Chapter 16
Animal Models for Ischemic Stroke

Liping Wang, Chuan Qin, and Guo-Yuan Yang

Abstract Developing reliable and reproducible animal model is of great importance in the therapeutic research of ischemic stroke. The location and volume of injury are varied in different animal models. Researchers choose different animal models according to the research purposes. In this chapter, we summarized the system of ischemic stroke models.

Keywords Animal model • Application • Distal • Embolic • Evaluation • Focal • Global • Ischemic stroke • Middle cerebral artery occlusion • Method and key point • Neonatal hypoxic-ischemic • Photochemically

Abbreviations

2-VO	Two-vessel occlusion
4-VO	Four-vessel occlusion
CBF	Cerebral blood flow
CCA	Common carotid artery
ECA	External carotid artery
EPCs	Endothelial progenitor cells
ICA	Internal carotid artery
MCAO	Middle cerebral artery occlusion
mNSS	Modified neurological severity score
PPA	Pterygopalatine artery
rt-PA	Recombinant tissue plasminogen activator

L. Wang • C. Qin • G.-Y. Yang (✉)
Med-X Research Institute and School of Biomedical Engineering, Shanghai Jiao Tong University, 1954 Hua Shan Road, Shanghai 200030, China
e-mail: gyyang@sjtu.edu.cn

P.A. Lapchak, G.-Y. Yang (eds.), *Translational Research in Stroke*, Translational Medicine Research, https://doi.org/10.1007/978-981-10-5804-2_16

16.1 Introduction

In order to study the pathophysiology and treatment of stroke, developing reliable and reproducible ischemic animal models is of great importance. The ideal animal model can excellently mimic features of human ischemic characteristics. Cerebral ischemic animal models could be divided into focal cerebral ischemic models and global cerebral ischemic models. Focal cerebral ischemic models include suture transient middle cerebral artery occlusion and permanent middle cerebral artery occlusion, embolic middle cerebral artery occlusion model, distal middle cerebral artery occlusion, photochemically induced middle cerebral artery occlusion model, and neonatal hypoxic-ischemic brain damage model. Global cerebral ischemic models include bilateral carotid artery ligation (2-VO), four-vessel occlusion (4-VO), and cardiac arrest-induced forebrain ischemia. This chapter outlines methods and key points, applications, advantages, and limitations of different animal models.

16.2 Focal Ischemic Stroke Model

16.2.1 Suture Transient Middle Cerebral Artery Occlusion (tMCAO)

16.2.1.1 Methods and Key Points

Adult male rats weighing 250–300 g or adult male mice weighing 30–35 g were ideal for this procedure. Isoflurane inhalation anesthesia and intraperitoneal anesthesia with ketamine (80–100 mg/kg) and xylazine (5–10 mg/kg) were the most commonly used methods of anesthesia. To continuously monitor arterial blood pressure and sample for analysis of blood gases and blood pH, a PE-50 catheter for rats or a PE-10 catheter for mice was inserted into the femoral artery. Body temperature is maintained at 37 ± 0.3 °C using a heating pad. Under the operating microscope, a midline incision on the neck is made. Then, the left common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) are exposed and isolated (Fig. 16.1). A 2.5-cm length of 4-0 suture for rats or a 1.5-cm length of 6-0 suture for mice coated with silicone was used. The suture was introduced into the transected lumen of the ECA and gently advanced from the ICA across to the opening of the MCA until a slight resistance was felt. The distance from the tip of the suture to the bifurcation of CCA was about 18 ± 1 mm in rats, while it was about 10 ± 1 mm in mice. A laser Doppler flowmetry was used to test the cerebral blood flow. The reduction of the cerebral blood flow down to 10% of the baseline indicates the success of the occlusion. For reperfusion, the suture was withdrawn to restore blood flow following 60–120 min of MCAO. The dissected skin was sutured, and the animal was returned to the cage when it recovers from the anesthesia [1–3].

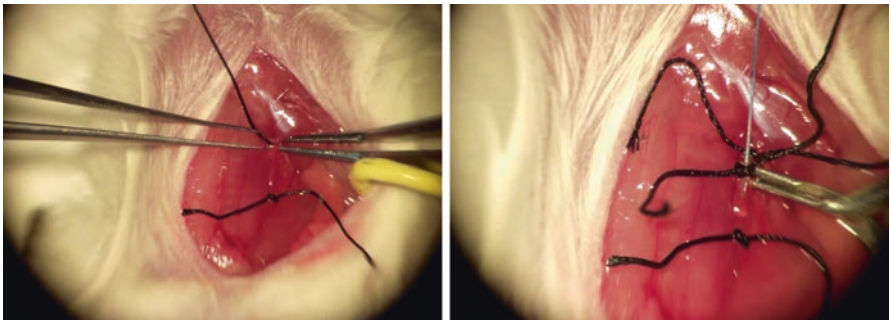

Fig. 16.1 Separation of cervical vessels and insertion of the suture under surgical microscope in the operation of suture MCAO model

16.2.1.2 Application of Transient MCAO

In recent years, many ischemic stroke animal models were established to mimic the clinical patients. In stroke research field, the aim of these stroke models was to understand the pathophysiological changes in the stroke development and the post-stroke recovery. We could use transient MCAO model to mimic the blood restoration following acute ischemia in the clinical situation. This was important to understand cellular and molecular mechanisms of stroke recovery even in the prognosis.

Transient MCAO model was worldwide used in the research trial since this model mimicked the human ischemic stroke and was highly reproducible. In addition, we could control the duration of ischemia and reperfusion, which was necessary for the study of mechanism of ischemia and reperfusion injury, as well as proper treatment during different time points.

The first main advantage of transient MCAO model was to understand the cellular and molecular mechanism in the process of cerebral ischemia. During the experiment, we could obtain the latest knowledge when the neurovascular unit was injured in the brain. We could make our hypothesis and develop a novel approach to attenuate ischemic brain injury and reverse the degeneration. Actually scientists have found many methods to inhibit or attenuate stroke occurrence and development such as signal pathway inhibitors, gene therapy, or stem cell therapy. Based on viral vector gene transfection, gene therapy was beneficial for stroke repair and recovery. Moreover, many kinds of stem/progenitor cells were effective for the stroke therapy. Endothelial progenitor cells (EPCs) were a kind of stem cells, which could differentiate into the endothelial cell for vascular repair after brain injury. EPCs could secret growth factors such as VEGF and FGF, which was necessary for the repair of vessels. The second advantage of tMCAO was this model could be used to study the restoration of blood flow during the stroke recovery stage. Recombinant tissue plasminogen activator (rt-PA) was the only proven drug for acute ischemic stroke; however, the clinical time window was very limited (within 4.5 h). The important advantage of intravenous rt-PA was that it could be used immediately

after clinical assessment. Although intravenous rt-PA has the evidence for the acute ischemic stroke therapy, the limitation including low rates of recanalization was driving people to find new approaches to prolong the therapeutic time window and to increase recanalization rates. Cerebral blood flow (CBF) was the critical issue for the stroke recovery. Effect of endovascular thrombectomy should be assessed by the recovery of blood flow. As we mentioned above, CBF changes following endovascular thrombectomy were similar to the changes observed in the transient MCAO models. Studies showed the similarities between the endovascular thrombectomy and transient MCAO animal procedure in CBF and pathophysiological characteristics, suggesting the rationale for the use of transient MCAO to mimic the potential outcomes of adjunct therapies for endovascular thrombectomy in humans. We could perform preclinical trials on animals and find more efficient drugs. Therefore, future clinical trials for neuroprotection should consider the transient MCAO model to assess preclinical efficacy.

