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Traumatic brain injury: pathogenesis, experimental models, prospects of cell-based therapy

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ABSTRACT

Traumatic brain injury is the brain damage caused by external mechanical force, for example: a severe impact as a result of a car accident, a blow of a blast wave, biomechanical damage to the brain as a result of a collision in contact sports, etc. This complex trauma with a wide range of symptoms became the main cause of death and disability in modern society throughout the world. The results of numerous therapeutic approaches to treating the consequences of injuries have shown promising perspectives in animal models of traumatic brain injury, but have not achieved any significant efficacy in clinical trials. In this review, we will consider the current topical issues of traumatic brain injury: a modern classification; general principles of the development of the pathological process; models of brain trauma in animals; therapy with the application of stem cells of various genesis.

KEYWORDS: *traumatic brain injury; animal models of traumatic brain injury; stem cells therapy*

Traumatic brain injury (TBI) is a damage to soft tissues of the head, the skull and/or the brain; the clinical state of the patient after an injury is manifested by the brain dysfunction [1, 2]. In fact, trauma is considered TBI when the initiating force affects the functioning of the brain, accompanied by one or more clinical signs, including loss or altered level of consciousness, amnesia, with or without neurological deficiency.

The problem of diagnosis and effective treatment of TBI is one of the most important in modern medical science. Any inhabitant of the Earth is at risk of TBI regardless of his age, place of residence and social status. In recent decades, there is the pandemic spread of TBI due to the increase in the pace of life, the increase in the number of high-speed vehicles, industrialization, as well as such phenomena as terrorism and local armed conflicts. In the world, about 5 million people die every year due to TBI [3]. Statistical indices indicate that the rate of traumatic brain injury is now higher than in any other diseases, including breast cancer, AIDS, Parkinson's disease and multiple sclerosis. [4]. For example, in the US, the TBI occurs every 15 seconds and the total number of victims reaches 1.7 million per year. Of these, 50,000 cases are fatal, and in 80,000 people, TBI causes different degrees of disability [5]. In Ukraine, about 100,000 people (2 per 1,000 people) get TBI, annually [6]. In Russia, about 600,000 TBI are registered each year (4 per 1,000 people) [7]. Despite significant investment in solving this problem, TBI is still one of the main violations of the brain functions, there is no effective pharmacotherapy and standard treatment protocols [8]. In order to develop and apply adequate therapy, it is necessary to understand the complex pathophysiology of TBI and to study the molecular mechanisms that occur at such brain damage.

CLASSIFICATION OF TRAUMATIC BRAIN INJURY

In the clinical practice, the classification of TBI is based on the Glasgow coma scale (GCS): mild (13-15 points), moderate (9-12 points) or severe (3-8 points). Recently, TBI was classified according to the duration of consciousness loss, change in consciousness and post-traumatic amnesia [2, 9]. Mild TBI is a concussion and a bruise of the brain of a mild degree; a moderate TBI is a moderate brain contusion, subacute and chronic compression of the brain; severe TBI is a severe brain contusion, diffuse axonal damage and acute compression of the brain. Naturally, in this context, only the overall spectrum of TBI severity is considered. In practice, this task is solved individually, taking into account the age of the victim, his/her premorbid, the presence of various trauma components (for example, when the extensiveness of scalp and/or skull injuries, even with a mild or moderate brain contusion, makes it necessary to qualify TBI as severe) and other factors.

GENERAL PRINCIPLES OF PATHOLOGICAL PROCESS DEVELOPMENT AT TBI

TBI is not just a one-time event, but also a process that includes multi-level cascades of primary and secondary brain damage, with immediate and delayed consequences. Understanding this has changed the approach to developing new treatments or improving of existing

treatment strategies [10]. Primary lesions are caused by the impact of traumatic force on the skull bones, shells and brain tissue, cerebral vessels and cerebrospinal fluid. Primary lesions include foci of brain contusions, primary brain stem contusions, diffuse axonal injuries and cerebral vascular injuries [11]. At primary brain damage, there is a mechanical damage to the integrity of neurons and neuroglia cells structure, the destruction of synaptic terminals, the destruction or thrombosis of the vessels. Complex cascades of metabolic, cellular and molecular events at a primary trauma collectively constitute a secondary trauma. They can develop from hours to months after the initial injury [12, 13].

