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REVIEW ARTICLE

A Review on Preclinical Models of Ischemic Stroke: Insights Into the Pathomechanisms and New Treatment Strategies

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Abstract: ***Background*:** Stroke is a serious neurovascular problem and the leading cause of disability and death worldwide. The disrupted demand to supply ratio of blood and glucose during cerebral ischemia develops hypoxic shock, and subsequently necrotic neuronal death in the affected regions. Multiple causal factors like age, sex, race, genetics, diet, and lifestyle play an important role in the occurrence as well as progression of post-stroke deleterious events. These biological and environmental factors may be contributed to vasculature variable architecture and abnormal neuronal activity. Since recombinant tissue plasminogen activator is the only clinically effective clot bursting drug, there is a huge unmet medical need for newer therapies for the treatment of stroke. Innumerous therapeutic interventions have shown promise in the experimental models of stroke but failed to translate it into clinical counterparts.

***Methods*:** Original publications regarding pathophysiology, preclinical experimental models, new targets and therapies targeting ischemic stroke have been reviewed since the 1970s.

***Results*:** We highlighted the critical underlying pathophysiological mechanisms of cerebral stroke and preclinical stroke models. We discuss the strengths and caveats of widely used ischemic stroke models, and commented on the potential translational problems. We also describe the new emerging treatment strategies, including stem cell therapy, neurotrophic factors and gut microbiome-based therapy for the management of post-stroke consequences.

***Conclusion*:** There are still many inter-linked pathophysiological alterations with regards to stroke, animal models need not necessarily mimic the same conditions of stroke pathology and newer targets and therapies are the need of the hour in stroke research.

Keywords: Stroke, preclinical models, brain ischemia, therapeutic target, pathophysiology, gut microbiome.

1. INTRODUCTION

Cerebral ischemic stroke is one of the most severe neurovascular disorders [1, 2]. Ischemic stroke is accounting for 80-87% of stroke cases, a disease that primarily curtails the blood flow leading to and within the brain due to occlusion of cerebral blood vessels by a thrombus or an embolus [3, 4]. Ischemia in any part of the brain results in a long-term disability (loss of neurologic function) and high rate of mortality [5-7]. The sudden neurological complications are related to the neuronal death (in core part of ischemia) within a couple of minutes after stroke onset, thus effective therapeutic intervention is immensely required. Importantly, the tissue surrounding the central core (penumbra), where cerebral blood flow (CBF) level falls under a functional threshold, is the salvageable brain region [8]. The restoration of CBF is believed to save the ischemic penumbra brain regions and neuro-substances by either promoting neuroprotective effects or triggering neuroplasticity.

The pathophysiology of ischemic stroke is multifactorial and dependent on several simultaneous alterations in the normal vascular homeostasis, leading to occlusion of the internal carotid artery and other major blood vessels that supply blood to brain regions [9]. These physiological events resulted in deprivation of oxygen and thus necrotic cell death of the neuronal cells. The elevated oxidative stress increases the rupture of weak vasculature and neighboring nerve cells. Ischemic zone in tissue increases the infiltration of immune cells and inflammatory mediators; this physiological change produces a profound debilitating impact after ischemic stroke [10]. The stroke prevalence is more in patients who are having pre-existing cardiovascular issues such as hypertension, diabetes, obesity and abnormal lipid profile [11]. The current review highlights critical underlying pathophysiological mechanisms and different experimental models of cerebral stroke, and also describe new therapeutic strategies for the management of stroke.

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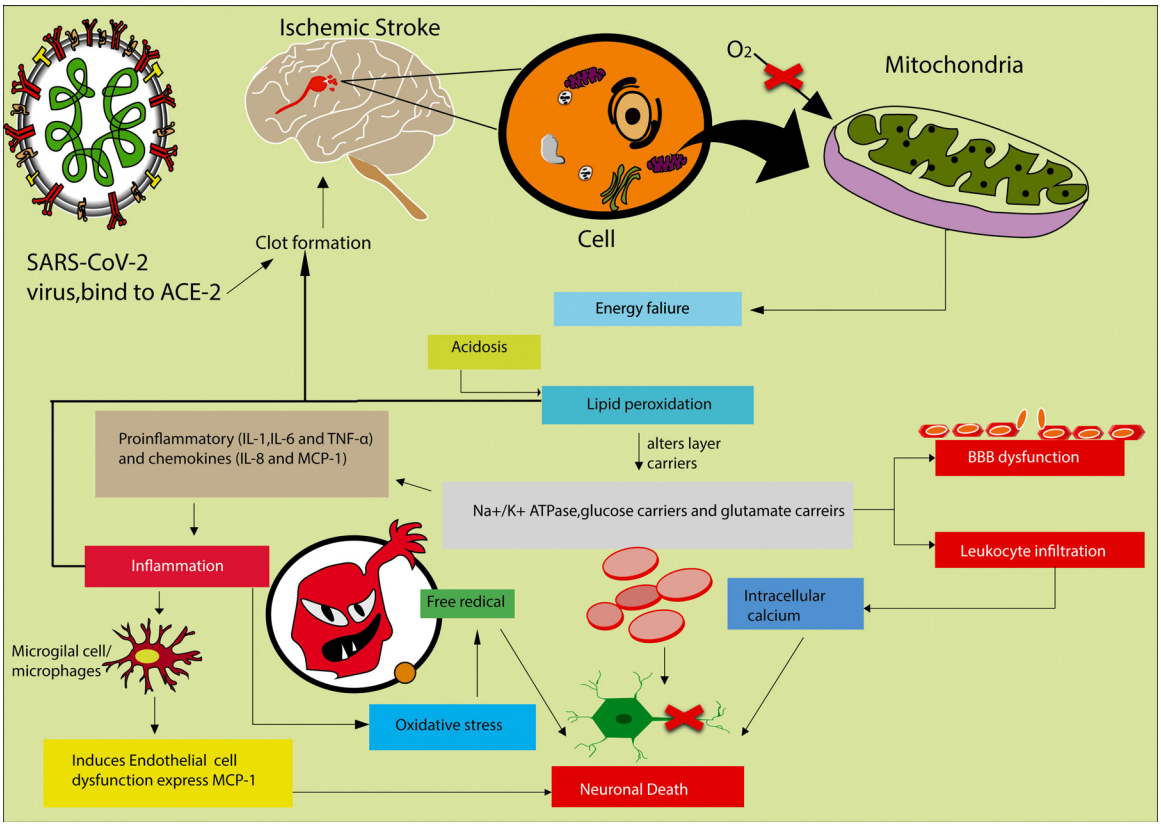

Fig. (1). Pathophysiology of ischemic stroke. Lack of oxygen and glucose supply after ischemic stroke triggers mitochondrial dysfunction, which resulted into the series of downstream reactions including lipid peroxidation, oxidative stress and inflammation that cause imbalances in the cellular homeostasis and neuronal death. SARS-CoV-2 virus also reported to triggers clot formation *via* ACE-2 receptors. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

2. ISCHEMIC STROKE PATHOPHYSIOLOGY

The pathophysiology of stroke is perplexing due to complex changes in the energy metabolism, ions homeostasis, calcium signaling, levels of cytokine, blood-brain barrier (BBB) integrity, inflammation, and glial cells [12]. These are interrelated and coordinated events, which influenced the ischemic-core region that later converted into necrotic cell death [13]. The occurrence of stroke and severity of post-ischemic consequences have been ascribed to several factors as described below and in Fig. (**1**).

