

Perspectives of use of bone marrow cells in future therapy of brain diseases

Możliwości wykorzystania komórek szpiku kostnego w przyszłej terapii chorób mózgu

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Key words

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Abstract

Medical problems associated with the increasing number of patients suffering from brain diseases have resulted in a constant search for effective therapeutics. Considering the complicated pathological processes occurring in diseases of the central nervous system and the limited capability of the neural tissue to regenerate, therapy of neurological diseases is extremely difficult. The lack of efficient medical treatment results in complex problems associated with rehabilitation and thus in functional disturbances, which prevent patients from restoring their independence and returning to complete, also professional, activity. Cell therapy has recently been considered as a possible approach in the treatment of brain diseases. Its aim is to supply pathologically changed brain tissue with factors promoting regeneration and with cells that may replace the damaged ones. Bone marrow cells have become a potential source of cells in this type of therapy. Bone marrow contains at least two major kinds of stem cells: haematopoietic stem cells, which give rise to the blood cells and mesenchymal stem cells, which can differentiate into cells of mesenchymal lineage and produce an array of growth factors essential for repair. The review presents the achievements of studies on use of bone marrow cells in the therapy of various brain diseases of traumatic or neurodegenerative aetiology.

Słowa kluczowe

komórki szpiku kostnego, terapia komórkowa, mózg, transplantacja, rehabilitacja

Streszczenie

Problemy medyczne, związane ze wzrastającą liczbą ludzi cierpiących na choroby mózgu różnego pochodzenia, są powodem ciągłych poszukiwań optymalnych metod terapeutycznych. Ze względu na złożoność procesów patologicznych zachodzących w schorzeniach mózgu oraz ograniczoną zdolność tkanki nerwowej do regeneracji, terapia chorób neurologicznych jest niezwykle trudna. Konsekwencją braku skutecznych metod ich leczenia są złożone problemy rehabilitacji oraz związane z tym zaburzenia funkcjonalne uniemożliwiające pacjentom powrót do pełnej samodzielności oraz aktywności, również zawodowej. W ostatnim czasie zwrócono uwagę na możliwość wykorzystania terapii komórkowej w leczeniu chorób neurologicznych, której celem jest dostarczenie do patologicznie zmienionej tkanki mózgowej czynników wspierających regenerację. Potencjalnym źródłem komórek w tego typu terapii stały się komórki szpiku kostnego. Poniższy przegląd przedstawia osiągnięcia w badaniach nad wykorzystaniem komórek w szpiku kostnego w leczeniu schorzeń mózgu pochodzenia urazowego oraz neurodegeneracyjnego.

INTRODUCTION

Pathological processes affecting the brain result in numerous signs associated with lesions to various anatomical structures, which subsequently induces functional disturbances in the patients and the associated problems

in activities of daily living and at work. These disturbances require application, apart from rehabilitation methods based on neurophysiologic principles, of knowledge on novel therapeutic approaches that may help in the therapy and prevention of sequelae of central nervous system dis-

eases in future. Cell therapy is one of the paths leading to creation of a new therapeutic strategy.

Cells that have a high division potential and are easy to obtain constitute a basis for studies that may, in future, pave the way for new therapeutic possibilities in the therapy of

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brain diseases. Cells derived from bone marrow are an example. Experimental studies provide information about numerous possible applications of these cells, depending on: site and timing of administration of bone marrow cells, source of transplanted bone marrow cells, conditions of culture of bone marrow cells and conditions of their administration to the pathologically changed tissue, as well as modification methods used in gene therapy.

Bone marrow cells are used in gene therapy because they contain stem cells that have the potential to differentiate into cells of the haematopoietic and mesenchymal lines (haematopoietic stem cells and mesenchymal stem cells, respectively) and because of properties of bone marrow interstitial stromal cells that produce numerous growth factors and cytokines promoting regeneration. Products of bone marrow stromal cells include macrophage and granulocyte colony stimulating factors. They also produce growth factors that control lymphopoiesis and factors inducing megakariopoiesis. Bone marrow interstitial stromal cells regulate thrombopoiesis and stimulate erythropoiesis. They may also inhibit haematopoiesis via release of transforming growth factor beta (TGF- β)¹. At present, bone marrow transplantation (transplantation of haematopoietic stem cells) is a routine procedure in the treatment of neoplasms and metabolic disorders of haematopoietic and immune systems. Overall, 70 different diseases of haematopoietic system are currently treated with bone marrow transplantation.

