Ischemic Stroke: Pathophysiology and Evolving Treatment Approaches

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ABSTRACT: Stroke remains a leading cause of mortality and disability, with ischemic stroke being the most common type. It occurs due to reduced cerebral blood flow, leading to a cascade of events initiated by oxygen and nutrient deprivation, triggering excitotoxicity, oxidative stress, and inflammation and finally culminating in neuronal injury and death. Key molecular players in ischemic stroke include glutamate receptors, acid-sensing ion channels, and purinergic receptors, exacerbating cellular damage through calcium influx, oxidative stress, and mitochondrial dysfunction. Understanding these mechanisms has shaped therapeutic strategies, such as neuroprotective agents and stem cell therapies. Current treatments such as tissue plasminogen activator (tPA) emphasize timely intervention, yet challenges persist in patient-specific variability and accessibility. This review provides an overview of ischemic stroke pathophysiology, emphasizing cellular responses to ischemia and current and future therapeutic approaches including stem cell therapies aimed at mitigating stroke-induced disabilities and improving long-term outcomes.

KEYWORDS: Ischemic stroke, ASIC, NMDA, ion channels, stroke therapy

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Introduction

One of the leading causes of disability in young adults is mediated by stroke. According to the World Health Organization (WHO), 15 million people get affected by this condition every year. Around 5 million people die while another 5 million are left with a permanent disability. According to Global Burden of Diseases, there has been a 26% increase in global stroke deaths in the last 2 decades, the number rising from 4.66 million in 1990 to 5.87 million in 2010.[1] Stroke, previously thought to be a disease affecting middle-aged and elderly patients, showed a remarkable shift in the trend, with a rise in stroke incidence in younger adults (18-44 years) according to a National Inpatient Sample (NIS) study in 2012. This NIS trend continues to remain the same demonstrating the rise in stroke-induced death and disability in young adults in 2017.[2]

Stroke can be broadly categorized into 2 types—Haemorrhagic stroke (10%-20% incidence) and ischemic stroke (80-90%). Around 8-% to 15% of people are affected in the United States of America, the United Kingdom, and Australia. The incidence is around 12% to 15% of cases per 100 000 per year. In Asia (15%-40%), the incidence rate of haemorrhagic stroke is higher than the developed nations (15%-20%).[3] Neuroimaging studies have revealed that the range of the ratio of infarction to hemorrhage is 1.86:1 to 2.21-14.4 in India which is higher than the average of 5:1 in western countries.[3,4]

Haemorrhagic stroke is induced by a rupture of a blood vessel leading to the accumulation of clots in the brain either in the intracerebral space (ICH; Intracranial hemorrhage) or into the subarachnoid space (SAH; Subarachnoid hemorrhage).[5-8] Stroke induced in this type have a higher morbidity rate[4,6] affecting more of the male population with a higher age group. The risk factors associated with haemorrhagic stroke include diabetes, hypertension, cardiovascular infection, smoking, dyslipidemia, and age.[9-14] Ischemic stroke is caused when there is less or no supply of blood and oxygen to the brain.[15] This generally happens due to embolism, where a blood clot inside the blood vessel forms elsewhere in the body and then travels to the brain resulting in a reduced supply of nutrients and oxygen to the brain.[15,16] Ischemic stroke also occurs when there is a narrowing of the blood vessels due to atherosclerosis.[15,17] Ischemic stroke is more prevalent than haemorrhagic stroke according to a study conducted.[18]

Presently, the existing reviews predominantly delve into either the intricate pathophysiology underlying ischemic stroke or the diverse treatment strategies employed in clinical settings. Recognizing this gap, my comprehensive review aims to harmoniously connect these crucial aspects. By elucidating the pathophysiological mechanisms central to ischemic stroke and identifying the pivotal ion channels involved, this review underscores their crucial role as therapeutic targets. Moreover, it endeavors to provide a cohesive narrative that integrates the complexities of pathophysiology with the targeted treatment strategies currently under exploration. Through this review, a complete perspective can be realized that not only enhances our understanding of the disease but also underscores the potential for targeted therapies to improve clinical outcomes in ischemic stroke management.

