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MESENCHYMAL STEM CELLS IN THE TREATMENT OF ISCHEMIC STROKE

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ABSTRACT

Over the past two decades, multiple preclinical studies have shown that transplantation of mesenchymal stem cells leads to a pronounced positive effect in animals with experimental stroke. Based on the promising results of preclinical studies, several clinical trials on the transplantation of mesenchymal stem cells to stroke patients have also been conducted. In this review, we present and analyze the results of completed clinical trials dedicated to the mesenchymal stem cells transplantation in patients with ischemic stroke. According to the obtained results, it can be concluded that transplantation of mesenchymal stem cells is safe and feasible from the economic and biomedical point of view. For the further implementa-tion of this promising approach into the clinical practice, randomized, placebo-controlled, multicenter clinical trials are needed with a large sample of patients and optimized cell transplantation protocols and patient inclusion criteria. In this review we also discuss possi-ble strategies to enhance the effectiveness of cell therapy with the use of mesenchymal stem cells.

Keywords: *cell therapy; mesenchymal stem cells; ischemic stroke; clinical trials.*

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INTRODUCTION

Ischemic stroke (IS) remains a crucial issue in modern medicine owing to its high morbidity, mortality, and patient disability rates [1–3]. According to the World Health Organization, IS and other acute circulatory disorders account for 11% of global mortality [4–6]. Over the last 5 years, ~430,000–470,000 cases of infectious diseases have been reported annually in Russia. The hospital mortality rate ranged from 17% to 21% [7].

Currently, the only effective IS treatments are intravenous thrombolysis and intravascular thromboextraction during the acute period to restore blood flow in cerebral arteries [8, 9]. However, these methods have limitations and contraindications, the most significant of which is the short time interval or “therapeutic window” for their application. Recent studies have shown that the therapeutic window ranges from 4.5 h for intravenous thrombolysis to 24 h for thromboextraction [10–12]. After IS, patients may experience lifelong neurological deficits, even after successful reperfusion therapy, due to the death of neurons and glial cells in the center of the brain infarction [13].

Recent analytical studies have revealed that in Russia, a high percentage of patients become permanently disabled after experiencing IS, resulting in reduced ability to work and contributing to economic cost. The state incurs an average cost of 0.9–1.2 million rubles per stroke case, and the economic damage during the first year after a stroke is equivalent to 0.3% of the country’s annual gross domestic product. This highlights the creation of a substantial socioeconomic burden for the state and thus confirms the need for new, effective methods and approaches for IS therapy in patients who miss the therapeutic window.

In recent years, there has been significant evidence supporting the potential of mesenchymal stem cell (MSC) transplantation as an IS therapy. MSCs are a subpopulation of mesenchymal stromal cells that meet the stemness criteria established by the International Society for Cellular Therapy (ISCT) [14]. The stemness criteria imply cell multipotency, i.e., the ability to

МЕЗЕНХИМАЛЬНЫЕ СТВОЛОВЫЕ КЛЕТКИ В ТЕРАПИИ ИШЕМИЧЕСКОГО ИНСУЛЬТА

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АННОТАЦИЯ

В последние два десятилетия накоплены данные о том, что трансплантация мезенхимальных стволовых клеток оказывает значимое положительное действие при экспериментальном инфаркте головного мозга у животных. Обнадёживающие результаты доклинических исследований сделали возможным проведение клинических испытаний по трансплантации мезенхимальных стволовых клеток пациентам с ишемическим инсультом. В настоящем обзоре приведены и проанализированы результаты завершённых клинических исследований, посвящённых клеточной терапии ишемического инсульта с помощью мезенхимальных стволовых клеток. На основании проведённого анализа можно заключить, что трансплантация мезенхимальных стволовых клеток является безопасной и целесообразной с патогенетической точки зрения процедурой. Для дальнейшего внедрения данной перспективной технологии терапии в клиническую практику необходимы продолжение рандомизированных плацебоконтролируемых многоцентровых клинических испытаний на большой выборке пациентов и оптимизация протоколов клеточной трансплантации и критериев включения пациентов в исследование. В настоящей работе обсуждаются также возможные стратегии для усиления клеточной терапии с использованием мезенхимальных стволовых клеток.