2.1. Genetic Aberrations in Stroke

Several studies highlighted the importance of genetic factors in the occurrence of ischemic stroke. A nested case control study reported the involvement of C-reactive protein (CRP) genetic variants in the prevalence of stroke, the gene variants rs1800947 in CRP gene and rs1169288 in the HNF1A gene [14]. A study conducted on the Chinese Han population for genetic risk of ischemic stroke reported polymorphisms in the three KALRN gene; SNP rs7620580, rs2289843 and rs1708303 [15]. TNF-α, an inflammatory marker that is ubiquitously expressed in ischemic stroke patients and can cause hyperinflammatory reaction, a meta-analysis study found that TNF-α 238G/A polymorphism is linked with an increased risk of ischemic stroke [16, 17]. Mammalian homolog of Drosophila diaphanous-1 (DIAPH-1) is an effector in Rho signaling pathway in humans; its effect is varied from vascular remodeling and inducing platelet formation that is implicated in the pathophysiological alterations following stroke episodes [18, 19], case controlled and cohort studies have reported that polymorphism associated with rs7703688 is linked to higher risk of ischemic stroke [20]. Thus, stroke is governed by genetic predisposition, as most of the genes that cause increased susceptibility to stroke are those of the inflammatory markers, which are highly overexpressed; these can cause potential oxidative and inflammatory damage to the regions associated with stroke and can further prove detrimental.

2.2. Influence of Age, Sex and Race on Stroke

Stroke incidence and severity are directly linked with the age and sex of an individual. The mortality rate of aged stroke patients is more irrespective of their sex [21]. The causal relation of hypercholesteremia, vasculature microenvironment and hypertension comorbidities with the occurrence of stroke episodes and severity was also reported in the aged population [22]. The stroke incidence doubled after an individual crosses the age of 55 years [23]. Adults, age group of 18-50 years, contribute 10-15% of all stroke cases [24]. However, the stroke incidences are stepping up in young adults also, which is possibly due to lifestyle alterations. For instance, 1008 young stroke patients in Finland were ascribed to stroke due to a number of vascular problems such as dyslipidemia, smoking and chronic hypertension [25, 26]. Moreover, a study on young adults concluded that the cases of stroke are more with individuals abusing methampheta-

mine, this is possibly due to the hypertensive crisis following the excessive use of the said drug [27].

It has been noticed that males are more susceptible to ischemic stroke, whereas a longer lifespan in females indicates high stroke prevalence [28]. The gender differences in stroke incidence were reported to be related to the immune responses. For instance, elevated levels of IFN-γ and T-cell functions were observed in male as compared to females [29]. The decreased levels of sex hormones the middle aged and menopause women have also been linked to ischemic stroke [30]. The risk of ischemic stroke is higher in the final trimester and early postpartum period of pregnancy [31]. Patent foramen ovale (PFO) related strokes are common in pregnant women. While PFO is of cryptogenic origins, this occurs mainly due to change in the hormonal and hemocoagulative parameters [32]. Females taking non-estrogenic oral contraceptives had lower risk of occurrence of ischemic stroke as compared to females taking high dose estrogen derivatives [33]. Another sex hormone, progesterone, was found to be neuroprotective in females in post-ischemic conditions, the effects may be due to the progesterone metabolite allopregnanolone which positively modulates $GABA_A$ receptors [34]. Post-menopause incidences of stroke episodes seem to increase due to lower levels of circulating estrogen, which offers neuroprotection when in optimum concentrations during the fertile age [35]. It is evident that stroke cases increase in the case of elderly and there is significant neuroprotection in the case of females. This can be a plausible reason in low occurrence of stroke incidences in females. Thus, these findings suggest that both age and sex factors are pertinent in the outcome and occurrence of stroke.

Apart from age and sex of an individual, it has been observed that race and ethnic background also determine the severity and mortality in stroke. Several studies documented that the burden of stroke-related mortalities is higher in black people as compared to the white population. Such a disparity has also been correlated with the higher co-morbidities and risk factors such as hypercholesteremia, high body mass index [BMI] and obesity [36, 37].

2.3. Hyperinflammatory Responses after Stroke

The endothelial cells, astrocytes, microglia, and neurons are known to increase the proinflammatory mediators after an ischemic insult. Reactive astrocytes are one of the hallmarks in the stroke pathology, which cause glial scarring, oxidative stress and inhibition of dendritic and axonal growth [38]. A significant part of neuroinflammation in the penumbral region is limited to microglial activation [39], by producing various proinflammatory cytokines and harmful metabolites. Invasion of macrophages into adipose tissue triggers the release of leptin and inflammatory cytokines, which provokes macrophages activation and disruption of endothelial cell [40]. Thus, considering the pathophysiological significance of microglia and macrophages in stroke, inflammation modulatory therapeutics may be developed [41, 42].

A range of studies indicated the involvement of leukocytes in the aftermath of stroke severity. The leukocytes interfere with reperfusion-initiated tissue injury and microvascular damage by releasing different proteases and causing oxidative stress, along with this, they also cause stiffening of the microvasculature due to occlusion by activated form of leukocytes [43]. Taken together, activation of brain endothelial cells, extravascular CNS cells (astrocytes, microglia, macrophages and neurons), and intravascular cells (platelets and leukocytes) are responsible for brain injury following ischemic stroke [44]. These augmented inflammatory reactions following stroke injury may negatively influence the BBB integrity. These complex alterations thus potentially lead to long-term damage of the CNS resident cells and increased death of the cells due to hyperinflammatory responses, thereby predisposing catastrophic outcomes.

2.4. Lifestyle and Ischemic Stroke

It is evident that sedentary lifestyle and unhealthy diets such as high salts and cholesterol containing foods increase the chances of cardiovascular diseases. The connection of cardiovascular diseases with stroke occurrence has been noted in several meta-analysis studies [45]. Indeed, the incidence of cardiovascular disease and ischemic stroke is remarkably low in the population who consume unprocessed plant-based products, polyphenols and olive oil rich in monounsaturated fatty acids, and the Mediterranean diet [46, 47]. The antioxidant property of these ingredients regulates the levels of inflammatory cytokines [47, 48]. Meta-analysis data indicated that increase intake of vitamin-B and omega-3 fatty acid reduces the incidence of stroke [49]. In contrast, cholesterol-rich foods and increased triglyceride levels provoke the endothelial dysfunction, oxidative stress, and inflammation that subsequently develops atherosclerotic plaques [50]. Smoking also increases the proinflammatory cytokines and platelet aggregation and stroke incidence by 2-4 folds [51, 52].