Ischemic Stroke: Cellular Pathology

Different physiological pathways encompassing several molecules are involved in the pathology of ischemic stroke. Stroke

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begins when a certain region of the brain called the "core" does not receive oxygen or nutrients for a time of 4 minutes.[19,20] This results in cellular acidosis, energy (ATP) depletion and disruption in ion homeostasis, excess intracellular calcium levels, free radical-mediated toxicity, generation of cytokines and arachidonic acid products, activation of glial cells, disruption of blood brain barrier and leukocyte infiltration, finally culminating in neuronal death.[21-23] The region surrounding this core is called the penumbra which is composed of cells with reduced/dysregulated electrical failure and potassium release. This region, although, is delicately time-sensitive, is also a region of medical interest and is highly salvageable in focal stroke. It is indeed a promising target in stroke scenarios as evidenced in clinical trials which highlighted that the success of treatment is higher in patients having a small infarct core and a larger penumbral tissue when compared to a bigger infarct size.[24,25]

So, what happens when there is a deficit of oxygen supply? Loss of oxygen and/or nutrients even for a few minutes can lead to cellular stress, dysregulated calcium levels and finally neuronal death.[21,23,26-28] Both apoptotic and necrotic pathways are believed to play a crucial role in ischemia-induced neuronal cell death. A drop in nutrient and oxygen levels in the brain leads to anaerobic glycolysis. Pyruvate gets converted to lactate with the release of a proton (H^+). This in turn reduces the cellular pH. At the same time, there is a significant rise in pCO_2, thereby aggravating this situation and creating lactic acid inside the cells leading to acidosis[29-33] (Figure 1).

Simultaneously, decreased ATP causes a dysfunction of ATP-dependent ion pumps in the ischemic core. One long-term question that prevails is what causes this decreased ATP and thereby dysfunction of ATP-dependent ion pumps? When the cells are reacting to this cellular insult of low oxygen levels, there is a consistent rise in cytosolic free Ca^{2+} ion and Na^+ ion concentration due to the release of excess glutamate from the neuronal cells.[34,35] A rise in Na^+ levels is detrimental to neuronal cells, however, more research points toward Ca^{2+} as one of the contributing factors in cellular death following ischemia.[21] The rise in (Ca^{2+}) activates calpains, caspase, and nitric oxide, free radicals and arachidonic acid metabolite-producing enzymes, which on one hand cleaves the protein and causes plasma membrane rupture and on the other hand, activates pro-apoptotic protein BID. The combined increase of (Ca^{2+}) and reactive oxygen species (ROS) activates the inner mitochondrial large conductance channel, MPTP (mitochondrial membrane transition pore). Loss of ATP and mitochondrial function happens with the opening of MPTP (Figure 1). The mitochondria swell up, cytochrome C is released, and apoptotic and necrotic pathways get activated resulting in cellular death.[35-39] The dying cells in the core and the penumbral region release signals that play a crucial role in post-ischemic inflammation.[34,35] Inflammation is a crucial component in the prevalence of ischemia and reperfusion-mediated neuronal damage. With the onset of hypoxia, changes in shear stress and ROS production, complement factors, platelets and endothelial cells get activated ensuring the beginning of the coagulation cascade.[35,40-43] Minutes after the ischemic attack, fibrin and P-selectin are translocated to the platelet and endothelial cell surface and trigger proinflammatory signaling molecules like adhesion molecules (ICAM1, VCAM1), chemokines (IL-1, IL-6, IL-10, IL-17, IL-20, TNF), matrix metalloproteinases (MMP2, MMP8, MMP9), prostaglandins and other small molecules.[44-46] Simultaneously, the production of nitric oxide (NO), a vasodilator is reduced, leading to further accumulation of intravascular clotting, thereby aggravating ischemic insult.[47] The combined effect of oxidative stress as well as inflammation cascade enhances the blood brain barrier permeability. Proteases get released, junctional proteins that seal endothelial cells are downregulated and proteins and cells are extravasated through a paracellular route.[42,48] Ischemia/Reperfusion also activates mast cells and macrophages which in turn releases histamine, proteases and pro-inflammatory cytokines, thus further damaging the BBB[49,50] promoting the infiltration of leukocytes.

