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Methods of Simulating the Pathology of the Nervous System Experimentally

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Abstract

Appropriate models of cerebral pathology contribute to detailed representations of the pathogenesis of these disorders and, in addition, allow to assess the development of damage and adaptive mechanisms of the brain, which serves as a fundamental basis for improving the methods of their diagnosis, treatment, and prevention. The data presented in the article can serve as a fundamental basis for further study of the brain in normal and pathological conditions with further extrapolation of the obtained data to humans.

Key words: methods; nervous system; experiment

Introduction

Appropriate models of cerebral pathology contribute to detailed representations of the pathogenesis of these disorders and, in addition, allow to assess the development of damage and adaptive mechanisms of the brain, which serves as a fundamental basis for improving the methods of their diagnosis, treatment, and prevention.

Leading positions in the structure of morbidity and mortality worldwide are occupied by cardiovascular and cerebrovascular diseases. About 7 million dies from cardiovascular diseases every year, and about 6 million from cerebrovascular diseases. Up to 85% of all strokes are due to cerebral ischemia. In Russia, the incidence of stroke ranges from 460 to 560 cases per 100,000 population [1]. According to the criteria of Trial of Org 10172 in acute stroke treatment (TOAST), presented at the Second World Stroke Congress (Washington, September 1992), the following pathogenetic subtypes of cerebral infarction are distinguished:

1. the atherothrombotic subtype occurs when there is a decrease or complete cessation of blood flow as a result of local thrombogenesis in the bloodstream of extra- or intracranial large arteries, caused by ulceration and instability of atherosclerotic plaques, or resulting from atherosclerotic stenosis of vessels;
2. the cardioembolic subtype is formed as a result of a blood clot from the heart in the pathology of its chambers, valves, atrial and interventricular valves defects (paradoxical cardiac embolism), rhythm disturbances (atrial fibrillation);
3. lacunar subtype is a small (less than 15 mm in diameter) deep infarction focus, which is formed as a result of occlusion of one of the penetrating arteries and eventually resolves into a small cyst - a lacuna;
4. stroke of another specific etiology;
5. stroke of uncertain etiology.

Cerebral ischemia is a serious neurodegenerative condition that, depending on the area involved in the pathological process, can prevent the realization of the cognitive and sensorimotor functions of the central nervous system. Even short-term ischemia leads to deep brain damage. The key links in the pathogenesis of cerebral ischemia are: lack of oxygenation of neurons, oppression of the aerobic and anaerobic pathways of glucose utilization in the brain, reduced energy production, violation of the transport of potential-determining ions, changes in the acid-base state, excitotoxicity, the occurrence of oxidative including nitrosative stress, involved nitric oxide (NO), activation of the inflammatory process, apoptosis. These processes cannot be modeled in vitro, and most research on ischemic brain injury are conducted on animals [2]. An

analysis of literature data has revealed a wide range of different methods for modeling cerebral ischemia, which makes it possible to use methods that correspond the pursued goal and technical capabilities. Based on the existence of various pathogenetic variants of ischemic brain damage, adequate methods for their modeling have been developed (Table 1).

Table 1: Methods for modeling cerebral ischemia.

Type of cerebral ischemia	Ways to implement
complete (total, global)	decapitation, cardiac arrest, or occlusion of the aorta and vena cava
incomplete (subtotal)	occlusion of both common carotid arteries with intracranial hypertension
partial	occlusion of one common carotid artery
focal (local)	occlusion of the middle cerebral artery (MCA) or its embolization with microspheres
multifocal	multifocal multiple embolization of the middle cerebral artery with microspheres, thromboembolism, photo thrombosis
hypoxic	ligation of the carotid artery followed by inhalation of a hypoxic mixture

Animal species differ in their sensitivity to ischemic effects on the brain. So, in rodents of the gerbil subfamily, the Wallisian circle is open, and a stroke develops when one of the common carotid arteries is occluded. The topography of the vessels of the Wallisian circle and the organization of the blood circulation of the brain in rats has a significant similarity with that in humans. In this regard, to extrapolate the results per person, it is advisable to use these animals for modeling cerebral pathology of vascular origin. In addition, inbred SHR (spontaneously hypertensive rats) rats have elevated blood pressure in 100% of cases and are often used to study cerebrovascular pathology due to the fact that their stroke develops spontaneously [3].