Ключевые слова: *клеточная терапия; мезенхимальные стволовые клетки; ишемический инсульт; клинические исследования.*

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differentiate into different mature cell types within one germinal leaflet (in the case of MSCs, mesoderm) along with the ability to actively proliferate. MSCs are commonly used in cell therapy and regenerative medicine because of their immunomodulatory, anti-inflammatory, angiogenesis-stimulating, and antiapoptotic effects [15, 16]. In addition to directly secreting cytokines and other regulatory molecules into the extracellular space, MSCs produce extracellular vesicles that can be taken up by target cells, facilitating the intracellular delivery of biologically active molecules. Exosomes are the most extensively studied MSC extracellular vesicles. They are 30–100 nm in size and can transport a diverse range of regulatory microRNAs and growth factors.

Phenotypically, MSCs express certain cell surface markers from the differentiation cluster (CD). The ISCT Mesenchymal and Tissue Stem Cell Committee proposed the minimum criteria for defining MSCs as cells that express CD105, CD73, and CD90 but do not express CD45, CD34, CD14, CD11b, CD79α, CD19, or HLA-DR. As adherent and actively proliferating cells, they can form a dense cell monolayer when cultured on adhesive polymer coatings under standard conditions. Additionally, under special conditions in vitro, MSCs can differentiate into adipocytes, chondroblasts, and osteoblasts [17] and into various cell types, such as pericytes, hepatocytes [18], cardiomyocytes [19], and other mesodermal cells. However, the scientific community currently questions

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the ability of MSCs to differentiate into neurons without additional induction [20].

MSCs can be isolated from various organs and tissues, including the bone marrow, placenta, umbilical cord, amniotic fluid, adipose tissue, skin, dental pulp, and stroma of parenchymal organs [21–26]. Importantly, MSCs obtained from different sources or donors may exhibit significant differences in gene expression patterns, resulting in varying regenerative potential [27]. MSCs have an immunomodulatory effect and are considered relatively immunologically inert because of their low HLA expression. This means that the risk of pathological immunosensitization during allogeneic MSC transplantation is low although it cannot be excluded [28].

The advantages of MSCs include their ease to procure from primary sources, such as the placenta, which is classified as biowaste, and the low cost of obtaining primary cultures and scaling up cell production for clinical use. Furthermore, their safety has been confirmed in preclinical and clinical studies. Throughout the period of MSC research, no cases of tumor formation or oncogenic transformation resulting from transplanted MSCs have been reported. Therefore, the oncological safety of these cells is not debatable [29, 30].

Numerous preclinical studies (PCSs) have found that transplantation of MSCs in animals with experimental brain infarction can have a significant positive therapeutic effect [31–33]. Studies have demonstrated that systemic (intravenous or intra-arterial) MSC transplantation and local intracerebral injection improve the survival rate of laboratory animals, reduce the severity of neurological deficits, and, in some cases, decrease the size of the brain infarction zone [34–40]. According to PCS data, the most favorable MSC transplantation method in IS, particularly in the acute and subacute phases, is systemic intra-arterial administration. This method allows MSCs to enter the brain microcirculatory bed first and potentially have the greatest systemic effect within the central nervous system [34, 41]. However, the optimal method of MSC transplantation remains unclear. Intravenous administration is the least invasive method of systemic administration. However, most cells are retained in parenchymal organs, primarily the lungs, liver, and spleen. This retention reduces the efficiency of MSC delivery to the brain, resulting in lower functional recovery compared with intra-arterial administration [42]. Intra-arterial transplantation has shown the highest therapeutic efficacy by delivering MSCs directly to cerebral arteries, bypassing peripheral organs [43]. To avoid embolic complications during intra-arterial injection, an appropriate dose and rate of MSC administration should be selected [44]. In recent years, endovascular surgery techniques have undergone significant development, making intra-arterial access more readily available for routine clinical use.