Role of Ion Channels in Stroke

As mentioned in the previous section, excessive glutamate release is associated with neuronal excitotoxicity. Few ion channels in the postsynaptic neuron are overstimulated like *N*-methyl-*D*-aspartate (NMDA) and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA) receptors which mediate cationic inward currents as a response to glutamate release by pre-synaptic neurons or reversal of uptake mechanism in astrocytes.[51-56] Opening of the NMDA receptor leads to an influx of sodium ions (Na+) and calcium ions (Ca2+). Influx of Na+ causes membrane depolarization while that of Ca2+ leads to the subsequent pathophysiological effects as discussed in the previous paragraph. AMPA receptors also contribute to neurotoxicity indirectly through membrane depolarizations removing just enough Mg2+ block and facilitating the Ca2+ channel activity.[57,58] The synergistic effect of ATP depletion and glutamate release activates another set of ion channels called the purinergic or P2 receptors. Prolonged activation can transform the cation permeable small pore to a nonspecific bigger pore.[59,60] Panx1 is another protein associated with P2X7 receptors and during ischemia NMDA receptor[61] activated Panx1 allows the rapid efflux of ATP into the extracellular milieu causing cytotoxicity which kills otherwise healthy or non-dying cells to succumb to cell death.[62,63] The transient receptor potential (TRP) channels are a tetrameric cation permeable channel associated with various signaling processes associated with Ca2+ influx.[64-66] TRP2 and TRP7 have been associated with neuronal death. TRMP2 gets activated by ROS, NO and adenine 5′-diphosphoribose (ADPR), while TRPM7 is activated by peroxynitrite, free radicals and change in extracellular pH during incidence of stroke. TRP7 produces a huge outward current in OGD (oxygen glucose deprivation) condition mimicking

