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REVIEW ARTICLE

The Role of Stem Cells in the Therapy of Stroke

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Abstract: ***Background*:** Stroke is a major challenge in neurology due to its multifactorial genesis and irreversible consequences. Processes of endogenous post-stroke neurogenesis, although insufficient, may indicate possible direction of future therapy. Multiple research considers stem-cell-based approaches in order to maximize neuroregeneration and minimize post-stroke deficits.

***Objective*:** Aim of this study is to review current literature considering post-stroke stem-cell-based therapy and possibilities of inducing neuroregeneration after brain vascular damage.

***Methods*:** Papers included in this article were obtained from PubMed and MEDLINE databases. The following medical subject headings (MeSH) were used: "stem cell therapy", "post-stroke neurogenesis", "stem-cells stroke", "stroke neurogenesis", "stroke stem cells", "stroke", "cell therapy", "neuroregeneration", "neurogenesis", "stem-cell human", "cell therapy in human". Ultimate inclusion was made after manual review of the obtained reference list.

***Results*:** Attempts of stimulating neuroregeneration after stroke found in current literature include supporting endogenous neurogenesis, different routes of exogenous stem cells supplying and extracellular vesicles used as a method of particle transport.

***Conclusion*:** Although further research in this field is required, post stroke brain recovery supported by exogenous stem cells seems to be promising future therapy revolutionizing modern neurology.

Keywords: Cell therapy, neurogenesis, stroke, stem-cells, extracellular vesicles, central nervous system.

1. INTRODUCTION

Stroke is one of the most common causes of disability and death worldwide. It remains one of the major challenges in neurology because of irreversible destruction of brain tissue due to vascular dysfunction. Stroke is a multifactorial disorder with plenty of well-known risk factors; many of them are modifiable. Despite multiple studies concerning pathogenesis, prevention and therapy, current methods of ischemic stroke treatment are limited by a narrow timeframe from onset to initial treatment and a lack of regenerative effects. Treatment following stroke is based on pharmacological therapies, mainly focused on secondary prevention, and prompt rehabilitation. Compared to therapies for progressive neurological diseases, stroke treatment has different requirements. An optimal therapy must take into account sudden onset, damage to neurovascular tissue, focal loss of various types of tissues, and the need to regenerate a variety of functionally specialized cells across various size lesions in diverse segments of the brain. There is a need to develop new approaches with greater accessibility and a wider therapeutic window to provide both neuroprotective and regenerative benefits. Stem-cell-based therapy seems as one such promising stroke therapy.

Cell death consequent to stroke is accompanied by excitotoxicity, mitochondrial dysfunction, abnormal protein folding, oxidative stress, and inflammatory reactions. In stroke, oligodendrocytes and damaged neurons cause a change in the chemical composition of the extracellular environment, which serves as a chemotactic stimulus for microglia and astrocytes. Secondarily, after ischemia and cell necrosis, an inflammatory reaction develops associated with microglia

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activation, tissue infiltration by neutrophils and macrophages from the blood, and damage to the Blood-Brain Barrier (BBB). To a large extent, the process is a consequence of toxic inflammatory mediators, including interleukin-1β (IL-1β), interleukin 6 (IL-6), and Tumor Necrosis Factor α (TNF-α) [1].

Stroke recovery usually is associated with some degree of endogenous recovery. Endogenous Stem Cells (SC), glial cells, even endothelial cells, and pericytes are involved in repair processes. Stroke has been associated with a significant change to the profiles of long non-coding RNA (lncRNA) and lncRNA-mRNA co-expression networks in Neural Stem Cells (NSC) [2].

There are two major pathways for endogenous neurogenesis in the adult mammal brain [3-6]. The first includes neuroprogenitor cells located in the subventricular zone (SVZ), from which precursors of nerve cells are formed, migrating along the rostral stream to the olfactory bulb. The second pathway of neurogenesis involves cells of the hippocampal subgranular zone (SGZ), which differentiate into the granular layer and then integrate with the CA3 field in the dentate gyrus. In the dentate gyrus, transcriptionally active cells are found including glial fibrillary acidic protein positive NSC, three types of which were differentiated in the SVZ [7].

