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·Review·

Experimental animal models and inflammatory cellular changes in cerebral ischemic and hemorrhagic stroke

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Stroke, including cerebral ischemia, intracerebral hemorrhage, and subarachnoid hemorrhage, is the leading cause of long-term disability and death worldwide. Animal models have greatly contributed to our understanding of the risk factors and the pathophysiology of stroke, as well as the development of therapeutic strategies for its treatment. Further development and investigation of experimental models, however, are needed to elucidate the pathogenesis of stroke and to enhance and expand novel therapeutic targets. In this article, we provide an overview of the characteristics of commonly-used animal models of stroke and focus on the inflammatory responses to cerebral stroke, which may provide insights into a framework for developing effective therapies for stroke in humans.

Keywords: ischemic stroke; hemorrhagic stroke; animal model; inflammatory cells

Introduction

Stroke is a leading cause of serious long-term disability and ranks fifth among all causes of death, exceeded by diseases of the heart, cancer, chronic lower respiratory disease, and unintentional injuries[1, 2]. Approximately 795 000 people continue to experience a new or recurrent stroke (ischemic or hemorrhagic) each year in the USA. In China, there are 2.5 million new stroke cases each year and 7.5 million stroke survivors, leading to a mortality rate of ~1.6 million, which has a large impact on the Chinese economy[3]. Appropriate animal models that mimic at least some features of human stroke would undoubtedly help to improve understanding of the diseases clustered as stroke, and to develop and test the effects of therapeutic strategies. Experimental stroke models have been developed since the 1970s[4].

In the cerebral ischemia stroke model, the oxygen and glucose supply to brain tissue is reduced[5]. Brain hemorrhagic stroke models mimic different aspects of clinical intracerebral hemorrhage (ICH), such as the physical injury caused by an expanding mass within the brain parenchyma, the role of blood components, and hematoma expansion[6]. Animal models of subarachnoid hemorrhage (SAH) provide a pathological condition in which arterial blood flows into the subarachnoid space, which is usually caused in patients by a ruptured aneurysm[7].

There are many animal models of stroke in a variety of species including primates, pigs, sheep, dogs, cats, Mongolian gerbils, rabbits, rats, and mice[4]. These models have been used to assess the pathophysiological consequences, test therapeutic strategies, and evaluate risk factors for stroke, as well as to investigate the effects of comorbidities on stroke outcome[8].

This review summarizes the characteristics of commonly-used animal models and inflammatory cellular

changes in ischemic or hemorrhagic stroke, and provides a framework for understanding the use of experimental models in stroke research.

Ischemic Stroke Models

Several focal cerebral ischemic stroke models have been developed in a variety of species; these include mechanical occlusion of the middle cerebral artery (MCA)[9-12], thromboembolic models[13-15], and photothrombotic models[16]. Global ischemia models, although not formally stroke, are divided into complete and incomplete models of ischemia, which are produced by occluding the cerebral blood flow (CBF) completely or incompletely[17].

Mechanical Middle Cerebral Artery Occlusion (MCAO) Models

MCAO models are most commonly used in stroke research for its close resemblance to stroke in patients. The MCA is directly occluded using surgical clips[18, 19], ligation sutures or snare ligatures[20], or electrocoagulation[11, 21] after craniotomy, when necessary. MCAO with an intraluminal filament is a widely-accepted and well-standardized model of cerebral ischemia and reperfusion injury that does not need a craniotomy[9, 12].

MCAO by surgical clip Tamura *et al.* developed the MCAO model with surgical clips in the proximal MCA of cats, but this had a high mortality rate[18]. They then developed the model in rats through a small subtemporal craniotomy, demonstrating technical feasibility and showing consistent histological results with clips[10]. They found ischemic damage in the cortex and basal ganglia: the frontal cortex, the lateral part of the neostriatum, the sensorimotor cortex, auditory cortex in most animals, and infrequently, the occipital cortex and medial striatum. The CBF decreased to 0.24 mL/g/min after MCAO, and the region of ischemic damage corresponded to the area with marked CBF reduction[22, 23]. This model is sufficiently reproducible to enable investigation of the pathophysiology of permanent ischemia or ischemia-reperfusion using autoradiographic and neurochemical methods[24]. However, it carries risks of subarachnoid hemorrhage, infection, and cerebrospinal fluid leakage because of the craniotomy.

MCAO by MCA ligation The ischemic model of MCA ligation in rats was introduced by Tamura[10]. However, this model was not popular due to the technical difficulty of the procedure, and because it was so invasive, its application was limited to acute experiments. Chen *et al.* later modified the model by interrupting blood flow to the right MCA territory by ligating the right MCA, and the right and left common carotid arteries (CCAs) in succession, resulting in a more consistent cortical infarction in the right MCA territory with low mortality[25]. The characteristic changes of ischemic necrosis are limited to the cortex, sparing subcortical structures and lacking motor deficits. This model has also been used in mice and achieved almost the same results, but failed in young rats[26, 27]. Proper performance requires a small burr-hole at the anterior junction of the zygoma and squamosal bones, which results in a consistent cortical infarction with low mortality[25].