Simulation of total (global) cerebral ischemia

There is a way to simulate total cerebral ischemia in rats by occlusion of four main arteries (two vertebral and two internal carotid arteries) supplying the brain. The disadvantages of the method are: a two-stage reproduction of the model (coagulation of the vertebral arteries at the first stage and clamping of the internal carotid arteries at the second stage of the experiment), the inaccessibility of the vertebral arteries, the risk of brainstem damage during coagulation of the vertebral artery. The subsequent modification of this method of modeling total (global) cerebral ischemia did not eliminate these significant drawbacks. A known method of modeling global cerebral ischemia in rats, which is a modification of the four-vessel model, which is reproduced in one step, with simultaneous occlusion of the common carotid arteries with the imposition of surgical micro clamps on the vertebral arteries between the second and third cervical vertebrae. However, this does not exclude the possibility of collateral blood supply to the brain in rats via the ventral spinal artery.
Global cerebral ischemia is modeled by extracranial occlusion of vessels supplying the brain (brachiocephalic trunk, left subclavian artery and left common carotid artery). This method of modeling cerebral ischemia is deprived of the disadvantages of the above methods: it allows one-stage operation, excludes collateral and reduced blood supply. The disadvantage of this method is its increased trauma due to the use of the approach to the aortic arch and the main arteries supplying the brain, through the opening of the chest. At the same time the pleural cavity is damaged, pneumothorax occurs, which cause the necessity of animal's artificial respiration. The severity of the implementation of this method shows high mortality rate of rats in the group of sham-operated animals [4].

Modeling of subtotal (incomplete) and partial cerebral ischemia

Most often, to study the consequences of ischemia on the brain, models of ligation of one of the common carotid arteries (CCA) - partial cerebral ischemia (PCI) or both common carotid arteries - subtotal cerebral ischemia (SCI) are used. Unilateral ligation of the CCA leads only to a decrease in the blood supply to the brain due to the presence of a closed circle of Willis in rats. Simultaneous bilateral ligation of the CCA (subtotal cerebral ischemia), which carry up to 90% of the blood to the brain, leads to circulatory disorders in the basin of the internal carotid artery and the middle cerebral artery, and the lethality of animals with bilateral ligation of the CCA, according to various authors, reaches 60-70 %. The method of subtotal cerebral ischemia according to Rozvadovsky V.D. (1985) is to ligate both CCA and subclavian arteries distal to the origin of the internal mammary arteries and proximal to the vertebral arteries. It is the easiest to use, has a low cost, but in this case, ischemia occurs not only in the carotid arteries, but also in other parts of the brain.
In addition, there is a method for simulating cerebral ischemia, in which the effects are simultaneously

performed in two ways: both CCAs are ligated and arterial pressure is artificially lowered to a level of 20-30 mm Hg. Art. At the same time, the minimum blood supply to the medulla oblongata, which supports the activity of the vasomotor and respiratory centers remains and the collateral circulation in the brain cannot be restored [5].

Modeling of focal (local) and multifocal cerebral ischemia

There is transient focal cerebral ischemia (with reperfusion) and persistent focal cerebral ischemia (without reperfusion).

There are the following methods of modeling focal cerebral ischemia:

1. Focal-occlusion of the middle cerebral artery OMCA.
    1) OMCA with craniotomy
        a) irreversible.
        b) reversible.
    2) OMCA without craniotomy
        a) Endovascular OMCA (irreversible and reversible).
        b) embolic OMCA (microsphere embolism).
2. Multifocal
    1) embolic OMCA (embolism with microspheres).
    2) thromboembolism.
    3) photo thrombosis.