16.2.1.3 Advantages

Intraluminal suture transient MCAO model was one of the widely used focal ischemia models. Suture-induced brain lesion is located both in the cortex and striatum. Postischemic restoration occurs frequently in human ischemic stroke induced by embolism. Transient MCAO model could mimic the clinical situation. It provides a unique tool for the mechanistic studies of cerebral ischemia in vivo. There was no need to perform craniotomy; therefore, the suture MCAO model was relatively simple.

16.2.1.4 Limitations

Approximately 10% of the animals died either during procedure even in experienced hands. The mortality was mainly due to subarachnoid hemorrhage, which was caused by inappropriate insertion of the suture.

Suture transient MCAO model had a high variation of infarct volume. Many factors contributed to the variation including brain and body temperature, method of anesthesia, physical properties of suture, duration, animal strain and weight, brain vascular anatomy, and collateral circulation [4, 5]. Many modifications were made to improve the stability.

Suture-induced thrombosis is a vital factor which causes the failure of reperfusion and reproducibility. Thrombosis could affect the infarct volume and neurobehavioral outcomes. Heparin injection 10 min before suture withdrawal could prevent thrombosis after suture withdrawal and does not reduce brain infarct volume or neurological score [2].

16.2.2 Suture Permanent Middle Cerebral Artery Occlusion

16.2.2.1 Methods and Key Points

The method for permanent MCAO is similar to that of transient MCAO described above. The only difference is that the suture stays inside the ICA without withdrawing it [6, 7].

16.2.2.2 Application of Permanent MCAO

The permanent MCAO was mainly established for the long-term ischemia of the brain [8]. In this model, the suture or other emboli were placed in the bifurcation of the artery to prevent the blood from flowing to the brain tissue. The ischemia period lasted a long time, and it could mimic the clinical pathology of stroke, which was the first leading cause of disabilities in the world. The model had a stable infarction area, but the damage to animal was so horrible at the same time that the mortality of this model was relatively high [9].

The huge territorial infarction and the considerable mortality rate in suture permanent occlusion were important features of the clinical malignant stroke, which was a devastating disease pattern and consists of 10% of all stroke patients. While neuroprotective therapies had frequently produced a significant reduction in filament-induced brain infarction following tMCAO, such protective effects were consistently absent in permanent ischemia [10]. Thus, the experimental model to mimic malignant stroke without high mortality rate was needed. Up to now, many modified permanent MCAO models had been explored to get a better animal performance and lower mortality rate [11]. These results showed that these new methods strongly reduce mortality. Using these methods, researchers could extend the studies over days or even weeks and study the mechanisms of tissue damage, edema resolution, and tissue remodeling.

By using this long-term period model, we could track the animal reactions during stroke. Collateral circulation was an important physiological accommodation; it could connect the vessels between artery and ischemic region where blood flow was needed [12]. In the transient middle cerebral artery occlusion model, it was harder to find the opening collateral circulation because there was no need to use the extra vessels when the artery still had function. So the permanent MCAO model was widely used to study the collateral circulation, the medicines which had the potential to open them, and the mechanisms during process.

As we claimed before, permanent MCAO model could mimic the clinical situation of stroke; many strategies were performed on this model. The pretreatment was the most widely ongoing research because we hoped to prevent the stroke from happening.

16.2.2.3 Advantages

Permanent MCAO suture model could mimic the cerebral injury caused by ischemic stroke without reperfusion. The stability of permanent MCAO suture model was better than that of transient MCAO suture model.

16.2.2.4 Limitation

Because the injured area of permanent MCAO was severer, the mortality of permanent MCAO was higher than that of transient MCAO. Furthermore, this model could not mimic the inflammation caused by reperfusion.

16.2.3 Embolic Middle Cerebral Artery Occlusion

16.2.3.1 Methods and Key Points

Sprague-Dawley rats, Wistar rats, and spontaneous hypertensive rats are the most common used strains for the procedure. First homologous clot is prepared 1 day before the experiment. Anesthetize a blood donor rat and expose the femoral artery. Insert the femoral artery with a 25-cm PE-50 tube. Fresh arterial blood is withdrawn by 2.5-ml syringe into the tube to form homologous clot. The clot is kept in the tube at room temperature for 2 h and subsequently stored at 4 °C for 22 h. The PE-50 tube containing the clot is cut to the length of 4 cm. Use a 2.5-ml syringe filled with saline to connect to the 4-cm tube and transfer the clot into a dish containing saline. Connect a 20-cm PE-04 with a 20-cm PE-50 tube. Inhale the clot into this PE-04-50 tube with a 2.5-ml syringe (Fig. 16.2).

Animals were anesthetized by isoflurane or ketamine and xylazine. Temperature was maintained 37 ± 0.3 °C during surgery by a heating pad. An incision was made in the middle of the neck of the rat, and the CCA, ICA, and ECA were exposed under the microscope, and then the bifurcation of the ICA and pterygopalatine artery (PPA) was exposed. The distal end of the ECA and the origin of the PPA were ligated by 5-0 suture. The proximal side of CCA and ICA was temporally clamped with microvascular clip, and then an incision was made at the ECA lumen close to the ligation. The PE-04 catheter containing a single fibrin-rich clot was introduced into the ECA lumen through the small incision. Then the clip was de-clamped at the ICA, and the catheter was advanced into the ICA. The tip of the catheter will be about 2–3 mm from the origin of MCA. The clot was gently injected with a 2.5-ml syringe filling with saline. After 5 min, the catheter was withdrawn from the ECA. The skin was closed with 3-0 suture. The animal was put back to the cage after it was awake from anesthesia [13–15].

