TASHKENT MEDICAL ACADEMY

100 TMA ANNIVERSARY

Journal of Educational and Scientific Medicine

Issue 1 | 2022

Lorem ipsum dolor sit amet, consectetur adipisicing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam, quis nostrud exercitation ullamco laboris nisiut aliquip ex ea commodo consequat.

Lorem ipsum dolor sit amet, consectetur adipisicing elit, sed do eiusmod tempor incididunt ut labore et dolore magna aliqua. Ut enim ad minim veniam,

OAK.uz
Google Scholar
ISSN: 2181-3175
3422

TASHKENT MEDICAL ACADEMY № 1 (05) 2022
JOURNAL OF EDUCATIONAL AND SCIENTIFIC MEDICINE

[1]Irisqulov B.U., [2,3]Abdullaev I.F., [2,4]Makhmudov A.R., [2]Baev A.Yu., [1,2]Sariev A.U.

REVIEW OF METHODS FOR MODELING ISCHEMIC STROKE IN VIVO

[1]Department of Physiology and Pathophysiology, Tashkent Medical Academy, Tashkent, [2]Laboratory of Experimental Biophysics, Center for Advanced Technologies, Tashkent, [3]Laboratory of Membrane Biophysics, Institute of Biophysics and Biochemistry, Tashkent, [4]Department of Chemistry of Natural Compounds, Faculty of Chemistry, National University of Uzbekistan, Tashkent, [5]Department of Biophysics, Faculty of Biology, National University of Uzbekistan, Tashkent

Abstract.
Ischemic stroke is a leading cause of permanent disability worldwide. It is accompanied by devastating long-lasting motor, sensory and cognitive deficits. Currently, there is no effective treatment for ischemic stroke except intravenous perfusion with tPA, which has a limited therapeutic window and success rate. Therefore, scientists have been developing animal models to investigate the pathophysiology behind ischemic damage and discover new therapeutic drugs and rehabilitation protocols to alleviate stroke-induced deficits in patients. Developing tests that can identify and quantify behavioral and histopathophysiological impairments after stroke is essential to expand the development of translational therapies. In this review, we will discuss current in vivo stroke models and examination methods to assess functional outcomes after induction of experimental stroke in laboratory animals.
Key words: stroke, animal model, ischemia, pathophysiology, MCAO

INTRODUCTION

Stroke continues to be a leading cause of death and disability worldwide. While advances in therapies for revascularization of occluded vessels have decreased mortality rate following ischemic stroke, the quality of life after stroke remains low due to functional impairments (Writing Group et al. 2016; Kolominsky-Rabas et al. 2001; Chung et al. 2014). Rodents serve as a cost-efficient and reliable animal model to investigate pathological mechanisms underlying brain ischemia.

The main goal of post-stroke interventions is the recovery of impaired neurological functions in patients. It is crucial to set a replicable and accurate method to induce ischemic stroke in an animal before testing the efficacy of treatment methods.

Although rodent models have provided valuable insight and understanding of the biological basis and functional outcome of stroke, the selection of individual tests is crucial for the success of translational research.

In this review, first, we will discuss experimental animal models of global and focal ischemia developed to study molecular mechanisms of ischemia-induced neuronal cell death and to find neuroprotective agents that may alleviate ischemia-induced neurological deficits in humans.

Next, we will describe behavioral and histopathological methods to evaluate the accuracy of stroke induction and the efficacy of applied treatment.

Global ischemia models

Global cerebral ischemia (GCI) occurs when the blood flow to the whole brain is greatly reduced or stopped. Even short-term GCI can result in severe brain damage and death. The most common reason is cardiac arrest; however, other conditions, such as carbon monoxide poisoning, hemorrhaging, or near-drowning can lead to GCI. While, focal cerebral ischemia (FCI) is caused by the occlusion of cerebral blood vessels, most commonly of the middle cerebral artery (ischemic stroke), and can induce development of infarction in localized areas of the brain and subsequent detrimental neuronal deficits.

Cardiac arrest by ventricular fibrillation

Ventricular fibrillation imitates cardiac arrest, and cardiopulmonary resuscitation is usually added to restore the heart function and blood flow to the brain (Berkowitz et al. 1991; Eleff et al. 1991). Defibrillation, with a combination of chest compressions and administration of epinephrine, is usually applied after cardiac arrest. This technique is generally used in large animals, but is expensive and extremely labor intensive during the first 24 to 48 hours after the arrest.

4-VO (four vessel occlusion)

4-VO is a widely used and well-established rodent model of reversible forebrain ischemia that mimics clinical cardiac arrest. This model is produced by coagulation of vertebral arteries through the alar

ISSUE 1 | 2022
ISSN 2181-3175

foramen on day 1, and transient occlusion of both common carotid arteries on day 2 (Pulsinelli and Brierley 1979; Small and Buchan 2000). In 4-VO, neuronal death mostly occurs in pyramidal neurons of the hippocampal CA1, usually three days after the procedure (Pulsinelli and Buchan 1988).

During 4-VO procedure, rat vertebral arteries are exposed and permanently cauterized. The CCAs are exposed and isolated with a silk thread, and the wound is sutured. 24 hours later, the wound is reopened and the common carotid arteries are subjected to temporary occlusion with microclamps (10 minutes for global ischemia). When the carotid arteries are occluded, blood flow is typically dramatically reduced in the hippocampus, striatum, and neocortex (Pulsinelli and Buchan 1988).