2.5. Oxidative Stress and Lipid Peroxidation

Several exploratory and clinical observations have indicated the free radical generation following stroke injury [53]. The superoxide formation causes additional tissue damage, and it is believed to be a significant trigger molecule for apoptosis after ischemic stroke [54, 55]. Lipid peroxidation seems to be of profound interest in the pathogenesis of stroke. A portion of these record factors prompts the declaration of provocative cytokines (for instance, IL-1, IL-6, and TNF-α) and chemokines (IL-8 and MCP-1), endothelial cell attachment molecules (selectins, ICAM-1 and VCAM-1), and other proinflammatory qualities (interferon-inducible protein-10] [39].

2.6. COVID-19 and Ischemic Stroke

COVID-19 is a viral respiratory disease that is caused by the severe acute respiratory syndrome-associated coronavirus-2 (SARS-CoV-2) variant, which has recently been declared as a pandemic by the WHO [56]. The level of D-dimer is found to be high in case of COVID-19 infected patients. The implications are increased coagulopathy that leads to thromboembolism causal for the occurrence of ischemic stroke [57, 58]. Along with this, enhanced levels of various proinflammatory cytokines such as IL-6, IL-1β and TNF-α (cytokine storm), which are associated with innate immune responses, increase the incidences of ischemic stroke [59]. The occurrence of stroke is also ascribed to in-

creased levels of both IL-6 and CRP which also led to stroke episodes, possibly by the release of tissue factors (TFs) and hypercoagulability [60]. Presence of antiphospholipid antibodies leads to a prothrombotic state [61]. The altered coagulative factors and endothelial dysfunction is seen in COVID-19 infected individuals [62]. While the putative mechanisms underlying the occurrence of stroke after COVID-19 infection is still in exploratory phase, vascular dysfunction due to depletion of angiotensin converting enzyme (ACE-2) receptors on the cerebral endothelial cells is one of the probable factors that can influence the occurrence of stroke episodes [61].

3. *IN VITRO* AND *IN VIVO* MODELS OF ISCHEMIC STROKE

3.1. *In Vitro* Models

Commonly, monocultures of brain capillary endothelial cells (BCECs) or co-culture of BCECs, astrocytes and glial cells are used, in these assays ischemia is mimicked by oxygen-glucose deprivation (OGD) method [63, 64]. Different cells are used to mimic stroke conditions *in vitro*, for instance, thin brain slices (400 μm) can be used of both human and murine origin where these are perfused with OGD artificial cerebral spinal fluid focally [65, 66]. Organotypic hippocampal slice cultures are also used coupled with OGD, particularly that of CA1 area of the hippocampus which is highly susceptible to stroke [67]. Primary cultures isolated from animals such as glial, pericytes or astrocytic cells are used for stroke studies [68-70].

3.2. *In Vivo* Models

3.2.1. Focal Cerebral Ischemic Models

Focal ischemic rodent models recapitulate several symptoms of human stroke, in which a minimum blood flow is allowed to the central core of the ischemic region *via* the vertebral arteries (Fig. **2**). Majority of focal cerebral ischemia procedures involve a transient or permanent occlusion of the middle cerebral artery (MCA) in rodents and large mammals [71, 72], which is commonly affected vessel in clinical ischemic stroke. A range of MCA occlusion (MCAO) models has been developed in rodents [73, 74]. Herein, some of these MCAO models are described below that are extensively used to produce focal cerebral ischemia in rodents.

3.2.1.1. Middle Cerebral Artery Occlusion (MCAO) Model

The filamentous arterial occlusion technique of MCA has been extensively used to study the pathophysiology of stroke and evaluation of new investigating molecules. As depicted in Fig. (**2**), this method can be employed for both permanent and transient focal cerebral ischemia. Koizumi *et al*. [75] and Longa *et al*. [76] have conducted pioneering studies using this method in rats. The procedure involves insertion of a filament from the external carotid artery (ECA) or carotid artery into the internal carotid artery (ICA) up to the branch of MCA to block the blood flow for 30-120 minutes, and then reperfusion can be achieved by retracting filament [76, 77]. It has been documented that blockade of MCA severely affects its innervated brain structures such as striatum, hippocampus, and cortex in the form of neuronal cell death by exacerbating the inflammatory response during reperfusion. While rats and mice are the most preferred species for this procedure, it can also be implemented on larger animals like primates using balloon catheters [78]. The pros and cons associated with this model are enlisted in Table **1**. The noteworthy part of the intraluminal filament occlusion model is that reperfusion (ischemic time) can be timely decided and thus this model plays an integral role in studies requiring reperfusion. Among the ischemic stroke models, the transient MCAO is the most popular due to its minimal invasive nature, absence of craniectomy, ease of performance and reproducible territory infarct volume. However, some pitfalls have also been associated to this procedure such as (i) it does not recapitulate the root cause of ischemia *i.e.*, thromboembolism [79], (ii) inadequate filament insertion till the opening of MCA or dislodged after insertion may lead to partial ischemia [80-82], (iii) may produce inadvertent subarachnoid hemorrhage (SAH) [76, 80, 83], (iv) involves instant blood surge during reperfusion in contrast to gradual recanalization of occluded vessel in stroke individuals [79], (v) a high rate of variability with neuroanatomical location and size of the lesion reported across animal species/strains [82, 84-87], (vi) chances of the intraluminal thrombus formation [75, 88], and (vii) intra-ischemic and post-ischemic hyperthermia may also occur [89]. Some of the procedural limitations can be avoided by inclusion of Laser Doppler Flowmetry (LDF) and using suitable size, shape and length of filament [81, 82, 84-86, 90-92]. Moreover, live imaging techniques such as magnetic resonance imaging (MRI) can be employed during surgery. Although inclusion of MRI during MCAO surgery becomes costlier to experimenter, the successful stroke and hemorrhagic complications in rats was 88% and 6% *vs*. 71% and 26%, respectively without MRI [93]. Vascular variability may be circumvented using suitable animal strain. For instance, Howells *et al*. [94] suggested that Wistar-Kyoto could be preferred for rat ischemic stroke models. Recently, a new way of mimicking the aged phenotype in young mice has been suggested by Spychala *et al*. [95]. They reported that fecal microbiota transplantation of aged mice into young generates the aged animal phenotypes. They also noted increased mortality, decreased performance in behavioral testing, and increased levels of cytokines following MCAO. It has been suggested that the unwanted effects of rapid blood reperfusion may be minimized using the gradual flow restoration in MCAO rats [96]. Long-term neurological deficits/recovery may be evaluated using several behavioral tests as described in Table **2** [97-99].

