO - It does not allow for the reperfusion that often occurs in stroke patients, making it less representative of the clinical scenario. The irreversibility of the occlusion limits the potential for studying interventions aimed at restoring blood flow.

Transient BCAO models involve the temporary occlusion of the common carotid arteries for a specific period of time, resulting in reversible ischemia and reperfusion. The occlusion is usually induced by clamping the arteries for a specific duration, which can vary depending on the experimental design. After the desired period of occlusion, the clamps are removed, allowing for reperfusion of the brain.

Advantages of Transient BCAO - It allows the study of both the acute and delayed effects of cerebral ischemia and reperfusion. The temporary nature of the occlusion enables the investigation of interventions aimed at restoring blood flow and reducing brain damage.

Limitations of Transient BCAO - The degree of cerebral ischemia may vary depending on the duration of the occlusion, making it more challenging to achieve standardized and reproducible results. There is a risk of injury during the occlusion or reperfusion process.

Overall, the choice between permanent and transient BCAO models depends on the specific research questions and goals of the study. Researchers often use a combination of these models to gain a comprehensive understanding of the mechanisms underlying cerebral ischemia and develop potential therapeutic strategies for stroke treatment.

Vessel Occlusion (2VO) model

The 2VO model is an experimental model used in research to simulate ischemic stroke in animals, typically rodents, first introduce by Eklöf and Siesjö in 1972 [19]. In this model, two major blood vessels supplying the brain, such as the common carotid arteries or vertebral arteries, are occluded or blocked. This leads to a reduction in blood flow to the brain, causing ischemia and subsequent damage to brain tissue. This model induces a more gradual reduction of blood flow and mainly affects the hippocampus and other vulnerable brain areas [18].

The 2VO model is commonly used to study the pathophysiology of stroke and to test potential therapeutic interventions. It allows us to examine the effects of reduced blood flow on the brain, including the development of neuronal injury, inflammation, and impaired neurological function [2,20,21].

Four-Vessel Occlusion (4-VO) model

The 4VO model is another experimental model used in stroke research, first introduce by Pulsinelli and Brierley in 1979 [22]. In this model, all four major blood vessels supplying the brain, including both common carotid arteries and vertebral arteries, are occluded or blocked [23]. This results in a more severe reduction of blood flow to the brain compared to the 2VO model. The 4VO model is typically used to study more severe and prolonged brain ischemia and the subsequent development of brain damage.

Both the 2VO and 4VO models provide valuable insights into the pathophysiology of stroke and can be used to assess new treatment modalities [6,20,24]. However, it is important to note that these animal models do not exactly replicate the complex nature of human stroke pathophysiology and outcomes.

Cardiac arrest-induced cerebral ischemia models

Cardiac arrest-induced global cerebral ischemia is a prevalent cause of morbidity and mortality [2]. Cardiac arrest-induced cerebral ischemia is a type of experimental model used in research to mimic the effects of oxygen deprivation in the brain following a sudden loss of cardiac activity, first introduce by Jackson and Dole in 1979 [25]. These models aim to study the impact of cerebral ischemia on brain function and explore potential interventions.

There are several classification systems for in vivo experimental models of cardiac arrest-induced cerebral ischemia. One common classification is based on the method used to induce cardiac arrest and subsequent cerebral ischemia [6,26-29].

Electrical induction models - These models involve the use of electrical shocks to induce cardiac arrest. For example, a direct current can be delivered to the heart through electrodes to stop its activity temporarily.

Chemical induction models - Chemical agents can be used

to induce cardiac arrest. For instance, potassium chloride can be injected intravenously to stop the heart.

Occlusion models - These models involve the occlusion of blood vessels supplying the heart to induce cardiac arrest. For example, a balloon catheter can be inserted into the coronary arteries and inflated to block the blood flow, leading to cardiac arrest and subsequent cerebral ischemia.

Ventricular fibrillation models - Ventricular fibrillation is an abnormal heart rhythm characterized by rapid and chaotic contractions of the ventricles. This model involves the induction of ventricular fibrillation, leading to cardiac arrest and subsequent cerebral ischemia.

Each of these models has its advantages and limitations. Researchers select the most appropriate model based on their specific research objectives and the availability of resources. The choice of model also depends on the desired duration of cardiac arrest and the method used to resuscitate the animal after cardiac arrest.

It is important to note that these experimental models may not completely replicate human cardiac arrest-induced cerebral ischemia. However, they provide valuable insights into the mechanisms and therapeutic opportunities for cerebral ischemia associated with cardiac arrest.

In summary, global cerebral ischemia models have significantly contributed to our understanding of the pathophysiology underlying this condition. Despite the limitations associated with these animal models, recent scientific advancements have improved their relevance and translational potential. Future research should focus on refining existing models, incorporating emerging technologies, and further exploring therapeutic interventions in order to develop effective treatments for global cerebral ischemia.

Focal cerebral ischemia models

Focal cerebral ischemia models involve inducing a localized interruption of blood flow to a specific region of the brain, which leads to ischemia and subsequent damage to the affected brain tissue.

Middle cerebral artery occlusion (MCAO)

This is the most widely used model and involves the temporary or permanent occlusion of the middle cerebral artery (MCAO), one of the major arteries supplying blood to the brain, first introduce by Tamura et al. in 1981 [30]. This can be achieved through surgical techniques or by inserting a nylon filament or suture into the artery to block blood flow [21,31].The suture is advanced until it occludes the vessel, leading to the blockage of blood flow to the surrounding brain tissue [32]. Reperfusion can then be achieved by removing the suture after a certain period of time. This model allows for controlled occlusion and reperfusion, mimicking the conditions of ischemic stroke.