The ideal timing for transplantation of MSCs and other types of stem cells in stroke patients remains unknown. However, unlike reperfusion therapy, the therapeutic window for cell therapy in IS is longer. The therapeutic effects of cell transplantation from several hours to several months after the onset of acute cerebral circulatory failure have been reported [40]. In comparing the therapeutic effects, the experimental animals showed the best functional recovery and faster reduction of the brain infarction center volume within 24–48 h from the onset of neurological symptoms [45, 46]. The mechanism of action of MSCs is associated with their anti-inflammatory and neuroprotective effects in brain ischemia, neuroinflammation, and blood–brain barrier damage. This association determines the maximum efficacy of MSC therapy within the first 48 h after IS [47]. The unique immunological properties of MSCs enable their allogeneic transplantation, which is significant from a socioeconomic perspective. This allows for the mass production of placental MSCs and their use during the acute and acute phases of IS when autologous cells are unobtainable [46, 48, 49].

The properties of MSCs described above and the encouraging results of PCSs have made it possible to conduct the first clinical trials (CTs) in foreign countries to study the effect of MSC transplantation on the course and outcomes of IS in humans.

The present study aimed to summarize the clinical trial experience of MSC transplantation in CTs, present the modern concept of MSC mechanism of action based on evidence-based medicine, and outline the methods of developing cell therapy for IS.

CLINICAL TRIAL RESULTS

Currently, the scientific literature presents the results of 14 CTs. These trials were conducted in foreign countries and evaluated the safety and efficacy of transplantation of MSCs obtained from different sources. Patients were transplanted with both autologous and allogeneic MSCs and genetically modified MSCs. In all studies, patients with IS were treated with standard therapy according to the clinical recommendations accepted in the country, in addition to cell therapy. Table 1 presents the results of the conducted studies [50–63].

Table 1

Results of major clinical trials on cell therapy for ischemic stroke

Study	Phase	Total patients/ MSC transplantation, *n*	MSC type/dose	Administration route	Follow-up period	Efficacy/adverse events, if identified
Bang et al. 2005 [50]	I/II	30/5	Autologous (bone marrow)/ two doses of 50 million cells	Intravenous	1 year	NIHSS improvement
Lee et al. [51]	I/II	52/16	Autologous (bone marrow)	Intravenous transplantation	5 years	Improved functional recovery, reduced mortality
Honmou et al. [52]	I	12	Autologous MSCs (bone marrow)/0.6 to 1.6×10^8 cells	Intravenous transplantation	1 year	Trend towards reduction in neurological deficits, significant reduction in size of infarct focus
Bhasin et al. [53]	I/II	12/6	Autologous MSCs (bone marrow) / $50–60\times10^6$ cells	Intravenous transplantation	6 months	Trend towards reduction in neurological deficits
Bhasin et al. [54]	I/II	40/ MSC 6/ 14 mononuclear SCs	Autologous MSCs (bone marrow)/ 50–60 million cells	Intravenous transplantation	6 months	Statistically significant improvement of mBI in the MSC therapy group
Fang et al. [55]	I/IIa	18/6/6 EPCs	Autologous MSCs (bone marrow)/ 2.5×10^6 cells + 2.5×10^6 cells/kg after 1 week	Intravenous transplantation	4 years	No statistically significant differences between groups
Hess et al. [56]	II	129/67	Allogeneic MSCs (bone marrow)/ 400 mln cells/kg or 1200 mln cells/kg	Intravenous transplantation	3 years	Positive therapeutic effect observed with early MSC transplantation within 12–36 hours
Levy et al. [57]	I/II	36	Allogeneic MSCs (bone marrow)/ 1.5 million cells/kg	Intravenous transplantation	12 months	Significant increase in mBI after 6 months and 12 months
Steinberg et al. [58]	I/IIa	18	MSCs (SB623 bone marrow line)/ 2.5×10^6, 5.0×10^6 or 10×10^6 cells	Intrathecal stereotactic	2 years	Meaningful functional improvement (ESS, NIHSS, F-M) from month 1