2-VO (two vessel occlusion)

Another method of inducing reversible global forebrain ischemia in rats is produced by occlusion of both common carotid arteries (CCAs), combined with hypotension (Smith et al. 1984). First, systemic hypotension is initiated by slowly withdrawal of blood from the femoral artery to drop the mean arterial pressure to 50 mmHg. Next, both common carotid arteries are clamped to restrict the blood flow to the brain. Rats can survive for 20-30 min under these conditions. Reperfusion is achieved by removing clamps from the CCAs, and infusing drawn blood into the jugular vein. These steps are sufficient to induce reproducible global cerebral ischemia. 2-VO-induced global ischemia inflicts a two-step insult on a brain. Sensitive pyramidal neurons of CA1 layer of hippocampus and 3-4 layers of neocortex are acutely affected first, followed by the delayed neuronal death that usually occurs after third day of reperfusion.

Focal ischemia models

While global ischemia models attempt to mimic the cardiac arrest by restricting the blood flow to the whole brain, focal ischemia models produce conditions within animal brains that resemble human ischemic stroke by restricting blood circulation in specific, localized areas of the brain. Since the vast majority of ischemic stroke cases in humans are due to the blockage of the middle cerebral artery (MCA), focal ischemia in laboratory animals is achieved by permanent or reversible MCA occlusion (MCAO) at proximal or distal parts of the artery.

There are several ways to occlude MCA: 1) by intraluminal suture (endovascular filament) inserted through external or common carotid artery (Koizumi 1989; Longa et al. 1989; Hill and Nemoto 2014); 2) endothelin-1-induced MCA constriction, 3) embolic MCAO; 4) transcranial MCAO with or without CCA occlusion; and 5) cerebrocortical photothrombosis.

MCAO using endovascular filament

In this method, developed by Koizumi and colleagues in 1986, a monofilament is fed to the MCA through the incision in CCA until it blocks the local blood circulation. This maneuver produces consistent ischemic insult to the cortex and large reproducible infarction volume. The CCA has to be permanently ligated to prevent bleeding through the incision site on CCA, but the whole brain still receives enough blood and oxygen through the other CCA and the circle of Willis. In 1989, Longa et al. modified this method (Figure 1), permanently ligating and inserting a silicon-coated monofilament through the external carotid artery (ECA). While both modifications of the method are used arbitrarily, Longa modification gained popularity due to the decreased mortality of the laboratory animals, lower post-operative inflammation and reproducibility of the infarctions (Smith et al. 2015).

Both Longa and Koizumi modifications offer only partial restoration of perfusion after the MCAO, because either CCA or ECA must be permanently ligated during the procedure. Hill and Nemoto in 2014 proposed a modification in Koizumi method, where the incision site on the CCA is sealed by cyanoacrylate tissue adhesive after filament removal. This allows for the establishment of the complete reperfusion and restoration of the vasculature to the preoperative state.

Endothelin-1-induced MCA constriction

Endothelin-1 is a potent long-lasting vasoconstrictor, and its application onto the exposed MCA can induce ischemic stroke with concentration-dependent severity (Macrae 2011; Sharkey, Ritchie, and Kelly 1993). In this method, laboratory animals are anesthetized with isoflurane and mounted on a stereotaxic frame, incision is made to expose the skull, a hole is made using a dental burr, and the peptide is injected onto MCA according to the coordinates determined based on the rat brain atlas. Endothelin-1-induced MCA constriction can last up to 22 hours post-injection followed by gradual reperfusion (Biernaskie et al. 2001). The induction of the ischemic stroke, continuous low cerebral blood

ISSUE 1 | 2022
ISSN 2181-3175

blood flow with gradual reperfusion and development of lesions resembles the time course of the development of the clinical ischemic stroke in patients (Trotman-Lucas and Gibson 2021). However, some drawbacks of this method include inexact drug delivery due to significant variability in brain anatomy, and difficulty ensuring consistent drug delivery and diffusion. Additionally, extreme care should be taken to ensure that peptide does not get into the ventricles, since it may cause barrel rolling or seizures (Macrae 2011).

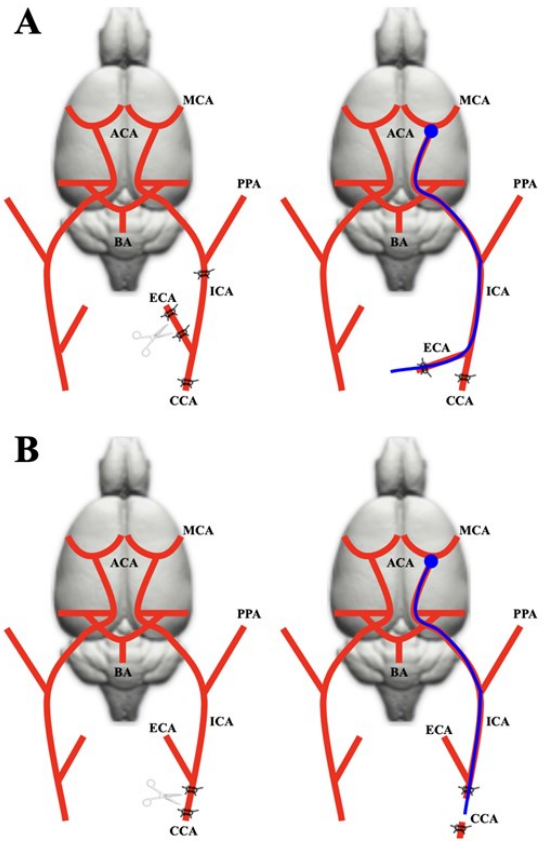

Figure 1. A. Koizumi middle cerebral artery occlusion surgery technique. B. Longa modification of middle cerebral artery occlusion surgery.