MCA occlusion mainly cause cortex and striatum insults, but the extent of infarction depends on the location and duration of occlusion and the amount of collateral blood of the MCA [6].

The MCAO model has provided valuable insights into the mechanisms underlying cerebral ischemia. Furthermore, the MCAO model has allowed researchers to study the role of various molecules and pathways in cerebral ischemia. For example, studies using this model have demonstrated the involvement of oxidative stress, mitochondrial dysfunction, and the activation of specific signalling pathways, such as the mitogen-activated protein kinase (MAPK) pathway and the nuclear factor-kappa B (NF-κB) pathway [33].

Photothrombotic ischemia

Photothrombosis involves the induction of focal cerebral ischemia through the administration of a photosensitive dye, such as Rose Bengal or erythrosin B into the bloodstream, followed by a laser illumination with a specific wavelength of light, first introduce by Watson et al. in 1985 [34]. The dye selectively binds to circulating plasma proteins, and upon exposure to light, produces reactive oxygen species that provoke local thrombosis causing blood clot formation, leading to local ischemia in the targeted area of the brain. This model allows precise and localized induction of cerebral ischemia and can be used to study specific brain regions or pathways affected by stroke [35].

Embolic and Endovascular coagulation stroke models

There are several models:

Microsphere model - In this model, microspheres with a diameter similar to that of blood vessels are injected into the bloodstream. These microspheres occlude the blood vessels, thereby causing a stroke-like condition [36,37]. These models involve injecting microspheres or blood clots into the cerebral circulation to block blood flow. The use of microspheres makes it possible to create a standardised impact size and position.

Thromboembolic model - This is one of the most commonly used embolic stroke models in rodents. In this model, a blood clot is formed and introduced into the brain vasculature through the carotid artery or middle cerebral artery. The clot then causes an obstruction in the blood flow, leading to an ischemic stroke [38-40].

Gas embolism model - Gas embolism, often caused by air bubbles introduced into the bloodstream, can also lead to stroke-like symptoms. This model involves introducing air bubbles into the circulation, which then block blood vessels and cause ischemia [41].

Fat embolism model - This model involves injecting fat particles directly into the bloodstream to mimic fat embolism, which can occur during fracture or trauma. Fat particles can obstruct blood vessels and result in a stroke-like condition [42].

Thrombin-induced model - In this model, a clot is formed within the cerebral circulation using endovascular techniques. Thrombin, is injected into a cerebral artery, causing coagulation and subsequent blockage of blood flow [40]. This mimics the formation of a clot in ischemic stroke and allows for the study of clot-related mechanisms in cerebral ischemia. Endothelin-1, a potent vasoconstrictor, can be injected directly into specific blood vessels in the brain to induce localized ischemia [43,44].

Coagulation-induced model - In this model, a coagulant substance such as ferric chloride is topically applied to the surface of a blood vessel, leading to clot formation and subsequent stroke [40].

Embolization model using autologous clots - In this model, a clot is collected from the animal's own blood and introduced into cerebral arteries [40]. This allows for the study of more natural clot compositions and their effects on stroke development.

Hypoxic models of cerebral ischemia

Hypoxic models of cerebral ischemia are experimental techniques used to induce temporary or permanent oxygen deprivation in the brain, leading to cerebral ischemia or stroke-like conditions [45-47]. There are several models:

Hypoxia-Ischemia model - This model involves reducing oxygen supply to the brain by interrupting blood flow to a specific brain region, usually by temporarily occluding or completely blocking the blood vessels supplying the brain. The duration and severity of the occlusion can be adjusted to induce different degrees of cerebral ischemia.

Global hypoxic model - In this model, the entire brain is exposed to a reduced level of oxygen, mimicking systemic hypoxia. It can be achieved by subjecting animals to low-oxygen environments or reducing the concentration of oxygen in the inhaled air.

Focal hypoxic model - This model involves targeting a specific brain region to induce focal cerebral ischemia. It can be achieved by injecting vasoconstrictors into specific blood vessels or by using microclamps to temporarily block blood flow to the targeted area.

Hypoxic preconditioning model - This model involves subjecting animals to short periods of mild hypoxia before inducing a more severe ischemic insult. This pre-exposure to mild hypoxia triggers endogenous protective mechanisms in the brain, resulting in increased tolerance to subsequent ischemia.

Neonatal hypoxic-ischemic model - This model is used to study cerebral ischemia in new born animals. It involves interrupting blood flow or reducing oxygen supply to the developing brain, which leads to brain injury similar to hypoxic-ischemic encephalopathy (HIE) seen in human infants.

Hypobaric hypoxia model

The hypobaric hypoxia model is commonly used to induce cerebral ischemia in animals. In this model, animals are exposed to reduced atmospheric pressure, leading to a decrease in oxygen availability. This reduction in oxygen availability mimics the conditions seen in ischemic stroke, where a disruption in blood flow leads to oxygen deprivation in brain tissue [48,49].

This model can be induced using a specialized chamber that can control atmospheric pressure. Animals are placed in the chamber and the pressure is gradually decreased to create a hypobaric environment. The animals are usually kept under hypobaric conditions for a specific duration to induce the desired level of cerebral ischemia [50].

The hypobaric hypoxia model allows us to study the impact of reduced oxygen availability on the brain, evaluate the effectiveness of potential therapeutic interventions, and investigate the underlying mechanisms of cerebral ischemia.