MCAO by electrocoagulation In other MCAO models, electrocoagulation is used to interrupt MCA blood flow[11, 20, 28-31]. A subtemporal craniotomy is required, like other direct MCAO models[10]. Based on the different sites of cauterization, MCAO distal and proximal to the lenticulostriate branches involve the cortex (distal occlusion) or cortex and striatum (proximal occlusion) at 24 h after electrocoagulation in rats[30]. CBF decreases gradually from rostral and caudal neocortex to the central core of the neocortex, leading to sensory, motor, and cardiac-autonomic dysfunction in mice, mimicking some of the changes seen clinically in stroke patients[28, 32].

MCAO by intraluminal filament The intraluminal filament MCAO model has been routinely used since the 1980s. This model reproduces cerebral ischemia and reperfusion injuries that involve both the frontoparietal cortex and the lateral caudoputamen without craniotomy in mice and rats[9, 12, 33-36]. In this model, a filament (mono-nylon suture, silicone/poly-*L*-lysine-coated suture, 4-0 for rats and 6-0 for mice) is inserted to the point of origin of the MCA through the internal carotid artery (ICA). The filament is then introduced into the external or the CCA and advanced to reach the proximal segment of the anterior cerebral artery (ACA) with a smaller diameter to block the MCA (17–20 mm for rats, and 10–11 mm for mice)[37-40]. The intraluminal suture blocks the origin of the MCA, occluding all blood flow from the ICA, ACA, and posterior cerebral artery. A major advantage of this model is that the nylon suture protrudes from the closed incision so that it can easily be

withdrawn for MCA reperfusion through the circle of Willis after different durations of occlusion. The most common durations of MCAO are 60, 90, and 120 min or permanent occlusion, but a minimum of 60–90 min is required to obtain a reproducible infarct volume in rats[41]. However, longer durations of occlusion usually result in larger infarcts involving both the cortex and striatum, and may be associated with some mortality[42]. The intraluminal MCAO model in rodents has facilitated the discovery of multitudes of mechanisms contributing to ischemic injury, restorative therapies, and neuroprotection[43-47], such as excitotoxicity and calcium overload, calcium dysregulation, angiogenesis, arteriogenesis, white-matter remodeling, and inflammatory responses. The disadvantages of this model in rats are that artery injury by inserting a filament to block the artery is unavoidable and it does not replicate the hemodynamic features found in stroke patients. In addition, there are several other side-effects of this model in mice, including subarachnoid hemorrhage, variation of lesion volume, and hypothalamic damage[48, 49].

Embolic Models

Most ischemic strokes in patients are caused by thromboembolisms[50, 51]. However, the intraluminal MCAO animal models do not reproduce thromboembolic occlusion. Thromboembolic animal models have been used for research on neuroprotection and re-canalization therapy after ischemic stroke. Tissue plasminogen activator is the only thrombolytic agent approved by the Food and Drug Administration (USA) for the treatment of ischemic stroke; it enhances reperfusion by re-canalizing the occluded arteries to improve the functional outcome after stroke[52]. In general, emboli of different amounts and sizes such as microspheres, thrombotic clots, and silicone-rubber cylinders have been injected to interrupt the CBF of target arteries in thromboembolic models using mice, rats, rabbits, and dogs[14, 53-56].

Thrombotic focal cerebral ischemia models Zhang *et al.* modified a rat model of thrombotic focal cerebral ischemia, in which the MCA was selectively occluded by a thrombotic clot made with autologous blood[15, 57]. In addition, magnetic resonance imaging (MRI) demonstrated thrombolysis of the occluded MCA by tissue plasminogen activator administration after ischemia in this model. In brief, a white embolus is made using arterial blood from a rat donor that is transferred to a modified PE-50 catheter with a 0.3-mm outer diameter filled with saline. The modified PE-50 catheter with a 25-mm clot is introduced into the external carotid artery (ECA) lumen, advanced to the segment of the ICA 2–3 mm proximal to the origin of the MCA and injected to block the MCA. CBF in the ipsilateral MCA territory was decreased to 43% of the pre-embolization level, and this decrease persisted for at least 2 h. Further MRI studies from our lab revealed persistent ~24–48 h of occlusion, with an autolysis rate and time-course similar to that in patients[58]. An embolus was detected in all rats sacrificed at 24 h after embolization, and 98% of all injected emboli were lodged at the origin of the MCA. This thromboembolic model accurately mimics the clinical conditions of ischemic stroke, and remains the best model for research on thrombolytic agents[15, 57]. Busch *et al.* performed intracarotid injection of 12 medium-sized, fibrin-rich autologous clots, but none achieved a stable proximal MCA occlusion[59]. In addition, Atochin *et al.* developed a dose-dependent microembolic model of stroke by injecting fibrin microemboli into the cerebral circulation of mice. In this way, the degree of injury was controlled by the dose of microemboli[55]. Zhang *et al.* also induced an embolic stroke model with a fibrin-rich allogeneic clot, which mimics human stroke[60].