Bone marrow cells' potential to differentiate into cells of mesenchymal line constitutes the principle for their use in future gene therapy. There is evidence that in favourable conditions, interstitial cells (stromal cells) may differentiate into osteocytes, chondrocytes, adipocytes, both *in vitro* and *in vivo*². Moreover, bone marrow stem cells have the potential to differentiate into hepatocytes, cardiomyocytes, epithelial cells and neural cells³⁻⁶. Implantation of these

cells into the pathologically changed tissue promote functional recovery after myocardial infarction (confirmed by cardiographic measurements)⁷ and improve bone regeneration due to favourable effects of transplanted cells on healing of bone fractures and defects⁸. A characteristic feature of bone marrow interstitial cells is their ability not only to differentiate into numerous cell lines (in *vitro*, after exposure to appropriate development and growth factors) but also to regenerate throughout the life span.

Simplicity of methods of setting up and handling bone marrow interstitial stromal cell culture that allows collection of abundant cell material within a short period of time as well as ease of performing auto- and allotransplantations of cultured groups of cells currently create new possibilities for the potential treatment of neurological conditions including brain injuries.

Traumatic brain injury

Brain injury, considered one of the main causes of mortality and morbidity in developed countries, is a complex condition involving numerous dynamic pathological processes observed immediately after the trauma (acute phase) as well as subsequently (post-acute phase). Trauma may result in changes including bleeding, oedema, axonal injury, hypoxia, and ischemia. At the site of mechanical brain injury, blood brain barrier is disrupted, electrolytic homeostasis is impaired and apoptotic or necrotic neuronal and oligodendroglial cell death occurs with activation of astrocytes and microglia. Sites of injury are also being infiltrated by white blood cells. Infiltrating macrophages, together with activated microglia, initiate an inflammatory reaction⁹, and, in cooperation with astrocytes and fibroblasts, lead to gliosis¹⁰. Course of gliosis depends on interactions between these cells and their cytokine production.

Apart from gliosis, delayed neuronal death is a process that impairs regeneration of the neural tissue. It is induced by inflammation that leads

to production of neurotoxic mediators¹¹.

Considering coexistence of multiple processes in traumatic brain injury, management aimed at one specific process may not be sufficient to prevent sequels of trauma. Currently, effective pharmacological neuroprotective treatments are being investigated that will improve function of the injured tissue, and, although many pre-clinical studies yield promising effects, current treatment of traumatic brain injury is still not sufficient and requires further improvement. Effects of COX2 (cyclooxygenase-2) inhibitors¹², calcium channel inhibitors¹³, dexamethasone¹⁴, cyclosporine A¹⁵ are currently being studied. Neural, embryonic and bone marrow stem cells are considered a potential source of factors inducing repair of the injured brain. Cell therapy is based on an assumption that neurological functions, lost as a result of brain injury, may be, at least in part, restored by substituting lost cells with new ones or by providing growth factors to the living cells that may improve their function and survival.

Colonization of the injured tissues of the brain and differentiation of bone marrow cells were observed after intravenous^{16,17} and intracerebral¹⁸ administration of these cells in rat models of brain injury. Only a few of the transplanted cells expressed neuronal (NeuN and MAP-2) or astrocytic markers (GFAP), while motor and sensory functions improved in the studied animals. There were no connections between the differentiated donor cells and the host cells and the number of differentiated cells was small. The researchers hypothesized that the observed functional improvement might be secondary to the interaction between the transplanted cells and the host tissue, related to the production of growth factors and cytokines.

There is evidence that bone marrow cells transplanted into the injured tissue produce a number of neurotrophic factors that induce regeneration. This phenomenon was observed both *in vitro*¹⁹ and in patho-

logical conditions in vivo, where increased expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) was observed in injured brain after intravenous administration of bone marrow stromal cells²⁰. Moreover, exposure of cultured stromal cells to growth factors (BDNF and NGF) increases their migration and colonization of the injured host brain tissues after transplantation. The observed improvement in motor function was greater than after transplantation of stromal cells cultured in standard conditions²¹.