Hyperbaric oxygen preconditioning model

The hyperbaric oxygen preconditioning model involves exposing animals to hyperbaric oxygen therapy (HBOT) prior to inducing cerebral ischemia. HBOT involves administering 100% oxygen at increased atmospheric pressure, which promotes the delivery of oxygen to tissues, including the brain [51,52].

In this model, animals are placed in a hyperbaric chamber and subjected to high-pressure oxygen treatment for a specific duration. This conditioning period is thought to induce various neuroprotective mechanisms in the brain, making it more resistant to subsequent ischemic injury.

After the preconditioning period, animals are then exposed to a stroke-inducing ischemic event, such as MCAO, to simulate the conditions of cerebral ischemia. The impact of hyperbaric oxygen preconditioning on the severity of ischemic injury and neurological outcomes can then be analysed [52,53].

The hyperbaric oxygen preconditioning model is useful for studying the potential benefits of HBOT as a therapeutic intervention for ischemic stroke. It can also shed light on the mechanisms by which HBOT exerts its neuroprotective effects and provide insights for developing novel treatments for cerebral ischemia.

Characterization and validation of experimental models of cerebral ischemia

Assessing the success of experimental model establishment is essential to ensure the accuracy and reliability of results. Characterization of experimental models of cerebral ischemia involves assessing the extent and duration of the resulting brain ischemia, as well as the behavioral and histological changes that occur as a result. This typically involves monitoring various parameters, such as cerebral blood flow, cerebral oxygenation, and neurologic deficits. Techniques such as laser Doppler flowmetry, near-infrared spectroscopy, and behavioral tests are used to evaluate the effects of experimentally induces cerebral ischemia.

Optical imaging techniques, such as near-infrared spectroscopy (NIRS) and fluorescence imaging, use light to visualize changes in cerebral blood flow and oxygenation [54,55]. Recent advancements in these techniques have improved sensitivity and allowed for real-time monitoring of brain function in experimental models of cerebral ischemia.

Preclinical imaging techniques, such as magnetic resonance angiography (MRA) and micro-CT (Computed Tomography), have been developed specifically for studying animal models of cerebral ischemia [56-58]. These techniques offer high-resolution imaging of blood vessels and allow for non-invasive assessment of cerebral blood flow and vascular structure in experimental models of cerebral ischemia.

Validation of experimental models of cerebral ischemia involves comparing the observed changes in the brain function and pathology, and behaviour, with known characteristics of cerebral ischemia and previous studies. This is important to ensure that the model accurately replicates the pathophysiological and functional changes seen in human brain ischemia. Validation may involve comparing the results of experimental models of cerebral ischemia with clinical data from stroke patients.

Validation also includes confirming the reproducibility of the experimental models of cerebral ischemia by ensuring consistent induction of ischemia and consistent outcomes across multiple experiments. This may involve controlling various factors, such as the duration and severity of the occlusion, the use of anesthesia, and the surgical technique used to perform the occlusion.

Overall, characterization and validation of experimental models of cerebral ischemia are critical for ensuring that these models accurately replicate the pathophysiology of brain ischemia and can be used effectively.

Behavioral assessments in experimental models of cerebral ischemia

Behavioral assessments play a crucial role in evaluating the functional outcomes of brain injury in animal models. In the context of brain-controlled assistive technologies, these assessments are particularly important in determining the effectiveness of the models in restoring motor and cognitive functions.

Motor function evaluation

Motor function evaluation aims to assess the ability of animals to perform coordinated movements and fine motor skills. Some commonly used assessments include Rotarod test - Measures the ability of animals to balance and stay on a rotating rod. Beam-walking test - Assesses the animal's balance and coordination while walking on a narrow beam. Cylinder test - Evaluates forelimb usage and asymmetry in activities like rearing and wall exploration. Grip strength test - Measures the force exerted by an animal's forelimbs while grabbing an apparatus [59-61].

Cognitive assessment

Cognitive assessments aim to evaluate various cognitive functions, including memory, learning, attention, and decision-making. Assessments that are common include Morris water maze - Tests spatial memory and learning by assessing the animal's ability to navigate and find a hidden platform in a pool of water. Novel object recognition test - Measures recognition memory by assessing an animal's preference for a novel object over a familiar one. Radial arm maze - Evaluates spatial working memory by assessing an animal's ability to remember and choose from multiple arms of a maze. Fear conditioning test - Assesses learning and memory by associating a neutral stimulus (e.g., sound) with an aversive stimulus (e.g., mild electric shock) [61-63].

Sensory and perceptual measures

These measures assess the animal's sensory and perceptual abilities, including vision, hearing, touch, and proprioception. Assessments that are commonly used include Visual cliff test - Evaluates depth perception in animals by assessing their willingness to approach the edge of a visual cliff. Prepulse inhibition - Assesses sensorimotor gating by measuring the animal's reduction in startle response to a stimulus preceded by a weak prepulse. Tactile sensitivity tests - Measure the animal's response to touch stimuli of varying intensities using von Frey filaments or other mechanical stimuli [61,62].

Neurobehavioral testing techniques in experimental models of cerebral ischemia

In addition to specific assessments mentioned above, experimental models of cerebral ischemia often require specialized techniques to evaluate neurobehavioral outcomes, such as Electroencephalography (EEG) - Measures the electrical activity of the brain to assess changes in brain function associated with motor and cognitive tasks or pathology. Functional magnetic resonance imaging (fMRI) - Provides insights into brain activity and connectivity during different tasks or stimulation.