Photothrombotic MCAO

In the photothrombotic stroke model, restriction of the cerebral blood flow in the MCA occurs when photosensitive dye Rose Bengal is injected in circulation, and then a laser beam illuminates the location at the MCA chosen by an investigator. Photochemical reaction between the laser and photosensitive dye causes local precise endothelial damage and platelet aggregation response that leads to the formation of the clot and occlusion of the artery (Yao et al. 1996; Qian et al. 2016). Incision is usually made between the right orbit and the external auditory canal. Following the intravenous injection of Rose Bengal, illumination is performed on the MCA using optic fiber connected to a laser with a wavelength of 532 nm. Reperfusion is achieved using optic fiber connected to an ultraviolet laser (with a wavelength of 355 nm) at one hour after occlusion. This method offers a very short time of the procedure (15 minutes) and no surgery-related mortality.

Methods to assess ischemic stroke model

To validate the level of relevance to the human disease animal model have to be assessed by a battery of physiological and laboratory methods. Since there is a big diversity of methods to induce ischemic stroke each of them has a different level of correlation for different aspects of stroke. While some of them are good to represent neurological dysfunction (transcranial occlusion, photothrombosis or endothelin-1 models), others are better to reproduce pathophysiological mechanisms (endovascular filament or embolic occlusion models). Methods to assess brain ischemia in laboratory animals could be divided into 3 main approaches: neurological, histological, and instrumental (Bouet et al. 2007; Baek et al. 2020).

Neurological assessment

The most clinically significant manifestation of the stroke is loss or alteration of neurological functions due to ischemic damage of cells in a specific brain region. These neurological dysfunctions are usually long-lasting and affect a patient's quality of life substantially. The type of neurological dysfunction and its severity highly correlates with the region of the brain responsible for these functions and the level of ischemic lesion. Therefore, in rodents, the region to induce ischemia must be clinically relevant and neurologically verifiable. Ischemic stroke occurs in the territory of the middle cerebral artery (Chung et al. 2014). In animal models motor, sensor, and cognitive function tests are performed to assess levels of ischemic injury quantitatively (Schaar, Brenneman, and Savitz 2010; Trueman et al. 2017; Baek et al. 2018). Bederson scale and more profound Modified Neurological severity score (mNSS) are two of the main tests to evaluate general post-ischemic state (Bieber et al. 2019; Bederson, Pitts, Tsuji, et al. 1986).

ISSUE 1 | 2022
ISSN 2181-3175

The Bederson scale include forelimb flexion, resistance to lateral push and circling behavior. A score from 0 to 5 is used to assess general state after stroke, where higher score correlates with wider brain damage. This scoring system became popular because of its simplicity and reliability. It can explain up to 48% of variance of infarct volume (Bieber et al. 2019), (Table 1).

Table 1.

Modified Bederson (0-5) scale	Score
No observable deficit	0
Forelimb flexion	1
Forelimb flexion and decreased resistance to lateral push	2
Forelimb flexion and circling	3
Circling and spinning around the cranial-caudal axis	4
No spontaneous movement.	5

One of the most common neurological scales used in stroke animal models is the mNSS score (Li et al. 2001; Chen et al. 2005). The mNSS rates neurological functioning on a scale of 14 or 18, depending on mice or rats, respectively. Each aspect of neurological state (motor, sensor, coordination, reflexes) is assigned a score, and the sum of individual scores represents severity (Table 2).

Motor Tests. Impairment of motor functions is the most devastating and common symptom of MCA occlusion. In laboratory animals, it can be tested by observation of natural behavior or performance of animals on specific motor tasks (Milgram 2002). Open-field, circle, cylinder, and rotation tests are most popular among observational measurements. In open-field (de Visser et al. 2006) and circle test (Uchida et al. 2009), the distance moved from initial point by animal is measured. The cylinder test (Schallert et al. 1982) allows to assess lateralization of injury or functional bias of limbs. Mice or rats are placed in a transparent cylinder and their activity is filmed. Animals tend to explore the environment by rearing and touching cylinder walls by forelimb. The degree of unilateral brain damage is determined by the asymmetry in the number of paw contacts (Figure 2). Another simple way to check functional asymmetry is the rotation test (Iancu et al. 2005; Nielsen et al. 1997). In this method, the difference between the number of clockwise and counterclockwise rotations of the animal is counted during a specific period of time in a closed arena.

These tests are highly sensitive to detect unilateral damage of brain motor areas, but less useful to assess bilateral motor dysfunctions. Overall advantage of observational methods is that they are easy to perform, emphasize natural behavior and do not require prior training to evaluate performance. However, they do not provide useful individual dependent variables of sufficient sensitivity to quantitatively discriminate specific functions.