Microsphere models Many calibrated compounds and artificial microspheres, such as collagen, viscous silicone, polyvinylsiloxane, and heterologous atheroemboli have been used as an alternative to induce ischemia in mice, rabbits, and primates through injection into the CCA or ICA[61-65]. Different sizes of emboli induce different ischemic events. Large (300–400 μm diameter) synthetic macrospheres induce a large lesion similar to that produced by permanent occlusion of the MCA. However, small (<50 μm) microspheres induce smaller injuries similar to the multifocal infarcts of cerebral small-vessel disease[56, 66], which accounts for 20%–30% of cases of ischemic stroke[67]. If the spheres used are small enough to penetrate arteries, the extent of infarction and neurological deficit correlates well with the distribution and number of trapped spheres. However, large numbers of microemboli can be created in the rat cerebral vasculature while only a few small areas of ischemic injury develop in these animals[68]. Lam *et al.* have shown that microemboli (20 μm) undergo

active extravasation, apparently removing the particulates into the perivascular space[69]. The possible mechanisms for cerebral microvascular re-canalization are embolus extravasation from the vessel lumen within 2–7 days after injection[69] and emboli washout by blood flow without induction of ischemic stroke[70].

Photothrombosis models Photochemically-induced focal cerebral thrombosis involves the focal illumination of the target cerebral vessel through the intact skull after intravenous injection of the photosensitive dye Rose Bengal[71]. It is also highly reproducible in terms of lesion size and location in mice and primates[72-74]. The mechanism of photothrombosis-induced injury involves singlet oxygen, focal endothelial damage, platelet aggregation, simultaneous microvascular occlusion throughout the irradiated area, and secondary ischemia[71, 75]. The first few days after ischemia is a critical period for brain damage and behavioral deficits[76]. This model has the advantages of long-term sensorimotor deficits with long-term survival. The procedure is also non-invasive, as the translucent skull is able to transmit the irradiated light[77]. Such models may facilitate behavioral studies of ischemia in specific anatomical-functional regions of the cortex, resulting from small infarcts with well-delimited boundaries. Furthermore, this model may be suitable for testing therapeutic agents that specifically influence the platelet response to endothelial damage and for studying the cellular and molecular responses underlying brain plasticity in transgenic mice[71, 74, 78]. However, photothrombosis-induced ischemia differs from stroke in patients. Photo-induced damage involves a large number of vessels in the illuminated area with a limited penumbra, whereas in most stroke patients, only a single terminal artery is blocked. In addition, the photothrombosis model does not produce reperfusion of blocked arteries to mimic the reperfusion injury seen in some stroke patients[74]. This model involves direct microvascular damage and direct parenchymal cell damage that are not secondary to vascular occlusion[79].

Endothelin-1 Models

Endothelin-1 (ET-1) exhibits a potent, yet reversible vasoconstrictive action on rodent vasculature[80]. ET-1 reduces focal CBF, and ischemic brain injury can be induced by direct application of ET-1 onto the exposed MCA[81, 82], adjacent to the MCA by stereotaxic intracerebral injection[83], or onto the cortical surface of the rat[84, 85], with a profound dose-dependent reduction (up to 93%) in local blood flow in those areas lying within the distribution of the MCA[86]. T2-weighted MRI has shown hyperintensity of this injury, reflecting the cytotoxic edema after ET-1 injection. This model provides a novel opportunity to assess the efficacy of novel neurorestorative and neuroprotective treatments in ischemic stroke that are targeted towards clinical trials. However, ET-1 *via* intracerebral injection produces little injury in mice, while combination of CCA occlusion with co-injection of ET-1 and NG-nitro-*L*-arginine methyl ester produces a lesion and results in a significant motor deficit. This demonstrates that ET-1 is much less potent for producing an infarct in mice than in rats[87]. Moreover, minimally invasive microinjection of ET-1 into the brain has provided no immunohistochemical evidence of an acute inflammatory response or breakdown of blood-brain barrier (BBB) integrity[88]. The ET-1 model also has disadvantages including the need for craniotomy and higher variability in stroke volume which can, however, be reduced by the use of laser Doppler flowmetry[89]. In addition, this model does not reflect the pathogenesis of human stroke and the associated vascular and BBB dysfunction.