When designing pre-clinical studies on use of bone marrow cells in the therapy of brain injury, number of cells and timing of implantation after the injury should be considered factors influencing efficacy of the treatment. Mahmood *et al.*²² administered intravenously bone marrow stromal cells at three different doses (2×10^6 , 4×10^6 , 8×10^6) one week after brain injury. Three months after the treatment, neurological functions improved in animals exposed to higher doses of stromal cells while no significant improvement was observed after administration of 2×10^6 cells. At all of the administered doses of bone marrow cells, levels of NGF and BDNF in the injured tissue significantly increased. However, the increase was lowest after injection of 2×10^6 cells.

Conditions of the culture and method of administration may also play an important role. Lu *et al.*²³ co-cultured stromal bone marrow cells and neural cells harvested from rat embryos. Stromal cells developed long projections and connections with embryonic cells. Moreover, increased growth of cultured bone marrow cells was observed. At the second stage cultured cells were administered directly to the site of injury. Their survival was increased in comparison with stromal and neural cells cultured separately. The diameter of the injured tissue decreased and motor functions significantly improved.

Mahmood and Lu²⁴ pointed out to the necessity of combining bone

marrow cell therapy with pharmacological treatment in future. Results of their research confirmed that in the treatment of traumatic brain injury, combination of bone marrow cell therapy and atorvastatine results in a better outcome in terms of motor functioning.

In experimental animal models of traumatic brain injury, the induced symptoms are similar to the clinical symptoms of traumatic brain injury. However, experimental injuries do not initiate all processes observed in humans and are more focal, which, regarding standardization of the research, may lead to significant discrepancy between results of clinical trials and results of experimental studies in animal models. Before bone marrow cells may become an approved clinical treatment in post-traumatic brain injury, it is necessary to study their effects on subsequent processes following injury and to determine the exact causes of the experimentally observed improvement in neurological function.

Ischaemic stroke

Morphologically, in the region of cerebral infarction, subsequent stages of cellular activity can be observed. Initially, neurons and some of oligodendrocytes swell, which leads to cell death. Then, progressive swelling of capillary endothelial cells, myelin and astrocytes occurs. Approximately 24 hours after ischemia, polinuclear leukocytes infiltrate pathologically changed tissues (leukocytic infiltration). Within 48 hours leukocytes are degraded and replaced by macrophages that cover the region of necrosis (resorption phase)²⁵. At the border of the infarction, there are numerous astrocytes which participate in resorption and demarcation. Phase of necrosis transits into the phase of glio-mesodermal organisation with the formation of cavities. Astrocytes and vessels survive and scars are the end phase of cellular activity^{25,26}.

Region of penumbra, where some of the neurons and remaining cells survive, is one of the targets of the

therapy in cerebral infarction because of the potential chance to spare additional neurons. Current pharmacological treatments may achieve this goal. Piracetam is a neuroprotective agent successfully used in experimental studies. It restores function of cellular membranes of neurons that survived in the region of penumbra, promotes synthesis of ATP and – thus – actively improves neurotransmission and activation of secondary signal transduction. Based on the results of the above-mentioned studies, piracetam is effective when used within 7 hours after cerebral infarction^{27,28}.

At present alternative approaches aimed at partial regeneration of the damaged tissues are being studied. Transplantation of bone marrow cells into the region of ischemia constitutes one of the options and the related studies aim at observation of both motor functioning, neurological status as well as histological improvement.

These studies are conducted in experimental animal model of cerebral infarction, where ischemia is produced by occlusion of the middle cerebral artery²⁹⁻³¹, leading to decreased blood flow and focal brain ischemia with all the secondary pathological changes. Fourteen days after implantation of human stromal cells into the site of cerebral infarction in rats, functional improvement (reduction of neurological deficits) was observed. Moreover, increased NGF and BDNF levels at the site of infarction as well as significant reduction of apoptosis in the region of selective necrosis and more active proliferation of lymphocytes except for the cytotoxic ones were reported³².