Electromyography (EMG) - Measures the electrical activity of muscles to assess changes in muscle function and control after brain injury. Eye-tracking - Measures the movement of the eyes to assess visual attention and oculomotor function in response to stimuli or tasks [10, 64].

These behavioral assessments and neurobehavioral testing techniques collectively provide valuable insights into the motor, cognitive, sensory, and perceptual abilities of animals in experimental models of cerebral ischemia. The data obtained from these assessments aid in characterizing the effectiveness of brain-controlled assistive technologies in restoring or enhancing function.

Therapeutic strategies in experimental models of cerebral ischemia

Hypothermia

Hypothermia, or cooling the body or brain to reduce body temperature, has been shown to be neuroprotective in experimental models of cerebral ischemia. Cooling the brain can reduce metabolic demands and decrease the release of excitatory neurotransmitters, ultimately protecting brain cells from ischemic injury. Therapeutic hypothermia has been investigated in both focal and global ischemia models and has shown promising results in improving neurological outcomes [65-67].

Pharmacological interventions

Pharmacological interventions in experimental models of cerebral ischemia aim to improve outcomes by attenuating the biochemical and cellular cascades associated with ischemic brain injury. However, the effectiveness of specific interventions may vary depending on the model, dose, timing, and duration of treatment. Evaluation of these interventions is essential to translate preclinical findings to clinical applications for stroke patients.

Neuroprotective agents

Over the years, researchers have been exploring various therapeutic approaches to reduce brain damage and promote recovery after global cerebral ischemia. Neuroprotection involves interventions aimed at preserving brain tissue during ischemia, while neurorecovery focuses on promoting the repair and functional recovery of the damaged brain. Some novel therapeutic approaches include the use of neurotrophic factors, stem cell transplantation, hypothermia, and pharmacological agents that target specific pathways involved in ischemic injury [68,21,2]. These advancements offer potential interventions to improve outcomes in global cerebral ischemia patients.

Neuroprotective agents often target various mechanisms involved in neuronal cell death, such as excitotoxicity, oxidative stress, and apoptosis. Examples of neuroprotective agents include NMDA receptor antagonists (e.g., memantine), free radical scavengers (e.g., edaravone), and anti-apoptotic agents (e.g., caspase inhibitors). The agents that can inhibit calcium influx, calcium channel blockers (such as nimodipine) [2,69].

Vasodilators, antithrombotic agents, and thrombolytics

Vasodilators and antithrombotic agents are aimed at improving blood flow to the affected brain regions and preventing further thrombus formation. These agents help to restore perfusion and decrease the risk of secondary ischemic events. Common vasodilators used in experimental models of cerebral ischemia include nitric oxide donors (e.g., nitroglycerin) and calcium channel blockers (e.g., nimodipine, nicardipine) [2,69]. Antithrombotic agents, such as antiplatelet drugs (e.g., aspirin) and anticoagulants (e.g., heparin), can also be used to prevent clot formation and promote blood flow [70]. Platelet aggregation and thrombus formation contribute to the formation and worsening of ischemic strokes. Antiplatelet agents, such as clopidogrel, or ticagrelor, inhibit platelet activation and aggregation, reducing the risk of further vascular occlusion [71].

Thrombolytics are drugs aim to dissolve blood clots and restore blood flow in ischemic stroke. They are typically administered shortly after the occlusion is established to reestablish cerebral blood flow. Common thrombolytics used include tissue plasminogen activator (tPA) and streptokinase [72].

Anti-inflammatory and immunomodulatory therapies

Inflammation plays a critical role in the pathogenesis of global cerebral ischemia. Recent studies have shown that the activation of immune cells and the release of pro-inflammatory molecules contribute to the progression of ischemic brain damage. Understanding the mechanisms underlying this inflammatory response is crucial for developing targeted therapies that can modulate the immune response and reduce brain damage in cerebral ischemia. Anti-inflammatory agents and immunomodulatory approaches are being explored as potential treatments in this context.

Anti-inflammatory and immunomodulatory therapies aim to reduce neuroinflammation and modulate immune responses to support neuroprotection and functional recovery. Drugs that target inflammatory pathways, such as nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids, may reduce inflammation and limit tissue damage [73,74]. Examples of these therapies include corticosteroids (e.g., dexamethasone), anti-inflammatory cytokines (e.g., interleukin-10), and immunomodulatory agents targeting specific immune cell populations [73-75].

Emerging therapies and strategies

There are ongoing efforts to develop new therapeutic approaches for experimental models of cerebral ischemia. These

include novel drug targets, such as neurotrophic factors (e.g., brain-derived neurotrophic factor), growth factors, and stem cell-based therapies [76,77]. Additional interventions include antioxidants (e.g., vitamin E), and acetylcholinesterase inhibitors (e.g., donepezil) to promote neuroprotection, neurogenesis, and cognitive improvement [78]. Emerging strategies also involve gene therapy techniques, nanoparticles for drug delivery, and neurostimulation techniques (e.g., transcranial magnetic stimulation) to enhance recovery [79,80].

Challenges and future directions of pharmacological interventions

Despite the progress made in understanding and treating experimental models of cerebral ischemia, several challenges remain. These include the development of more selective and effective drugs, optimization of treatment time windows, addressing potential side effects of pharmacological interventions, and translating preclinical findings into clinical trials.

Future directions in experimental models of cerebral ischemia research involve a multidisciplinary approach that integrates advances in neuroscience, pharmacology, genetics, and technology to improve the outcomes for individuals affected by stroke. Additionally, there is a need for better understanding of the complex interplay between molecular, cellular, and systemic responses in experimental models of cerebral ischemia to identify novel therapeutic targets.