3.2.1.2. Internal Carotid Artery Occlusion (ICAO) Model

This is an alternative method of MCA occlusion, which bypasses the ligation of ECA. The major strength of ICAO model is that it may avoid the unwanted neurological deficits such as impeded food intake and weight loss seen after MCAO surgery due to disruption of the blood supply to mastication muscles [100]. Similar to MCAO procedure, the ICAO involves the occlusion of the ICA for approximately 90 min followed by reperfusion. This model generates mild to moderate lesion in the striatum that resembles human stroke pathology [101]. It is anticipated that this model is more robust and confer more similarity to human stroke episodes due to precise targeting of the ICA.

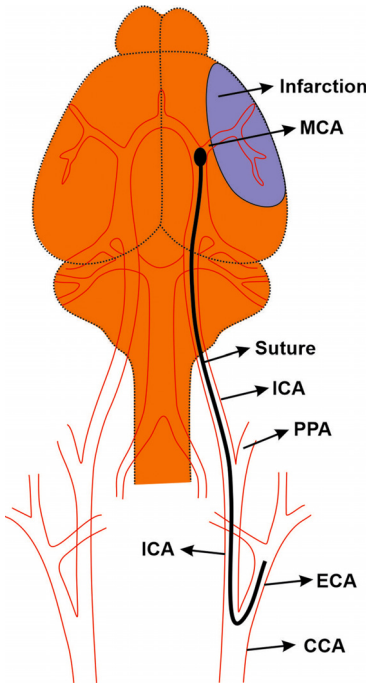

Fig. (2). Schematic representation of MCAO procedure. The procedure involves insertion of a filament from the ECA into the upto the branch of MCA to block the blood flow, and then reperfusion by retracting filament: Abbreviations: CCA: Common Carotid Artery; ECA: External Carotid Artery; ICA: Internal Carotid Artery; PPA: Pterygopalatine Artery.

3.2.1.3. Embolic Model

Since ischemic strokes in humans are mostly caused by thromboembolism [102, 103], animal stroke models recapitulating the clinical thromboembolism conditions are considered relevant [104]. These models are useful for assessing neuroprotective activity and re-canalization (thrombolytic) therapy after ischemic stroke [105, 106]. In embolic paradigms, emboli (such as thrombotic clots, microspheres and photothrombotic) of different sizes and quantity are injected to interrupt the CBF of targeted arteries in mice, rats, rabbits, pigs, and dogs [107-110]. These animal models are suitable to detect the additive or synergistic effects of new treatments with thrombolytic drugs (like rt-PA) or to screen new thrombolytic agents.

3.2.1.4. Thrombotic Model

In thrombotic focal cerebral ischemic model, the MCA is selectively occluded by introducing a thromboembolic clot made up of autologous blood [111, 112] or a clot of human blood [113, 114]. This thromboembolism model has higher relevance to human stroke [115]. While decreased CBF in the ipsilateral MCA territory for at least 2 h indicates successful modelling, multiple fibrin-rich allogeneic clots are also injected simultaneously into the ECA to avoid the problem of spontaneous recanalization [116, 117]. MRI can be used to visualize the persistent ~24-48 h of occlusion and thrombolysis of occluded MCA by rt-PA administration after ischemia [118]. Atochin *et al*. [107] injected fibrin microemboli into the cerebral circulation of mice to generate a microembolic model of stroke. In 2007, a mouse model of thromboembolic stroke was developed using in situ microinjection of purified murine thrombin to the distal branches of MCA on the cortical surface [119]. Topical application of ferric chloride on brain area overlying the MCA or common carotid artery (CCA) is reported to generate a more robust thrombotic MCAO [47, 120]. The important advantages pertaining to this model are higher reproducibility, real time *In-vivo* evaluation of the cortex feasible using laser speckle flowmetry or 2-photon microscopy, and useful for testing thrombolytic drugs [121].

3.2.1.5. Microsphere Model

The microsphere embolic stroke is a multifocal model of permanent occlusion [122] that occurred primarily by depositing microemboli in the pial vasculature (around 40%) and among the remaining [60%) by entering into penetrating arteries [123]. In a non-human primate model, microspheric emboli are reported to distribute in the watershed areas [124]. In a recent study, ischemic damage is induced by injection of microspheres into the ICA that showed a cortical watershed-pattern embolism [125]. In this method, the severity of stroke and lesion volume can be controlled by the size and number of the injected microspheres [126-128]. Several different validated compounds and artificial microspheres, such as collagen, viscous silicone, polyvinylsiloxane, TiO2, Al2O3 and heterologous atheroemboli have been employed to induce ischemia [126, 129-131]. Sodium alginate microspheres [100-300 μm) were also found useful to induce ischemic stroke in miniature pigs by rete mirabile occlusion [132].

The clearance of microemboli is a major challenge faced by this model (Table **1**). Lam *et al*. [133] suggested that microemboli undergo active extravasation from the vessel lumen within 2-7 days of injection, which resulted in the clearance of emboli without inducing the ischemic stroke [134]. This technique is advantageous and unlike intraluminal suture MCAO model, it did not show hypothalamic infarction and hyperthermia in animals [126, 135]. Moreover, lesion development occurs at a slower rate than the MCAO model [136].

3.2.1.6. Photothrombosis Model

The Rose Bengal model was initially proposed by Rosenblum and El-Sabban [137] and later on in 1985, photothrombotic stroke was generated by producing a blood vessel occlusion. A systemic injection of photosensitizing dye (Rose Bengal or erythrosin B) with an irradiating beam of light at a wavelength (560 nm) is administered within a specific cerebral blood vessel to induce cortical infarct [138]. The reaction between light and the photoactive dye generates singlet oxygen species that cause peroxidation of endothelial lipids and blood elements with subsequent platelet aggregation or thrombi formation and microvascular occlusion within the irradiated area [111, 138]. Studies have also confirmed the mechanics of targeted green laser in the noninvasive occlusion of pial arterioles and venules at the brain surface [139, 140]. This protocol demonstrated the production of a larger, highly reproducible infarction in terms of lesion size and location in rats, mice and primates like marmosets [141-143] by adjusting both intensity of beam light and the concentration of photoactive dye. This method *i.e.,*

Table 1. Summary of brain ischemia animal models.