It was found that co-administration of stromal cells and nitric oxide (NONOate) promotes angiogenesis, neurogenesis and increases expression of vascular-endothelial growth factor (VEGF) and fibroblast growth factor (bFGF) in the subventricular area and at the site of ischemia³³. Use of combination of stromal cells and NONOate led to a better functional improvement (both motor and sensory) in experimental models

compared with the results of therapy with stromal cells alone. These results suggest that combined treatment with both pharmacological agent and cell preparation may yield significant benefits in terms of functional neurological improvement after cerebral infarction.

Additional mobilisation of the host bone marrow cells after the infarction may become an adjunctive therapy of this condition. Administration of granulocyte colony stimulating factor (G-CSF) reduces the region of ischemic injury and improves motor function. Furthermore, it promotes migration of bone marrow cells into the brain, improves neuronal plasticity and neovascularization^{34,35}.

Borlongan *et al.*³⁶ were studied effects of xenogenic stromal cells on restoration of the cerebral circulation and the blood-brain barrier disrupted as a result of cerebral infarction. Transplantation was performed within 15 minutes following the experimental ischemia. The authors observed that certain doses of transplanted stromal cells may promote restoration of blood-brain barrier and improve cerebral circulation in the region of penumbra. Levels of growth factors from the TGF-beta superfamily increased at the site of transplantation³⁶. Chen *et al.*³⁰ administered stromal cells 1 day and 7 days after the occlusion. The implanted cells survived and colonised the region of ischemic injury. Some of the implanted stromal cells expressed neuronal markers³⁰. In another study, the region of ischemia was assessed 4 months after transplantation of labelled stromal cells. Increased number of oligodendrocytes and astrocytes that showed positive reaction to the applied marker as well as increased gliogenesis were observed in the region of the injured hemisphere³⁷.

Studies on glial scar, especially in the late phase after the transplantation, indicate that transplantation of bone marrow cells early after cerebral infarction may preserve survived neurons in the region of penumbra. During the later phase, new neurons

and astrocytes are expected to appear in this region. The aim of a therapy potentially restoring the lost motor function, would be axonal regeneration in the region of ischemia. Moreover, according to the published literature, numerous factors should be considered when planning preclinical studies, i.e. site and timing of transplantation, number of injected cells, use of additional factors³⁸. Autologous transplantation of bone marrow cells 3, 6, 12, 24, 72 hours after the experimental ischemia reduced the size of injury. Earlier transplantation, however, was associated with better healing³⁹.

Recently, clinical trials on autologous transplantation of mesenchymal bone marrow cells were performed that included five patients with severe neurological deficits secondary to ischemic stroke in the territory supplied by the middle cerebral artery⁴⁰. Influence of the transplanted cells on healing was evaluated using three scales: NIHSS (*National Institutes of Health Stroke Scale*), Barthel Index and Rankin Scale. One year after the transplantation, the degree of functional independence of post-stroke patients significantly improved according to the Barthel Index scores and there was a decrease of the degree of disability as evidenced by Rankin Scale scores. However, the degree of neurological deficits secondary to cerebral infarction, assessed using NIHSS, did not change significantly. These results confirm preclinical observations, that condition of the studied animals improved after cell transplantation, but – considering small number of studied patients – their interpretation must be cautious. Moreover, there is a remaining question as to the main mechanism of healing of injured brain tissues after cell transplantation and what requirements must be fulfilled by clinical trials to fully confirm results of previous studies. The degree of functional independence, as a measure of improvement, raises a question whether inclusion of the intensive, neurophysiology-based rehabilitation to the therapeutic programme of transplantation of autolo-

gous mesenchymal bone marrow cells in patients with cerebral infarction, may, in future, create a new model of treatment aimed at reducing motor and cognitive deficits.

Parkinson's disease

Degeneration and depletion of melanin positive cells of the substantia nigra pars compacta, resulting in a reduction of dopamine content, are the main morphological changes observed in the brain. Active gliosis affects the region of degeneration and the striatum. Apart from the active gliosis, numerous cavities with macrophages are observed. Pathological process involves neurons producing substance P and enkephalins. There is also imbalance between the dopaminergic and cholinergic systems secondary to the degeneration of cells producing GABA. These cells project from the striatum to the substantia nigra.