Table 2.

Modified neurological severity score (mNSS) for rat	Points
Motor tests **Raising by the tail** **1** forelimb flexion **1** hindlimb flexion **1** head moved >10° to vertical axis within 30 s **Placing rat on the floor** (normal=0; maximum=3) **0** Normal walk **1** Inability to walk straight **2** Circling toward the paretic side **3** Fall down to the paretic side	**3** **3**
Sensory test **1** Placing test (visual tactile test) **2** Proprioceptive test (deep sensation, pushing the paw against the table edge)	**2**
Beam balance test (normal=0; maximum=6) **0** Balance with steady posture **1** Grasps side of beam **2** Hugs the beam and one limb falls down from the beam **3** Hugs the beam and two limbs fall down from the beam, or spins on beam (>60 s) **4** Attempts to balance on the beam but falls off (>40 s) **5** Attempts to balance on the beam but falls off (>20 s) **6** Falls off: No attempt to balance or hang on the (<20 s)	**6**
Reflexes absent and abnormal movements **1** Absence of corneal reflex (eye blink when lightly touching the cornea with cotton) **1** Absence of startle reflex (motor response to a brief noise) **1** Seizures, myoclonus, myodystonia	**3**
Maximum score points	**18**

ISSUE 1| 2022
ISSN 2181-3175

Specific motor tasks involve balance beam, rotarod, grid walking, grip strength, reaching task, etc. Most of these tasks require pre-test habituation and training to evaluate pre-stroke performance. After stroke, most tests show a significant decline in performance associated with the level of neuronal cell death. These tests are sufficient to compare the effects of various treatments on functional recovery after an ischemic stroke. The most popular test among them is the balance beam walking (Wallace, Krauter, and Campbell 1980).

Rats or mice are trained to run on a 1- or 1.5-meter-long horizontal beam. The time to reach the end of the beam and the number of foot slips are taken into account to measure performance. In addition to evaluating motor functions, this test shows an animal's ability for motor coordination and balance. Another test to assess motor coordination and balance is the rotarod test (Dunham and Miya 1957), where the animal is put on a rotating rod and time to fall is registered. Reaching task (Whishaw, O'Connor, and Dunnett 1986) and staircase test (Montoya et al. 1991) are designed to assess fine motor skills of the forelimb to get a food pellet with various degrees of difficulty. This battery of motor tests could be easily used to assess motor dysfunction not only after stroke, but also for conditions affecting motor activity, such as Parkinson's disease and Huntington's disease.

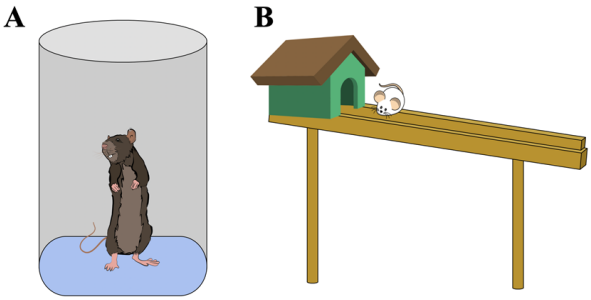

Figure 2. A. Cylinder test. B. Balance beam test

Sensorimotor tests. A degree of damage to the sensory cortex could be assessed by forelimb flexion, adhesive removal, corner, von Frey filament test. Most tests can reveal both sensory and motor function losses. In the adhesive removal test (Schallert et al. 1982), a small patch is attached to the paw of the animal bilaterally, and the duration of time to detect and remove the patch is recorded. This test assesses overall sensorimotor functions of forelimbs as well as limb asymmetry. Another interesting method to evaluate sensory function is von Frey filament test (de Sousa et al. 2014; Bradman et al. 2015). Set of filaments, each able to exert a different force, is applied to the animal paw, from the weakest to the strongest, until the paw is withdrawn (Figure 3).

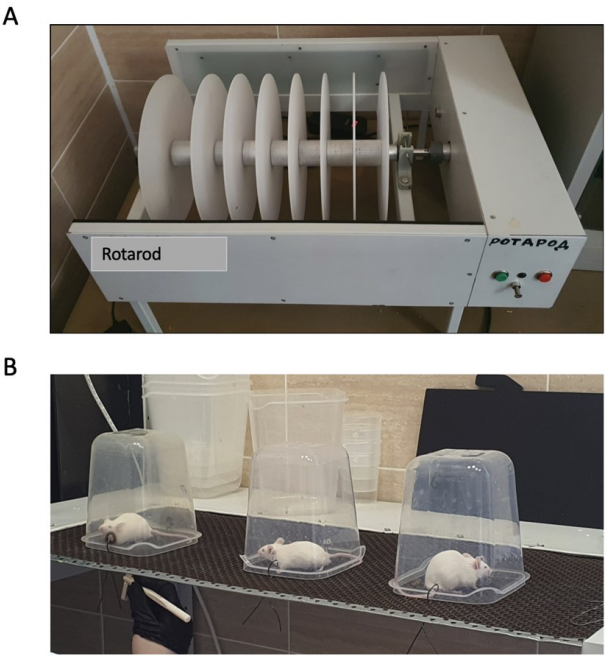

Figure 3. A. Rotarod. B. von Frey filament test.