Global Cerebral Ischemia Models

Global cerebral ischemia with critical reduction of CBF in the whole brain induces selective neuronal injury in the CA1 region of the hippocampus, among Purkinje cells, and in the frontal neocortex if the duration of ischemia is limited[17, 90, 91]. Global cerebral ischemia models were introduced to mimic the ischemic injury after CBF reduction and subsequent reperfusion, most commonly caused by cardiac arrest. In general, two models of global cerebral ischemia are widely used: the four-vessel occlusion (4-VO) model in rats[92] and the two carotid artery (2-VO) occlusion model[93-96]. There are several other models of global cerebral ischemia, such as the ventricular fibrillation[97], the three-vessel occlusion (3-VO)[98], the neck tourniquet[99, 100], and the decapitation ischemia models[101].

The 4-VO model The 4-VO model was developed to study reversible bilateral forebrain and brainstem ischemia with highly-predictable brain damage in conscious, freely-moving rats[92]. The original model involves two-stage surgery with permanent occlusion of the vertebral arteries on the first day followed by transient occlusion of the

CCAs on the following day. In this model, CBF changes are correlated with both the distribution and progression of neuronal damage. CBF to the forebrain was characterized by 5–15 min hyperemia after 30-min moderate to severe ischemia, and then fell below normal and remained low for up to 24 h[102]. Given the side-effects of bleeding and significant trauma leading to high mortality, the classic 4-VO model was developed in mice[103]. This model is induced by occlusion of the bilateral CCAs and the left subclavian artery together with right subclavian artery stenosis under controlled ventilation, and the CBF is also reduced to <10% of the pre-ischemic value[104]. This model has several advantages including reproducible cerebral ischemic insult, sufficient reperfusion, and low mortality rate (10%), and can be used to study global cerebral ischemia/reperfusion injury in mice.

The 2-VO model The 2-VO model is an alternative to the 4-VO model with a combination of bilateral CCA occlusion and systemic hypotension to produce reversible forebrain ischemia[96, 105]. Smith *et al.* developed a method of inducing global brain ischemia by combining carotid clamping and hypotension (reducing the mean arterial pressure (MAP) to 50 mmHg)[91]. This method produces ischemia throughout vulnerable areas of the forebrain such as the CA1 pyramidal neurons of the hippocampus, caudoputamen, and neocortex, resulting in a pattern of brain damage that closely mimics that of cardiac arrest survivors. Atlasi *et al.* reported that the 4-VO model results in more ischemic lesions in CA1 neurons of the rat hippocampus than 2-VO after 24 h reperfusion[106]. CA1 neuronal death can be quantified on day 7 after reperfusion, with inflammatory cells and activated glial cells[93]. High-grade CA1 neuronal loss is dependent on the reduction of MAP in global ischemia: reduction of MAP to 37 mmHg results in 90% CA1 neuronal loss, while reduction of the MAP to 45 mmHg results in 50% CA1 neuronal loss[107]. MAP was reduced to 30 mmHg ± 1 mmHg for 8 min in a further refined method for ischemia with a low mortality rate[108]. The 2-VO model has also been adapted for mice, resulting in reductions of CBF in forebrain structures including the cortex, hippocampus, and caudoputamen[109, 110]. Compared to 4-VO, the 2-VO model is easier to perform and is fully reversible, and the less-intrusive surgical intervention allows greater scope for recovery experiments[111].

The 3-VO model The 3-VO model combines occlusion of the basilar artery with temporary bilateral CCA occlusion in rats and mice[98, 112, 113]. The 3-VO with or without neck ligation offers consistent results without additional manipulation or selection of the animals[98]. However, the infarct size may be determined by the reduction of CBF in the periphery of the MCA territory during 1 h of focal ischemia[114]. In addition, this model has surprisingly high reproducibility of the intra-ischemic blood flow reduction and post-ischemic cell death. The intra-ischemic blood flow declined without exception to <15% of baseline[113]. A longer delay of cortical and striatal neuronal death (by at least 24 h) than hippocampal neuronal death, which is evident at 6 h, has been reported in this model[113].

Ventricular fibrillation models In adult patients, global cerebral ischemia is usually caused by cardiac arrest with ventricular fibrillation[97]. The ventricular fibrillation model is used to study the mechanisms of cardiac arrest-induced delayed neuronal death and the efficacy of neuroprotective drugs because they mimic the “square-wave” type of insult (rapid loss of pulse and pressure) commonly encountered in adults at the onset of cardiac arrest[115-118]. Cardiac arrest is induced by injecting KCl *via* a jugular catheter, and confirmed by an immediate MAP drop[116]. However, this model is not popular because of the difficulty of the procedure and poor animal survival.

Neck tourniquet ischemia models The neck tourniquet model is induced by inflating a high-pressure cuff (~600–700 mmHg) around the neck of an anesthetized rat, leading to a reduction of CBF to <1% of control throughout the brain[100]. Meanwhile, the arterial blood pressure is regulated at 60 mmHg during ischemia by blood withdrawal/infusion. In this model, the bilateral carotid arteries and veins are occluded and other cervical structures are subjected to great pressure, which can lead to variable ischemic outcomes. However, this model has not been widely used to induce global cerebral ischemia in the last few decades.