Despite the advance in the pharmacological therapy, treatment of Parkinson's disease remains symptomatic and does not prevent progression of the disease. Positive effects of Levodopa on motor function are still limited. During the course and progression of the disease, levodopa becomes less effective and patients experience sudden changes in motor functioning (the "on-off" phenomenon). In order to decrease the on-off effect and to postpone its occurrence, therapeutic preparations contain both Levodopa and benserazide – decarboxylase inhibitor. Other medications used to treat Parkinson's disease include dopamine agonists⁴¹ and MAO B (monoamine oxidase type B) inhibitors⁴², an enzyme that metabolises dopamine.

Considering pathological processes observed in Parkinson's disease, its therapy should be aimed at prevention of neuronal death, regeneration of the destroyed structures and restoration of their lost function as well as concomitant induction of synthesis of dopamine and other important neuromodulators.

One of the methods to obtain cells that produce dopamine is trans-

plantation of embryonic stem cells, with the aim to improve dopaminergic neurotransmission^{43,44}. Implanted cells, apart from synthesis of dopamine and restoration of the normal reactivity of receptors, may release neural growth factor, NGF, that may play important role in the stimulation of the remaining cells in the substantia nigra to produce new neural processes projecting towards the striatum⁴⁵. Lindvall *et al.*⁴⁶ observed a reduction of symptoms in a patient who had underwent transplantation of embryonic stem cells. Improvement in bradykinesia and spasticity was noted. Importantly, on-off effect was minimised while maintaining optimal pharmacotherapy. The most prominent improvement was observed contralaterally to the side of transplantation. In clinical studies that involved three patients who underwent cell transplantations, reduction of dyskinesia was observed up to four years after the procedure. The most significant improvement in motor function was observed one year after the transplantation⁴⁷. Nevertheless, application of this method in humans remains ethically controversial. Regarding motor improvement observed in studies in humans that involved application of embryonic stem cells, next therapeutic approach – involving simple access to the cellular material – that may bring functional improvement, is transplantation of stromal cells.

Li *et al.*⁴⁸ conducted a study in mice, in which parkinsonian symptoms were produced by injection of MPTP (1-methyl-4-phenyl-1,2,3,4,6-tetrahydropyridine). The authors observed not only expression of tyrosine hydroxylase (TH), but also an improvement in motor function. Cells that were transplanted into the striatum survived at least 4 weeks after the procedure.

Other studies on bone marrow stem cells aimed at selection of cell lines in culture that were able to synthesize dopamine. There was also an attempt to genetically transduce stromal cells so that they were able to produce Levodopa and to implant these cells into the striatum^{49,50}. Ret-

rovirus with a gene encoding TH and GTP cyclohydrolase I was introduced into the human and rat stromal cells. In the culture, these cells produced Levodopa while demonstrating preserved multipotential properties. After implantation into the rat striatum, the cells survived more than 80 days, and the expression of the transgene was observed in the first 10 days after the procedure⁴⁹. The level of dopamine in the pathologically changed striatum after the administration of MSCs expressing TH was higher than in control animals. Concomitantly, asymmetrical rotations in parkinsonian rats after injection of apomorphine decreased⁵¹, indicating a possibility of restoration of normal symmetry and sequence of alternating movement.

Bone marrow cells were also transduced with the gene encoding glial cell line-derived neurotrophic factor (GDNF). After transplantation of these cells into the brain of the MPTP-treated mouse, increase of TH, GDNF and improvement in motor functions were observed. These results suggest that bone marrow cells might be used in gene therapy of Parkinson disease^{52,53}.

Considering the lack of ethical limitations and the fact that bone marrow stromal cells are easy to obtain, there is a realistic chance to use this method more commonly in patients suffering from Parkinson disease in future. Transplantation of bone marrow cells may be a novel, less invasive method of administration of favourable factors into the lesioned region of the brain.