In fact, regarding the time after injury, secondary damage is divided into three overlapping phases: early, intermediate and late [14]. The early phase, usually 24 hours after the injury, is caused by the interruption of normal local blood flow, leading to an ischemic cascade that begins with the accumulation of lactate, which causes a metabolic transition from the usual aerobic process to anaerobic glycolysis [15]. Due to the primary injury, access to adenosine triphosphate is reduced and the permeability of the cell membrane (membrane pump) is impaired [11]. This leads to the depolarization of the neuronal membrane and the release of excitatory neurotransmitters (glutamate and aspartate). Linking to glutamate receptors, neurotransmitters not only cause a powerful influx of calcium ions into the cell, but also their release from intracellular depot [16]. Calcium-activated phospholipases, proteases and endonucleases break down lipids, carbohydrates, proteins and nucleic acids, which leads to disruption of the integrity of the cell membrane and the mitochondrial membrane, the interruption of oxidative phosphorylation, protein synthesis, and the formation of free radicals [17]. Moreover, increasing the concentration of intracellular Ca^{2+} activates caspases and calpains, which trigger necrosis or apoptosis of cells and lead to their death and accumulation of cellular debris [18].

In addition, significant injury factors after TBI are activation of immune inflammation and a deficiency of neurotrophic factors. In the intermediate phase of damage, the integrity of the blood-brain barrier is disrupted, and as a result, many immunological and inflammatory processes are induced [14, 19]. This is evidenced by the activation of resident immune cells, which release a number of cellular mediators, such as IL-1β, IL-6 and TNFα, associated with the formation of anti-inflammatory cytokines, mainly IL-10 and TGFβ, along with prostaglandins, free radicals, chemokines and complement system factors [14, 20]. A powerful stimulus that triggers the development of immune inflammation is the presence of cellular debris in the bloodstream because of the destruction of the blood-brain barrier during primary damage. The molecules of the major histocompatibility complex, numerous cytokines and chemokines are expressed in the endothelium of capillaries and on the surface of activated leukocytes, namely granulocytes, macrophages, monocytes and lymphocytes.

The most important for the development of inflammation are tumor necrosis factor and interleukins [21]. In fact, inflammation has a dual role in the brain: beneficial – regeneration processes are triggered; and negative – further damage is intensified. In the latter case, TNFα signaling through the TNFR1 receptor is positively associated with increased regulation of the aquaporin protein AQP4, which belongs to the family of membrane water channels. This protein enhances the formation of edema, contributing to increased intracranial pressure in the brain and cerebrospinal fluid, which leads to further degeneration of neurons. In addition, AQP4 promotes an increase in the number of microfilaments in astrocytes, inducing the formation of scar tissue. The formation of a glial scar, on the one hand, limits the area of further neuronal damage, and on the other hand, prevents sprouting of neuronal processes [11, 14].

An important role in the inflammatory response in brain damage is given to glial cells [22]. TBI is accompanied by the activation of glial cells, which develops into chronic astro- and microgliosis [23]. Activation of glial cells is characterized by the proliferation of astrocytes and microglia and a change in their structure (hypertrophy of soma cells and their processes, the appearance of numerous vacuoles and lysosomes). Activation of astrocytes is accompanied by an increase in the expression of glial fibrillary acidic protein GFAP, which in clinical studies is considered as a potential marker of TBI. Microglial cells are resident macrophages in the central nervous system and can interact with macroglial cells, neurons, and immune cells, through multiple signaling pathways. Microglial cells express receptors classically described for binding to neurons, such as neurotransmitter receptors, and those that are first detected as specific for immune cells mediators such as cytokines and chemokines. Microglial cells are considered the most susceptible sensors of brain pathology. Activated microglial cells, like leukocytes and astrocytes, can migrate to the site of injury, proliferate and phagocytize cellular debris [24, 25].

Along with the processes of secondary damage to the brain tissue at TBI, the processes of neuroregeneration and neuroprotection are triggered. In particular, astrocytes and microglia express the brain-derived neurotrophic factor (BDNF) and other neuroprotective factors, namely nerve growth factor (NGF), neurotrophin-3; produce anti-inflammatory cytokines and chemokines (CD206, CD163, FcyR, Arginase 1, TGFβ) [26, 27].