Type of Model	Experimental Models	Preferred Species and Procedure	Strengths	Caveats
Focal Ischemia	Intraluminal filament occlusion model	Rat, mouse and several other species including primates, Procedure: Introduction of intraluminal thread	▪ Gold standard method for mechanistic studies of cerebral ischemia. ▪ Resembles to clinical stroke (face validity). ▪ Suitable for both permanent and transient MCAO. ▪ Reperfusion time can be managed accurately. ▪ Experienced surgeon can achieve high success rate. ▪ Low mortality and highly reproducible. ▪ Infarction and penumbra regions clearly visible. ▪ No craniectomy requires which preclude the impact of physical injury to the brain.	▪ Risk of partial (incomplete) occlusion of the MCA. ▪ Challenging surgical procedure for inexperience person. ▪ Not mimic pathophysiology of clinical stroke (constructive validity) where typically gradual blockade of blood vessel and recanalization occurs. ▪ Not reproduce thromboembolic occlusion or examine the thrombolysis. ▪ Infarction size highly dependent on the anatomy of the circle of Willis and degree/duration of occlusion. ▪ Chances of SAH and hyperthermia Application of modern techniques like DWI, MRI, MR angiography are expensive.
	Endothelin-1 (ET-1]	Rat, mouse, monkey Procedure: Topical administration of ET-1 to the abluminal surface of the exposed MCA or stereotaxic injection of ET-1 into tissue adjacent to the MCA	▪ The surgery is quick and straightforward for targeting the MCA and avoids damage to the facial muscles. ▪ Brain region-specific delivery of ET-1 is possible by implanting guide cannula. ▪ By targeting specific neuroanatomical areas, for instance white matter tracts, internal capsule *etc.*, a specific behavioural deficit can be achieved. ▪ ET-1 can be injected in conscious animals, thereby removing any confounds of anaesthesia. ▪ Low invasiveness, low mortality ▪ Reproducible, possible without opening skull.	▪ Require surgical skills to carry out the craniectomy and expose the MCA without causing significant bleeding or damage to the underlying cortex. ▪ High variability in lesion volume linked to variability in the response of the blood vessels to ET-1. ▪ Production of penumbral tissue is unclear. ▪ Occlusion time is uncertain. ▪ Induces astrocytosis and axonal sprouting. ▪ During proximal occlusion may damages temporalis muscle which can cause eating disturbances. ▪ Inconsistent with single vessel theory of lacunar stroke.
	Embolic	Thrombotic: Rats, mice, rabbits, and dogs Procedure: Injection of clots (fibrin-rich emboli) into cerebral vessels	▪ Recapitulate the clinical situation of ischemic stroke. ▪ Reproduce the clinical condition observed with rt-PA induced reperfusion (predictive validity). ▪ New thrombolytic agents and neuroprotective agents can be evaluated Higher reproducibility and suitability for testing thrombolytic drugs either alone or alongside adjunct therapies. ▪ *In-vivo* real time recording of the cortex possible using laser speckle flowmetry or 2-photon microscopy.	▪ Display higher mortality and variability in lesion size. ▪ High probability of multifocal ischemic lesions ▪ Spontaneous recanalization occurs. ▪ Challenging procedure to position the clot to the MCA-origin. ▪ Show high incidence of gross haemorrhage.
		Microspheres: Rats, mouse, rabbits, and dogs Procedure: Injection of calibrated microspheres into MCA	▪ Represent the clinical situation linked to rt-PA induced reperfusion. ▪ Higher reproducibility, low mortality and suitability for testing thrombolytic drugs either alone or alongside adjunct therapies. ▪ *In-vivo* real time recording of the cortex possible using laser speckle flowmetry or 2-photon microscopy. ▪ New thrombolytic agents can be evaluated. ▪ Ischemia severity can be managed using different sizes of microspheres. ▪ No hyperthermia like MCAO method, and also avoids hypothalamic damage.	▪ Challenging to induce the neurological/sensorimotor deficits because of the small size and location of the infarct. ▪ Partial to complete reperfusion occurs by spontaneous recanalization after occlusion. ▪ Low rate of successful induction of stroke ▪ Highly variable histologic and behavioural outcome. ▪ rt-PA dose in rodents is 10 times more as compared to humans.

(Table 1) contd….

Type of Model	Experimental Models	Preferred Species and Procedure	Strengths	Caveats
-		Photothrombosis: Rats, mice, marmoset, piglets, rabbit Procedure: Inject photosensitive dye	▪ Produces thrombi similar to the thrombi observed in human stroke. ▪ Relatively simple and quick surgical procedure. ▪ Lower rate of variability. ▪ Circumscribed lesions possible with anatomical precision. ▪ Impact of reperfusion with thrombolysis (*i.e.* rt-PA) and/or neuroprotectants is doable. ▪ Cellular mechanisms can be studied using application of MRI, 2-photon microscopy *etc.* ▪ High throughput assay.	▪ Vasogenic edema and BBB breakdown occurs. ▪ End-arterial occlusive nature of the model. ▪ Not suitable for investigating neuroprotective agents. ▪ Creates narrow ischemic penumbra. ▪ Not suitable to screen anti-thrombotic agent.
	Electrocoagulation	Rat, cat, monkey, dog, rabbit and pig Procedure: Surgical occlusion of MCA by electrocoagulation	▪ Distal or proximal occlusion of the MCA can be planned in order to induce a stroke affecting cortical or both cortical and sub-cortical territory. ▪ Display good reproducibility and less variability in lesion size. ▪ Avoid damages to hypothalamus, hippocampus and mid-brain. ▪ Low mortality rate. ▪ Owing to the craniectomy required to visualise and occlude the MCA which limits the effects of oedema.	▪ It induces permanent MCAO. ▪ Reperfusion studies are not possible. ▪ May damage the underlying cortex or lead to the rupture of blood vessels. ▪ Perturb the intracranial pressure and BBB function.
Global Ischemia	4-vessel occlusion	Rat Procedure: Atraumatic clamps loosely placed around both CCA and electro-cauterization of vertebral arteries	▪ Perform on awake and freely moving animals. ▪ Induces reversible bilateral forebrain and brainstem ischemia.	▪ Visualisation of vertebral arteries is difficult ▪ Neurological effects highly dependent on animal strain.
	2-vessel occlusion or Bilateral carotid occlusion	Rat and gerbil Procedure: Bilateral CCA occlusion	▪ Simple surgical procedure. ▪ Better histological assessments. ▪ Rapid screening technique. ▪ Induces reversible forebrain ischemia.	▪ Induces hypotension. ▪ Use of anaesthesia complicates the assessment of results.
	Decapitation	Rat and mouse Procedure: Decapitation after anaesthetizing animals	▪ Simple surgical procedure.	▪ Produce irreversible global ischemia without recirculation. ▪ Obsolete method.
	Cardiac arrest	Rat, cat, dog and monkey Procedure: Intra-cardiac injection of KCl or other cardioplegic agents or introducing a hook into the chest	▪ Mimic a common cause of ischemic stroke in humans. ▪ Can be performed in different strain of animals.	▪ Display high variability. ▪ Complicate the systemic effects and increases morbidity.
	Systemic hypotension and hypoxia	Procedure: Hypoxia is induced by 4% oxygen and 96% nitrogen and for hypotension different pharmacological agents can be used	▪ No surgical procedure involved.	▪ Reproducibility issue in terms of infarct size.

photothrombosis of MCA was also tried on infant piglets for ischemic stroke [144]. Recently, Clark *et al.* [145] demonstrated the artery-targeted photothrombosis to create selective infarcts in the forelimb regulating region of motor cortex that resulted in a deficit of forelimb motor function.