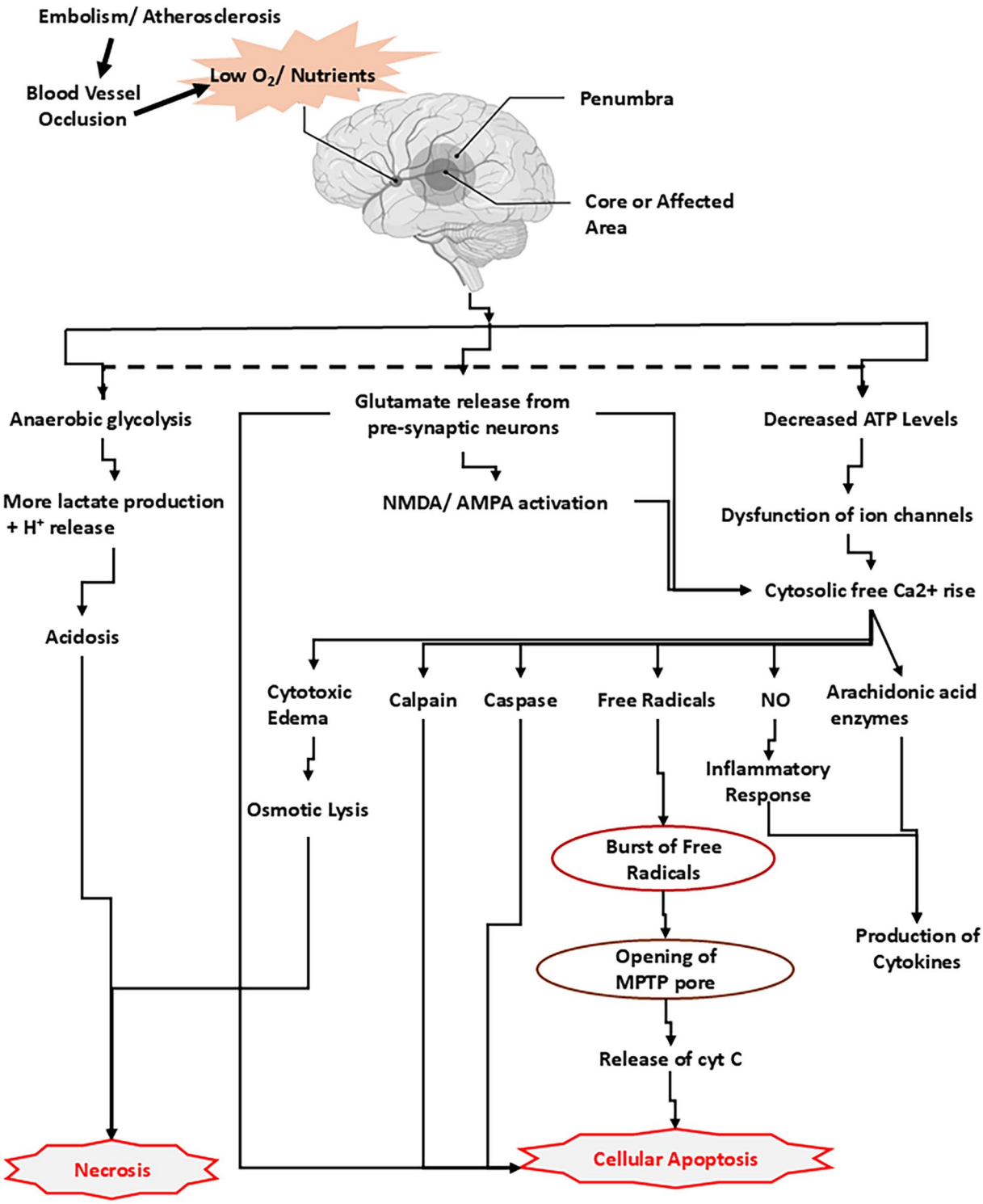

Figure 1. Schematic representation of the biological events in ischemic stroke. The figure explains the events happening during an ischemic stroke finally culminating in cellular death (apoptosis or necrosis).

ischemic situation which is detrimental to the survival of the neurons.[67] TRP2 induces neuronal cell death by aiding in the uptake of Ca2+ which is induced due to oxidative stress or TNFα.[68] Another group of ion channels called acid-sensing ion channels (ASIC) are activated at a low pH caused due to ischemic stress. ASICs lead to Na+, Ca2+ influx, thereby dysregulating the ionic balance.[69,70]

Treatment: Current and Possible Future

We have already stated that stroke pathophysiology is a complex process with many key players contributing to the severity of the outcome. Similarly, stroke recovery therapeutics also include different intervention methods and treatment targets. Personalized medicines also offer a promising future that considers inter-patient differences like infarct location, stroke