At the late stage, the most notable feature is the appearance, and then the increase in the number of sudden single seizures, and in more severe cases – the development of epilepsy, which correlates with the type and severity of the trauma [28]. It is known that excitotoxicity is a contributing factor in the development of epilepsy, accompanied by seizures; TBI-induced decrease in the expression of Kv.4.2 potassium channel protein makes neurons more excitable and, consequently, leads to an increase in seizures. Moreover, TBI contributes to seizures activity by reducing the inhibitory current [14].

The variety of pathophysiological processes that develop over time lead to irreversible damage and death of cells and, consequently, to functional brain disorders. In addition, the pathological processes leading to the development of injuries after TBI, as well as those that contribute to recovery are extremely complex and often overlap. Depending on the type of the damage, the pathophysiological mechanisms may be different and even vary with time, and therefore it is very important for neuroprotective therapy to be developed and implemented considering these variables.

Despite the fact that in recent decades, the results of TBI studies have brought our understanding of the development and treatment of the consequences of injuries closer, the dynamics of neurodegenerative processes remains poorly understood, which makes it necessary to use animal models in the experiment.

MODELS OF TRAUMATIC BRAIN INJURY ON ANIMALS

Due to the heterogeneous origin of the TBI, there was a necessity to develop various models of TBI in animals. Basically, laboratory mice and rats are used because of their affordable cost and, most importantly, short life expectancy, which enables to trace the long-term consequences of injuries. Four of them are considered the most widely used: the model of TBI caused by fluid percussion injury (FPI); model of TBI caused by blast injury; a type of TBI caused by a weight drop injury; a TBI model applied with a controlled cortical impact. Along with the above-mentioned models of TBI, other models, which are their modification, are used [12].

Model of fluid percussion brain injury. After cutting the scalp and performing a craniotomy, the trauma is caused by a bolus of fluid from the tube attached to the exposed area of the dura mater, through a quick compression using the plunger. The impact causes a short-term displacement and deformation of the brain tissue, and the severity of the injury depends on the force of compression. In this model, brain damage reproduces clinical TBI without damage to the skull, but the average and severe TBI in humans is often associated with a fracture of the skull and contusion of several cerebral convolutions that cannot be reproduced in this model. However, this model can lead to intracranial hemorrhage, brain edema and progressive damage to gray matter [29].

Model of brain trauma caused by a blast wave (blast-induced injury). The device for modelling a TBI caused by the impact of a blast wave consists of a tube of a large diameter, in which a blast occurs at one end, and on the other, there is an animal. A blast does not directly influence the

experimental animal because of the length of the tube, but the shock wave propagating through it affects the animal. The need for a TBI simulation by blast occurred when it was discovered that many military personnel who were exposed to a blast wave and did not have external injuries were diagnosed with rather severe TBI. This TBI model confirmed the effectiveness of Kevlar® fibers as a material for protective helmets. These studies showed that Kevlar® helmets reduce animal mortality, but the degree of diffuse axonal trauma increases, which proves the appearance of TBI under the influence of blast waves. Minor lesions under the action of the blast wave have the following pathophysiological manifestations: diffuse cerebral edema, severe hyperemia and vasospasm (these symptoms are characteristic for TBI in both humans and animals). A model of TBI with a simulated blast causes significant neuronal dysfunction, cognitive deficit and reduces intracranial pressure in rats [30].

A model of weight drop injury. In the free falling model, the head of the experimental animal is subjected to a shock directed by a freely falling load. The severity of the injury is regulated by the weight of the load and the height from which it is lowered [12, 31]. The varieties of this model are in most cases designed to reproduce diffuse TBI. In this model, there are also violations of motor and cognitive functions [32]. According to the widespread scheme of diffuse axonal damage, developed by Marmarou et al. (impact acceleration model) [33], an impact is applied in the vertex surface of the skull (the skin is previously cut along the median line). A protective metal disc is attached to the impact site to avoid fractures of the skull. Varieties of the model can differ according to the following criteria: whether a craniectomy is necessary, the damage is diffuse or focal, centrally or laterally, whether the animal is fixed in the device or not, etc. [12, 31].