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Table 1

Continued

Study	Phase	Total patients/ MSC transplantation, *n*	MSC type/dose	Administration route	Follow-up period	Efficacy/adverse events, if identified
Qiao et al. [59]	I	8	Allogeneic MSCs (umbilical cord)/ 0.5×10^6 cells/kg intravenously or 5×10^6 cells of MSCs + 6×10^6 cells of NSCs/NPCs intrathecally	Intrathecal MSC transplantation by 4 injections or co-transplantation of 1 dose of MSC intravenously and MSC + NSC intrathecally	2 years	Functional improvement (NIHSS, mBI, and mRS) more pronounced after co-transplantation
Jaillard et al. [60]	I/II	44/31	Autologous MSCs (bone marrow)/ 1×10^6 cells/kg	Intravenous transplantation	90 days	Significant improvement in F-M scale and restoration of interhemispheric and ipsilateral conduction pathways
Jaillard и др. [61]	II	31/16	Autologous MSCs (bone marrow)/ 1×10^8 cells or 3×10^8 cells	Intravenous transplantation	2 years	Significant improvement in motor function
De Celis-Ruiz et al. [62]	IIb	30/15	Allogeneic MSCs (adipose tissue)/ 10^6 cells/kg	Intravenous transplantation	2 years	Improvement trend in NIHSS score
Bang et al. [63]	II	54/39	Autologous MSCs (bone marrow)/ 1×10^6 cells/kg	Intravenous transplantation	90 days	Significant increase (~5-fold) in blood levels of extracellular vesicles and microRNAs associated with neurogenesis/ neuroplasticity 24 hours after transplantation, correlating with recovery of motor function and pathway

Note: MSCs — mesenchymal stem cells; EPCs — endothelial progenitor cells; SC — stem cells; NIHSS — National Institutes of Health Stroke Scale; mBI — modified Barthel Index; mRS — modified Rankin scale; ESS — European Stroke Scale; F-M — Fugl-Meyer scale; NSCs/NPCs — neural stem/progenitor cells.

Transplantation of autologous MSCs

In 2005, Bang et al. have conducted one of the first CTs on cell therapy for IS using MSC transplantation. The study involved intravenous transplantation of autologous bone marrow MSCs at a dose of 100 million cells (using cell culture media containing fetal bovine serum) in five individuals within 5–7 weeks from the onset of the disease. No serious side effects were found, and the transplantation was deemed safe. Furthermore, patients who received cell therapy demonstrated persistent clinical improvement and a reduction in neurological deficit severity at 3, 6, and 12 months post-transplantation compared with 25 controls.

Following the success of their initial study, Bang et al. have performed a larger blinded phase II study with 52 patients [51]. The study involved intravenous MSC transplantation in two stages: an initial dose of 50 million cells followed by an additional 50 million cells 2 weeks later. Furthermore, the study confirmed the safety of intravenous MSC transplantation and reported that using bovine serum to culture MSCs did not result in zoonosis or any other adverse effects. The cell therapy group showed higher functional recovery and lower mortality than the control group.

Owing to concerns within the scientific community regarding prion disease transmission through animal-derived media and sera used for culturing MSCs, Honmou et al. have conducted a study in which MSCs were cultured in autologous serum [52]. They performed intravenous transplantation of bone marrow-derived MSCs in patients 36–133 days after IS. The study confirmed the safety and feasibility of culturing autologous bone marrow MSCs using autologous human serum. No significant adverse events were detected. In the patients who received cell therapy, a decrease in neurological deficits and significant decrease in the size of the brain infarction focus were observed 1 week after intravenous transplantation.

In 2011, Bhasin et al. transplanted autologous bone marrow MSCs into patients with IS for the first time. MSCs were cultured on serum-free medium. In the phase I/II study, MSCs were administered intravenously to 12 patients with IS in the early and late recovery period (from 3 months to 1 year from disease onset). The data were compared with those of the control group (6 patients). In the group that received MSC cell therapy, the severity of neurological deficits decreased; however, these changes were not statistically significant when compared with those in the control group. In 2013, Bhasin et al. initiated another clinical trial [54] to compare the efficacy of MSCs with that of hematopoietic/mononuclear cells. The results confirmed the safety and feasibility of cell therapy using MSCs. No significant differences were found between the controls and patients who showed improved neurological deficit.