Irreversible transcranial OMCA

This method is quite invasive and traumatic, because it requires a craniotomy. When modeling irreversible transcranial OMCA, the artery is ligated or subjected to electrocoagulation. This method can be combined with interim or permanent CCA occlusion. The method according to Tamura A. (1981) lies in ligation of the middle cerebral artery through a trephination hole in the area between the foramen ovale and the optic nerve foramen. This method mostly approximated to the development of ischemic stroke in humans. However, it is complicated in execution. When using the method described in the work of Gill R. et al. (1987), the middle cerebral artery is ligated through a trephination hole at the intersection of the nasal cleft. This method of modeling cerebral ischemia is also one of the most adequate, as well as the Tamura method, however, it is complicated and has a high cost [6].

Reversible transcranial OMCA

This method of modeling ischemic brain damage is similar to the previous one. The difference lies in the restoration of blood flow through the carotid artery after a certain period of ischemia.

Endovascular OMCA

For implementing the method, a surgical thread is inserted into the internal carotid artery until it obturates it, which will lead to the cessation of blood flow and the development of cerebral infarction in the basin of this artery. In endovascular OMCA, after a certain period of time, the sutures is removed (reversible endovascular OMCA), providing reperfusion of the ischemic area, or retain occlusion (irreversible endovascular OMCA). The proposed method Smrĉka M. (2002) consists in introducing a monofilament fiber through the aortic bifurcation incision into the internal carotid artery, and then intracranially, and therefore is very difficult to perform. The disadvantages of this method of modeling cerebral ischemia include: insufficient occlusion of the carotid artery, the possibility of developing subarachnoid hemorrhage, hyperthermia, and necrosis of extracranial tissues from the ipsilateral side [7].

Embolic OMCA

In this way, modeling of focal and multifocal cerebral ischemia is carried out. In this case, OMCA is achieved by the introduction of blood clots (thromboembolic occlusion) or artificial micro- and microspheres. The thromboembolic model is closest to the cardioembolic pathogenetic variant of human stroke according to the TOAST classification. Blood clots in thromboembolic OSMA are formed either in vitro or by endovascular instillation of thrombin in situ. When spheres are introduced into the cerebral circulation, the pattern of cerebral infarction is determined by their size: the introduction of microspheres (300-400 mcm) induces cerebral infarction, which is characteristic of occlusion of the proximal section of the carotid artery, while the introduction of microspheres (~ 50 mcm) leads to diffuse embolism of distal vessels circle of Willis. However, the volume of the simulated cerebral infarction with this modeling method is very variable, since it is determined by the rate of spontaneous lysis of the injected blood clots [8].

Photo thrombosis

To implement a photothrombotic model of cerebral ischemia, local photocoagulation of the vessels of limited areas of the cerebral cortex is used. After intravenous injection of light-sensitive dyes ("Bengal rose"), the brain is irradiated, which leads to photochemical occlusion of the irradiated vessels and the development of ischemia of the brain zone [9].

Modeling of hypoxic cerebral ischemia

The most commonly used models of hypoxic cerebral ischemia were described in 1960. For its

implementation, 7-day-old rat pups conducted unilateral ligation of the common carotid artery, followed by 3-hour inhalation of a hypoxic mixture containing 8% oxygen. In this case, a unilateral infarction occurs in the cerebral hemispheres, and the area of damage is localized in the periventricular regions of the brain, more often in the neocortex and hippocampus. In our opinion, models of total cerebral ischemia are suitable for reproducing an atherothrombotic pathogenetic variant, while models of focal, multifocal, and hypoxic ischemia allow studying the development mechanisms and consequences of cardioembolic and lacunar types of cerebral infarction increasingly [10].