Huntington's disease

Currently, use of bone marrow cells in the treatment of Huntington disease, a congenital neurodegenerative disease, is being studied. In the initial phase, progressive cell death of GABA-ergic neurons in the striatum is observed, subsequently followed by the degeneration of neurons involving other regions of the brain, including the hypothalamus and the hippocampus. Cell transplants may become useful in the therapy of this

condition, although it is difficult to evaluate their effectiveness at present, especially as the precise mechanism of neurodegeneration remains to be uncovered. Leuscadron *et al.*⁵⁴ studied effects of transplanted bone marrow stem cells in the animal model of Huntington's disease. While only scarce cells implanted into the striatum expressed neuronal markers, a significant reduction in memory deficits was observed. This improvement might be secondary to the production – by the transplanted cells – of the factors that promote cell survival and exert neuroprotective effects on pathologically changed tissues. In contrast, replacement of destroyed cells with the pool of implanted cells may play a minor role.

Multiple sclerosis

Demyelination plaques, macroscopically recognisable on transverse sections of the brain, are the characteristic neuropathological feature of multiple sclerosis. The plaques have various colours which is related to the hyperaemia of the demyelisation foci^{55,56}.

Observation of brain slice preparations stained for myelin suggests that the process of myelin degradation may progress from inside, where there is often a blood vessel, toward the peripheral part of the plaque.

In regions of active degradation of myelin, there are numerous macrophages that participate in both degradation and repair. There are also astrocytes there participating in repair and substitution, as well as in removal of myelin degradation products⁵⁷. In pathological foci that are not active, macrophages are observed mostly perivascularly together with high density of glial fibres. Astrocytes are also less abundant and oligodendrocytes are very uncommon⁵⁷.

Treatment of multiple sclerosis is mainly symptomatic. Despite numerous studies, there is still no medication that would have a specific action because none of the currently known pharmacological therapies causes remyelination of the dam-

aged neurons. In animal experiments, similarity of multiple sclerosis and experimentally evoked EAE-chronic autoimmune encephalomyelitis is taken into account⁵⁸. Relationships between these conditions enable, based on studies in animals, construction of models of treatment of inflammatory demyelination diseases.

One of the newer approaches in the treatment of severe multiple sclerosis is transplantation of stromal cells. In a study conducted by Suzuki *et al.*⁵⁹, stromal cells were used as a multipotential source of neurons, astrocytes and oligodendrocytes. Prerequisites for use of this form of treatment are also the autoimmune aetiology of multiple sclerosis and the fact of use of bone marrow transplants in the treatment of immune system diseases. La Nasa *et al.*⁶⁰ reported a case of a 57-year-old male treated for multiple sclerosis and large granular lymphocyte (LGL) leukaemia. Therapy for LGL leukaemia, involving implantation of allogenic haematopoietic stem cells (ASCT), also improved neurological status 36 months after the transplantation. Progression of the disease was

attenuated and there was an improvement in the EDSS score (Extended Disability Status Scale)⁶⁰. Significant clinical improvement was also observed in patients with severe multiple sclerosis, who underwent autologous transplantation of haematopoietic stem cells⁶¹. In three patients, improvement in the EDSS and relative regression of lesions were observed. Additionally, transplantation produced long-term stabilisation of neurological condition during a two year follow-up in two patients and during more than one year of follow-up in the third patient. Considering the results of these clinical observations, haematopoietic stem cells may be considered one of the immunosuppressive treatment methods in multiple sclerosis.

Sasaki *et al.*⁶² studied potential of stromal cells to differentiate into Schwann cells. They experimentally produced demyelination in rats in the region of spine using X-ray irradiation preceded by administration of ethidium bromide. Currently, Schwann cells may be used in the process of regeneration of peripheral nerves to correctly direct growing fibers^{63,64}.

There is evidence that bone marrow cells transplanted into the site of lesion differentiate into astrocytes, oligodendrocytes and myelin-producing cells. Because of these properties, stromal cells may potentially become a new therapeutic tool in demyelinating diseases of the central nervous system. However, exact conditions necessary to achieve effective differentiation of stromal cells must be further investigated.

Improvement in neurological functioning as an effect of the therapy using bone marrow cells – implications for rehabilitation

The aim of preclinical and clinical studies, searching for effective methods in the treatment of diseases of the brain, is to achieve improvement in the following neurological functions: motor, sensory and cognitive. Results of the studies on potential use of bone marrow cells in the therapy of diseases of the brain indicate that these cells contribute to reducing neurological deficits and improving motor function.