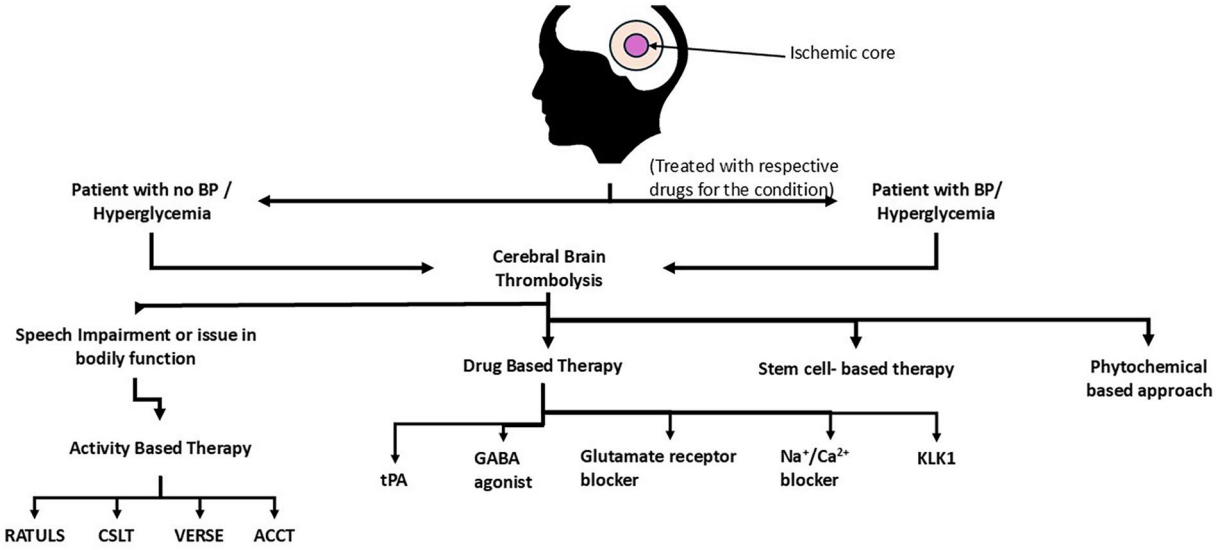

Figure 2. The three different types of stroke treatment strategies. The figure explains the patient care and the different types of therapeutic strategies received after the onset of the disease.

severity as well as co-morbidity factors like diabetes, hypertension, and previous stroke incidence records. As a primary step toward the standard of care, patients with high blood pressure (value > 185/105 mm Hg) or a higher hyperglycemia (value > 155 mg/dl of glucose) are treated with the same. Another target is the administration of anticoagulants and antiplatelets to patients suffering from cerebral brain thrombosis unless there is a contraindication of anticoagulants.[71-73] Several clinical trials are conducted every day to re-establish neuronal as well as motor function ability in patients post-ischemic stroke. Many drugs and different cell-based approaches have been established with varying success rates in vitro, in vivo as well as clinical trials. Stroke therapeutics are therefore, broadly categorized into 3 types based on their mode of action—activity-based, drug therapies and cell therapies as described in Figure 2. Tables 1 lists out some of the drugs used to target specific proteins in alleviating stroke symptoms.

The activity-based therapies are intended for patients who have lost partial or total impairment of speech as well as motor bodily functions as a result of ischemic stroke. Clinical trials such as Robot-assisted training for the upper limb after stroke (RATULS),[74] home-based speech and language therapy (CSLT),[75] Very Early Rehabilitation for Speech (VERSE),[76] Adaptive conjunctive cognitive training (ACCT)[77] are some of the activity-based therapies intended to improve patient conditions. Robot-assisted training or RATULS enables a patient to use their upper arms to perform repetitive movements in order to perform their daily tasks. Patients suffering from speech difficulty are categorized based on aphasia severity and were enrolled 14 days post-stroke in VERSE clinical trial.

Tissue plasminogen activator, or tPA, is a group of drug-based therapeutics that are to date most widely used along with surgery. However, if the administration of this drug gets delayed from the onset of ischemia, then this drug is not

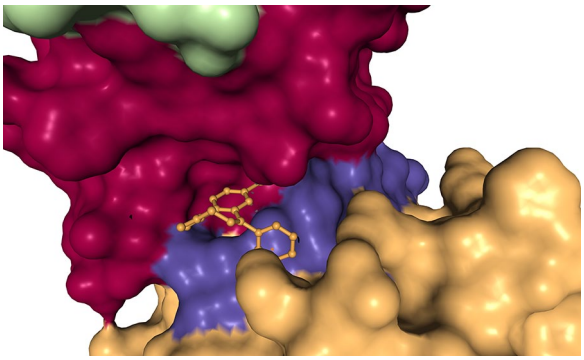

Figure 3. Cavity-detection guided Blind Docking study of GABA receptor agonist (diazepam) and GABA receptor molecule. The GABA structure (pdb format) is selected and docked blindly with Diazepam known structure and then with the CB-dock software were analyzed together. The final image represents the drug (ball and stick structure, yellow) nicely binding with the GABA receptor. The Vina score of the interaction is –6.7 which represents the most favorable binding affinity between the drug diazepam and 2 chains of the GABA molecule.