Cognitive tests are mainly intended to examine motor learning and working memory functions. They include but not limited to Morris water maze (Morris 1984), radial arm maze (Volpe et al. 1984), and sequence learning (Cho et al. 2007) tests. While in Morris water maze animals are required to find a platform under opaque water, in radial arm maze animals should learn reward locations. Although such tests require well developed set up and training, they are able to estimate stroke-induced changes in the higher functions of the brain, which can in some extent relate to human cognition.

Histological assessment

The main advantage of using animal models of human disease is the ability to explore pathophysiological processes at tissue and cellular levels. Also, it helps to objectively test the effectiveness of various treatments to decrease malfunctions of organisms. The first set of methods is designated to evaluate brain infarct volume after ischemia (Hossmann 2008; Li et al. 2014; Sommer

ISSUE 1 | 2022
ISSN 2181-3175

2016). Commonly used techniques are chemical staining with Hematoxylin eosin (H&E), Nissl, Silver (Vogel, Mobius, and Kuschinsky 1999), 2,3,5-triphenyltetrazolium hydrochloride (TTC) (Bederson, Pitts, Germano, et al. 1986) and immunohistochemistry staining of microtubule-associated protein (MAP2) or DNA-binding neuron-specific protein – NeuN (Wolf et al. 1996). Generally, 6-24 hours after an animal is exposed to ischemic stroke its brain is extracted and cut into 1-2 mm thick equal slices. Following staining protocol slices imaged via microscope and volume of infarct calculated by demarcation contrast area multiplied by thickness of slices. Since most staining methods label unaffected brain areas, lesioned areas defined as unstained regions. Among them TTC (Bederson, Pitts, Germano, et al. 1986) staining allow to directly assess mitochondrial oxidative stress. In cells with functioning mitochondria, TTC is reduced to red-colored formazan, thus marking live cells. This is a good method to mark neuronal as well as glial cell death, however, after 24 hours its quality can be affected by the migration of inflammatory cells. The next set of methods allows evaluating the recovery rate after stroke by marking cell proliferation at the penumbra zone. Bromodeoxyuridine (Brdu) (Surugiu et al. 2018) stains proliferation of cells in the penumbra, with the highest rate in post ischemic days 3–4, and higher rate of proliferation in the region immediate to the ischemic core than in the distant region. Similarly, GFAP+ is used to label proliferating astrocytes and Iba1+ is used to label proliferating microglia/macrophage observed in the penumbra.

Another set of tests designated to check level of inflammation over affected region of the brain after stroke(Wu et al. 2014).

The immune response to acute cerebral ischemia is a major factor in stroke pathobiology and outcome.

While the immune response starts locally in occluded and hypoperfused vessels and the ischemic brain parenchyma, inflammatory mediators generated in situ propagate through the organism as a whole. Immunohistochemical methods allows to detect propagation of immune cells in the cerebral penumbra and necrotic area. Microglial cells in the infarcted region well stained by Iba-1 antigen (Zamanian et al. 2012), while neutrophils could be labeled by myeloperoxidase (MPO) antigen (Matsuo et al. 1994).

It was demonstrated that stroke will lead to impairment of (Huang et al. 2012) the blood-brain barrier (BBB) function.

The blood-brain barrier is dynamic and regulates leukocyte infiltration from the blood compartment into the brain.

Disruption of the blood-brain barrier allows leukocytes to migrate into the brain, leading to neuronal death and apoptosis. Permeability of the blood-brain barrier is evaluated by stains of reagents (Lenzser et al. 2007), which normally would not pass through blood-brain barrier (Evans blue or Na-fluorescein). Inflammation and increased permeability of BBB usually lead to brain swelling – edema, which by itself can cause severe damage to the brain, because of limited volume for brain expansion in the skull. In laboratory animals brain edema is calculated by comparison of dry and wet weights of affected and unaffected brain hemispheres (Bederson, Bartkowski, et al. 1986; Keep, Hua, and Xi 2012).

CONCLUSION

Although animal models can have brought a lot of valuable information, they should be treated always with caution. As much biological model are close to human as much it can be reliable.

Although cellular models play an enormous role to uncover the biological basis of pathology, they are usually less efficient while translating results to humans. On the other hand, studies on primates give reliable evidence on the effectiveness of various treatment methods, however, these works are ethically unfavorable and cost-inefficient.

Therefore, investigation of human-related diseases has commonly proceeded on rodent models. Thus, the selection of individual tests is crucial for the success of translational research. It is imperative to choose tests that are sensitive to both the area of the brain damage and the interventions that are being applied. In ischemic stroke, mostly sensorimotor and cognitive functions are impaired.

Rodent models can be assessed mainly for simple neurological functions. However, animal models allow application of a huge battery of histo-pathophysiological tests, which helps to precisely dissect the mechanism of disease and find suitable treatment. In conclusion, we can emphasize that animal models of human disease are a great approach to advance Medicine.

ISSUE 1| 2022
ISSN 2181-3175

ACKNOWLEDGEMENT

This work was supported by A-ИРВ-2021-465 grant from the Ministry of Innovative Development of the Republic of Uzbekistan.

This work is devoted to each animal sacrificed for the sake of science and humanity.