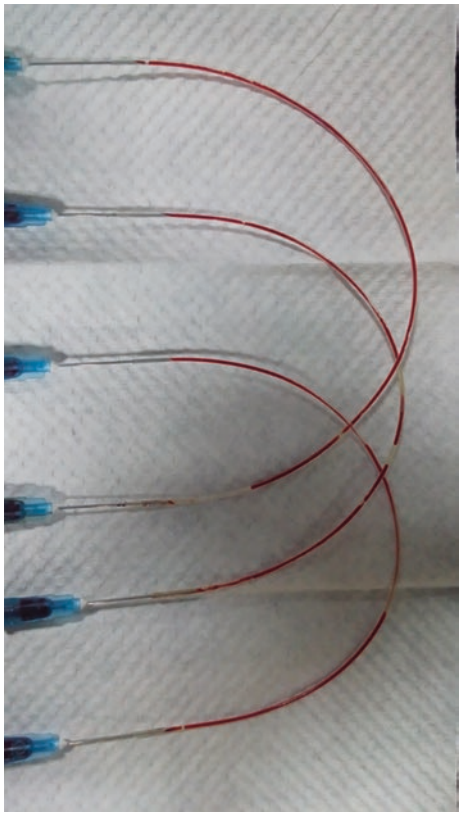

Fig. 16.2 Preparation of thrombus

16.2.3.2 Application of Embolic MCAO

When human stroke was mostly caused by cerebral thromboembolism, a number of animal models had been developed to mimic the embolic occlusion of brain arteria. Embolic stroke models could be performed by injecting some large or small synthetic microspheres into the internal carotid artery to the bifurcation of middle carotid artery. In the first case, large infarcts were similar to those produced by the permanent occlusion of the MCA which could be induced. But in the latter case, some smaller and multifocal infarcts could occur [16, 17]. To study thrombolytic therapies (a clinically very relevant therapeutic issue), several models of vascular occlusion were developed using autologous blood clots that are injected into the internal carotid artery [18]. Embolic stroke models were variable infarcts, which made it more difficult to assess the neuroprotective results; and meanwhile they were well suited to study the reperfusion therapies.

Thromboembolic models of focal cerebral ischemia were mostly used to reestablish human thromboembolic stroke, particularly to investigate the thrombolytic medicine for acute focal cerebral ischemia. In order to find new strategies that may

increase thrombolytic efficiency and reduce the fail rate, the reproducible animal model is required.

Embolic models were more closely representative of the pathophysiology of human ischemic stroke; they offered potential to test more thrombolytic agents [19], assess reperfusion damage after thrombolysis [20], and study potential combination therapies, such as thrombolytic agents combined with neuroprotective drugs [21, 22]. At first, researchers had willings to use the human blood clot suspension of homologous small clot fragments [23]. However, they induced infarcts where spontaneous recanalization took place, and later on autologous clot attracted researcher's attention [24]. Some researchers had described a model of microsphere embolic stroke which had been proved to reduce variability in lesion and more close to the evolution of human disease [25, 26]. However, a definitive microsphere model remains to be defined, because one half of microspheres failed to place in the cerebral arterial circulation [27].

The thromboembolic stroke model showed an excellent reproducibility, and the purified thrombin could be performed to the MCA with good precision [28]. Previously this model was given poor consideration in neuroprotection studies after several failures in applying animal results in human. Moreover, the supraphysiological thrombin content in clots generated by this method might confer resistance to currently available thrombolytic agents [29]. Compared with the mechanical models that had greater accuracy, thrombolytic model did reflect hemodynamic characteristics of blood reperfusion and influencing factors of brain tissue responding to neuroprotective agents.

16.2.3.3 Advantage

Embolic MCAO model in rats could be used to mimic human thromboembolic stroke. So this reproducible animal model could be applied to investigate thrombolytic therapies for cerebral embolism.

16.2.3.4 Limitation

The infarct volume of this model was not stable. The variation was relatively high because of the properties of thrombus. One main reason for failure was spontaneous vascular thrombolytic recanalization.

16.2.4 Distal Middle Cerebral Artery Occlusion

16.2.4.1 Methods and Key Points

Adult male Sprague-Dawley rats weighing 250–300 g were anesthetized by isoflurane or ketamine and xylazine, and an incision was made between the orbit and the tragus under an operation microscope. The temporal muscle was retracted laterally, and a 3-mm diameter craniotomy was made just rostral to the foramen ovale. The dura was incised with a tiny hook, and the MCA was exposed. The arachnoid was then opened, and the MCA was ligated for the permanent MCAO.

For adult male CD-1 mice weighing 30–35 g or adult male C57BL/6 mice weighing 25–30 g, the process was similar to that of rats. Mice are anesthetized by isoflurane or ketamine and xylazine, and a 1-cm incision is made between the orbit and the tragus. The arachnoid is opened, and the MCA is ligated for the permanent MCAO. The reduction of the cerebral blood flow is verified by a laser Doppler flowmetry. The incised skin is sutured. The infarct produced by this model restricts to the cerebral cortex.

16.2.4.2 Application of Distal MCAO

Compared with the other models of MCAO, distal model was the most stable model in all of them, and the infarct area of distal MCAO model was always in the cortex whatever degree of injury was, while the infarct size was affected by whether the MCA and CCAs are permanently or transiently occluded. Cerebral ischemia induced by this method damaged most of the frontal, parietal, temporal, and rostral occipital cortices, the underlying white matter, and a marginal part of the striatum. This technique avoided the thalamic, hypothalamic, hippocampal, and midbrain damage that was seen in the suture transient MCAO model [30]. In the distal MCAO model, it was more common now to ligate the MCA at a more distal location and leave the zygomatic arch intact. This made the surgery less intrusive and speed up recovery time; this procedure induced a smaller infarct compared to the suture MCAO model.

There were several advantages of this model, which has good reproducibility in infarct size and neurologic deficits, low mortality, and visual confirmation of successful MCAO surgery. The main disadvantage was the craniectomy technique, which might lead to injury of the underlying cortex or rupture of a vessel by drilling or electrocoagulation. Additionally, this technique allowed subsequent reperfusion of ischemic tissue. Furthermore, this procedure affected intracranial pressure and blood-brain barrier function and required significant surgical skills to perform it. In order to overcome this limitation, researchers preferred to induce distal MCAO using a photochemical approach through the intact skull of the mouse, which was another kind of MCAO model.

Cortex injury induced by the distal MCAO model was much more stable than other models, so the distal model was becoming more and more widely used not only for its stability but also the convenience in animal experiment. And in some cases where we just want to create a cortex injury excluding the striatum, distal model is the best choice; it is enough to produce the damage that we want.

16.2.4.3 Advantage and Limitation

The distal MCAO model had high stability and strong repeatability. The operation was relatively simple. This model produced ischemic brain injury that was restricted to the cortex of the ipsilateral hemisphere and similar to the pattern of cerebral ischemia in human. Although craniotomy was performed, the overall mortality rate remained low. The limitation was that the infarct volume might be too small to evaluate the therapeutic effect of intervention.