Genetic and molecular approaches in experimental models of cerebral ischemia

Genetic and molecular approaches have been widely used in experimental models of cerebral ischemia to understand the underlying mechanisms and develop potential treatments for cerebral ischemia.

Molecular approaches in experimental models of cerebral ischemia involve the analysis of gene expression, protein levels, and signalling pathways involved in cerebral ischemia [81-83]. Techniques like polymerase chain reaction (PCR), Western blotting, and immunohistochemistry can be used to measure changes in gene expression or protein levels in the brain after experimental cerebral ischemia. These approaches can help identify specific genes or proteins that are involved in the pathogenesis of cerebral ischemia, as well as potential therapeutic targets.

Transgenic and knockout models

Genetically modified animal models allow us to study specific genes and their role in the development and progression of ischemic stroke, providing valuable insights into potential therapeutic targets. Transgenic animal models have been developed to mimic specific genetic mutations or alterations associated with cerebral ischemia. These models enable us to study the role of specific genes or signalling pathways in stroke pathogenesis and test potential therapeutic targets. Transgenic models involve the introduction of a foreign gene into the genome of an animal, while knockout models involve the deletion or inactivation of a specific gene [84]. These models enable us to manipulate specific genes and observe their effects on experimental cerebral ischemia development and progression. For example, we may introduce or delete genes associated with blood vessel growth, inflammation, or other processes involved in experimental cerebral ischemia [82,83].

Gene therapy approaches

Gene therapy is a promising approach for treating experimental cerebral ischemia. It involves the delivery of therapeutic genes to target cells to correct genetic abnormalities or promote protective mechanisms. In the context of experimental cerebral ischemia, gene therapy aims to restore or enhance blood vessel growth, reduce inflammation, or promote other processes that can improve blood flow to the brain. Various methods, such as viral vectors or lipid nanoparticles, can be used to deliver therapeutic genes to targeted cells in the brain [85,86].

MicroRNA regulation and therapeutic potential

MicroRNAs are small RNA molecules that play a crucial role in regulating gene expression. Dysregulation of microRNA expression has been implicated in several diseases, including experimental cerebral ischemia [87-89]. The therapeutic potential of targeting specific microRNAs to modulate gene expression and potentially promote blood vessel growth, reduce inflammation, or protect brain cells are explored [87-89]. This approach involves delivering synthetic microRNA molecules or using gene editing techniques to manipulate endogenous microRNA expression levels.

Proteomic and metabolomic analyses

Proteomic and metabolomic analyses involve studying the proteins and metabolites present in a biological sample, respectively. These approaches can provide valuable insights into the molecular changes that occur during experimental cerebral ischemia [90,91]. By comparing the protein or metabolite profiles of healthy and ischemia-affected brains, we can identify potential biomarkers of the disease, as well as gain a better understanding of the underlying molecular mechanisms. This information can aid in the development of targeted therapies and the identification of novel therapeutic targets.

Cell-based therapies for experimental models of cerebral ischemia

Cell-based therapies have been explored as potential treatments for experimental models of cerebral ischemia, aiming to restore damaged brain tissue and improve functional recovery [76,77,92].

Stem cell transplantation

Stem cell therapy involves the transplantation of stem cells into the brain to promote tissue repair and regeneration. Stem cells have the ability to differentiate into various cell types and can potentially replace damaged cells in the brain. Various types of stem cells, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells (MSCs), have been investigated for their potential to treat experimental models of cerebral ischemia [76,92]. These stem cells can differentiate into different cell types, secrete neurotrophic factors, and modulate the immune response, which may promote brain tissue regeneration and repair [77,92]. Stem cell therapy holds promise for treating cerebral ischemia by promoting neuroprotection, angiogenesis, and neurogenesis.

Cell replacement strategies

Cell replacement strategies involve the transplantation of specific cell types into the brain to replace damaged cells and restore function. For experimental cerebral ischemia, this may involve transplanting neural stem cells (NSCs) derived from fetal or adult brain tissue, or other cell types, such as astrocytes or oligodendrocyte progenitor cells, into the affected brain regions [93]. These cells have the ability to differentiate into neurons and glial cells, potentially replacing damaged or lost cells in the brain. They may also promote tissue repair through their secretion of various growth factors and cytokines [94]. These transplanted cells can integrate into the existing brain circuitry and contribute to functional recovery.

Emerging cell-based therapies

Several emerging cell-based therapies are being explored for experimental models of cerebral ischemia. These include using mesenchymal stem cells (MSCs) derived from various sources, such as bone marrow or adipose tissue, to promote neuroprotection and tissue repair [95]. Other emerging therapies include using immune cells, such as regulatory T cells or macrophages, to modulate the immune response and reduce inflammation in the brain [73]. Additionally, gene therapy approaches are being investigated, where specific genes or proteins are delivered to the brain using viral vectors or nanoparticles to promote neuroprotection and functional recovery in experimental models of cerebral ischemia [96]. These emerging cell-based therapies hold promise for improving outcomes in cerebral ischemia.

Genetic modification of cells

Researchers have explored genetically modifying cells to enhance their therapeutic potential in experimental models of cerebral ischemia. For example, stem cells or neural precursor cells can be engineered to express specific growth factors or factors that promote angiogenesis, the formation of new blood vessels [76]. This approach aims to enhance tissue regeneration and improve functional recovery.