Models of total, subtotal and partial cerebral ischemia are quite simple to implement, but their results are more difficult to extrapolate to humans, because focal ischemia is more typical for them. The model of total cerebral ischemia is suitable for reproducing anoxic brain damage during cardiac arrest. Adequate models of cerebral ischemia contribute to detailing the pathogenesis of cerebrovascular diseases and are the basis for improving their diagnosis, treatment and prevention, but the validation of these models still remains an important problem, since they are not always able to reflect all those disorders of higher nervous activity, that occur when the human cerebral cortex is damaged, especially those associated with the second signaling system. It is important to note that the most adequate models are not always doable due to the lack of technical capabilities, while easy achievable models (subtotal and partial cerebral ischemia) are less adequate to the pathology developing in humans [11].

Alcohol brain injury simulation

It is believed that ethanol reduces the survival rate of neurons and disrupts the function in two main ways: 1) as a neurotoxin, causing oxidative stress, DNA damage and mitochondrial dysfunction; 2) suppresses insulin signals essential for viability, metabolism, synapse formation, and acetylcholine production. During ontogenesis, alcohol causes defects in many molecular, neurochemical, and cellular processes that occur during normal brain development, disrupts the functions of neuroglia, alters the regulation of the expression of genes and molecules involved in intercellular interactions, and increases the formation of free radicals. Alcohol acts on specific receptor membrane proteins, ion channels (Ca2+ L-type channels) and signaling pathways. These effects may form the basis of a wide range of behavioral disorders caused by ethanol [12].

In a model of alcoholic brain injury, animals consume ethanol with drinking water as the only source of fluid, as well as with food. For dosed introduction of alcohol, a gastric tube is used. Perhaps the introduction of ethanol in the form of intraperitoneal and subcutaneous injections or vapors. The development of alcohol dependence in animals can be achieved by creating permanent intoxication when used at the maximum tolerated dose of ethanol [13].

Experimental Alzheimer's disease modeling

Alzheimer's disease (AD) is a common neuro-degenerative disease and the creation of adequate models of it is an important direction of modern medicine. One of the hypotheses for the occurrence of AD is based on the intracerebral deposition of amyloid or amyloid precursor protein (APP). A shift in the pH of the intercellular environment to the acid side, insufficiency of mitochondrial oxidation processes, an increase of harmful free radicals, and a decrease in the activity of lysosomal hydrolases lead to the transition of soluble APP to insoluble.

Amyloid is deposited in the walls of cerebral vessels and in the parenchyma of the brain in the form of "senile plaques". In neurons located near senile plaques, calcium channels are activated, oxidative stress develops, followed by the expression of apoptosis inducer genes. Experimental AD models are divided into 2 main groups: cell culture models and animal models.

Cell culture models

Animal (including transgenic) and human cell cultures are used to model AD. There are two- and three-dimensional cell cultures, of which three-dimensional more accurately reflects the structure of the nervous tissue. The method is quite complicated, since the creation of a culture of neurons takes several days, the culture remains viable only for a few weeks and needs constant monitoring. The method makes it possible to study the influence of hereditary factors on the development of the disease, since the culture can be created based on cells of genetically modified animals.

There are several ways to obtain a culture of neural stem cells:

1. epigenetic programming and genetic modification of fibroblast skin stem cells;
2. microfluidic (microflow) cellular model of diffusion delivery of oligomeric aggregates of beta amyloid into neurons;
3. a cellular microfluidic system for creating a porous medium for cell cultures, where one cell population with defined specific property of the disease is in

direct contact with a cell population that is not affected by the development of the pathological process. This model makes it possible to observe morphological and electrophysiological changes in neurons in dynamics;

4. generation of induced pluripotent stem cells from patients with familial AD with subsequent differentiation. At the same time, beta-amyloid accumulated in pluripotent stem cells causes destruction of organelles in mature neurons, which makes it possible to use this model to analyze the pathogenesis of AD and performance evaluation of experimental correction.

Experimental models of AD in animals

Animal models allow to study the pathogenesis and the impact of experimental correction. They are divided into 2 main groups: stereotaxic and transgenic. Stereotactic models are carried out by stereotaxic injection of β-amyloid into the animal's brain (the most common target is the hippocampus).