Table 1

List of the most important results obtained in pre-clinical and clinical studies on the effects of bone marrow cells on healing and repair processes occurring in the brain during various pathological conditions

Disease affecting CNS	Effects of bone marrow cells transplantation observed in pre-clinical studies	Effects of bone marrow cells transplantation observed in clinical studies
Traumatic brain injury	<ul style="list-style-type: none"> – colonisation of the site of injury by bone marrow cells – differentiation of bone marrow cells into neurons and astrocytes – increased levels of neurotrophic factors in the injured tissue – improvement in neurological functions (motor and sensory) 	–
Ischaemic stroke	<ul style="list-style-type: none"> – migration of cells into the region of ischaemia – increased levels of NGF and factors from the TGF-β superfamily at the site of ischaemic injury – inhibition of apoptosis – differentiation of bone marrow cells into neurons and astrocytes – improvement in neurological functions – motor and sensory 	<ul style="list-style-type: none"> – functional improvement in patients measured according to Barthel and Rankin scales
Demyelination diseases - Multiple sclerosis	<ul style="list-style-type: none"> – differentiation of stromal cells into astrocytes, oligodendrocytes 	<ul style="list-style-type: none"> – improvement in patients' neurological status assessed using the EDSS – stable neurological status 2 years after the transplantation
Neurodegenerative diseases: Parkinson's disease	<ul style="list-style-type: none"> – improvement in motor function – increased expression of TH in the tissue – increased level of Levodopa in the tissue resulting from the presence of transduced bone marrow cells 	–
Huntington's Disease	<ul style="list-style-type: none"> – reduction of memory deficits 	–

In the experimental traumatic brain injury and cerebral infarction, the degree of recovery of the studied animals was most commonly assessed using *Neurological Severity Scale*, NSS. NSS was created based on scales used in humans, i.e. GOS (*Glasgow Outcome Scale*) and DRS (*Disability Rating Scale*). NSS includes motor tests (muscle tone, abnormal movements), sensory tests (vision, touch, proprioception) and reflexes⁶⁵. According to this scale, recovery in animals after transplantation of bone marrow cells involved an improvement in the ability to flex the extremities, to maintain balance of the body, in limb and corneal reflexes, and in responses to stimuli.

In addition to the above-mentioned parameters, in preclinical studies, spatial orientation of the animal and motor learning abilities (*Morris Water Maze Test*)³⁹, resistance to exercise and locomotion (*Rotarod Test*)^{16,17,18,48}, somatosensory impairment based on timing of stimulus localization (*Adhesive-removal Somatosensory Test*)³³, and imbalance (*Beam Balance*)²² were evaluated.

Results of the conducted studies – mostly preclinical – suggest that improvement in motor and sensory functions may also be achieved in humans.

The aim of the majority of therapeutic interventions in diseases of the brain is protection against either destruction or degeneration of the present neural structures. Current therapeutic strategies focus – in addition – on development and integration of new neuronal connections that may replace injured region or structure. The question remains, whether newly implanted cells will integrate with the present neuronal net and if so – how fast and to what extent this integration will occur.

Development of new and preservation of the existing neuronal connections and stimulation of neural structures to produce neurotransmitters are closely related to the potential improvement of impaired functions. Training conducted in animals and appropriate rehabilitation in humans may influence neurogenesis,

neovascularisation and neural reorganisation⁶⁶. There is evidence that animals kept in an improved environment (requiring higher motor activity) earlier achieve functional recovery^{67,68}. This observation suggest that transplantation of bone marrow cells as a therapy of diseases of the brain require neurophysiology-based rehabilitation, similarly to the physiotherapy in the majority of brain diseases.

Summary

Results of numerous pre-clinical studies demonstrate the ability of bone marrow cells to migrate, to colonise pathologically changed brain tissues, and to differentiate into cells morphologically similar to resident cells, which is accompanied by an improvement in neurological functions. The perspective of use of bone marrow cells in the therapy of diseases affecting the brain seems to be closer and closer, but all the related phenomena require further studies on the conditions of use of cell therapy, combination of cell therapy with effective pharmacotherapy, currently used animal models of brain diseases, methods of analysis of the material and results, as well as tests used to assess neurological functions.

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