Presence of poststroke active cell proliferation in ipsilateral subventricular zone was proven unambiguously in humans [8]. Spontaneous, transient increase in the number of precursor cells begins on day 2 after stroke and reaches a maximum in about 2 weeks, decreasing over the next 3-5 weeks and returning to baseline at about 6 weeks [9, 10]. However, some authors report that slightly higher mitotic activity in SVZ persists over the next year [11]. Experimental studies have shown that in ischemic stroke, endogenous NSC respond with proliferation and migration towards the lesion [4, 12, 13]. After ischemia, neuroblasts outside SVZ can migrate at a relatively high speed of 17.98±0.57 μm/h [10]. As compared with resting state, the production of neuroblasts after stroke is increased significantly and can even result in a 31-fold increase in the number of newborn neurons in the striatum ipsilateral to the damage [14]. Regulation of neurogenesis is provided by a network of neurotrophic signals that can act in an autocrine or paracrine manner. It can, *inter alia*, be secreted by endothelium and pericytes [15]. Migration is thought to be caused by stimuli sent from the ischemic zone in two ways: by changes in the composition of the cerebrospinal fluid or by diffusion of signals through the blood vessels [9]. The exact mechanism has not been established, but it is likely these processes involve Retinoic Acid (RA), Sonic Hedgehog (SHH), Bone Morphogenic Protein (BMP), Nerve Growth Factor (NGF), Brain Derived Neurotrophic Factor (BDNF), Glial cell line-Derived Neurotrophic Factor (GDNF), Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), Epidermal Growth Factor (EGF), erythropoietin (EPO), Transforming Growth Factor (TGF)-α, and granulocyte-colony stimulating factor [4, 11, 16]. As an example, EPO activates endothelial cells after ischemia that in turn promote migration of endogenous neuroblasts [11]. Up-regulation of hypoxia-inducible factor-1 alpha (HIF-1α) gene can promote the proliferation, migration and differentiation of endogenous NSC [17]. Moreover, the redirection of neuroblasts process involves stromal cell-derived factor-1 (SDF-1α) and its CSCR4 receptor, monocyte chemoattractant protein-1 (MCP-1), and metalloprotease matrix (MMP)-9 released by neuroblasts themselves [9, 18].

Stroke causes not only long-term changes in NSC but changes in Ventricular-Subventricular Zone (V/SVZ) neurogenic niche vascular architecture [12]. Stroke increases V/SVZ endothelial cell proliferation from 2% in non-ischemic mice to 12% and 15% after 7 and 14 days after ischemia, respectively. In contrast, the volume of vessels in V/SVZ increased from 3% of the total volume before stroke to 6% at 90 days after stroke. Brain pericytes contribute to the formation of new neurons in response to ischemia [19]. In contrast, VEGF secreted by endothelial cells and pericytes is one of the most important neurotrophic factors that stimulate cell proliferation in SVZ and facilitate the migration of immature neurons towards the lesion.

In stroke, peri-infarction astrocytes undergo reactive astrogliosis and are involved in modulating adaptive responses in neurons as well as affecting neurological regeneration [20]. Astrocyte subpopulations located a few hundred μm from the lesion proliferate and participate in the development of glial scars. The cells within the given distance of the infarction are involved in controlling the extracellular environment and release proteins and other molecules that can promote neuronal plasticity and improve neurological function. These compounds include *inter alia*, BDNF, GDNF and NGF [21, 22].

Experimental studies have shown that after stroke, astrocytes can be a source of new neurons [14, 23]. In rats, the striatal astrocytes differentiated into immature neurons after 1 week and into mature neurons 2 weeks after Middle Cerebral Artery (MCA) occlusion [24]. After 13 weeks, they formed synapses with other neurons and were able to trigger action potentials and receive synaptic signals.

Animal studies demonstrated that subpopulation of astrocytes originating from SVZ acts as neuroblasts progenitor in mammalian brain. Newborn neurons migrate to the olfactory bulb to incorporate into existing circuits [25]. Astrocyte neuronal precursor cells can be also found in SGZ of hippocampus [25]. Hippocampal neurons generated in SGZ are located in granule layer of dentate gyrus [26]. Microglia play a key role in neurogenesis [14]. They are a source of neurotrophic factors, including Insulin-like Growth Factor 1 (IGF-1), which is essential for the survival and proliferation of new neurons. In response to stroke, the microglia are polarized to the pro-inflammatory phenotype M1 or anti-inflammatory phenotype M2 [25, 27]. Activated microglia M1 releases proinflammatory cytokines, such as IL-6, IL-1β, interleukin 23 (IL-23), interleukin 12 (IL-12), TNF-α, interferon gamma (IFN-γ), *etc*., and Reactive Oxygen Species (ROS). These M1 molecules can cause secondary brain damage by inducing neuronal death, reducing the number of synapses, damaging the BBB, and inhibiting neurogenesis.