In 2019, Fang et al. [55] showed results of a clinical randomized placebo-controlled phase I/IIa study. The study involved the intravenous transplantation of autologous bone marrow MSCs to patients with IS. MSCs were cultured using fetal bovine serum. The efficacy of the treatment was evaluated 1 week after the onset of the disease. First, bone marrow was collected to isolate and culture autologous MSCs. These cells were then administered intravenously in two stages: the first dose was 2.5 million cells per kilogram of body weight, given after 4 weeks, followed by a second dose of the same amount after 1 week. The study compared therapeutic efficacy and safety between two groups: one group (6 people) received MSC transplantation and the other group (6 people) received a placebo that was intravenously injected with autologous endothelial progenitor cells. The follow-up period was 4 years, and the study was considered safe. No significant differences were found between the two groups regarding survival and degree of functional recovery. One possible reason for the insufficient therapeutic effect may be the introduction of MSCs during the subacute period of IS. To solve this problem, allogeneic stem cells should be transplanted in patients during the acute phase of IS.

Transplantation of allogeneic native MSCs

MSCs can be easily multiplied in culture and weakly express antigens of the major histocompatibility complex HLA-ABC, making their allogeneic transplantation possible [64, 65, 66]. This type of transplantation has advantages for the treatment of neurological diseases. First, it significantly reduces costs and simplifies production, allowing for the creation of a standardized bank of MSCs that can be prepared in advance and used in the acute period of IS as needed. Second, the age of the donors is a crucial factor to consider. Epidemiological studies have indicated that over 75% of all cases of IS occur in individuals aged >65 years [67]. Obtaining MSCs from bone marrow in this age group is challenging, and the regenerative potential of such MSCs is significantly lower than that of MSCs obtained from young donors. This difference is due to natural aging processes [68].

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The largest randomized double-blind placebo-controlled phase II MASTERS trial to date, which studied the effect of allogeneic MSC transplantation in IS, was conducted across 33 medical centers in the USA and the UK [56]. This study investigated the safety and efficacy of the MultiStem cell product, which consists of allogeneic bone marrow MSCs obtained from adult donors [69]. Patients were administered MSCs intravenously at a dose of either 400 million or 1.2 billion cells 24–48 h after disease onset. The safety of this technology was confirmed when both doses of MSCs were administered. However, the primary endpoint of achieving the expected degree of improvement in the functional status of patients 90 days after IS was not met when comparing the cell therapy group with the placebo group. A retrospective analysis of the results obtained in some patients with functional recovery still showed statistically significant improvement. The researchers used this data to initiate the next phase of the clinical trial, which is a prospective randomized placebo-controlled double-blind phase III study (MASTERS-2). The study began between 18 and 36 h after the onset of neurological deficit and is currently ongoing. The results have not yet been published.

In a phase I/II study, Levy et al. [57] have evaluated the safety and efficacy of allogeneic bone marrow MSCs obtained from a single healthy donor. MSCs were transplanted intravenously at a dose of up to 1.5 million cells per kilogram of body weight to 38 patients in the late recovery period of IS (>6 months from disease onset). The study revealed the safety of MSC infusion, and all patients demonstrated significant functional recovery according to the Barthel scale. However, this study did not include a control group.

Transplantation of modified allogeneic MSCs

In phase I/IIa, Dr. Steinberg and a team of scientists studied the safety and efficacy of transplanting a modified line of allogeneic bone marrow MSCs, known as SB623, in patients with IS. SB623 is a modified line of bone marrow-derived MSCs. These cells were transiently transfected with a plasmid containing the intracellular domain of Notch1, resulting in Notch1 overexpression. Notch1 plasmid does not replicate during mitosis and is rapidly lost during cell division. In PCSs, Notch1-modified MSCs were found to have a neurotrophic effect, improve survival and maintain neuronal viability in cerebral ischemia, improve neoangiogenesis, and have an anti-inflammatory effect [70–73]. In experimental IS models, SB623 MSCs demonstrated both functional recovery and neuroprotective effects on neurons in the peri-infarct area. However, the transplanted cells did not have a direct substitutive effect and were not preserved in the recipient's body for an extended period [74]. In CTs, the SB623 cell line was stereotactically injected into the brains of 18 patients during the late recovery period of IS (6 months to 3 years from disease onset) at doses of 2.5, 5, or 10 million cells. Each patient received 20 stereotactic injections in the brain infarction areas. The authors attributed adverse events, such as headache, nausea, and vomiting, to neurosurgery rather than the effects of the transplanted cells. No graft rejection reactions were observed. The study demonstrated significant functional recovery in patients at 3, 6, and 12 months, with sustained clinical improvement 2 years after transplantation [75]. A larger randomized phase IIb clinical trial has been initiated because of the promising results; however, its findings have not yet been published.