recommended.[78,79] GABA or gamma amino butyric acid receptor agonists (diazepam and clomethiazole; Figure 3) have shown huge promise in stroke models. Glutamate receptor blockers are another set of drugs that are used to target N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptors. These ion channels are mainly responsible for excitotoxicity and blocking these has not only paved the way for a treatment process but also proved to be beneficial with fewer side effects.[15,80-82] Sodium and Calcium channel blockers are used extensively in mice models, however, not all these blockers are successful. While Amiodarone is unsuccessful since it elicits aggravating brain injuries, Amlodipine has shown a 13.5% reduction in stroke risk factors (PMID: 20227347). Fibrinogen depleting agents, obtained from pit viper venom, are used to decrease blood viscosity and increase

Table 1. Drugs that target a few cellular signaling pathways that are involved in the pathophysiology of stroke.

DRUG/THERAPY USED	SIGNALING PATHWAYS
GluN2BCT1292-1304	DAPK1/GluN2BPSD95-nNOS
Geniposide	GluN2A/AKT/ERK
Genipin	UCP2-SIRT3
Resveratrol	TLR4/NF-Kb/STAT3
Stevioside	TLR/NF-kB
Dexmedetomidine	HMGB1/TLR4/NF-kB
Tak242	TLR4
Isoquercetin (Figure 4)	
Quercetin	Sirt
Patchouli alcohol	MAPK
DL-3n-butylphthalide (NBP)	MAPK/AQP4/MMP9
Selenium	PI3K/Mtor/Akt
DHL	
Melatonin	PI3K-Akt
Diosgenin	STAT2/HIKESHI
Stem cell-secreted vesicles	STAT3
MTMR14	PTEN
Sevoflurane	PTEN/AKT1/Mtorc1
Remote ischemic preconditioning	PTEN/AKT1
Electropuncture	Wnt
SMXZF	AMPK-mTOR
Puerarin	AMPK/Mtorc/ULK1
BML-275	AMPK
Glycine	AMPK/GSK-3β/HO-1
CTRP3	AMPK/SIRT1-PGC-1α
Rosuvastatin	Sirt1/NF-kB
Stem cell therapy	SIRT-NFkB
	MiRNA-29b/SIRT1/PGC-1

flow, are another kind of drug. The clinical trial, although, not successful yet, shows a promising direction for the future to remove clots from blood vessels. The most recent drug that shows potential is DM199, a recombinant protein of human tissue kallikrein-1 (KLK1). The modus operandi of this drug is to improve blood flow in capillaries and form new capillaries over time with better tolerance in phase I and II clinical trials.[83] (NCT03290560).

Stem cell therapy is a reliable and effective method of stroke treatment. The degree of effectiveness depends on the type of stem cells administered. Neural stem cells when administered can generate all types of glial cells and neurons.[84] Quite similar to neural stem cells, hematopoietic stem cells are also gaining the limelight in stroke treatment and are often utilized in neurogenesis investigations.[84,85] The last category of stem cells are human umbilical cord-derived mesenchymal stem cells which have been used in many experiments and have been found to be of therapeutic efficacy.[86]

Another group of compounds that are gaining importance is phytochemicals. Phytochemicals are secondary metabolites produced by plants and have potential anti-inflammatory and anti-oxidative properties. This group includes various phenolic compounds, terpenoids and alkaloids. Many of these phytochemicals have the ability to target more than one molecule or pathway and therefore are considered by many to be more beneficial in treating multifactorial disease conditions like stroke.[87]