In contrast, M2 microglia promote brain repair. Perhaps under ischemia/hypoxia, Peroxisome Proliferator-Activated

Receptor γ (PPARγ), a transcription factor with anti-inflammatory properties, is activated and mobilized from the nucleus to the cytoplasm of microglia cells [28]. This in turn leads to the activation of M2 microglia, which release anti-inflammatory cytokines such as bFGF, BDNF, and interleukin 4 (IL-4), IL-10, chitinase-3-like protein 3 and TGF-β [14, 24]. These compounds stimulate brain repair processes.

M1 microglia markers showed an upward trend during the first 14 days after stroke, after which they decreased. Expression levels of M2 microglia markers increased from day 1 after stroke, peaked on days 5-7 and decreased to day 42 [24]. However, data obtained from animal models cannot be simply transferred to humans. For example, rodent microglia do not fully reflect human microglia [29]. Human and mouse microglia have been shown to age differently under normal and diseased conditions and many of the immune genes identified in human microglia are not expressed in rodents. The schema of the endogenous repair processes is shown in Fig. (**1**).

Although there is evidence that ischemia-induced endogenous neurogenesis promotes brain regeneration to some extent, it is not sufficient: neuroregenerative capacity is not adequate to replace damaged functional nerve tissue [11, 13]. The reason for this is perhaps the limited survival of NSC, since most newly formed neurons die in the early weeks after stroke, and only about 10% of endogenous NSC survive long enough and mature into functional cells [30, 31]. In addition, the mobilization of NSC from neurogenic niches is transient and their integration in damaged brain circuits remains incomplete. There is no adequate biological structure to enable new cells to occupy the damaged area. In addition, a large proportion of NSC differentiate into glial cells [32] and approximately 5%-10% of newborn granule cells reveal substantial morphological anomalies making them unfit [14].

Finally, there are studies indicating that acute ischemia may impair endogenous neurogenesis in the brain and may inhibit the proliferation of neural progenitor cells in SVZ [1]. In the group of patients with ischemic stroke, a statistically significant decrease in transcription active cell density in SVZ was demonstrated ($p = 0.001$) [7].

Evidence of endogenous post-stroke neurogenesis, despite its insufficiency, suggests possibility of SC based therapy enabling not only minimizing the post-stroke deficits and secondary prevention, but, hopefully, full recovery of lost functions. The aim of this study is to review current research considering SC based therapies in stroke suggesting further directions in this field of science.

2. MATERIALS AND METHODS

A literature search was performed with the use of PubMed and MEDLINE databases. Authors of the study included papers written in English with particular reference to studies published in the last 6 years (2015 – 2020), which covers about 60% of cited literature. The following medical subject headings (MeSH) were used: “stem cell therapy”, “post-stroke neurogenesis”, “stem-cells stroke”, “stroke neurogenesis”, “stroke stem cells”, “stroke”, “cell therapy”, “neuroregeneration”, “neurogenesis”, “stem-cell human”, “cell therapy in human”. Articles were included after a manual review of the obtained reference list.

3. RESULTS AND DISCUSSION

3.1. Methods of Supporting Neurogenesis after Stroke

Previous research has attempted to stimulate endogenous SC to support neurogenesis. Formulations modulating microglia activation and inflammatory lesions, neurotrophic factors, and signaling pathways (*e.g.* associated with apoptosis) have been administered [27]. Intranasal treatment with cocaine- and amphetamine-regulated transcript used from day 3 after MCA closure in rats facilitated the proliferation and migration of Neural Progenitor Cells (NPC) from SVZ, infarct resolution, and reinnervation and angiogenesis [33]. Administration of the p53 inhibitor from day 6 of experimental stroke increased the survival of endogenous NPC and improved motor function in rats [34]. Injections of the Neural Cell Adhesion Molecule (NCAM) derived peptide FG Loop (FGL) resulted in a significant increase in the mobilization of endogenous NSC in neurogenic niches [35, 36]. The use of recombinant IL-6 (rIL-6) or anti-IL-6 neutralizing antibodies (anti-IL-6 mAb) in mice showed that rIL-6 increased the proliferation and differentiation of NPC neurons in ipsilateral stroke SVZ as well as functional recovery; while anti-IL-6 mAbs produced opposite effects [37]. After stroke, lower neuronal loss, pro-angiogenic effect, and improved motor function were found in fingolimod-treated mice [38]. This drug polarized the microglia towards the salutary M2 phenotype.

One proposed method for safe and effective treatment of stroke is to use natural or synthetic biomaterials [9, 39, 40]. These materials can be carriers of bioactive molecules, can act as a protective barrier for these molecules, and can provide cells with an appropriate environment for survival, proliferation, differentiation and extracellular matrix formation. Of course, they must be delivered such that they are biologically accepted by the host and do not to create harmful immune or inflammatory responses [36-38].