REFERENCES

1.Baek, H., A. Sariev, M. J. Kim, H. Lee, J. Kim, and H. Kim. 2018. 'A neuroprotective brain stimulation for vulnerable cerebellar Purkinje cell after ischemic stroke: a study with low-intensity focused ultrasound', Annu Int Conf IEEE Eng Med Biol Soc, 2018: 4744-47.

2.Baek, H., A. Sariev, S. Lee, S. Y. Dong, S. Royer, and H. Kim. 2020. 'Deep Cerebellar Low-Intensity Focused Ultrasound Stimulation Restores Interhemispheric Balance after Ischemic Stroke in Mice', IEEE Trans Neural Syst Rehabil Eng, 28: 2073-79.

3.Bederson, J. B., H. M. Bartkowski, K. Moon, M. Halks-Miller, M. C. Nishimura, M. Brant-Zawadski, and L. H. Pitts. 1986. 'Nuclear magnetic resonance imaging and spectroscopy in experimental brain edema in a rat model', J Neurosurg, 64: 795-802.

4.Bederson, J. B., L. H. Pitts, S. M. Germano, M. C. Nishimura, R. L. Davis, and H. M. Bartkowski. 1986. 'Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats', Stroke, 17: 1304-8.

5.Bederson, J. B., L. H. Pitts, M. Tsuji, M. C. Nishimura, R. L. Davis, and H. Bartkowski. 1986. 'Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination', Stroke, 17: 472-6.

6.Berkowitz, I. D., H. Gervais, C. L. Schleien, R. C. Koehler, J. M. Dean, and R. J. Traystman. 1991. 'Epinephrine dosage effects on cerebral and myocardial blood flow in an infant swine model of cardiopulmonary resuscitation', Anesthesiology, 75: 1041-50.

7.Bieber, M., J. Gronewold, A. C. Scharf, M. K. Schuhmann, F. Langhauser, S. Hopp, S. Mencl, E. Geuss, J. Leinweber, J. Guthmann, T. R. Doeppner, C. Kleinschnitz, G. Stoll, P. Kraft, and D. M. Hermann. 2019. 'Validity and Reliability of Neurological Scores in Mice Exposed to Middle Cerebral Artery Occlusion', Stroke, 50: 2875-82.

8.Biernaskie, J., D. Corbett, J. Peeling, J. Wells, and H. Lei. 2001. 'A serial MR study of cerebral blood flow changes and lesion development following endothelin-1-induced ischemia in rats', Magn Reson Med, 46: 827-30.

9.Bouet, V., T. Freret, J. Toutain, D. Divoux, M. Boulouard, and P. Schumann-Bard. 2007. 'Sensorimotor and cognitive deficits after transient middle cerebral artery occlusion in the mouse', Exp Neurol, 203: 555-67.

10.Bradman, M. J., F. Ferrini, C. Salio, and A. Merighi. 2015. 'Practical mechanical threshold estimation in rodents using von Frey hairs/ Semmes-Weinstein monofilaments: Towards a rational method', J Neurosci Methods, 255: 92-103.

11.Chen, J., C. Zhang, H. Jiang, Y. Li, L. Zhang, A. Robin, M. Katakowski, M. Lu, and M. Chopp. 2005. 'Atorvastatin induction of VEGF and BDNF promotes brain plasticity after stroke in mice', J Cereb Blood Flow Metab, 25: 281-90.

12.Cho, Y. H., S. Delcasso, A. Israel, and Y. Jeantet. 2007. 'A long list visuo-spatial sequential learning in mice', Behav Brain Res, 179: 152-8.

13.Chung, J. W., S. H. Park, N. Kim, W. J. Kim, J. H. Park, Y. Ko, M. H. Yang, M. S. Jang, M. K. Han, C. Jung, J. H. Kim, C. W. Oh, and H. J. Bae. 2014. 'Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification and vascular territory of ischemic stroke lesions diagnosed by diffusion-weighted imaging', J Am Heart Assoc, 3.

14.de Sousa, M. V., C. Ferraresi, A. C. de Magalhaes, E. M. Yoshimura, and M. R. Hamblin. 2014. 'Building, testing and validating a set of home-made von Frey filaments: a precise, accurate and cost effective alternative for nociception assessment', J Neurosci Methods, 232: 1-5.

15.de Visser, L., R. van den Bos, W. W. Kuurman, M. J. Kas, and B. M. Spruijt. 2006. 'Novel approach to the behavioural characterization of inbred mice: automated home cage observations', Genes Brain Behav, 5: 458-66.

16.Dunham, N. W., and T. S. Miya. 1957. 'A note on a simple apparatus for detecting neurological deficit in rats and mice', J Am Pharm Assoc Am Pharm Assoc, 46: 208-9.

17.Eleff, S. M., Y. Maruki, L. H. Monsein, R. J. Traystman, R. N. Bryan, and R. C. Koehler. 1991. 'Sodium, ATP, and intracellular pH transients during reversible complete ischemia of dog cerebrum', Stroke, 22: 233-41.

18.Hill, J. W., and E. M. Nemoto. 2014. 'Transient middle cerebral artery occlusion with complete reperfusion in spontaneously hypertensive rats', MethodsX, 1: 283-91.