A model of the controlled cortical injury. Compared to the weight drop model, the controlled cortical injury is less consistent with the actual conditions for TBI, but is much more reproducible due to a number of parameters that can be adjusted, such as the depth of damage, time and speed [12]. The principle of device consists in pneumatic or electromagnetic action on a limited area of the dura mater of the brain [34, 35], which leads the destruction of the cortical tissue, axonal damage, concussion, dysfunction of the blood-brain barrier and even coma. The controlled cortical damage in all cases provides exposure of a dura mater. The localization of the application of controlled cortical impact to the brain tissue is most suitable for the reproduction of focal trauma.

CURRENT METHODS OF TBI TREATMENT

Modern methods of TBI treatment have been developed for each pathological stage of secondary damage. Pharmacotherapy is usually restricted to drugs that improve metabolism in the brain tissue (piracetam, picamilon), and vascular drugs (vinpocetine, cinnarizine). Unfortunately, very often the positive effects of treatment for TBI in animals did not confirm the expected results on patients with TBI. In particular, early clinical studies in patients with TBI have confirmed the moderate efficacy of nimodipine, a calcium channel blocker. At the same time, TBI models in rodents have provided strong evidence for the use of nimodipine as a potential therapeutic agent [36, 37]. Similar results were obtained with the use of the tirilazad – an inhibitor of lipid peroxidation, often used to treat cerebral edema. In laboratory studies on animal models of TBI using this drug, it was shown that it has neuro- and angioprotective properties. However, the use of tirilazad in the clinic for patients with moderate and severe TBI did not improve their neurologic status [38].

Laboratory studies on the use of steroids for the treatment of TBI consequences, in particular glucocorticoids, have shown that they reduce the amount of free radicals and have a neuroregenerative effect. However, the use of dexamethasone in the treatment of severe head trauma showed good results for some indicators in surviving patients. Nevertheless, there was an increase in the number of vegetative state patients, who did not have the favorable outcome of treatment [39]. The use of the preparation selfotel – NMDA antagonist and an inhibitor of excitatory amino acids – in clinical trials showed the development of psychoactive behavioral effects in patients with TBI, which caused the stop of clinical trials. [40]. Here are just a few examples of the treatment of TBI patients with those drugs that showed a potential therapeutic effect in animals on the TBI models.

Considering the global impact of TBI on the population, it is actual that research and development of new methods of therapy continue to develop actively in this area.

STEM CELLS APPLICATION IN TREATMENT OF TBI OUTCOMES

Recently, it has become possible to use stem cells (SCs) as a potential therapy for TBI, based on their neuroprotective and neuroregenerative properties [41]. Numerous studies on animal TBI models are conducted using stem cells of various sources, namely: endogenous neural stem cells, embryonic and fetal SCs, induced pluripotent SCs, and mesenchymal SCs.

Activation of endogenous neurogenesis at TBI. It is known that in two parts of the central nervous system of an adult organism there is a process of neurogenesis: the subventricular zone [42], situated throughout the lateral ventricle, and the dentate gyrus of the hippocampus [43]. Stem cells in these zones have self-renewal properties and are able to differentiate into neurons and glial cells [44, 45].

It was shown that the endogenous NSCs from the subventricular zone affect the microenvironment and promote the survival of neurons and glia after TBI. The studies were performed on transgenic mice expressing thymidine kinase gene of herpes simplex virus under the control of the nestin promoter, which expression increase was observed in the model of controlled cortical damage [46]. In such mice, the endogenous SCs of the subventricular zone were removed. Two weeks after TBI in mice with a deficiency of endogenous SCs, a decrease in the level of neuronal survival and a reduction in the number of glial cells in the cortex was detected, however, hypertrophy of the soma cells in the site of injury was noted. The results showed that endogenous NSCs play an important role in maintaining the microenvironment in the cortex and thereby contribute to the survival of neurons and glial cells after TBI. Considering obtained results, endogenous NSCs can exhibit protective properties after trauma [46]. Thus, endogenous stem cells located in the neurogenic niche form a potential pool of cells involved in brain repair. The functional role of these new cells depends largely on the number of newly generated cells, their potential for differentiation, survival and integration into existing neural networks.

Studies have shown that TBI significantly increases the proliferation and differentiation of resident stem cells in both subventricular zone and dentate gyrus in adult injured mice and rats [47]. Regenerated neurons in these areas can integrate into neural networks of the brain and replace the lost neurons in the damaged parts of the brain. In particular, the newly created granular cells in the dentate gyrus of the hippocampus have the ability of mature neurons of this zone to generate action potentials and form functional synapses [45]. Therefore, the ability to stimulate the proliferation and differentiation of endogenous NSCs is an attractive strategy for restoring the damaged brain.