The synthetic compounds most commonly used in stroke therapy include co-polymers of lactide and glycolide. They were, *inter alia*, used in the form of nanoparticles cholic acid-coated poly lactic-co-glycolic acid to carry EPO, which in this form penetrated the BBB and reduced the infarct volume and cellular apoptosis [39]. Persistent protective effects of pH sensitive polyethylene glycol-conjugated urokinase nanogels, administered beyond the usual time frame were seen in rats with MCA occlusion [40]. Nanogels released urokinase at specific pH values. Maintenance of BBB integrity and inhibition of apoptosis and excitoneurotoxicity were observed with therapy. A promising therapy is the use of a biomimetic nanocarrier comprising a platelet (PLT) membrane envelope loaded with l-arginine and γ-Fe_2O_3 magnetic nanoparticles [41]. This carrier has the natural properties of PLT. It reaches the ischemic lesion under the direction of an external magnetic field. After releasing l-arginine at the thrombus site, endothelial cells secrete NO and the blood vessels expand, which creates the possibility of restoring blood flow.

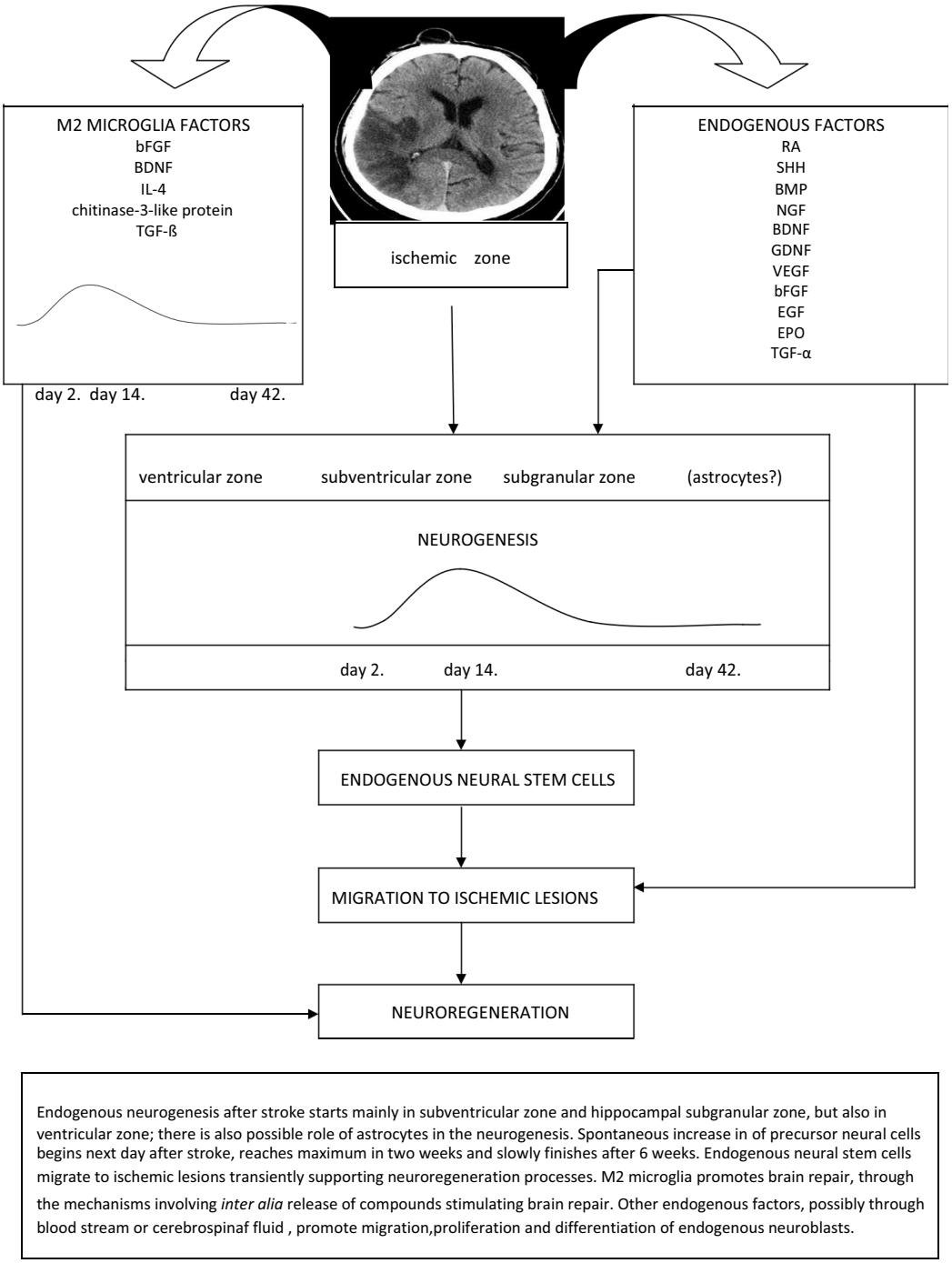

Fig. (1). Endogenous repair processes after stroke. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