Treatment Strategies in Ischemic Stroke

According to the National Health Service (NHS),[88] UK or Indian Medical Association, India,[89] the present treatment plan for ischemic stroke patients includes the intravenous administration of clot-busting or thrombolytic medicines like alteplase which helps in the reduction or dissolving of blood clots and thereby restoring circulation. Alteplase, a recombinant tissue plasminogen activator, increases plasmin activity and thereby dissolves blood clots by hyperfibrinolysis.[90-92] However, as mentioned before, tPAs like alteplase should be given to patients as soon as possible to the occurrence of the stroke. Medical associations around the world agree that before treating patients with tPAs, medical practitioners must confirm the occurrence of stroke either by conducting blood tests (a time-consuming process) or by carrying out imaging tests like CT-scan, MR-angiography, or diffusion weighted-MRI.[92] This is to ensure that the patient is truly suffering from ischemic stroke and not from haemorrhagic stroke. In a few cases, if the clot is in the bigger arteries, a thrombectomy is carried out. A small catheter is inserted into the artery attached with a device and then it is guided through the arterial system till it reaches the region of blockage and is either dissolved or suctioned out. Patients are also given aspirin, clopidogrel and dipyridamole. Aspirin or salicylic acid is a non-steroidal anti-inflammatory drug that acetylates serine residues of cyclo-oxygenases and hinders the production of prostaglandins, platelet aggregations and inflammation, thereby acting as anticoagulant drug.[93-97] Clopidogrel is another drug widely used to treat stroke patients as an alternative to aspirin. Clopidogrel binds to the platelet receptor for adenosine diphosphate (ADP) and irreversibly alters it, thereby inhibiting the ADP-mediated activation of glycoprotein complex GPIIb/IIIa and subsequently the platelet adhesion and aggregation.[98,99] According to medical professionals, clopidogrel, in