The study conducted on the cerebral cortex of the affected person with TBI found out an increase in the number of cells expressing SCs markers, such as DCX, TUC4, PSA-NCAM, SOX2, NeuroD [48]. It is known that induced endogenic neurogenesis promotes acceleration of regenerative processes after TBI. In particular, serotonin, glucocorticoids and growth factors significantly increase the proliferation and maturation of cells in the subventricular zone and dentate gyrus [44, 47]. Intraventricular administration of the basic fibroblast growth factor (bFGF) or epidermal growth factor (EGF) enhances endogenous neurogenesis, increases the TBI-induced cell proliferation in the subventricular zone, hippocampus and significantly improves cognitive recovery [49].

Regenerative responses to focal brain damage have shown that cell proliferation in the dorsolateral subventricular zone increased twice to 48 hours in the injured brains of young animals compared with the missing proliferative response in the brains of adults [50]. Moreover, the progenitors of the subventricular zone in the injured brain of young animals formed twice as many neurospheres and proliferated much faster than

animals of the same age, which brain did not suffer [50]. Nevertheless, such a reparative response did not lead to a significant regeneration of neurons. Stimulation of endogenous neurogenesis through NSCs activation is not an easy task. It should be considered, that endogenous stem cells cannot be equally stimulated because of the multicomponent environment in which the cells are located [51, 52].

Other studies have shown that the formation of neurons from endogenous NSCs after TBI and their integration into the existing network takes about 10-14 days [47]. Certain drugs and changes in behavioral responses in the context of a more complex environment can enhance endogenous neurogenesis [53]. Antidiabetic drug metformin increases endogenous neurogenesis in the modeling of ischemic trauma. The administration of metformin activated the proliferation, migration and differentiation of endogenous NSCs in the damaged brain of newborn mice. In the neurospheres formed by NSCs in such mice, more oligodendrocytes and neurons were detected in the presence of metformin compared to the animals in the control group. In addition, the administration of metformin contributed to the improvement of sensory-motor functions [54].

Numerous results of the study of endogenous NSCs role for successful recovery of a damaged brain undoubtedly showed their positive potential. An important task is to create conditions for the proliferation of endogenous NSCs and their differentiation into a sufficient number of mature neurons and glia that can integrate into the damaged neural network of the brain.

Different types of exogenous stem cells used at TBI. The brain of adult mammals and humans has a limited ability to generate new cells, in particular, neurons and glial cells at various types of damage. Therefore, transplantation of exogenous SCs is a promising therapy for treatment of the consequences of TBI and can replenish the pool of lost neurons and glial cells [55]. The use of exogenous stem cells as a potential cell therapy for various types of TBI is based on their ability to differentiate and integrate into the recipient's neuronal networks, as well as to produce a variety of trophic factors [55, 56, 57].

Embryonic and fetal stem cells. Stem cells derived from an adult organism have a limited ability to differentiate into many types of cells. In contrast, embryonic stem cells from the inner cell mass of the blastocysts are pluripotent, and fetal SCs are multipotent and are able to differentiate into all types of neural cells [58].

Fetal SCs can be isolated from the ganglionic eminence of mouse embryos on 14-16th day of pregnancy, and from the area of active neurogenesis – the subventricular zone of the lateral ventricle [59, 60]. Due to their high plasticity, such SCs are an ideal source of cells for transplantation, as they can differentiate into different types of neural tissue cells, migrate and integrate into different parts of the brain [61]. They also have a trophic effect. Namely, transplanted cells can secrete a wide range of chemokines, cytokines, growth factors, immunosuppressive molecules and other trophic factors that actively influence the microenvironment and, accordingly, the response from host cells at the site of the injury [62]. Embryonic SCs can also secrete neurotrophic factors GDNF and BDNF produced by glial and nerve cells [62].