16.2.5 Photochemically Induced Cortical Ischemia

16.2.5.1 Methods and Key Points

Male C57BL/6 mice (20–25 g) were used in the photothrombotic cortical ischemia model. The animal was anesthetized by isoflurane or ketamine and xylazine. The body temperature was monitored continuously and maintained at 37 ± 0.5 °C during surgery using a heating pad. Under an operational microscope, an incision was made between the right orbit and the external auditory canal. A fiber-optic bundle of a KL1500 LCD cold light source with a 4-mm aperture was focused on the selected arteriole. Immediately after the intravenous injection of rose bengal, the brain was illuminated through the exposed intact skull for 2 min. Finally, the surgical wound was sutured [26, 31, 32].

16.2.5.2 Application, Advantage, and Limitation

One of advantages of this model was its high reproducibility and the minimal variation in infarct size combined with a very low mortality. This made it a predestined model to study repair mechanisms and related long-term functional outcome. Another advantage of this model was that the researchers could select a specific cortical brain region of interest to make the ischemia by using stereotactic coordinates [33]. The major issues with this experimental model were fundamental differences compared to the situation in acute human stroke. In this kind of stroke model, cytotoxic and vascular edema and the rapid breakdown of blood-brain barrier were all in an acute phase [34].

But in this model we could not reopen the vessel that was blocked by the thrombus induced by light. It could only be a permanent ischemic model. Compared to the other focal stroke models, this model involved the nonphysiological treatment that made a lesion [35]. Further, because of the damage of the vessels induced by the "photothrombosis," substantial local vasogenic edema formed early after infarction; this was not similar to a clinical patient.

16.2.6 Neonatal Hypoxic-Ischemic Brain Injury

16.2.6.1 Methods and Key Points

Originally 7-day-old Wistar or Sprague-Dawley rats weighing 14–18 g were commonly used in this model. Isoflurane or halothane was delivered from a vaporizer in a mixture of oxygen and nitrous oxide (1:1), 3% for induction and 1.0–1.5% for maintenance. The duration of anesthesia should be less than 5 min. A cervical incision was made, and the common carotid artery was exposed. The common carotid artery using two sutures was ligated; the artery was cut off between the two ligatures. The wound was sutured and infiltrated with a local anesthetic. The animal was kept at around 33 °C temperature before and after procedure. The animal was not anesthetized during hypoxia. The animal was allowed to recover for 1 h. After recovery, the animal was put in the incubator, and the temperature was kept strictly to 36 °C by flowing heated, moisturized air through the incubator for 10 min. The gas flow rate was kept at 3.0 L/min and switched from 7.8% air for rats to 10% oxygen for mice balanced with nitrogen for 50 min. The hypoxic gas mixture was heated and moisturized prior to flow through the incubator and kept at a constant flow of 3.0 L/min. The hypoxia time should be modified according to the lab conditions and different animal strains. Gas flow was switched from 7.8% for rats or 10% for mice oxygen to air for another 10 min. The animals were put back after ischemia [36, 37].

16.2.6.2 Application

In the last decades, researchers had found some similarities in the mechanisms of brain injury and damage evolution between rodents and human infants with cerebral palsy during HI (hypoxia/ischemia) [38]. Modified HI models successfully reproduced hypoxic-ischemic conditions, white and gray matter damage in apoptotic-necrotic pattern, and neuromotor impairments. However, the biggest limitation of the HI rodent models was the difference between rodents and humans in the overall complexity of brain organization and the discrepancies compared with human in the rate of maturation in any period of time [39].

Hypoxia-ischemia rodent models could be used to induce brain injury at the similar stage of cellular development: between 24 and 32 weeks of gestation in human. Most of the oligodendroglial lineage cells were at the stage of pre-OLs at these time points, and they were maximally vulnerable to hypoxic-ischemic injury.

16.2.6.3 Advantage

The neonatal rodent cerebral hypoxia-ischemia model was the most widespread perinatal brain injury model. Ischemic brain damage was produced by unilateral common carotid artery ligation, which induced moderate hypoxia.

The operation was technically simple; this model produced reproducible brain injury with low mortality. This model had proven to be an excellent tool to study physiological and molecular mechanisms of perinatal hypoxia-ischemia brain damage and test potentially protective treatments.

16.2.6.4 Limitation

The variation of the injury degree of brain tissue was considerable. So in order to compensate for the variation, a large number of animals were needed in each group [40].

Another disadvantage was that this model could not produce white matter lesions easily. As white matter lesions were often observed in premature infants, this model had the limitation in precisely mimicking the clinical situation.

16.3 Global Cerebral Ischemia

16.3.1 Bilateral Carotid Artery Ligation (2-VO)

16.3.1.1 Methods and Key Points

Male Wistar rats weighing 250–300 g were commonly used for the procedure. After the rat was anesthetized, the neck skin was shaved and scrubbed with alcohol. A midline cervical incision is made. Both common carotid arteries were gently exposed and isolated from nerve fibers and the surrounding tissues. Then bilateral common carotid arteries were ligated with 5-0 suture. The skin was closed with 3-0 suture. The body temperature was kept at 37 ± 0.5 °C during the procedure until the animal recovery from anesthesia [41].

16.3.1.2 Application of 2-VO

Two-vessel occlusion was the most common model for the chronic cerebral hypoperfusion [42]. In clinic, the chronic ischemic represented many disease situations like Alzheimer. In human, the most common state of aging was the hypoperfusion of the brain tissue, especially the functional cortex that controls the movement and memory [43]. Due to the importance of learning and memory, it was meaningful to perform the chronic hyperfusion research. Though there were several models mimicking the chronic hypoperfusion situation, the two-vessel occlusion was the most widely used model. The aim of two-vessel occlusion was to produce a global ischemic damage in which the onset and the reversal of ischemic process were very rapid. The damage that is produced by the two-vessel occlusion changes the cell morphology in the ischemic brain areas including the hippocampal CA1 subfield, striatum, and cortex. And it had been well documented that the two-vessel occlusion was neither to bring the cerebral blood flow down below the ischemic threshold nor to upset the energy state of the brain tissue to an extent to induce the cell death [44]. With the management of the two-vessel occlusion, most of the brain areas had similar levels of damage, except the Purkinje fibers of the cerebellar brain stem.

With the establishment of the two-vessel occlusion model, pharmacology of many medicines had been studied in the rat or mouse. A number of results were found, and most of these experiments could be divided into these parts. The first purpose was to detect the difference of the region blood flow between treatment and control [42]. The trials were mainly wanted to study the effect of the regulation ability of a medicine or chemical compound. And the other aim of the model was to study the protection mechanism of some medicine or compound. The ischemia often led to the trouble of learning and memory ability, for the decrease of brain blood flow reduced the activity of brain. Based on the theory, the 2-VO rat or mouse was used as the learning- and memory-deficient model; scientists used this model to study methods that could relieve or even reverse the symptom. The second purpose of the model was to mimic the hypoperfusion microenvironment and assess the ability of some treatment that regulates the cerebral blood flow and the function of specific region of the brain like the cortex and striatum. For example, researcher reported effects of chronic guanosine treatment on hippocampal damage and cognitive impairment of rats induced by chronic cerebral hypoperfusion [45]. This study aimed at the reperfusion of the cerebral blood flow in the whole brain and how these treatments could increase the perfusion level, such as the collateral circulation or other way.