Few drawbacks of this technique are (i) if adopted for distal MCAO, it leads to infarction in the cortex [146], which is not mimicking the clinical stroke *i.e.* in basal ganglia [147], (ii) it creates a small ischemic penumbra [148] thus it is challenging to study the cellular mechanisms of recovery associated with improved functional outcomes [149], (iii) it

does not replicate the inflammatory and protective milieu of MCAO model [150], and (iv) non-specific application of illumination affects a large number of vessels in brain, whereas a single artery is affected in clinical stroke.

The advantage of this stroke model is that (i) it produces thrombi similar to the clinical cases by occluding the pial vessels around the illuminated zone, (ii) it is a non-invasive technique in which animals display long-term sensorimotor deficits and survival [151], (iii) it can create a circumscribed lesion in discrete brain structures like cortex which may facilitate reliable behavioural impairments [138, 148], and (iv) it is useful for identifying new therapeutic agents for endothelial damage and examining molecular mechanisms underlying brain plasticity in transgenic mice [138].

3.2.1.7. Models Requiring Craniectomy (Electrocoagulation)

Another invasive procedure of MCAO includes a craniotomy surgery to coagulate or ligate the MCA at the proximal or distal end [152]. It involves direct occlusion of the MCA by electrocoagulation, microaneurysm clips or ligatures. Apart from successful implementation in rats and mice for inducing experimental stroke [153], this procedure has also been used in non-human primates, cats, dogs, rabbits and pigs [154, 155]. The infarction size depends on whether the MCA and CCA are permanently or transiently occluded [156]. The cortical lesion is induced by a distal occlusion of the MCA, whereas a proximal occlusion leads into a larger sub-cortical and cortical infarct.

As summarized in Table **1**, advantages of this model include low mortality, visual confirmation of successful MCAO, reproducible infarct size, reperfusion possible in ischemic region, and occurrence of noticeable neurologic deficits. Upon comparison of the intraluminal suture model to the craniotomy model, the latter produces smaller infarcts as well as avoids damage to the hypothalamus, hippocampus and mid-brain. This method mostly targets the cortical and striatal regions and produces ischemia. However, the major concern with this model is the surgical damage inflicted to the cortex or the rupture of blood vessels, which affects the intracranial pressure and blood brain barrier (BBB) function [157]. To overcome this problem, photochemical application through intact skull was suggested to induce a distal MCAO in mouse model [158]. Furthermore, application of MRI during MCAO may prevent non-specific brain damage [159].

Despite promising efficacy of investigational molecules in the experimental preclinical models, only few compounds achieved success in the clinical trials. Thus, question on the predictive validity of animal experimental models has been raised to preclinical consortium. However, lack of efficacy in clinic may be due to the multifaceted nature of stroke pathophysiology and presence of multiple comorbidities. To address this translational problem, Stroke Therapy and Academic Industry Roundtable [STAIR] have defined some guidelines for conducting the preclinical studies [160, 161]. For instance, in 2009, STAIR recommended the use of female, hypertensive, aged, and diabetic animals in preclinical stroke research to better simulate human stroke [160]. They also suggested testing the efficacy in at least two independent laboratories performed in blinded and randomized manner, with supported histological and functional data. The STAIR committee also advised researchers to focus on the test compound's therapeutic time window, route of administration, pharmacokinetics and dose-response relationships. Nonetheless, yet most of the animal models of stroke are too dissimilar to the human stroke and implementation of the STAIR recommendations do not satisfy the clinical demand in stroke. Therefore, more refined preclinical testing is still warranted to achieve successful stroke therapies in the future.

4. NOVEL EXPLORATORY TARGETS IN ISCHEMIC STROKE

Current therapeutic interventions for ischemic stroke are mainly aimed at reopening the blocked blood vessel using mechanical or pharmacological (thrombolysis) strategies. To date, only one thrombolytic drug, recombinant tissue plasminogen activator (rt-PA), has been approved by the U.S. Food and Drug Administration for acute ischemic stroke [162]. However, rt-PA treatment has few limitations like narrow time window (*i.e.* mostly useful when administered within 6 h of stroke onset), exhibits a risk of a cerebral hemorrhage in some cases [163], and found effective only in a small subset of patients. Thus, there is an unmet medical need in this area. Several new treatment modalities have been tested in experimental stroke models [164], and many of these also demonstrated substantial preclinical success. For instance, restorative treatment using stem cell therapy showed a dramatic reduction of neurological disability [165]. But, till today, no therapeutic entity showed promising clinical recovery in stroke patients. Thus, there is a pressing need for developing newer and improved therapies for the management of post-stroke neurological and neuropsychiatric aberrations.

The alteration in tight junction proteins and transporters such as Mrp1, Mrp2 and Mrp4 represented the compromised BBB integrity following stroke injury [166]. The antioxidant drug such as Tempol has shown a great promise in preserving the integrity of tight junction proteins. The Mrp inhibitors also showed promising effects in *in vivo* models of stroke [167]. Therefore, antioxidant drugs that repair the compromised BBB integrity can be developed for post-stroke complications.

Circular RNAs (circRNAs) are endogenous and stable RNAs in the CNS, which are reported to be overexpressed in case of patients with ischemic stroke. The knockdown of this protein reduces the post-stroke infarct size in mice [168]. Similarly, circTLK1 was reported to upregulate in patients with acute ischemic stroke, and injection of shRNA circTLK1 lentivirus reduces the plasma levels of this circRNA variant and thereby reducing the infarct zone [169]. Thus, circRNA can be one of the possible targets for neuroprotection after an ischemic injury. However, the underlying mechanisms are still unclear and need further investigation.

Caveolin-1 is a cholesterol binding protein important for neuronal survival and growth [170]. In this connection, drugs that upregulate caveolin-1 have been reported to promote neuronal plasticity and growth *via* PI3K and GSK3β signaling [171]. In recent times, the role of fatty acid binding protein 4 isoform (FABP4) has also been envisaged in ischemic

Table 2. Behavioral models for neurological deficits testing in stroke research.