Embryonic NSCs isolated from human fetuses and mice were used as a source for transplantation in various animal models of TBI, and after transplantation, motor and cognitive functions were restored [47]. Transplanted embryonic NSCs obtained from a 10-week human brain in the rat brain after weight drop model of TBI survived and differentiated into neurons and astrocytes after migration to the contralateral cerebral cortex of rats [60]. In studies on the transplantation of differentiated human NSCs into injured rat brains after controlled cortical impact demonstrated a temporary increase in angiogenesis and neuronal survival in the lesion with a decrease of astrogliosis and the volume of damaged brain tissue [59]. Moreover, NSCs isolated from the brain of mouse embryos and transplanted into the injured brain of adult mice significantly improved the motor and cognitive functions of such animals, since these cells differentiated into neurons and glial cells that contributed to the repair of damaged tissues [59].

Nowadays, numerous protocols have been developed for the obtaining of embryonic NSCs and their directed differentiation with varying degrees of success. A huge disadvantage of application such cells is the ethical issues of their obtaining. The fact that transplanted embryonic or fetal NSCs can integrate into the nerve tissue of the recipient makes it easier to use them in cell therapy for traumatic injuries of the nervous system.

Adult neural stem cells. Along with embryonic and fetal NSCs in the cell therapy of TBI, neural stem cells isolated from an adult organism are used. The use of embryonic and fetal SCs in cell therapy is complicated by many technical and ethical problems, the risk of tumor formation and a greater probability of immunological rejection [63]. To overcome these complications, neural stem cells derived from an adult organism are considered a promising potential source of therapy for TBI [64]. Such cells are isolated from the subventricular zone of the lateral ventricles of adult humans or animals. These SCs are unipotent cells, which have low potential for self-renewal and differentiate only into one cell type of the tissue, where they are located. Thus, the adult NSC can differentiate into cells of the neural tissue and, therefore, they can be used as a possible treatment for the consequences of the TBI.

After transplantation into the injured brain of rats, the adult NSCs showed sufficient survival for a long period and differentiated into tissue-specific cells of the recipient [65, 66]. Such cells migrate to short distances from the site of administration, where they expressed markers of neurons and glial cells, such as astrocytes and oligodendrocytes, indicating their ability to differentiate into both neurons and glia [67]. Another study showed that NSCs isolated from the human spinal cord and transplanted into the injured spinal cord of adult rats differentiated into neurons and integrated into the recipient's neural networks [68].

The therapeutic potential of neural stem cells was studied to treat the diffuse pathology of white matter after traumatic brain injury. Neuronal SCs isolated from the subventricular zone of an adult mouse were transplanted into the lateral ventricle of the mouse brain two weeks after TBI. SCs transplantation significantly reduced reactive gliosis in the corpus callosum, and thereby reduced neuroinflammation [69].

There have been several clinical studies related to the use of NSCs in human. Namely, isolated and purified human NSCs were transplanted to patients with chronic spinal cord injuries [70]. NSCs were integrated into the nerve parenchyma and obtained results give hope for the continued application of such cells as a cell therapy for traumatic injuries of the nervous system.

At present, there is a need to use SCs with a certain type of differentiation for the treatment of various types of TBI. So, for example, at diffuse TBI, unlike focal TBI, axons are damaged, which leads to their demyelination. For this type of damage, it is most likely that you will need to use combined therapy with SCs, which were previously differentiated not only into neurons, but also into oligodendrocytes.

Induced pluripotent stem cells (iPSCs). Reprogramming of differentiated somatic cells into pluripotent CSs can be accomplished by ectopic expression of four transcription factors: Oct4, Sox2, c-Myc and Klf4 [71]. Such induced pluripotent stem cells can self-renew and differentiate into different cell types, similar to embryonic NSCs. The possibility of iPSCs application opens new prospects for cell therapy and has a huge advantage: it avoids the use of embryonic SCs for ethical reasons and generates autologous cells from the patients, and thus does not cause any immunological rejection during their transplantation [47].

The cells survived and contributed to the improvement of cognitive and motor characteristics at iPSCs transplantation into a damaged rats' brain on a model of controlled cortical impact. However, a complete cognitive recovery was recorded only in a complex treatment with a complicated residence environment in which rats were found and with iPSCs transplantation [72].

A protocol for the generation of iPSCs is known by Sendai virus transduction of fibroblasts isolated from the dura mater of a 60-year-old patient with severe cognitive impairment [73]. Cells of the dura mater can

be easily isolated during neurosurgery, which makes them an effective source for the generation of iPSCs. It was shown that iPSCs clones express some SCs markers, such as Nanog, the transcription factors Sox2 and Oct4 [73]. Thus, similar iPSCs are potential candidates for autotransplantation, which can be effectively used in cell therapy.