19.Hossmann, K. A. 2008. 'Cerebral ischemia: models, methods and outcomes', Neuropharmacology, 55: 257-70.

20.Huang, P., C. M. Zhou, Hu Qin, Y. Y. Liu, B. H. Hu, X. Chang, X. R. Zhao, X. S. Xu, Q. Li, X. H. Wei, X. W. Mao, C. S. Wang, J. Y. Fan, and J. Y. Han. 2012. 'Cerebralcare Granule(R) attenuates blood-brain barrier disruption after middle cerebral artery occlusion in rats', Exp Neurol, 237: 453-63.

21.Iancu, R., P. Mohapel, P. Brundin, and G. Paul. 2005. 'Behavioral characterization of a unilateral 6-OHDA-lesion model of Parkinson's disease in mice', Behav Brain Res, 162: 1-10.

22.Keep, R. F., Y. Hua, and G. Xi. 2012. 'Brain water content. A misunderstood measurement?', Transl Stroke Res, 3: 263-5.

23.Koizumi, JI. 1989. 'Experimental studies of ischemic brain edema: Effect of recirculation of the blood flow after ischemia on post-ischemic brain edema', Jpn J Stroke, 11: 11-17.

24.Kolominsky-Rabas, P. L., M. Weber, O. Gefeller, B. Neundoerfer, and P. U. Heuschmann. 2001. 'Epidemiology of ischemic stroke subtypes according to TOAST criteria: incidence, recurrence, and long-term survival in ischemic stroke subtypes: a population-based study', Stroke, 32: 2735-40.

25.Lenzser, G., B. Kis, J. A. Snipes, T. Gaspar, P. Sandor, K. Komjati, C. Szabo, and D. W. Busija. 2007. 'Contribution of poly(ADP-ribose) polymerase to postischemic blood-brain barrier damage in rats', J Cereb Blood Flow Metab, 27: 1318-26.

26.Li, H., N. Zhang, H. Y. Lin, Y. Yu, Q. Y. Cai, L. Ma, and S. Ding. 2014. 'Histological, cellular and behavioral assessments of stroke outcomes after photothrombosis-induced ischemia in adult mice', BMC Neurosci, 15: 58.

27.Li, Y., J. Chen, L. Wang, M. Lu, and M. Chopp. 2001. 'Treatment of stroke in rat with intracarotid administration of marrow stromal cells', Neurology, 56: 1666-72.

28.Longa, E. Z., P. R. Weinstein, S. Carlson, and R. Cummins. 1989. 'Reversible middle cerebral artery occlusion without craniectomy in rats', Stroke, 20: 84-91.

29.Macrae, I. M. 2011. 'Preclinical stroke research--advantages and disadvantages of the most common rodent models of focal ischaemia', Br J Pharmacol, 164: 1062-78.

30.Matsuo, Y., H. Onodera, Y. Shiga, M. Nakamura, M. Ninomiya, T. Kihara, and K. Kogure. 1994. 'Correlation between myeloperoxidase-quantified neutrophil accumulation and ischemic brain injury in the rat. Effects of neutrophil depletion', Stroke, 25: 1469-75.

31.Milgram, Norton W. 2002. 'Central Nervous System Diseases: Innovative Animal Models from Lab to Clinic', Journal of Psychiatry and Neuroscience, 27: 292-93.

32.Montoya, C. P., L. J. Campbell-Hope, K. D. Pemberton, and S. B. Dunnett. 1991. 'The "staircase test": a measure of independent forelimb reaching and grasping abilities in rats', J Neurosci Methods, 36: 219-28.