Behavioral Test	Type of Assessment	Merits	Demerits	Clinical Relevance
Neurological deficits scoring	Sensorimotor screening	Simple testing; Good for acute neurological scoring.	Subjective; Not useful for long-term assessment.	Post-stroke early onset phenotypes: weakness of arms/legs, gait disturbances and muscle strength.
Rotarod	Motor coordination and balance	Simple testing; Good for acute neurological scoring.	Pre-training required; Not suitable for long-term neurological evaluation.	Gait disturbances and muscle strength.
Adhesive label	Forelimb sensorimotor asymmetry	Helps to differentiates between sensory and motor functions at both short- and long-term intervals.	Removal time affected by motor learning and repeated exposure.	Numbness or loss of sensations of arm/leg.
Cylinder test	Spontaneous use of forelimbs	Simple testing; No pretraining required; Sensitive to long-term neurological evaluation.	Not suitable for time-dependent repeated testing.	Numbness or loss of arm/leg sensations.
Grid walking (Foot fault test)	Forelimb and hindlimb coordination	No pretraining; Assessment of both fore- and hindlimbs.	Severely sick animals scrumbled on grid.	Gait disturbances and motor coordination.
Wire hanging	Muscle strength, balance and endurance	Simple testing; Good for early and late neurological scoring.	Pre-training required; Stressful to animals.	Numbness/weakness of arms/legs or muscle strength.
Beam walking test	Test hindlimb function	Easy assessment; Sensitive to detect hindlimb placing deficits.	Pre-training required.	Gait disturbances and motor coordination.
Corner Test	Turning preference and asymmetry	Simple testing; Sensitive to long-term neurological evaluation.	Not perform when tested repeatedly.	Numbness/weakness of arm/leg or muscle strength.
Skilled reaching tasks	Skilled forelimb use	Detailed analysis of movements and compensatory strategies.	Laborious pretraining.	Numbness/weakness of arm/leg or muscle strength.
Montoya's staircase	Forelimb reaching and grasping	Independent use of both forelimbs; Sensitive to lesion size.	2-week pretraining necessary; Food deprivation.	Numbness/weakness of arm/leg or muscle strength.
Open Field test	Motor function and normal exploratory locomotion	Simple testing; Automation possible.	Habituation decreases the movement.	Gait disturbances and motor activity.
Single pellet reaching	Skilled forelimb use	Detailed analysis of movements and compensatory strategies.	Laborious, Pretraining required, Food deprivation.	Numbness/weakness of arm/leg or muscle strength.
Pole test	Motor function and coordination, bradykinesia	Simple testing; Good for acute neurological scoring.	Anxiety and learning effects performance.	Numbness/weakness of limb or muscle strength.
Nest-building deficit test	Sensorimotor indicator	Natural and spontaneous activity.	Long-term assessment feasible.	Numbness/weakness of arm/leg or muscle strength.
Catwalk	Accurately record gait	Automatically record data on several variables; Long term sensitivity.	Body weight, light intensity, and walking speed of animals affects results.	Gait disturbances and motor coordination.
Kinematics	Fine digit control	Differentiation between true recovery and compensation.	Time consuming.	Numbness/weakness of arm/leg and muscle strength.

stroke. FABP4 increases the COX-2, inflammatory cytokines and chemokines, and subsequently provokes the inflammasome formation in the macrophages and aggravates the post-stroke brain injury [172]. Moreover, upregulation of FABP4 after stroke resulted in the disruption of BBB by the enhanced levels of MMP-9. Thus, FABP4 has been suggested as a potential target for the treatment of post-ischemic deleterious effects [173]. Post-ischemic release of platelet activating factor led to neuronal damage by increased intracellular Ca^{2+} levels and triggering inflammatory cascades

[174]. Administration of platelet activating factor receptor (PAF-R), antagonist LAU-0901 showed an improved ischemic infarct 2 h post-MCAO injury [175]. Adropin, a small polypeptide, is downregulated in stroke. Administration of synthetic adropin was reported to show neuroprotective effects and reduction of infarction by activating the eNOS [176]. Similarly, another active peptide cerebrolysin may reverse post- stroke deleterious consequences by increasing neuronal survival and reversing glutamate-induced necrotic and apoptotic cell death [177, 178].

Macrophages and microglial transformation to active form play a key role in the progression of post-stroke brain injury. M1 microglia is believed to exacerbate the injury by releasing the proinflammatory cytokines, whereas M2 phenotype was found to be protective [179]. Several repurposed drugs are under clinical trials, for instance, drugs that promote M1 to M2 polarization (Rosuvastatin), decrease M1 macrophages (Fingolimod, Minocycline and Erythropoietin) and promote M2 phenotype-like responses (Rapamycin and Xinomiline) [180]. Minocycline, a second-generation tetracycline drug, has been repurposed for neuroprotective and anti-apoptotic responses. It inhibits the COX, MMPs and inflammatory mediators and reduces inflammation and microglial activation [177, 181]. Edaravone is a free radical scavenger and has been implicated in treating acute ischemic stroke; it works by inhibiting the free radical formation and anti-apoptotic effects [182, 183]. Inhibition of astrocytes activation by fluorocitrate has shown promising effects in a focal ischemic mice model [184]. Thus, targeting reactive astrocytes may render greater plasticity and neuronal recovery [185]. The cilostazol which offers neuroprotective effects and is generally safer than aspirin and significantly reduces the infarct size in patients receiving the drug [186]. Some other drugs that are extensively used in the clinical setup are anti-thrombotic agents such as argatroban, rivaroxaban, apixaban and dabigatran etexilate [187].

4.1. Neuroprotective Approaches

4.1.1. Neurotrophic Agents

Neurotrophins belong to a class of growth factors which dimerize to form the biologically active species [188, 189]. This family includes 5 prominent members such as BDNF, NGF, neurotrophin 3 (NT3), neurotrophin 4/5 (NT4/5) and neurotrophin 6 (NT6). These neurotrophins are involved in the regulation of neuronal differentiation, axonal and dendritic growth, and synaptic plasticity. A profound increase in neurotrophic factors such as NGF and BDNF was reported after brain injury [190]. Administration of neurotrophins reported to shift the expressions of antioxidant enzymes [191, 192] and repair injured brain tissue [193-195]. In Table **3**, we summarized few key findings relating to the use of endogenous and exogenous neurotrophins such as BDNF and NGF in the management of stroke complications in *In-vitro* and *In-vivo* animal models.

4.1.2. Neurogenesis in Ischemic Stroke

Neurogenesis centers in the brain *i.e.*, subventricular zone (SVZ) and subgranular zone (SGZ) of the dentate gyrus (DG) that may offer neuroblast migration to the ischemic boundary [204]. Neurogenesis process is regulated by the levels of glucocorticoids, excitatory neurotransmission, nerve growth factors and physiological stress [205]. It can also be controlled by pharmacological interventions [206]. In adults, the occurrence of focal cerebral ischemia induces the proliferation and differentiation of neural stem and progenitor cells in the SVZ and SGZ. Many evidence showed that vascular (niche) were progenitor cells which regulate the stem cell-renewal, progenitor differentiation and neuroblast migration [207]. In adults SVZ region is composed of A, B and C types of cells [208, 209]. It has been reported that stroke triggers these SVZ cells during neurogenesis after stroke [210, 211]. Endothelial cells release neurotrophic factors like brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), which play a role in stimulating the self-renewal of adult neural stem cells, and promote the production of neurons [212, 213]. These reports suggest that neurogenesis increases after ischemic stroke induction.