Combined transplantation of MSCs and other stem/progenitor cells

In the context of PCSs, MSCs secrete various paracrine factors and exhibit trophic, neuroprotective, anti-inflammatory, and immunomodulatory effects [46, 76]. It is hypothesized that joint transplantation of MSCs with other types of stem/progenitor cells can improve graft engraftment and enhance the therapeutic effects of cell therapy. Animal studies using models of experimental brain infarction have confirmed that combined transplantation of MSCs with neural [77] or endothelial progenitor cells [78] can have a more pronounced effect than monotherapy with only one type of stem/progenitor cells [76]. This may be due to the synergy between the therapeutic effects of the two different stem cell types. Several CTs have been conducted on the combined transplantation of MSCs and other types of stem cells on patients with IS based on encouraging preclinical data.

In a pilot clinical study, Chen et al. [79] transplanted allogeneic MSCs obtained from umbilical cord blood, along with various fetal cells of neural origin (neural progenitor cells, cells of olfactory lining, Schwann cells isolated from sciatic nerve), in 10 patients with ischemic or hemorrhagic stroke in the late recovery period (from 6 months to 20 years). MSCs were transplanted intravenously, whereas the other cell types were injected intracerebrally or intrathecally. The study results demonstrated an improvement in the clinical condition of patients without significant adverse

reactions. However, the sample size in each group was negligible.

Qiao et al. [59] have assessed the safety and feasibility of transplanting human fetal neural progenitor cells and allogeneic MSCs isolated from umbilical cord blood in 8 patients with IS at different stages of the disease (ranging from 1 week to 2 years after onset). The first group of patients received four intravenous injections of MSCs at 500,000 cells per kilogram of body weight. The second group received a single intravenous infusion followed by three consecutive injections of MSCs and neural progenitor cells into the brain cisterns. The combined transplantation of MSCs and NPCs was deemed feasible and safe. Each treated patient showed clinical improvement, which was sustained for 2 years after transplantation. No oncogenic transformation of organs was observed during this period.

To confirm these findings, further randomized placebo-controlled multicenter CTs on a larger sample of patients with IS are recommended.

Mechanisms of MSC action

Currently, numerous preclinical studies have investigated the efficacy and mechanisms of MSCs in experimental IS [80]. The following presents generalized data on the therapeutic mechanism of MSCs from an evidence-based medicine perspective.

Although MSCs are multipotent stem cells capable of differentiating into various mesodermal cells, some scientists revealed that MSCs have low or no potential for transdifferentiation in the ectodermal direction, specifically into neural or glial cells in the brain infarction area. Therefore, a direct substitutive mechanism of action is unlikely. Single cases have been described in which, after intracerebral injection, some of the transplanted MSCs lost expression of specific markers and acquired a neuron-like phenotype [81]. Simultaneously, a significant amount of data indicated that the therapeutic potential of MSCs is linked to their paracrine action. This refers to their ability to secrete anti-inflammatory cytokines, growth factors, and exosomes loaded with biologically active substances such as microRNAs, cytokines, and growth factors [82]. Furthermore, several studies have shown that MSCs can fuse with other cells, which may be a mechanism of tissue regeneration [83]. Transplanted MSCs have a direct neuroprotective effect by transferring mitochondrial and cytoplasmic components to the recipient nerve and glial cells [84]. Several studies have reported enhancement of endogenous neurogenesis in recipient animals after MSC transplantation [85–87].