Factors such as temperature control and the age of animals can interfere with the reproducibility of cerebral ischemic lesions. Temperature control is a well-known factor in maintaining consistent pathological effects in animals with global cerebral ischemia. Lowering the brain temperature by only a few degrees during ischemia has a markedly protective effect[119]. In addition, differences

between old and young animals have been found in the time-courses of neuroinflammation and apoptosis after ischemic damage, suggesting that neuroinflammation is an age-dependent event rather than a vulnerability of the hippocampus and cerebral cortex. These two factors should be taken into account in searching for therapeutic targets in global cerebral ischemia[120].

In the general population, men experience ischemic strokes more frequently, while strokes in women tend to be more severe[121, 122]. Experimental studies have also shown that young female animals have smaller infarcts after induced strokes than young males[123]. A possible reason for this difference is that the female hormone estrogen, *via* estrogen receptors (ERs) such as ERα, ERβ, or ERx[124, 125], exerts anti-inflammatory[126] and antioxidant actions[127] and enhances angiogenesis[128] and neurogenesis[129] after ischemic damage. Sex differences should be taken into account in experimental investigations of ischemic stroke.

One major problem of experimental stroke models is that studies are mostly conducted in young animals without any comorbidity. However, there is a high incidence of coexisting medical disorders among patients with stroke, such as hypertension, diabetes mellitus (DM), hypercholesterolemia, and atrial fibrillation[130]. Hypertension is a well-recognized risk factor for stroke, and ranks first of the comorbidities in stroke patients with poor outcomes[131]. Stroke-prone hypertensive rats with acquired hypertension have been introduced to mimic stroke patients with hypertension. This model closely mimics human hypertension in cerebrovascular pathology and physiology after ischemic stroke[132]. DM patients rapidly develop vascular disorders[133] and suffer significantly worse outcomes[134] with poor long-term recovery due to recurrent strokes[135]. Our studies have elucidated the differences in the lesions as well as functional outcomes and responses to treatment between DM and non-DM stroke models[45, 136-139]. There is a compelling need to develop therapeutic approaches specifically designed to reduce neurological deficits after stroke in the DM population.

The characteristics and means of induction of ischemic stroke models are listed in Table 1.

Inflammatory Responses after Ischemic Stroke

Cerebral ischemia activates the innate and adaptive immune systems, compromises the BBB, and leads to a massive migration of peripheral leukocytes into the brain that orchestrates focal inflammatory responses, catalyzes tissue death, and worsens the clinical outcome[140].

Microglia, the resident immune cells of the central nervous system, are rapidly activated within 24 h after stroke and play a prominent role in phagocytosis as a response to the loss of normal interactions with adjacent neurons in the ischemic brain[141, 142]. Microglial proliferation is the main mechanism underlying the early increase in phagocyte numbers in the ischemic brain after MCAO[141]. The severe injury associated with 60-min MCAO leads to a markedly reduced proliferation of resident microglial cells. Reduced numbers of microglia after MCAO are associated with more severe injury[141].

In animals with experimental stroke, recruitment of peripheral leukocytes to the brain occurs following cerebral ischemia with a sequence of neutrophils first, followed by monocytes, and then lymphocytes[143]. The neutrophil infiltration into the ischemic brain occurs in the first few hours after focal cerebral ischemia[144]. Infiltrating leukocytes affect the development of tissue damage by releasing a series of mediators including purines, reactive oxygen species, and danger-associated molecular patterns such as high-mobility group box-1 protein, heat-shock protein 60, β-amyloid, and DNA or RNA immune complexes[145]. The accumulation of neutrophils in the brain is correlated with poor neurological outcome and the severity of damage in most studies[146, 147]. However, Price *et al.* showed that neutrophil accumulation is not related to stroke severity and outcome[148].

Blood-borne monocytes recruited from the periphery are present in ischemic brain tissue from 24 h to up to 14 days after stroke[149]. Monocytes may play a detrimental role in the acute phase, but a reparative role in the chronic phase of cerebral ischemia[150]. $CD4^+$ T-cells, $CD8^+$ T-cells, and natural killer (NK) cells are among the lymphocyte subsets that respond to cerebral ischemia. T-cell infiltration after 60 min of transient MCAO peaks around day 3, whereas in different MCAO models, infiltration peaks at around days 5–7 following permanent MCAO[151, 152]. $CD8^+$ cells contribute more to the inflammation and thrombogenesis during cerebral ischemia than $CD4^+$ cells[153, 154]. In addition, depletion of $CD8^+$ T-cells is more protective against experimental stroke than $CD4^+$ T-cell