On the model of damaged spinal cord in adult monkeys, it has been shown that transplanted human iPSCs survived and differentiated into neurons, astrocytes and olidodendrocytes, enhanced axonal regeneration and prevented their demyelination, while not showing an oncogenic effect. Transplanted iPSCs not only promoted regeneration, but also exhibited immunomodulatory and neuroprotective properties to prevent further tissue damage [74].

At present, the technology of using iPSCs has some serious limitations. There is a risk of tumor formation due to the use of viral vectors and a low reprogramming efficiency in the production of these cells. It is extremely important to study carefully the safety of application iPSCs in the clinic, since the induced cells genetic and epigenetic background is unclear [75]. Further systematical study of iPSCs is necessary for prospective cell therapy.

Multipotent mesenchymal stromal cells (MMSCs). MMSCs are multipotent stem cells that can be isolated from bone marrow and other adult tissues [76]. MMSCs are characterized by the expression of various surface molecules, such as CD105, CD73 and CD90, and the absence of hematopoietic markers, including CD45, CD19 and CD34 [65, 77]. They can be easily isolated from rodent and human tissue, cultured and used in *in vitro* and *in vivo* models. MMSCs have considerable plasticity, high level of self-renewal, proliferation and differentiation. These characteristics allow the use of MMSCs in cell therapy for the treatment of a wide range of diseases, including neurological trauma, such as TBI [76, 77]. Cultured MMSCs can be injected intravenously or directly to the injury site, where they will release growth factors, thereby restoring damaged tissue and inducing angiogenesis [78]. At intravenous administration, MMSCs penetrate the blood-brain barrier and produce trophic factors in the brains of rats after TBI. The most known secreted factors of MMSCs are BDNF, NGF, vascular endothelial growth factor (VEGF) and glial cell line-derived neurotrophic factor (GDNF) [79].

Due to the high adhesive characteristics and the ability to differentiate into many cell types, MMSCs were proposed as candidates for treatment of TBI. Indeed, cells expressing markers such as beta tubulin III and GFAP can be obtained from MMSCs when they are cultured with bFGF and hEGF. In the co-culture of astrocytes with human MMSCs isolated from the umbilical cord (hUC-MSCs) the proliferation of MMSCs and their differentiation into neurons were increased [80].

It was shown that the transplanted human MMSCs to rats after TBI improved the neurological function based on the values of the rotarod test and evaluation of the severity of the neurological disorders. In addition, rats's cognitive features were improved, which were assessed using Morris water maze test [81].

Currently, MMSCs therapy has shown promising effects in the treatment of many diseases. In particular, the experimental study was carried out on 10 patients with severe TBI using neural stem cells obtained from autologous bone marrow-derived mesenchymal stem cells. Phase I clinical study was conducted to assess the safety, validity and biological effect of intravenous or intrathecal transplantation. As a result of the injection of such cells, there were no signs of neurological worsening in all patients. Moreover, in remote periods after transplantation, 7 patients had an improvement in neurological function compared to pre-transplantation values [82].

Due to the relative easiness of obtaining of multipotent mesenchymal stromal cells and their ability to differentiate into a specific type of neural cells, MMSCs are the most promising candidates for treating the outcomes of TBI.

CONCLUSION

Despite the increase in the number of studies on the diagnosis, treatment and prevention of TBI, the main question remains unanswered: what is the most effective method of restoring brain function after TBI? Discovery of the SCs and their detection in the brain of embryos and adults changed our understanding of the plasticity of the central nervous system; however, given their complex genetic variability, the final answer will be far from simple.

Stem cells are good candidates for the treatment of TBI and, therefore, a hope for many people who have survived the TBI. Despite the early successes in the study of the SCs, there are still many questions and problems requiring comprehensive research. The main of them are the following: 1) the ability of the SCs to cause the development of malignant tumors after transplantation into the recipient; 2) the ability of the SCs to unexpected differentiation and the difficulties of effective directed differentiation; 3) the immune incompatibility of SCs with the recipient in case of their allogeneic application.

Without an adequate solution to the problems associated with SCs biology, the effective use of these cells in clinical practice is impossible.

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