Exosome treatment

Exosomes are small vesicles secreted by cells that contain various bioactive molecules, such as proteins, lipids, and nucleic acids. They have been shown to have a crucial role in intercellular communication and can carry therapeutic cargo to target cells [95,97]. Exosome treatment involves delivering exosomes derived from specific cell types, such as stem cells or immune cells, to promote brain recovery and reduce inflammation in experimental models of cerebral ischemia [95,97]. Exosomes have shown promise in preclinical studies for improving functional recovery and reducing brain damage in experimental models of cerebral ischemia.

Microglial cell transplantation

Microglial cells are the resident immune cells of the central nervous system. In experimental models of cerebral ischemia, transplantation of microglial cells has been investigated as a means to modulate inflammation and promote tissue repair [98,99]. The aim is to transplant microglial cells with anti-inflammatory properties to reduce tissue damage and improve functional outcomes.

Combination therapies

Some studies have explored combining different cell-based therapies with other treatments, such as pharmacological agents or physical rehabilitation, to enhance the therapeutic efficacy in experimental models of cerebral ischemia [44,96]. These combination therapies aim to target multiple mechanisms involved in brain tissue repair and functional recovery.

Overall, cell-based therapies show promise in experimental models of cerebral ischemia by promoting brain tissue repair, modulating inflammation, and improving functional outcomes. However, further research is needed to optimize the cell type, delivery methods, and combination approaches to increase the therapeutic potential of these cell-based therapies.

Repurposing existing drugs

In vivo experimental models can be used to explore the therapeutic potential of repurposing existing drugs for neurodegenerative diseases. By utilizing animal models of specific neurological disorders, researchers can administer drugs that are currently used for other indications and evaluate their effects on disease progression, symptom alleviation, or disease modification [70,96]. This approach can identify potential candidates for drug repurposing and accelerate the development of new treatment options for neurological conditions.

Moreover, the effects of drugs or other treatments can be assessed in experimental models of cerebral ischemia by measuring changes in gene expression, protein levels, or histopathological findings in the brain [91].

In summary, in vivo experimental models play a pivotal role in exploring and evaluating various therapeutic strategies for cerebral ischemia. They provide a platform for testing the effectiveness of neuroprotective agents, investigating neurorestorative approaches, assessing stem cell transplantation, and exploring drug repurposing. These models help bridge the gap between laboratory research and clinical applications, ultimately leading to the development of novel treatments for stroke.

Strengths and limitations of in vivo experimental models of cerebral ischemia

In vivo models of cerebral ischemia face several strengths and limitations. Strengths are:

Relevance to Human Physiology - In vivo experimental models use living organisms (animals) to study brain condition and interventions. This allows us to investigate the effects and safety of potential treatments or therapies in a setting that closely mimics human physiology, increasing the chances of obtaining clinically relevant results. Researchers can administer pharmacological agents or test new interventions like neuroprotective drugs, stem cell therapies, or gene therapies in the animal models. By observing improvements in functional outcomes, such as reduced neurological deficits or increased survival rate, it can be determine the clinical relevance and potential translational impact of these interventions.

Complex Interactions - In vivo models also provide a platform to study and understand the complex interactions between various organs, tissues, and systems in a living organism. One major advantage of in vivo models is their ability to replicate the complex pathophysiological cascade that occurs during cerebral ischemia. By studying these processes in animal models, we can identify key molecular and cellular targets for potential therapeutic interventions.

Real-Time Observation - In vivo models enable us to observe the effects of cerebral insult or interventions in real time. This allows for a better understanding of the underlying mechanisms and dynamics of processes occurring in the brain during ischemia, such as changes in blood flow, oxygen and glucose metabolism, and neurochemical alterations.

Therapeutic windows - In vivo models have provided insights into the time-dependent nature of ischemic injury and the narrow therapeutic windows for intervention. Understanding the optimal timing for treatment initiation is critical for translating preclinical findings to the clinic.

Behavioral outcomes - In vivo models have allowed us to assess long-term functional outcomes after cerebral ischemia. This has provided valuable information on the impact of ischemic injury on cognitive, motor, and sensory functions, guiding the development of interventions that aim to improve post-stroke recovery.

Translational Potential - In vivo models offer a realistic representation of the in vivo environment encountered by drugs or interventions in humans. This allows for a better prediction of efficacy and safety, increasing the chances of successful translation from bench to bedside.

On the other hand, limitations are: Ethical considerations - The use of animals in research raises ethical concerns, and there is a need for strict guidelines and regulations to ensure their welfare. There is ongoing debate about the moral justification of using animals for research purposes.

Variability - In vivo models can be subject to inherent variability among animals, including differences in genetics, physiology, and responses to ischemia, which can affect the reliability and reproducibility of results.

Complexity and Cost - In vivo experiments can be complex, time-consuming, and expensive to conduct. They require specialized facilities, resources, and personnel to manage and care for the animals involved. This can be a limiting factor for small research groups or institutions with limited resources.

Recent advancements in in vivo experimental models of cerebral ischemia

One of the significant advancements in global cerebral ischemia modelling is the development of more sophisticated animal models. Traditionally, rats and mice have been commonly used in these models, but recent advancements have introduced new animal species, such as pigs and non-human primates, to better mimic the human brain [100]. These larger animal models provide a closer representation of the human physiology and allow us to study more complex aspects of cerebral ischemia. Non-human primate models provide a more accurate representation of human neurophysiology and anatomy compared to rodent models. Recent advancements in non-human primate models of cerebral ischemia include the development of techniques for inducing focal or global ischemia in primates, allowing for translationally relevant preclinical studies.