Transgenic models are carried out in several ways:

1. insertion into the animal genome of new genes that are expressed simultaneously with the organism's genes, which are homologues of the introduced one;
2. selective modification of a certain gene.

Most often, pathological genes of the beta-amyloid precursor protein (APP), presenilin-1, 2 and τ-protein are introduced [14].

Experimental modeling of Parkinson's disease

Parkinson's disease (PD) belongs to extrapyramidal disorders, is a hyperkinetic-akinetic syndrome. The etiopathogenesis of PD is based on a combination of genetic predisposition and the influence of adverse environmental factors. Its occurrence is associated with damage to the substantia nigra, where dopamine is formed. Also, PD is characterized by an excess of acetylcholine. Substances that selectively disrupt the functioning of the catecholaminergic system-reserpine, methamphetamine, 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, rotenone and paraquat are used for its experimental reproduction. Reserpine is a central sympatholytic, its systemic administration leads to depletion of the dopaminergic system. At the same time, hypokinesis in animals develops already 2 hours after the administration of reserpine and persists for about 4 days.

After the introduction of 6-OHDA into the substantia nigra in the striatum, dopamine decreases to 80-90%, severe neurological and behavioral disorders occur, which makes it possible to evaluate the effectiveness of pharmacological correction and neuro-transplantation of dopaminergic neurons. The absence of the formation of cytoplasmic inclusions specific for PD-Lewy bodies is the disadvantage of the model. Rotenone is a lipophilic compound that crosses the blood-brain barrier. Its chronic exposure leads to inhibition of mitochondrial cytochromes in brain neurons and selective degeneration of the nigrostriatal dopaminergic pathway, damage to the striatum, and the formation of ubiquitin-positive inclusions in substantia nigra neurons. At the molecular level, rotenone disrupts the synthesis and assembly of cytoskeletal proteins, DNA replication, and vesicular mediator transport. The rotenone-induced model makes it possible to reproduce most of the links in the pathogenesis of PD, but behavioral disorders are not expressed in it.

Experimental modeling of Huntington's disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by a polyglutamine-coding mutation in the HTT gene. The mutant form of the Huntingtin protein is characterized by a pathologically increased number of copies of glutamine residues, which gives it toxic properties and leads to disruption of mitochondrial functions, excitotoxicity, oxidative damage to neurons and their death. The most vulnerable are GABAergic neurons of the striatum and glutamatergic pyramidal neurons of the cerebral cortex layers IV, V and VI, which is accompanied by motor disorders, dementia of the subcortical type and affective-behavioral disorders. To study the pathogenesis and methods of HD correction, animal models are used, which can be divided into genetic (transgenic) and non-genetic.

Genetic models are based on the introduction of the pathological HTT gene into the animal genome, while non-genetic models are based on the use of selective toxins, one of which is 3-nitropropionic acid (3-NPA). The mechanism of action of 3-NPK is based on the irreversible inhibition of succinate dehydrogenase in the mitochondrial electron transport chain (complex II), especially in striatal neurons. This leads to impaired glucose metabolism, decreased ATP synthesis, formation of reactive oxygen species, excitotoxicity, hyperactivation of NMDA receptors, excessive entry of Ca^{2+} ions into the cytoplasm, oxidative stress, and apoptosis.

3-NPA crosses the blood-brain barrier (BBB) and can be administered systemically to laboratory animals, unlike quinoline acid, which is also used in HD modeling but

does not cross the BBB. Chronic systemic administration of 3-NPK intraperitoneally or subcutaneously makes it possible to more accurately provide the daily dosage, taking into account the weight of the experimental animal, which makes it possible to vary the degree of damage to striatal neurons and the nature of neurological disorders. This model is able to simulate both hypokinetic and hyperkinetic symptoms of HD depending on the regimen of 3-NPK administration, which makes it possible to test new experimental approaches to the correction of HD, including those based on neuro-transplantation of induced pluripotent stem cells, using this model [15]. The data presented in the article can serve as a fundamental basis for further study of the brain in normal and pathological conditions with further extrapolation of the obtained data to humans.

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