ISSUE 1 | 2022
ISSN 2181-3175

33.Morris, R. 1984. 'Developments of a water-maze procedure for studying spatial learning in the rat', J Neurosci Methods, 11: 47-60.
34.Nielsen, D. M., K. E. Visker, M. J. Cunningham, R. W. Keller, Jr., S. D. Glick, and J. N. Carlson. 1997. 'Paw preference, rotation, and dopamine function in Collins HI and LO mouse strains', Physiol Behav, 61: 525-35.
35.Pulsinelli, W. A., and J. B. Brierley. 1979. 'A new model of bilateral hemispheric ischemia in the unanesthetized rat', Stroke, 10: 267-72.
36.Pulsinelli, W. A., and A. M. Buchan. 1988. 'The four-vessel occlusion rat model: method for complete occlusion of vertebral arteries and control of collateral circulation', Stroke, 19: 913-4.
37.Qian, C., P. C. Li, Y. Jiao, H. H. Yao, Y. C. Chen, J. Yang, J. Ding, X. Y. Yang, and G. J. Teng. 2016. 'Precise Characterization of the Penumbra Revealed by MRI: A Modified Photothrombotic Stroke Model Study', PLoS One, 11: e0153756.
38.Schaar, K. L., M. M. Brenneman, and S. I. Savitz. 2010. 'Functional assessments in the rodent stroke model', Exp Transl Stroke Med, 2: 13.
39.Schallert, T., M. Upchurch, N. Lobaugh, S. B. Farrar, W. W. Spirduso, P. Gilliam, D. Vaughn, and R. E. Wilcox. 1982. 'Tactile extinction: distinguishing between sensorimotor and motor asymmetries in rats with unilateral nigrostriatal damage', Pharmacol Biochem Behav, 16: 455-62.
40.Sharkey, J., I. M. Ritchie, and P. A. Kelly. 1993. 'Perivascular microapplication of endothelin-1: a new model of focal cerebral ischaemia in the rat', J Cereb Blood Flow Metab, 13: 865-71.
41.Small, D. L., and A. M. Buchan. 2000. 'Animal models', Br Med Bull, 56: 307-17.
42.Smith, H. K., J. M. Russell, D. N. Granger, and F. N. Gavins. 2015. 'Critical differences between two classical surgical approaches for middle cerebral artery occlusion-induced stroke in mice', J Neurosci Methods, 249: 99-105.
43.Smith, M. L., G. Bendek, N. Dahlgren, I. Rosen, T. Wieloch, and B. K. Siesjo. 1984. 'Models for studying long-term recovery following forebrain ischemia in the rat. 2. A 2-vessel occlusion model', Acta Neurol Scand, 69: 385-401.
44.Sommer, Clemens. 2016. 'Histology and Infarct Volume Determination in Rodent Models of Stroke.' in Ulrich Dirnagl (ed.), Rodent Models of Stroke (Springer New York: New York, NY).
45.Surugiu, R., D. Glavan, M. Popescu, O. Margaritescu, R. Eugen, and A. Popa-Wagner. 2018. 'Vasculature Remodeling in a Rat Model of Cerebral Ischemia. The Fate of the BrdU-Labeled Cells Prior to Stroke', Front Neurol, 9: 1014.
46.Trotman-Lucas, M., and C. L. Gibson. 2021. 'A review of experimental models of focal cerebral ischemia focusing on the middle cerebral artery occlusion model', F1000Res, 10: 242.
47.Trueman, R. C., C. Diaz, T. D. Farr, D. J. Harrison, A. Fuller, P. F. Tokarczuk, A. J. Stewart, S. J. Paisey, and S. B. Dunnett. 2017. 'Systematic and detailed analysis of behavioural tests in the rat middle cerebral artery occlusion model of stroke: Tests for long-term assessment', J Cereb Blood Flow Metab, 37: 1349-61.
48.Uchida, M., J. M. Palmateer, P. S. Herson, A. C. DeVries, J. Cheng, and P. D. Hurn. 2009. 'Dose-dependent effects of androgens on outcome after focal cerebral ischemia in adult male mice', J Cereb Blood Flow Metab, 29: 1454-62.
49.Vogel, J., C. Mobius, and W. Kuschinsky. 1999. 'Early delineation of ischemic tissue in rat brain cryosections by high-contrast staining', Stroke, 30: 1134-41.
50.Volpe, B. T., W. A. Pulsinelli, J. Tribuna, and H. P. Davis. 1984. 'Behavioral performance of rats following transient forebrain ischemia', Stroke, 15: 558-62.
51.Wallace, J. E., E. E. Krauter, and B. A. Campbell. 1980. 'Motor and reflexive behavior in the aging rat', J Gerontol, 35: 364-70.
52.Whishaw, I. Q., W. T. O'Connor, and S. B. Dunnett. 1986. 'The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat', Brain, 109 (Pt 5): 805-43.
53.Wolf, H. K., R. Buslei, R. Schmidt-Kastner, P. K. Schmidt-Kastner, T. Pietsch, O. D. Wiestler, and I. Blumcke. 1996. 'NeuN: a useful neuronal marker for diagnostic histopathology', J Histochem Cytochem, 44: 1167-71.
54.Writing Group, Members, D. Mozaffarian, E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S. R. Das, S. de Ferranti, J. P. Despres, H. J. Fullerton, V. J. Howard, M. D. Huffman, C. R. Isasi, M. C. Jimenez, S. E. Judd, B. M. Kissela, J. H. Lichtman, L. D. Lisabeth, S. Liu, R. H. Mackey, D. J. Magid, D. K. McGuire, E. R. Mohler, 3rd, C. S. Moy, P. Muntner, M. E. Mussolino, K. Nasir, R. W. Neumar, G. Nichol, L. Palaniappan, D. K. Pandey, M. J. Reeves, C. J. Rodriguez, W. Rosamond, P. D. Sorlie, J. Stein, A. Towfighi, T. N. Turan, S. S. Virani, D. Woo, R. W. Yeh, M. B. Turner, Committee American Heart Association Statistics, and Subcommittee Stroke Statistics. 2016. 'Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association', Circulation, 133: e38-360.
55.Wu, L., K. Zhang, G. Hu, H. Yan, C. Xie, and X. Wu. 2014. 'Inflammatory response and neuronal necrosis in rats with cerebral ischemia', Neural Regen Res, 9: 1753-62.
56.Yao, H., S. Ibayashi, H. Sugimori, K. Fujii, and M. Fujishima. 1996. 'Simplified model of krypton laser-induced thrombotic distal middle cerebral artery occlusion in spontaneously hypertensive rats', Stroke, 27: 333-6.
57.Zamanian, J. L., L. Xu, L. C. Foo, N. Nouri, L. Zhou, R. G. Giffard, and B. A. Barres. 2012. 'Genomic analysis of reactive astrogliosis', J Neurosci, 32: 6391-410.

ISSUE 1 | 2022
ISSN 2181-3175