In recent years, significant progress has been made in refining and advancing global cerebral ischemia models. Advances in molecular techniques, as well as the exploration of non-coding RNAs and rehabilitation strategies, have further expanded our understanding of global cerebral ischemia and opened new avenues for therapeutic interventions [2]. The continuous refinement and

optimization of these models will undoubtedly provide further advancements in our knowledge of global cerebral ischemia and guide the development of effective neuroprotective therapies.

The Transient Bilateral Common Carotid Artery Occlusion (TBCCAO) model is widely used to study global cerebral ischemia and its long-term cognitive impairments. Recent studies have focused on optimizing this model to capture different durations of ischemia and investigate the underlying mechanisms contributing to neurodegeneration. Zhang L. et al. [84] utilized the TBCCAO model to elucidate the role of microRNA-34a in neuronal apoptosis and cognitive deficits following global cerebral ischemia, suggesting its potential as a therapeutic target. Furthermore, investigations into the effects of exercise and environmental enrichment on neurogenesis and cognitive recovery have highlighted the importance of rehabilitation modalities in global cerebral ischemia models [101].

The 4-VO model, initially proposed by Pulsinelli and Brierley in 1979 [22], has become widely adopted to induce global cerebral ischemia in rodents. Recent studies have focused on modifying this model to improve reproducibility and reduce variability. One such modification involves inhalational anesthetics, such as isoflurane, which can attenuate the severity of ischemia without compromising post-ischemic outcomes [5]. Additionally, the use of normobaric hyperoxia during reperfusion has shown neuroprotective effects by reducing oxidative stress and inflammation [102]. Advances in molecular and cellular techniques, including transcriptomics and proteomics, have further contributed to our understanding of the molecular pathways involved in the 4-VO model [96].

Several recent studies have provided valuable insights into cardiac arrest-induced global cerebral ischemia model. Stetler et al. in 2020 [103] investigated the involvement of long non-coding RNAs (lncRNAs) in the pathogenesis of global cerebral ischemia using a rat cardiac arrest model. They found that lncRNAs play a crucial role in neuronal death and the inflammatory response after global cerebral ischemia. This study highlights the potential of lncRNAs as therapeutic targets in global cerebral ischemia. Furthermore, animal models of cardiac arrest have been utilized to test various intervention strategies aimed at reducing ischemic injury, including therapeutic hypothermia [65] and stem cell-based therapies [104].

Advancements in area of focal ischemia models include the use of microsurgical techniques to create precise, reproducible occlusions in specific brain regions. Advancements in MCAO models include the use of novel techniques such as laser or photothrombotic MCAO (pt-MCAO), which allow for more precise control of the occlusion site and duration [105].

Advances in neuroimaging techniques have greatly advanced the field of cerebral ischemia research. These advancements continue to contribute to the field of cerebrovascular research and have the potential to improve patient outcomes in the future. The field of in vivo modelling of cerebral ischemia is constantly evolving, and there are several promising emerging technologies that can address the current challenges. Advancements in imaging techniques such as MRI, CT, and PET have enabled the development of imaging-guided models of cerebral ischemia. These models allow for real-time monitoring of cerebral blood flow, infarct volume, and other biomarkers, aiding in the evaluation of therapeutic interventions [61,106,107]. These techniques allow non-invasive evaluation of brain structure and function, providing valuable information on the extent and location of ischemic damage.

One significant advancement in experimental models of cerebral ischemia is the improved reproducibility and standardization of the models. Researchers have developed standardized protocols and guidelines for inducing cerebral ischemia, ensuring consistency across experiments and allowing for better comparison between different studies.

Another important advancement is the incorporation of comorbidities and risk factors into the experimental models. Cerebral ischemia often occurs in individuals with certain comorbid conditions, such as hypertension, diabetes, or obesity. It has been developed models that mimic these conditions to study the effects of cerebral ischemia in a more realistic and clinically relevant context [2,44].

Additionally, the use of stem cells, gene-editing techniques, and organ-on-a-chip technology show promise for more accurate modelling and potential therapeutic interventions.

Furthermore, computational modelling has seen considerable progress in recent years. Computational models can simulate the complex physiological processes occurring in the brain during ischemia and help researchers understand the interplay between different factors [108]. These models provide a platform for testing hypothetical scenarios and predicting the outcomes of potential treatments, which can guide experimental studies and improve the development of therapeutic strategies.

Overall, these advancements in in vivo experimental models of cerebral ischemia have contributed to a better understanding of the disease, its underlying mechanisms, and potential therapeutic interventions. They have also facilitated the translation of basic science findings to the clinic, ultimately improving patient outcomes.

Challenges and future directions for in vivo models of cerebral ischemia

There are several challenges and future directions in the development and use of in vivo models of cerebral ischemia.

One challenge is the heterogeneity of the ischemic insult and the resulting brain damage. Cerebral ischemia can occur through various mechanisms, including thrombotic or embolic occlusion of blood vessels or systemic hypoperfusion [2,100]. These different mechanisms can lead to varying degrees and patterns of brain damage. Therefore, it is essential to develop models that accurately mimic the specific pathophysiological processes of interest.

The biochemical and behavioral differences observed between left- and right-hemispheric cerebral ischemia suggest that the side of ischemia can be selected according to the experimental purpose - left for motor dysfunction and right for memory impairment. Hence, the choice of left-sided vs. right-sided ischemia cannot be ignored in the generation of animal models. Additionally, patients with left-hemispheric ischemia have a shorter time to hospital admission and a higher incidence, and this difference is positively correlated with the age of the patients and negatively correlated with the severity of the symptoms [109]. The uneven distribution of patients with clinical left- and right-hemisphere ischemic stroke suggests that preclinical studies can preferentially select the left-hemisphere ischemic animal model to promote the clinical translation of the results. The diagnosis insufficiency of patients with right-sided cerebral ischemia is a reminder that researchers should pay increased attention to the right-sided ischemia model. In addition, there are very few studies on the differences between left- and right-sided cerebral ischemia [110].