Currently, the therapeutic effect of MSCs is primarily due to their paracrine effect, rather than their ability to differentiate into other cell types [88, 89]. MSCs secrete factors that influence tissue regeneration, reduce inflammation, and promote angiogenesis. These factors include cytokines that modulate the immune response, such as interleukin (IL)-6, and regulatory molecules, including vascular endothelial growth factor (VEGF), IGF-1, IGF-2, and PDGF-AA. These molecules were detected in the conditioned medium in which MSCs were cultured. Moreover, a transcriptome analysis showed that human MSCs differ from fibroblasts, osteoblasts, chondrocytes, and adipocytes, among other types of differentiated cells, because of increased expression of certain molecules. Specifically, the levels of brain-derived neurotrophic factor (BDNF) are approximately 20-fold higher, IL-6 is 60-fold higher, and VEGF is 20-fold higher [90]. Experimental evidence confirms the neuroprotective role of BDNF secreted by human MSCs [91]. Furthermore, MSC transplantation can stimulate recipient cells to produce VEGF and basic fibroblast growth factor, as demonstrated in a rat model of brain ischemia [92]. IL-6 plays a pro-inflammatory role [93] and a crucial role in regeneration and stemness. It helps maintain the “stem” phenotype of MSCs [94] and can activate axon regeneration in mature retinal ganglion cells under certain conditions [95].

Currently, researchers are attempting to determine the molecular composition of biologically active substances in exosomes secreted by MSCs. The exosomes of MSCs from adipose tissue contain 591 proteins, which are mainly involved in signal transduction (GO:0007165), cell adhesion (GO:0007155), positive regulation of proliferation (GO:0008284), and immune response (GO:0006955), according to Gene Ontology [96]. Exosomes contain 489 microRNAs from various families, including the mir-515 and mir-10 families. A significant portion of microRNAs in MSC-secreted exosomes are believed to exist as microRNA precursors [79]. Bioinformatics indicates that some of these precursors are involved in regulating inflammatory processes, such as microRNAs hsa-let-7g-5p, hsa-miR-16-5p, and hsa-miR-92a-3p, or in negatively regulating macrophage activation, such as hsa-miR-124-3p [97]. Furthermore, microRNA targets of secreted MSCs in exosomes include the Wnt signaling pathway, profibrotic signaling cascades such as TGF-β (transforming growth factor-β) and PDGF (platelet-derived growth factor), and signaling pathways that regulate cell proliferation and apoptosis [98].

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The immunomodulatory effect of MSCs is based on their ability to influence the polarization of macrophages in the area of injury. This polarization can lead to the M2 phenotype, which is anti-inflammatory, in response to cytokines such as IL-4, IL-10, and IL-13. Polarization plays a crucial role in central nervous system damage. Macrophages, depending on their phenotype, can either contribute to secondary tissue damage or aid in its repair [99]. This process is influenced by the activation of pro-inflammatory M1 or anti-inflammatory M2 macrophages. Research has demonstrated that MSCs can influence the polarization of macrophages toward M2 through regulatory molecule secretion [100]. Remarkably, this polarization is regulated by both cytokines and nucleic acids found in exosomes secreted by MSCs. For instance, a long noncoding RNA called LncGm37494 possesses such a function. It can promote macrophage polarization in the M2 direction by inhibiting miR-130b-3p microRNA and stimulating PPARγ expression [101]. MSCs can influence the balance of T-cell subpopulations, particularly Treg and Th17, as demonstrated *in vitro* [102]. This effect can lead to the repair of damaged tissue. MSCs also affect Breg cells although the mechanism of this effect is poorly understood [103]. Both Treg and Breg cells are key suppressors of inflammation and autoimmune reactions.

MSCs can affect the state of the extracellular matrix, which is a crucial component of the cellular microenvironment that regulates cell differentiation, migration, and tissue repair. MSCs produce and deposit fibronectin, a component of the extracellular matrix, which can promote tissue regeneration, as demonstrated in a model of spinal cord injury [104]. Additionally, MSCs secrete proangiogenic factors, which have been extensively covered in recent publications. Factors secreted by MSCs include growth factors, such as EGF, FGF-2, ANGPT1, ANG, PDGF, TGF-α, TGF-β, and VEGF [105], and regulatory nucleic acids that affect angiogenesis. Among these nucleic acids are proangiogenic microRNAs, including miR-30b [106].