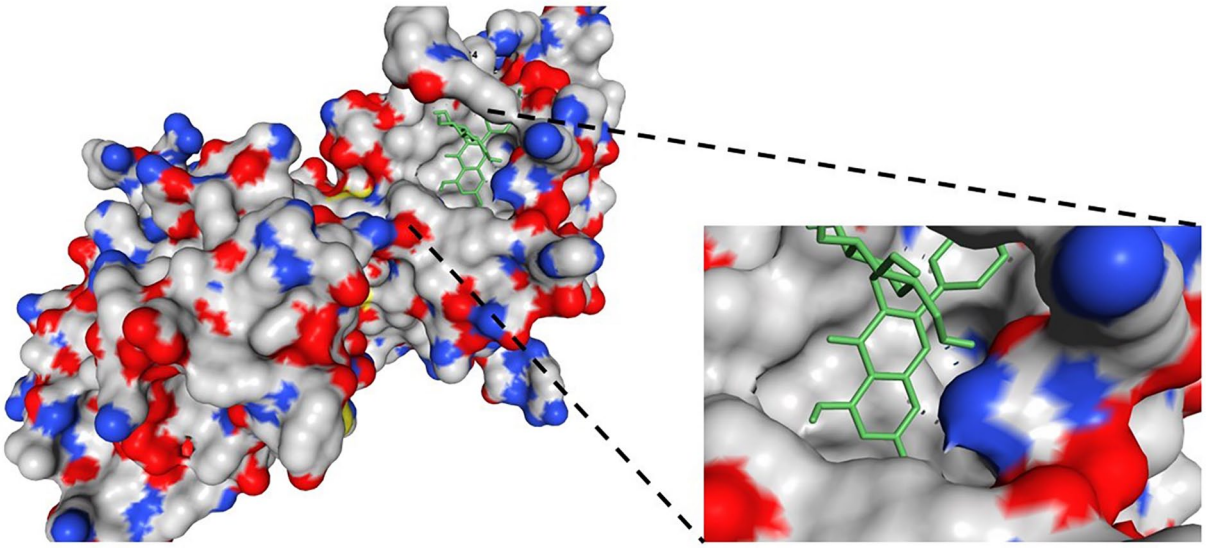

Figure 4. Cavity-detection guided Blind Docking study of TLR4 ligand Isoquercetin and TIR domain of Toll-like receptor 4 (TLR4) molecule. The TIR domain of TLR4 pdf format is tested against the isoquercetin molecule and analyzed using the docking software in a blinded fashion. The final image represents the drug (ball and stick structure, green) strongly bound to the protein having a Vina score of –7.2.

some cases, works better than aspirin. Some studies show that dual administration of aspirin and clopidogrel can prevent recurrent stroke in patients with transient ischemic attack (TIA) or mild stroke.[100,101] The third drug that is also used is dipyridamole which inhibits the uptake of ADP by endothelial cells and platelets. This inhibition results in the accumulation of cAMP (cyclic adenosine monophosphate) and prevents platelet aggregation.[102] Dipyridamole also acts as a vasodilator routinely used in thromboembolism prophylaxis.[103] The combination of aspirin and extended-release dipyridamole is extensively used to treat stroke patients as well as patients suffering from intolerable headaches.[103-105] Other than these, some other anticoagulants are routinely used called heparin (for short-term use) and warfarin, apixaban, dabigatran, edoxaban and rivaroxaban (for long-term used). If plaques are formed in the carotid artery, then carotid endarterectomy is performed to release this plaque and allow free circulation in the carotid artery, thus ensuring, blood flow to the brain will not be interrupted. Craniotomy is performed in some cases when it is an emergency and blood vessels need to be repaired. Blood pressure and cholesterol levels are maintained to keep the prognosis favorable.[88,89,92]

Conclusion

In conclusion, stroke remains a significant global health challenge, causing substantial mortality and long-term disability. The epidemiological landscape of stroke has evolved, with a notable increase in incidence among younger adults, highlighting the need for heightened awareness and preventive strategies across all age groups. The pathophysiology of stroke is complex and varies between ischemic and haemorrhagic types, each presenting distinct mechanisms of neuronal injury and cellular death.

Ischemic stroke, the more prevalent type, results from compromised blood flow to the brain, triggering a cascade of events including excitotoxicity, oxidative stress, and inflammation. These processes ultimately lead to neuronal cell death in the core ischemic region, while the surrounding penumbra represents a critical target for therapeutic intervention due to its potential for salvageable tissue.

The role of ion channels, particularly glutamate receptors, acid sensing ion channels and purinergic receptors, underscores the neuronal vulnerability during ischemia, contributing to the pathogenesis of stroke. Advances in understanding these molecular mechanisms have paved the way for targeted therapeutic approaches, ranging from pharmacological agents to emerging stem cell therapies aimed at restoring neurological function and promoting recovery.

Current treatments, such as tissue plasminogen activator (tPA) and neuroprotective drugs targeting ion channels, have shown efficacy but also highlight the challenges of timely intervention and patient-specific variability. Ongoing research into novel therapeutic modalities, including activity-based therapies and personalized medicine, holds promise for improving outcomes and reducing the burden of stroke-related disabilities.

Existing reviews predominantly explore either the intricate pathophysiology underlying ischemic stroke or the diverse treatment strategies in clinical settings. Recognizing this gap, my comprehensive review has aimed to bridge these critical aspects. By elucidating the central pathophysiological mechanisms of ischemic stroke and identifying key ion channels involved, this review underscores their pivotal role as potential therapeutic targets. In conclusion, while stroke pathophysiology remains multifaceted, ongoing advancements in research and clinical practice offer hope for enhanced treatment

strategies and improved patient outcomes. Addressing the intricate molecular pathways and optimizing therapeutic interventions will be crucial in mitigating the profound impact of stroke on individuals and healthcare systems worldwide.

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