16.3.1.3 Advantage and Limitation

The surgical procedure was relatively simple. The success rate of this model is more than 90%. This model could induce high reproducible ischemic damage with great animal survival rate. This model had selective neuronal vulnerability and delayed

neuronal death. For example, typically CA1 pyramidal neurons of the hippocampus were most vulnerable.

The limitation was that brain ischemia could not be induced in awake rats, and it required systemic control of hypotension.

16.3.2 Four-Vessel Occlusion (4-VO) Model

16.3.2.1 Methods and Key Points

Male Wistar rats weighing 250–300 g were used in this model. The animal was anesthetized, and a dorsal neck incision was made from the occipital bone to the second cervical vertebra. The paraspinal muscles were separated to expose the alar foramina of the first cervical vertebrate. The vertebrate arteries were occluded by electrocautery. On the following day, the bilateral common carotid arteries were occluded with aneurysm clips for 10 min to induce ischemia. The animal lost its righting reflex within 30 s, and its pupils were dilated and unresponsive to the light. After 10-min ischemia, the aneurysm chips were removed for reperfusion. The body temperature was maintained at around 37 °C during the procedure [46].

16.3.2.2 Application of 4-VO

The 4-VO was mainly used to produce a global cerebral ischemic pathology. Moreover, this model was always compared with the two-vessel occlusion model; the difference was that the posterior circulation is still working in two-vessel occlusion model; the post-cerebral artery blood could be compensatory supplied to the hindbrain, which only lead to forebrain ischemia [47]. When there is no compensatory blood from the post-cerebral artery in the occlusion of vertebral artery, we could create a permanent global cerebral ischemia and give the reperfusion after a short time in the occlusion of the bilateral common carotid arteries.

There were always some collateral circulations or blood compensation involved in other ischemic model, and that is why we could only induce a regional ischemic model. In order to perform the global ischemic model, the four-vessel occlusion was proposed. In the complete non-blood environment, apoptosis of neuron in the cortex or hippocampus was studied. The neuropathological examination of the model shows a good repeatability of the ischemic damage in the target brain region. The vulnerable brain regions to ischemia were the CA1/2 region of the hippocampus, subiculum, the CA3 region of hippocampus, the CA4 region of hippocampus, superior pyramidal lobe and inferior pyramidal lobe of dentate gyrus, and neocortex in order. The CA1/2 hippocampus and subiculum were most vulnerable to ischemic damage. With the increase of ischemic duration, the damage of the neurons from the outer segment of CA1/2 hippocampus to subiculum was intensified gradually.

Ischemic damage of the neurons in neocortex varied greatly in different individuals; no evident change appeared even after 15 min of ischemia in some animals.

Combining 4-VO with arterial hypotension could considerably enhance the severity of the ischemia by nearly eliminating all of CBF for sustaining tissue. Indeed, total circulatory arrest in the brain was observed only when systemic hypotension was employed and never when the blood pressure was normal. Global cerebral ischemia caused by 4-VO in the rat shows variation in its severity among regions of the brain. Fortunately, the blood supplied to the cardiorespiratory centers in the medulla is relatively spread, which maintained the viability of the animal. Arterial hypotension during 4-VO considerably enhanced the degree of ischemia and permits complete circulatory arrest to be achieved in the cerebrum [48].

16.3.2.3 Advantage

4-VO model in rat was well established and produces reproducible neuropathological results. The cost in purchase and maintenance was relatively low. 4-VO model induced transient forebrain ischemia that mimicked cardiac arrest in clinical situations. This model produced reliable outcome with selective, delayed cell death. For example, CA1 pyramidal neurons in the hippocampus died 2–3 days after ischemia, while CA3 neurons survived after the same insult.

16.3.2.4 Limitations

A common complication of this model was seizure. The incidence of seizures had positive correlation with the severity and duration of ischemia. Ten percent of the animals died during ischemia or after reperfusion. One major complication that contributed to the animal's death during ischemia was the respiratory failure during the first 2–3 min of occlusion. Death after reperfusion might have correlation with severe ischemia. The electrocauterization of the vertebral arteries which are hidden underneath the alar foramina was a difficult task. Unsuccessful electrocauterization was the major reason for incomplete ischemia.

The damage of 4-VO might vary among animals. Many factors, including cerebral collaterals, brain temperature, anesthesia, etc., influenced the ischemic outcome. So in order to obtain stable and consistent results, strictly control variables and optimize experimental conditions in the surgical process.

16.3.3 Cerebral Ischemia Induced by Cardiac Arrest

16.3.3.1 Methods and Key Points

Male adult Sprague-Dawley rats weighing 300–350 g were used in the asphyxia cardiac arrest model. Anesthesia was induced with 4.0% halothane in oxygen, which was then titrated to 1.5% halothane during the surgery. ECG electrodes were put on each forelimb and hind limb. The left femoral area was shaved and prepared with povidone-iodine. The femoral artery and vein were dissected apart carefully. The left femoral vein and artery were inserted with catheters. Arterial blood pressure and blood gases were monitored. The rat was paralyzed by slowly infusing vecuronium (2 mg/kg) intravenously in order to induce asphyxial arrest. During administration of vecuronium, halothane was discontinued. The oxygen source was disconnected, and ventilation with room air was allowed for 2 min. Asphyxia was induced by turning off the ventilator at end expiration. Blood pressure and heart rate would begin to decrease within 30 s. There was no pressure gradient when arterial pressure fell to near 20 mmHg or when pulsatile signals were absent from the arterial tracing. Complete circulatory arrest occurred reliably within 180 s after onset of asphyxia. After 8 min, the ventilator was reconnected, and ventilation resumed with 100% oxygen at a rate of 60 breathes/min. Rapidly administer the resuscitation drugs and deliver chest compressions at a rate of 200 beats/min. Chest compressions continued until spontaneous cardiac activity was detected. The rat would require ventilator support for at least 60 min. When the rat breathes well on its own, the tubes were withdrawn. Rats would not feed properly and might be in a coma for several days after cardiac arrest. Subcutaneous fluids (e.g., 5% dextrose in 0.9% saline) were administered (20–40 ml/kg/day) until rats could feed independently [49, 50].

16.3.3.2 Application

The brain injury associated with cardiac arrest was more global with evidence of neuronal injury in multiple brain regions. Thus, rats surviving cardiac arrest might be expected to have deficits in a variety of behavioral tests in addition to memory [49]. The global assessment of neurological function used here had more relevance to the clinical situation of cardiac arrest, which caused coma followed by global dysfunction.