Strategies for developing cell therapy using MSCs

None of the CTs conducted to date in patients with IS after MSC transplantation have revealed serious adverse events. All studies have noted a tendency toward improvement in patients' condition and a reduction in the severity of neurological deficits. In studies involving humans, where patients were randomized and a control group was included, not all patients demonstrated a statistically significant positive effect of MSC cell therapy compared with the controls. Possible reasons for insufficiently expressed clinical effects include suboptimal parameters of cell transplantation and criteria for patient inclusion in trials, such as a limited therapeutic window and variation of several months regarding MSC infusion. Moreover, therapy is often performed in the late recovery period of IS, and the comparison group may include patients with a large age difference (from childhood/youth to old age) or those who receive autologous MSCs from elderly donors. The choice of transplantation method and the frequency of MSC administration should be considered. In most studies, MSCs were administered intravenously once or twice during the therapy period. Recent preclinical studies have demonstrated the high efficiency of the intra-arterial method of stem cell administration. This method allows for targeted delivery of transplanted cells to cerebral vessels, bypassing parenchymal organs [44]. Selecting the most effective therapeutic window and transplantation method is dependent on the presumed mechanisms of stem cell action, which require further study.

To further develop cell therapy for IS, one strategy is to modify and unify protocols for MSC use for further optimization. Currently, randomized placebo-controlled multicenter CTs with modified protocols of cell transplantation are underway in several countries. The results of these trials will be available within the next few years [107].

In addition to continuing CTs, an essential strategy for developing cell therapy using MSCs is to continue translational basic research on experimental brain infarction models in laboratory animals. This study aimed to clarify the mechanisms of action of transplanted stem cells and optimize transplantation protocols for their subsequent introduction into clinical practice. When conducting translational studies, evaluating the efficacy of MSC therapy in animals of different genders and ages and in those with concomitant pathologies such as diabetes mellitus and arterial hypertension is advisable [108, 109]. Additionally, investigating the mechanisms of MSC therapeutic action and ways to enhance it is critical. For example, combining MSC transplantation with neuronal progenitor cells obtained through modern methods that do not involve the use of human embryo or fetal tissues [34, 110] may be effective.

To assess the efficacy of MSC cell therapy in preclinical studies, objective quantitative methods

of therapy evaluation, in particular the degree of neurological deficit severity, such as kinematics of movements of the paralyzed limb and assessment of the volume of the brain infarction focus, should be used. Wider implementation of new methods at the preclinical level will allow selecting the best ways to objectify the results of cell therapy evaluation and use them in the design of CTs. Evaluation of the brain infarction center volume is a critical objective quantitative parameter for assessing the quality of cell therapy. Morphometric analysis of brain infarction can be performed in experimental studies through histological examination [111, 112] and/or magnetic resonance imaging (MRI) [111, 112]. In preclinical studies on experimental models of cerebral ischemia, MRI allows for the assessment of changes in the volume of the brain infarction without the need to remove animals from the experiment for histologic analysis at each time point. When transferred to computer systems, MRI enables objective assessment of the dynamics of the pathological process in the brain before and after treatment throughout the patient's lifetime [113–115]. For quantitative assessment of MRI data, a promising method is fully automatic parametric image analysis [116] or convolutional neural networks [117]. These methods minimize the subjective contribution of the operator when assessing the data. However, to increase the objectivity of the assessment, the process of morphometric analysis of the brain infarction focus should be standardized for optimal use upon the patient's hospital admission. One of the methods is the segmentation method [118] by selecting the region of interest on a series of images. This approach allows avoiding errors due to the subjectivity of focal volume assessment by an operator or several operators without averaging their assessment.

The development and testing of methods for automatic objective assessment of cell therapy efficacy in cerebral infarction in PCSs and CTs can create a solid foundation for qualitative assessment of the results obtained.

CONCLUSIONS

Based on the analysis of the conducted CTs of the safety and efficacy of cell therapy for IS, it can be concluded that MSC transplantation is a safe and effective procedure from a pathogenetic perspective.

Continuing research in this direction, including the initiation of the first CTs in Russia, is recommended. To introduce IS therapy into clinical practice, CTs on a large sample of patients with randomization and adequate selection of a control group should be conducted. This should include criteria modification for patient inclusion in the study and protocols of MSC transplantation corresponding to a high degree of evidence. Further fundamental research on the mechanisms of cell therapy action and the selection of the optimal time window, methods, and frequency of stem cell administration is warranted.

ADDITIONAL INFORMATION

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