Table 1. Animal models of ischemic stroke

Types	Means of induction	Characteristics
Focal ischemic models		
Mechanical MCAO models		
Surgical clips	Clip proximal MCA	Technical feasibility and consistent histological results
MCA ligation	Ligate the MCA	Lesion limited to cortex
Eletrocoagulation	Interrupt MCA blood flow by electrocoagulation	Induces distal and proximal MCAO
Intraluminal filament MCAO	Insert filament to origin of MCA	Reproduces cerebral ischemia and reperfusion injury
Embolic		
Thrombotic	Occlude MCA by thrombotic clot made with autologous blood	Best model for study of thrombolytic agents
Microspheres	Inject calibrated compounds and artificial microspheres into MCA	Different sizes of emboli induce different ischemic events
Photothrombotic	Inject photosensitive dye	Facilitates behavioral studies of ischemia in specific anatomical-functional regions
Endothelin-1	Apply ET-1 to exposed MCA	Induces dose-dependent reduction in local blood flow
Global ischemic models		
4-VO	Ligate carotid artery and vertebral arteries	Induces reversible bilateral forebrain and brainstem ischemia
2-VO	Ligate bilateral carotid arteries	Induces reversible forebrain ischemia
3-VO	Occlude common carotid and basilar arteries	Better control of CBF than 4-VO
Ventricular fibrillation	Induce cardiac arrest with ventricular fibrillation	To study mechanisms of CA-induced delayed neuronal death
Neck tourniquet	Inflate high-pressure cuff to occlude bilateral carotid arteries	Leads to variable ischemic outcomes

depletion[155]. γδT-cells are a small subset of T-cells that possess a distinct T-cell receptor (TCR) on their surface and are the major source of IL-17[156]. A significant reduction in infarct volume occurs in mice treated with TCR-γδ-specific antibody, as well as in TCR-γδ-knockout mice[157]. NK cells recruited by ischemic neuron-derived fractalkine determine the size of lesions in a T- and B-cell-independent manner[158]. In a permanent MCAO model, infiltration of NK cells into the ischemic infarct region peaks at 12 h after ischemia. Fu *et al*. found that the sphinogosine-1-phosphate receptor modulator fingolimod, which inhibits the egress of lymphocytes from lymph nodes and limits their recirculation, reduces the numbers of $CD4^+$ T-, $CD8^+$ T-, and $CD56^+$ NK cells in peripheral blood, decreases microvascular permeability, attenuates neurological deficits, and promotes functional recovery in patients with cerebral ischemia[159]. Further studies are needed to investigate the therapeutic inflammatory cell targets in cerebral ischemia.

Inflammatory cell responses after stroke usually occur in the intraluminal filament MCAO model in rodents. In addition, studies have been performed in thromboembolic and thermocoagulation stroke models[160, 161]. In the thromboembolic stroke model, significant infiltration of lymphocytes as well as cells with a lymphocyte-like morphology into the ischemic brain occurs at 25 h after stroke induction, and no significant numerical increase of microglia has been found in the ischemic brain[160]. In the thermocoagulation model, primarily neutrophils and peripheral monocytes are found along the meninges on the first day after stroke, whereas the situation is

dominated by microglia, macrophages, lymphatic dendritic cells, and T-cells, with an almost complete decline of intracerebral and peripheral neutrophils to control levels 4 days after stroke[161]. Furthermore, studies on permanent MCAO and transient MCAO animal models have shown differences in inflammatory cell infiltration into the ischemic hemisphere. Neutrophils and several cell types (monocytes, macrophages, B lymphocytes, $CD8^+$ T-lymphocytes, and NK cells) are increased at 3 h, whereas others ($CD4^+$ T-cells, NK T-cells, and dendritic cells) remain unchanged at 3 h, but increase by 24 h after permanent MCAO. Neutrophils are the predominant cell type entering the brain after stroke[162, 163]. Moreover, there are fewer infiltrating leukocytes at 24 h after transient MCAO than after permanent MCAO, while microglia are bilaterally increased in both models. In experimental stroke models, immune cell infiltration is more evident after permanent than transient occlusion of the MCA[162, 163].

As noted above, the thromboembolic model mimics ischemic stroke in humans, especially the response to thrombolytic agents. Regarding translational issues, infiltration of inflammatory cells in thromboembolic models may provide an opportunity to investigate stroke-related pathophysiology in more detail, and to test potential treatment strategies for ameliorating brain injury and improving functional recovery after stroke. Additional studies on the inflammatory responses to stroke with thromboembolic models are warranted.

Intracerebral Hemorrhagic Models

Experimental ICH models have been available since the 1960s and involve the intracerebral injection of autologous blood[164] or bacterial collagenase into the cerebrum[165, 166], balloon inflation[167, 168], or cerebral blood vessel avulsion[169]. Many species have been used to mimic the pathophysiology of ICH in patients, such as rodents, rabbits, cats, dogs, pigs, baboons, and other primates[170-175]. The most common models in preclinical studies are the intracerebral injection of autologous blood or bacterial collagenase into the cerebrum.