4.2. Stem Cell Therapy in Stroke

Neural stem cells (NSC) envisage to form new synaptic connections and protect neurons after ischemic brain injury [214]. NSC therapy also stimulates the secretion of endogenous neurotrophins like BDNF [215], which may protect BBB integrity and avoid subsequent damage [216]. Apart from NSC, induced pluripotent stem cells (iPSCs) and mesenchymal stem cells (MSCs) are popular stem cell-based treatments. Administration of bone marrow derived MSC is reported to increase angiogenesis and neuronal growth by releasing angiogenic factors and neurotrophins [217]. Introduction of BDNF gene into human bone marrow derived MSCs reported to increase the neurogenesis in a rat MCAO model [218]. Administration of bone marrow stem cells induces astrogliosis in the MCAO mouse model [219]. Injection of human induced pluripotent stem cells showed an increase in neurogenesis and decreases of microglial activation in a pig model of stroke [220]. Adult pluripotent like olfactory stem cells have also shown beneficial effects in stroke models of pig *via* their differentiation into neuronal, glial cell types in the injury site and thus better functional recovery post intracerebral injection [221]. Newer therapies targeting both the areas of interest and other peripheral mechanisms that are altered in ischemic attack can be of some clinical importance, such as neurotrophins and stem cell therapy [10]. Stem cell-based treatments definitely seem to be effective in preclinical models, but variability in responses due to the source of the cells and biochemical modifications that can potentially alter the properties can be a definite challenge. The time window for stroke therapy is narrow, and stem cell therapy is a long process. Thus some clinical validations in this regard need to be answered. Moreover, conditions for transplant differ and may not be possible logistically, thus there are still several unanswered doubts before stem cell therapy turns out to be a golden tool for the management of stroke patients.

4.3. Management of Stroke by Gut-Microbiome Enrichment

It has been proposed that increased proinflammatory mediators, compromising BBB integrity and activation of microglia following ischemic insult are linked with gut dysbiosis [222, 223]. Moreover, perturbation of gut microbiota and metabolites of the intestine are accompanied by low levels of

Table 3. Importance of BDNF and NGF in the management of ischemic stroke.

Neurotrophic Agents	Outcomes	Reference
BDNF	Emphasis on critical role of BDNF-AKT/cAMP-responsive element-binding (CREB) pathway in the neuroprotection and proneurogenic effects of tetramethylpyrazine nitrone in MCAO model.	[196]
	Demonstrated a potential influence of the Val66Met polymorph of BDNF gene in the functional recovery of aphasia in patients of stroke and concluded that the polymorph has no significant difference in terms of activity and that it promotes recovery in a similar way.	[197]
	The expression of BDNF and NGF mRNA in the hippocampus is regulated by glutamate and GABA, and involved in the activity-dependent synaptic plasticity in ischemic stroke.	[198]
	Progesterone improved neurological deficits and neuroprotective responses in the MCAO model by triggering an endogenous BDNF system.	[199]
	The neuroprotective effects of neural stem cells/neural progenitor cells are reported to be mediated by release of BDNF.	[200]
NGF	The increase levels of NGF are noted for 1-3 days post injury and important for tissue repair process.	[201]
	Nanocarriers with the NGF can cross the artificial BBB and promote neurite outgrowth in PC12 cells, the nanocarrier loaded NGF and small molecule MEK inhibitor U0126 reduced the infarct size as compared to the control group of animals in a transient MCAO rat model.	[202]
	Proline-rich AKT substrate phosphorylation and its interaction play a role in neuroprotection which is mediated by the NGF in apoptotic neuronal cell death after stroke.	[203]

short-chain fatty acids (SCFAs) may represent a decrease in neuroprotective effects [223, 224]. The reduced levels of anti-inflammatory cytokines such as IL-10 and TGF-β after stroke have been linked with gut dysbiosis [225]. The migration of T cells from Peyer's patches to peri-infarct zone of brain occurs after stroke episode, linking a possible role of the immune system in the stroke pathophysiology and its relationship with gut dysbiosis [226]. Perturbation of gut microbiota is also linked to the formation of atherosclerotic plaques, which is one of the pathological features of ischemic stroke [227]**.** Thus, restoration of gut microbiome could be an emerging target in the modulation of neurological disorders [228].

4.3.1. Probiotics

Oral administration of probiotics like *Lactobacillus casei*, *Bifidobacterium breve*, *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* was reported to reduce the infarct size and normalize the TNF-α levels in rodent MCAO model [229]. Administration of butyric acid also enhances the α-diversity of gut microbiome and offers neuroprotective effects following stroke [230]. Lone effects of Tong-Qiao-Huo-Xue decoction and FMT of the treatment groups to stroke induced rats led to increased expressions of IL-10 and reversed the increase in the detrimental population of Bacteroides, thus promoted functional recovery [231]. The principal target is the production of metabolites *viz*- SCFAs which are transported to the brain *via* the vagal nerve, which are good neuroprotectants [232]. Thus, probiotic administration confers normalisation of the gut microbiome or causes a "gut-eubiosis" and is beneficial for prophylaxis and treatment of stroke patients. The therapeutic outcomes are i) increas.ed neuroprotection ii) correction of imbalances in immune responses, and iii) reduction in cytokine production.

4.3.2. Fecal-microbiota Transplantation (FMT)

In recent years, importance of FMT has been growing in the treatment of various diseases. FMT confers normalization of the α-diversity, which is reported to increase the neuroprotection *via* production of SCFAs [233]. Changes in the infarct size were noted in the antibiotics-treated mice which indicates a key role of gut microbiota in the regulation of post-stroke consequences. Indeed, FMT obtained from antibiotics-treated mice showed neuroprotective effects in preclinical stroke model [234]. Secondly, FMT collected from sodium butyrate treated mice was also reported to reverse neurological deficits and confer neuroprotection *via* reduction in apoptosis and neuronal loss in MCAO mice [235]. Interestingly, transplantation of young mice microbiota into the aged animals showed rapid recovery of stroke-generated problems and reduced the inflammatory processes in the gut and brain [236]. Thus, replenishment of commensal microbiota with the FMT may be a preferable choice for the neuroprotective effects. Moreover, identification of personalized microbiome approaches is also growing in this field.

CONCLUSION

Stroke is one of the most prevalent neurovascular diseases and cause of mortality and locomotor disability. Indeed, a root cause of stroke incidence in humans is heterogeneous in nature and entails complex pathophysiology, thus replication of all the traits of human stroke into a single animal model looks challenging. However, refilling this gap in the translational experimental stroke models may reverse the paucity of effective therapeutics available to stroke patients. This review comprehensively summarizes the strengths and caveats associated with the currently available preclinical stroke models. An ideal experimental stroke model should mimic

the characteristics of clinical relevance, ease of experimental procedure, reproducibility, and absence of collateral effects unrelated to ischemia. Newer targets and therapies are pressing the need of the hour as there is low to insignificant clinical translation of preclinical candidates. The deeper insights into the neurobiology of stroke and newer experimental stroke models may provide new avenues for development of newer therapeutics.

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CONFLICT OF INTEREST

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