An important limitation of many porcine cardia arrest models was that juvenile, disease-free pigs were generally used. In clinical settings, patients who experience cardiac arrest typically were elderly and suffered from chronic disorders such as hypertension, atherosclerosis, congestive heart failure, diabetes, emphysema, or end-stage renal disease [51]. The Ossabaw swine, which was predisposed to develop metabolic syndrome when consuming a high-fat diet, provided a unique, clinically relevant experimental model; it was suitable for studying cardiac arrest and

resuscitation superimposed on metabolic syndrome [52]. Indeed, under anesthesia, these swine developed severe arrhythmias, responsive to amiodarone, that might deteriorate into cardiac arrest.

16.3.3.3 Advantage

Sudden cardiac arrest resulted in neurological injury, affecting the majority of person who initially had restoration of pulses. The rat asphyxial cardiac arrest was an established model which mimicked neurological injury after cardiac arrest. This model could reproduce many aspects of neurological injury, for example, transient coma, evolving motor deficits, persistent sensorimotor deficits, and the systemic metabolic disruptions.

16.3.3.4 Limitation

The process of cardiac arrest requires 3 h of special care. The animals need 2–3 days' care after operation. Acute death is not common. Most of the death time is 1–5 days after cardiac arrest which is related to the severity of neurological impairment. The main cause of death is respiratory failure.

16.4 Methods of Evaluation

16.4.1 Cresyl Violet Staining

Cresyl violet staining was used for infarct volume measurement. Following MCAO, the animals were killed, and the brains were removed and frozen immediately in isopentane at −42 °C for 5 min. A series of 20-μm-thick coronal sections from anterior commissure to hippocampus were cut. The distance between adjacent sections on the slide was 200 μm. The sections were dried and stained with Cresyl violet [7]. The infarct area that could not be stained by Cresyl violet (Fig. 16.3) was calculated. Using Image J software, the ischemic area was delineated, and the infarct volume was calculated by multiplying the infarct area by the thickness of the section.

16.4.2 2,3,5-Triphenyltetrazolium Chloride (TTC) Staining

Another method of detecting infarct area was TTC staining which was simple and fast. After MCAO, animal brains were removed immediately and six (for rats) or four (for mice) coronal slices were dissected using a brain slicer. The brain slices

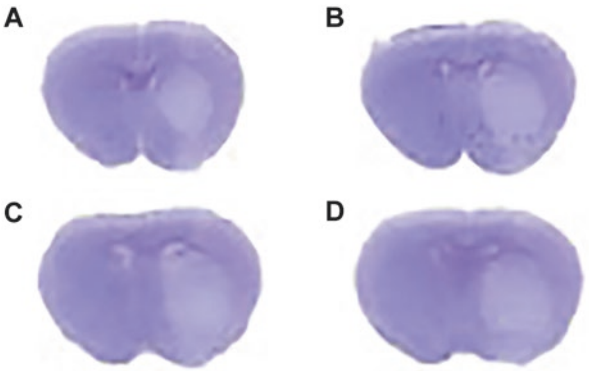

Fig. 16.3 A set of Cresyl violet-stained mouse brain coronal sections. The mouse underwent 90 min of MCAO, followed by 24 h of reperfusion

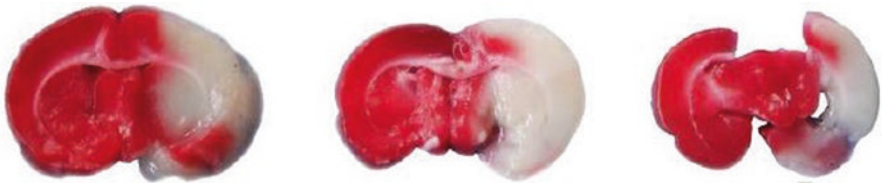

Fig. 16.4 A set of TTC-stained mouse brain coronal sections of a rat 24 h after embolic MCAO

were stained with 2% TTC in Dulbecco's phosphate buffer (pH 7.4) at 37 °C for 20 min [53]. The ischemic lesion area could not be colored red by electron transport in active mitochondria. The volume of infarction is calculated by multiplying the distance between sections (Fig. 16.4).

16.4.3 Brain Water Content Assay

Rats or mice were sacrificed at 1 or 3 days after acute ischemia. Brain samples were weighted before and after dehydration in an oven at 95 °C for 24 h. Brain water content was calculated using the formula: ((wet tissue weight – dry tissue weight)/wet tissue weight) ×100%. The water content of normal brain tissue was about 78% while that of the ischemic brain tissue might elevate to 82–85% [54].

16.4.4 Neurological Severity Scores

modified Neurological severity score (mNSS)	
Raising rat by the tail	
Flexion of the forelimb	1
Flexion of the hind limb	1
Head moved >10° to vertical axis within 30 s	1
Placing rat on the floor (normal=0; maximum=3)	
Normal walk	0
Inability to walk straight	1
Circling toward the paretic side	2
Fall down to the paretic side	3
Beam balance tests (normal=0; maximum=6)	
Balances with steady posture	0
Grasps side of beam	1
Hugs the beam and one limb falls down from the beam	2
Hugs the beam and two limbs fall down from the beam or spins on beam (>60 s)	3
Attempts to balance on the beam but falls off (>40 s)	4
Attempts to balance on the beam but falls off (>20 s)	5
Falls off: No attempt to balance or hang on to the beam (<20 s)	6
Reflexes absent and abnormal movements	
Corneal reflex (eye blink when lightly touching the cornea with cotton)	1
Startle reflex (motor response to a brief noise from snapping a clipboard paper)	1

16.4.5 Rotarod Test

Mice or rats were placed on a rotating rod at a fixed speed for 1 min to make an adaptation. The rod was slowly accelerated from 20 to 40 rpm in 5 min. Duration that mice stayed on the rod (fall latency) was recorded and analyzed as mean duration of three trials on the rotarod. Before performing stroke model, mice or rats were trained for 3 consecutive days to get stable baseline values. The baseline values represented normal neurobehavioral function. Rotarod test could be performed at 7 days, 14 days, 21 days, or 28 days after stroke [1, 55, 56].

16.4.6 Morris Water Maze Test

Water maze test was used to detect the capacities of spatial learning and memory. The water maze was located in a large room with several visual signs around the tank to show the orientation. The visual signs were used by the rats or mice for

spatial orientation. The position of the cues remained unchanged throughout the experiment. The round water tank is divided into four quadrants (north, south, east, and west). Water was filled to a height of 50 cm. The water temperature was kept at 21–24 °C. A circular platform was placed in the center of one of the four quadrants and was 1–2 cm for rats and 0.5–1 cm for mice under the water surface, hidden from the animal's view.