Autologous Blood Injection Models

The most straightforward method for the introduction of blood and hematoma formation in the brain is a single injection. Blood is taken from a superficial vessel and stereotactically injected into the striatum at different volumes to establish a hematoma model. The rapid accumulation of intraparenchymal blood is relevant to ICH in patients[164, 171]. CBF is reduced both around the hematoma and in the surrounding brain. This change is strongly volume-dependent and is not accompanied by significant alterations in cerebral perfusion pressure[165]. The volume of injected blood varies among studies and corresponds to the average hematoma size in humans[168, 176-178]. A slow injection of 50 μL blood with a Hamilton syringe over 5 min is recommended for good reproducibility of hematoma volumes[179]. Some investigators have also performed double or multiple injections, which produces consistent neurological deficits, brain swelling, and cortical hypoperfusion[180, 181]. This technique has been adapted to mice and is used widely[181].

Collagenase Injection Models

Collagenases are proteolytic enzymes that exist within cells in an inactive form and are secreted at sites of inflammation by mononuclear cells[182]. Brain tissue contains collagen in the basal lamina of blood vessels[183]. Hematoma expansion and vasogenic edema following ICH have been considered to result from elevated local concentrations of collagenase released from injured cells[184]. Injection of collagenase leads to disruption of the extracellular matrix in the basal lamina. To study the pathophysiology of ICH, Rosenberg *et al.* introduced the collagenase-induced ICH model that generally evolves over hours[166, 185]. This model is reproducible for the study of spontaneous intracerebral bleeding that develops over several hours and tests the effects of hematoma and brain edema in preclinical studies[85, 186]. Both models yield consistent hemorrhagic infarcts and are basic methods for preclinical ICH research with intrastriatal injection of autologous blood (30 μL) or bacterial collagenase (0.075 U) leading to reproducible neurofunctional deficits in mice[186].

Balloon Inflation Models

Balloon inflation models are used to study the mass effects of a hematoma and its removal on brain injury. It is an unusual experimental model of ICH[167, 187, 188]. A modified procedure has been developed in piglets, in which supratentorial ICH is induced with a balloon introduced into the right striatum through a burr hole[188].

Avulsion of Cerebral Blood Vessels

This is a simple but infrequently-used model of cortical injury. It involves stripping the cortical surface of blood vessels, where avulsion of the veins creates cortical hemorrhages[169, 189]. However, cortical vessel avulsion by pial stripping causes a mixed form of injury with non-perfusion ischemia and hemorrhage.

Inflammatory Responses after ICH

Rapid activation of resident microglia within minutes followed by infiltration of circulating inflammatory cells is a characteristic of inflammatory responses to ICH. Microglia are thought to be the first inflammatory cells to react to various pathological conditions after ICH[190, 191]. They play neuroprotective roles by clearing the hematoma and damaged cell debris through phagocytosis. However, excessive microglial activation promotes ICH-induced inflammatory injury by releasing pro-inflammatory mediators, which subsequently induce the infiltration of peripheral inflammatory cells[191-193]. Therefore, inhibition of microglial activation attenuates BBB leakage as well as edema and damage in experimental models of ICH[194].

Recruitment of peripheral leukocytes to the brain occurs in the early stages of experimental ICH[191]. Neutrophils are the earliest leukocytes associated with acute inflammation presenting in the hematoma[191]. The increased neutrophil counts may be an independent risk factor for early functional deterioration in patients with ICH, and the suppression of neutrophils appears to be a promising target in the treatment of ICH[195]. The deletion of neutrophils in collagenase-induced ICH reduces matrix metalloproteinase-9 expression, blood vessel disruption, BBB leakage, axon damage, and astrocytic and microglial/macrophage activation[196]. In addition, the deletion of neutrophils decreases tissue plasminogen activator-induced ICH in ischemic models[197]. In both mouse and rat ICH models, neutrophil infiltration occurs at the early stage and peaks at ~3 days after ICH[191, 198].

$CD8^+$ T-cells and $CD4^+$ T-cells are increased and contribute to inflammatory damage after ICH[189, 199]. Fingolimod treatment decreases both cell types in ICH patients[200] and the total counts of T-cells in the peri-hematomal area of mouse models of ICH[201], improves neurological function, and reduces edema after ICH. The number of reactive astrocytes increases significantly in the peri-hematomal area, and this contributes to ICH-induced injury in both autologous blood and collagenase injection ICH models[202]. Reactive astrocytes participate in edema *via* the induction of matrix metalloproteinase-9 in an ICH mouse model[203]. Inhibition of astrocyte activation is associated with an improvement in neurological function and a reduction of brain edema[202]. Mestriner *et al*. compared long-term GFAP-positive astrocyte morphology after ischemic and hemorrhagic stroke, and found similar astrocyte plasticity in both stroke subtypes for all evaluated measures (regional and cellular optical density, astrocytic primary process ramification and length, and density of GFAP-positive astrocytes) in the perilesional sensorimotor cortex and striatum[204]. These results suggest that microglial activation, neutrophil infiltration, increases of $CD8^+$ and $CD4^+$ T-cells, and astrocyte activation have detrimental effects after ICH.