One of the major challenges in using animal models of cerebral ischemia is the difficulty in translating the findings from these models to human clinical trials. The challenge is the difficulty in recapitulating the complexity of the human brain and there can be species-specific differences in the response to ischemic insults. Animal models, such as rodents, are typically used due to their size and availability, but their brains differ in terms of anatomy and function from the human brain. While animal models provide valuable insights into the mechanisms underlying ischemic injury and potential therapeutic interventions, there are often significant differences between animal models and human patients in terms of disease pathology, genetic factors, and drug metabolism. Bridging this translational gap is essential to ensure the effectiveness of therapeutic interventions in human patients. Therefore, it is crucial to consider the limitations of animal models and develop strategies to translate findings from animals to humans. In vivo experimental cerebral ischemia models that perfectly mimic human stroke are still not available, and the translation of successful treatments from animal models to human patients is not always straightforward.

Another challenge in *in vivo* models of cerebral ischemia is the lack of inclusion of sex and age-related differences. There is growing evidence that sex and age can significantly influence the outcome and response to treatment in cerebral ischemia [111]. However, many animal models fail to consider these differences, leading to a limited understanding of the disease mechanisms and inadequate evaluation of potential therapies. There is emerging evidence suggesting that there may be sex differences in the susceptibility to and outcomes of cerebral ischemia. Several preclinical studies have reported differences in brain damage, neuroinflammation, and neuroprotective responses between male and female animals exposed to ischemic insult [112,113]. Investigating these sex differences may lead to the development of sex-specific therapeutic strategies for the prevention and treatment of global cerebral ischemia. Additionally, understanding the underlying mechanisms of these differences could provide insight into the neuroprotective mechanisms that are more effective in one sex compared to the other, and ultimately improve patient outcomes. It is crucial to include both male and female animals, as well as different age groups, in order to capture the true complexity of cerebral ischemia and effectively develop targeted therapeutic strategies.

Furthermore, it is important to consider the ethical issues associated with using animals in research. Researchers should strive to minimize the number of animals used and refine experimental protocols to reduce animal suffering.

Additionally, there is a need for improved methods to assess brain damage and functional outcomes in animal models. Current methods, such as histological examination and behavioral tests, provide limited information about the extent and mechanisms of brain damage. Developing advanced imaging techniques and biomarkers that can accurately measure brain damage and monitor recovery will enhance the utility of in vivo models.

Collaboration among researchers and institutions is essential to overcome the challenges in in vivo modelling of cerebral ischemia. By pooling resources, expertise, and data, researchers can improve the design and standardization of animal models, as well as facilitate the translation of findings into clinical trials. Data sharing initiatives, such as open-access repositories and collaborative research networks, can facilitate the dissemination and exchange of knowledge, leading to faster progress in the field.

Future directions in the field include the development of more specific and targeted therapeutic interventions. Currently, treatments for cerebral ischemia primarily focus on reducing overall brain damage and improving general outcomes. However, with advances in our understanding of the cellular and molecular mechanisms of ischemic injury, there is a need for targeted therapies that can modulate specific pathways involved in brain damage.

On the other hand, non-pharmacological treatments such as neurostimulation, rehabilitation therapies, and lifestyle modifications may also have a significant impact on functional

recovery after cerebral ischemia [79]. Future studies could explore the efficacy of these non-pharmacological interventions and their potential combination with pharmacological treatments.

Translational potential and clinical relevance

In vivo models of cerebral ischemia have significant translational potential and clinical relevance by providing a valuable platform for studying the complex mechanisms of cerebral ischemia and testing potential interventions. However, careful interpretation and further validation of findings in human subjects are necessary to ensure successful translation into clinical practice.

One of the main objectives of in vivo models of cerebral ischemia is to bridge the gap between preclinical studies and clinical trials. Animal models play a critical role in providing insights into disease mechanisms, discovering potential therapeutic targets, and evaluating the efficacy of novel interventions. The translational potential lies in successfully translating these findings into human clinical trials.

Incorporating patient-derived cells and genetic modifications in animal models can provide a more personalized and clinically relevant approach. However, bridging this gap is challenging and requires careful consideration of multiple factors, including appropriate dose translation, understanding species differences, and aligning experimental protocols. Future studies should focus on optimizing the translational process and conducting well-designed clinical trials to assess the safety and efficacy of promising interventions.

In vivo models have highlighted the heterogeneity of cerebral ischemia, with variations in the location, severity, and temporal dynamics of the ischemic insult. Future approaches could focus on developing personalized treatment strategies based on each patient's specific characteristics and needs.

Additionally, the development of humanized animal models or in vitro models using patient-derived cells holds promise for better mimicry of the human condition.

Conclusion

Despite the challenges and limitations, in vivo models of cerebral ischemia continue to be crucial for advancing our understanding stroke and developing therapeutic strategies.

Future directions involve a multidisciplinary approach that integrates advances in neuroscience, pharmacology, genetics, and technology. The translational potential lies in successfully translating these findings into human clinical trials. That would facilitate the translation of basic science findings to the clinic, ultimately improving patient outcomes.

Overall, ongoing advancements in technology and research methodologies provide exciting opportunities for enhancing the clinical relevance of in vivo cerebral ischemia models.

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