In the test, the animals were first trained to find the hidden platform for 5 days. The animal was placed in the water facing the wall at a start position in any of the four areas. Each animal was allowed to find the platform. If the animal failed to find the hidden platform within 120 s for rats and 60 s for mice, it was placed on the platform for 15 s. The procedure was repeated for all the four start locations. On the sixth day, the platform is removed and the animal is placed in a novel starting position. The number of platform-site crossovers, time, and distance spent in the target quadrant was recorded [57–59].

16.5 Conclusion and Future Directions

Ischemic stroke models need high level of operation to ensure the model's success and stability. Patience, carefulness, and practice are required. Each model remains huge space for development, and stroke models need continuously being improved.

References

1. Li Q, Tang G, Xue S, He X, Miao P, Li Y, et al. Silica-coated superparamagnetic iron oxide nanoparticles targeting of EPCs in ischemic brain injury. Biomaterials. 2013;34(21):4982–92.
2. Lin X, Miao P, Wang J, Yuan F, Guan Y, Tang Y, et al. Surgery-related thrombosis critically affects the brain infarct volume in mice following transient middle cerebral artery occlusion. PLoS One. 2013;8(9):e75561.
3. Chen C, Lin X, Wang J, Tang G, Mu Z, Chen X, et al. Effect of HMGB1 on the paracrine action of EPC promotes post-ischemic neovascularization in mice. Stem Cells. 2014;32(10):2679–89.
4. Guan Y, Wang Y, Yuan F, Lu H, Ren Y, Xiao T, et al. Effect of suture properties on stability of middle cerebral artery occlusion evaluated by synchrotron radiation angiography. Stroke. 2012;43(3):888–91.
5. Yuan F, Tang Y, Lin X, Xi Y, Guan Y, Xiao T, et al. Optimizing suture middle cerebral artery occlusion model in C57BL/6 mice circumvents posterior communicating artery dysplasia. J Neurotrauma. 2012;29(7):1499–505.
6. Li Y, Huang J, He X, Tang G, Tang YH, Liu Y, et al. Postacute stromal cell-derived factor-1alpha expression promotes neurovascular recovery in ischemic mice. Stroke. 2014;45(6):1822–9.
7. Huang J, Li Y, Tang Y, Tang G, Yang GY, Wang Y. CXCR4 antagonist AMD3100 protects blood-brain barrier integrity and reduces inflammatory response after focal ischemia in mice. Stroke. 2013;44(1):190–7.
8. Shanbhag NC, Henning RH, Schilling L. Long-term survival in permanent middle cerebral artery occlusion: a model of malignant stroke in rats. Sci Rep. 2016;6:28401.

9. Fluri F, Schuhmann MK, Kleinschnitz C. Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther. 2015;9:3445–54.
10. Shimazu T, Inoue I, Araki N, Asano Y, Sawada M, Furuya D, et al. A peroxisome proliferator-activated receptor-gamma agonist reduces infarct size in transient but not in permanent ischemia. Stroke. 2005;36(2):353–9.
11. Spratt NJ, Fernandez J, Chen M, Rewell S, Cox S, van Raay L, et al. Modification of the method of thread manufacture improves stroke induction rate and reduces mortality after thread-occlusion of the middle cerebral artery in young or aged rats. J Neurosci Methods. 2006;155(2):285–90.
12. Armitage GA, Todd KG, Shuaib A, Winship IR. Laser speckle contrast imaging of collateral blood flow during acute ischemic stroke. J Cereb Blood Flow Metab. 2010;30(8):1432–6.
13. Ishrat T, Pillai B, Ergul A, Hafez S, Fagan SC. Candesartan reduces the hemorrhage associated with delayed tissue plasminogen activator treatment in rat embolic stroke. Neurochem Res. 2013;38(12):2668–77.
14. Zhang RL, Chopp M, Zhang ZG, Jiang Q, Ewing JR. A rat model of focal embolic cerebral ischemia. Brain Res. 1997;766(1–2):83–92.
15. Zhang Z, Zhang RL, Jiang Q, Raman SB, Cantwell L, Chopp M. A new rat model of thrombotic focal cerebral ischemia. J Cereb Blood Flow Metab. 1997;17(2):123–35.
16. Gerriets T, Li F, Silva MD, Meng X, Brevard M, Sotak CH, et al. The macrosphere model: evaluation of a new stroke model for permanent middle cerebral artery occlusion in rats. J Neurosci Methods. 2003;122(2):201–11.
17. Miyake K, Takeo S, Kaijihara H. Sustained decrease in brain regional blood flow after microsphere embolism in rats. Stroke. 1993;24(3):415–20.
18. Kilic E, Hermann DM, Hossmann KA. A reproducible model of thromboembolic stroke in mice. Neuroreport. 1998;9(13):2967–70.
19. Overgaard K. Thrombolytic therapy in experimental embolic stroke. Cerebrovasc Brain Metab Rev. 1994;6(3):257–86.
20. Brinker G, Franke C, Hoehn M, Uhlenkuken U, Hossmann KA. Thrombolysis of cerebral clot embolism in rat: effect of treatment delay. Neuroreport. 1999;10(16):3269–72.
21. Krafft PR, Bailey EL, Lekic T, Rolland WB, Altay O, Tang J, et al. Etiology of stroke and choice of models. Int J Stroke. 2012;7(5):398–406.
22. Zhang L, Zhang ZG, Zhang C, Zhang RL, Chopp M. Intravenous administration of a GPIIb/IIIa receptor antagonist extends the therapeutic window of intra-arterial tenecteplase-tissue plasminogen activator in a rat stroke model. Stroke. 2004;35(12):2890–5.
23. Papadopoulos SM, Chandler WF, Salamat MS, Topol EJ, Sackellares JC. Recombinant human tissue-type plasminogen activator therapy in acute thromboembolic stroke. J Neurosurg. 1987;67(3):394–8.
24. Takano K, Carano RA, Tatlisumak T, Meiler M, Sotak CH, Kleinert HD, et al. Efficacy of intra-arterial and intravenous prourokinase in an embolic stroke model evaluated by diffusion-perfusion magnetic resonance imaging. Neurology. 1998;50(4):870–5.
25. Rapp JH, Pan XM, Yu B, Swanson RA, Higashida RT, Simpson P, et al. Cerebral ischemia and infarction from atheroemboli <100 microm in size. Stroke. 2003;34(8):1976–80.
26. Ahn SM, Kim HN, Kim YR, Choi YW, Kim CM, Shin HK, et al. Emodin from Polygonum multiflorum ameliorates oxidative toxicity in HT22 cells and deficits in photothrombotic ischemia. J Ethnopharmacol. 2016;188:13–20.
27. Zhu L, Hoffmann A, Wintermark M, Pan X, Tu R, Rapp JH. Do microemboli reach the brain penetrating arteries? J Surg Res. 2012;176(2):679–83.
28. Orset C, Macrez R, Young AR, Panthou D, Angles-Cano E, Maubert E, et al. Mouse model of in situ thromboembolic stroke and reperfusion. Stroke. 2007;38(10):2771–8.
29. Niessen F, Hilger T, Hoehn M, Hossmann KA. Differences in clot preparation determine outcome of recombinant tissue plasminogen activator treatment in experimental thromboembolic stroke. Stroke. 2003;34(8):2019–24.