Similar temporal inflammatory profiles of cell death, inflammatory cell infiltration, and microglial reaction following ICH are present in the autologous blood, collagenase, and cerebral vessel avulsion models. However, there are quantitative histological differences among the models. There is more necrosis, less hemorrhage, and less neutrophil infiltration in the cerebral blood vessel avulsion model than in both the autologous blood and collagenase injection models[189]. In addition, the collagenase-induced ICH model exhibits more peri-hematomal neutrophil infiltration than the autologous blood injection model[189, 198].

The reproducible experimental model of spontaneous ICH mimicking clinical ICH is an invaluable tool for improving our understanding of the mechanisms underlying inflammation following ICH-induced injury. Further studies on the differences among these experimental ICH models or ischemic and hemorrhagic stroke models are required and may lead to the development of therapeutic targets.

Subarachnoid Hemorrhage Models

SAH is a pathological condition in which arterial blood flows into the subarachnoid space, usually caused by a ruptured aneurysm. Methods used in experimental animals to mimic human SAH include injecting blood into the cisterna magna once (single injection) or twice (double injections), and endovascular perforation of an intracranial artery in the anterior circulation[205-207].

Blood Injection Model

An average of 300 μL whole blood is injected into the cisterna magna in the single-hemorrhage model to induce SAH[205, 208]. In most of the double-hemorrhage models, the second injection with autologous arterial blood is given 48 h after the first[206]. The single- and double-hemorrhage models have been performed in dogs, rabbits, rats, and mice with acceptable mortality rates[206], and the models are fairly reproducible because a fixed amount of blood is injected into the subarachnoid space[209].

Endovascular Puncture Model

In endovascular puncture, the ECA and all of its branches are identified, dissected, cauterized, and divided. A suture is inserted into the ECA and advanced through the ICA up to the MCA where the vessel is punctured. The suture is then withdrawn through the ICA into the ECA, allowing reperfusion and producing an SAH that mimics the clinical situation as closely as possible[210]. The endovascular puncture model is mainly performed in rats, although mice have also been used in a few studies[206].

The characteristics and means of induction for hemorrhagic stroke models are listed in Table 2.

Inflammatory Responses after SAH

SAH triggers reactive astrogliosis and upregulates microglial activation, which impact the brain parenchyma in the SAH model[211]. Microglia may be both necessary and sufficient to cause vasospasm in both the early and late phases of SAH in animal models[212]. In an intracranial aneurysm study, macrophages and $CD3^+$ T-lymphocytes were present at high frequency in the wall of the aneurysm but were rare in control basilar arteries[213]. However, there are few studies on the inflammatory cellular changes in SAH. Additional investigations in this area are required.

Conclusions

In this review, we have described in detail various cerebral ischemia and stroke models and their characteristics. In addition, the inflammatory responses in subtypes of these stroke/ischemia models are reviewed. Inflammatory cells infiltrate the brain and exhibit distinct temporal profiles for microglia, neutrophils, T-cells, astrocytes, and NK cells, with a variety of quantities and peak times after ischemic or hemorrhagic stroke. Furthermore, these inflammatory cells play similar roles in brain injury and neuroprotection in ischemic and hemorrhagic stroke. Unfortunately, few studies have compared the inflammatory responses and pathological patterns among stroke subtypes and experimental models[85, 204]. Clarifying the abilities of these models to mimic the conditions of post-stroke patients will assist in understanding the underlying mechanism of inflammatory responses to stroke-induced brain injury and may lead to the development of neuroprotective and neurorestorative therapeutic approaches to clinical stroke.

Table 2. Animal models of hemorrhagic stroke

Types	Means of induction	Characteristics
Intracerebral hemorrhagic models		
Autologous blood injection	Stereotactically inject different blood volumes into the striatum	Lesion strongly volume-dependent
Collagenase injection	Inject collagenase	Disruption of extracellular matrix
Balloon inflation	Place a balloon stereotactically, then inflate it	To study mass effects of hematoma
Avulsion of cerebral blood vessels	Strip the cortical surface of blood vessels	A mixed form of injury with non-perfusion ischemia and hemorrhage
Subarachnoid hemorrhage models		
Blood injection	Inject whole blood into the cisterna magna	Fairly reproducible
Endovascular puncture	Puncture a suture through the MCA	Closely mimics the clinical situation

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