30. Wayman C, Duricki DA, Roy LA, Haenzi B, Tsai SY, Kartje G, et al. Performing permanent distal middle cerebral with common carotid artery occlusion in aged rats to study cortical ischemia with sustained disability. J Vis Exp. 2016;108:53106.
31. Qian C, Li PC, Jiao Y, Yao HH, Chen YC, Yang J, et al. Precise characterization of the penumbra revealed by MRI: a modified Photothrombotic stroke model study. PLoS One. 2016;11(4):e0153756.
32. Liao LD, Bandla A, Ling JM, Liu YH, Kuo LW, Chen YY, et al. Improving neurovascular outcomes with bilateral forepaw stimulation in a rat photothrombotic ischemic stroke model. Neurophotonics. 2014;1(1):011007.
33. Frauenknecht K, Diederich K, Leukel P, Bauer H, Schabitz WR, Sommer CJ, et al. Functional improvement after Photothrombotic stroke in rats is associated with different patterns of dendritic plasticity after G-CSF treatment and G-CSF treatment combined with concomitant or sequential constraint-induced movement therapy. PLoS One. 2016;11(1):e0146679.
34. Scalzo F, Nour M, Liebeskind DS. Data science of stroke imaging and enlightenment of the penumbra. Front Neurol. 2015;6:8.
35. Bacigaluppi M, Comi G, Hermann DM. Animal models of ischemic stroke. Part two: modeling cerebral ischemia. Open Neurol J. 2010;4:34–8.
36. Wang X, Zhu C, Wang X, Gerwien JG, Schrattenholz A, Sandberg M, et al. The nonerythropoietic asialoerythropoietin protects against neonatal hypoxia-ischemia as potently as erythropoietin. J Neurochem. 2004;91(4):900–10.
37. Bona E, Johansson BB, Hagberg H. Sensorimotor function and neuropathology five to six weeks after hypoxia-ischemia in seven-day-old rats. Pediatr Res. 1997;42(5):678–83.
38. Colver A, Fairhurst C, Pharoah PO. Cerebral palsy. Lancet. 2014;383(9924):1240–9.
39. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. Early Hum Dev. 1979;3(1):79–83.
40. Hagberg H, Bona E, Gilland E, Puka-Sundvall M. Hypoxia-ischaemia model in the 7-day-old rat: possibilities and shortcomings. Acta Paediatr Suppl. 1997;422:85–8.
41. Park JA, Lee CH. Time-course change of Redd1 expressions in the hippocampal CA1 region following chronic cerebral hypoperfusion. Cell Mol Neurobiol. 2016;37(3):563–9.
42. Karpiak SE, Tagliavia A, Wakade CG. Animal models for the study of drugs in ischemic stroke. Annu Rev Pharmacol Toxicol. 1989;29:403–14.
43. Ohta H, Nishikawa H, Kimura H, Anayama H, Miyamoto M. Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. Neuroscience. 1997;79(4):1039–50.
44. Tsuchiya M, Sako K, Yura S, Yonemasu Y. Cerebral blood flow and histopathological changes following permanent bilateral carotid artery ligation in Wistar rats. Exp Brain Res. 1992;89(1):87–92.
45. Ganzella M, de Oliveira ED, Comassetto DD, Cechetti F, Cereser VH Jr, Moreira JD, et al. Effects of chronic guanosine treatment on hippocampal damage and cognitive impairment of rats submitted to chronic cerebral hypoperfusion. Neurol Sci. 2012;33(5):985–97.
46. Gu L, Huang B, Shen W, Gao L, Ding Z, Wu H, et al. Early activation of nSMase2/ceramide pathway in astrocytes is involved in ischemia-associated neuronal damage via inflammation in rat hippocampi. J Neuroinflammation. 2013;10:109.
47. Pulsinelli WA, Buchan AM. The four-vessel occlusion rat model: method for complete occlusion of vertebral arteries and control of collateral circulation. Stroke. 1988;19(7):913–4.
48. Furlow TW Jr. Cerebral ischemia produced by four-vessel occlusion in the rat: a quantitative evaluation of cerebral blood flow. Stroke. 1982;13(6):852–5.
49. D'Cruz BJ, Logue ES, Falke E, DeFranco DB, Callaway CW. Hypothermia and ERK activation after cardiac arrest. Brain Res. 2005;1064(1–2):108–18.
50. Neumar RW, Bircher NG, Sim KM, Xiao F, Zadach KS, Radovsky A, et al. Epinephrine and sodium bicarbonate during CPR following asphyxial cardiac arrest in rats. Resuscitation. 1995;29(3):249–63.

51. D'Cruz BJ, Fertig KC, Filiano AJ, Hicks SD, DeFranco DB, Callaway CW. Hypothermic reperfusion after cardiac arrest augments brain-derived neurotrophic factor activation. J Cereb Blood Flow Metab. 2002;22(7):843–51.
52. Cherry BH, Nguyen AQ, Hollrah RA, Olivencia-Yurvati AH, Mallet RT. Modeling cardiac arrest and resuscitation in the domestic pig. World J Crit Care Med. 2015;4(1):1–12.
53. Bederson JB, Pitts LH, Germano SM, Nishimura MC, Davis RL, Bartkowski HM. Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke. 1986;17(6):1304–8.
54. Tang G, Liu Y, Zhang Z, Lu Y, Wang Y, Huang J, et al. Mesenchymal stem cells maintain blood-brain barrier integrity by inhibiting aquaporin-4 upregulation after cerebral ischemia. Stem Cells. 2014;32(12):3150–62.
55. He X, Li Y, Lu H, Zhang Z, Wang Y, Yang GY. Netrin-1 overexpression promotes white matter repairing and remodeling after focal cerebral ischemia in mice. J Cereb Blood Flow Metab. 2013;33(12):1921–7.
56. Tang Y, Wang J, Lin X, Wang L, Shao B, Jin K, et al. Neural stem cell protects aged rat brain from ischemia-reperfusion injury through neurogenesis and angiogenesis. J Cereb Blood Flow Metab. 2014;34(7):1138–47.
57. Han H, Wu LM, Han MX, Yang WM, Wang YX, Fang ZH. Diabetes impairs spatial learning and memory and hippocampal neurogenesis via BDNF in rats with transient global ischemia. Brain Res Bull. 2016;124:269–77.
58. Choi SH, Woodlee MT, Hong JJ, Schallert T. A simple modification of the water maze test to enhance daily detection of spatial memory in rats and mice. J Neurosci Methods. 2006;156(1–2):182–93.
59. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006;1(2):848–58.