Balasubramanian *et al.* [42] have bioengineered functionalized porous silicon nanoparticles (PSiNP) conjugated with a specific antibody against polysialylated neural cell adhesion molecule (PSA-NCAM). PSA-NCAM was bound specifically to neuroblasts in the brain and delivered to them a small molecule drug, SC-79, capable of increasing the activity of the Akt signaling pathway and promoting further neurogenesis. Within a few days, PSiNP are degraded to non-toxic silicic acid. Natural biomaterials are compounds present in the extracellular matrix of the brain, among them the most commonly used are fibrin, HA-methylcellulose, chitosan, and collagen [9]. A combination of Hyaluronic Acid and Methyl Cellulose (HAMC) was given in stroke models with good effect [43-45]. For example, by using the HAMC hydrogel, it was possible to administer cyclosporin and EPO locally and gradually to the brain [43]. Both drugs diffused into the niche of the subcortical stem and progenitor cells, where they were present for at least 32 days after the stroke. Cyclosporin increased striatal plasticity, while EPO stimulated endogenous progenitor cells. Biomaterials can be drugs or SC [46, 47] as well as bioactive molecule carriers (*e.g.* VEGF, angiopoietin-1) [48]. Administration of intrac-

erebroventricular injection of neural stem or progenitor cells together with chondroitinase ABC reduced brain damage compared to administration alone in the hypoxia model of neonatal rats [49].

3.2. Exogenous Stem Cells

Despite numerous preclinical and clinical studies, it remains uncertain which types of NSC will be most effective in stroke therapy. When choosing cells, safety of the treatment, possible host immune response, availability and utility of the cells should be taken into consideration.

SC can be divided into two broad classifications: Embryonic SC (ESC) and adult-derived stem cells – Adult SC (ASC), also known as Somatic SC (SSC) [50]. The first type consists of highly undifferentiated cells isolated from early embryos or primary gonads. ESC are able to proliferate unrestrictedly with self-renewal and multidirectional differentiation. Some of them, after induction, express neuron-specific antigens, others antigens characteristic of glia [51]. The embryonic origin of NSC is associated with heterologous transplants and therefore with the possibility of rejection. Their clinical use in stroke therapy is limited by the risk of developing teratoma. The use of ESC may raise ethical questions. In the animal model of ischemic stroke, implanted ESC migrated to the opposite hemisphere towards the damaged areas, resulted in histological and behavioral improvement, and restored damaged synaptic connections [52].

ASC can be found in a variety of adult tissues, including NSC, Hematopoietic SC (HSC), Mesenchymal SC (MSC), and so forth. NSC can be further divided into neuroectodermal cells (neural tube epithelium), neuroblasts (primary nerve cells), and neural precursors. It is difficult to obtain human neural progenitor cells due to their limited availability and location in the brain. HSC consist of cells derived from hematopoietic tissues, including bone marrow, embryo liver, peripheral blood, and umbilical cord blood. They play an important role in the treatment of hematological diseases. Experimental studies have shown that transient occlusion of the MCA in mice lead to the activation of HSC of the bone marrow [53]. CD34+ hematopoietic stem/ progenitor cells are involved in vasculogenesis; hypoxia is a strong stimulus activating this ability [54].

MSC are a type of pluripotent cells that originate from the early development of mesoderm and can be obtained from readily available sources such as bone marrow, adipose tissue, umbilical cord tissue, and placenta. MSC properties and functions vary depending on the source [55, 56]. These cells can differentiate into various tissues, including neural tissue, and currently constitute the main source of SC used in most both preclinical and clinical stroke studies. MSC derived from Bone Marrow (BM-MSC) are frequently used. These cells can easily be obtained from the host, thereby avoiding the immune response and rejection of the transplant. In addition, BM-MSC are able to pass through BBB without damaging it [50]. It has been shown that BM-MSC actions involved neuroprotective mechanisms [57]. These cells do not contribute to simple neuronal replacement; however, they secrete factors that promote neurogenesis and suppress inflammation, reduce the secretion of IL-12 and TNF-α, and increase the secretion of regenerative IL-10. In mouse stroke models, BM-MSC have been shown to migrate to ischemic areas where they regulate the production of neurotrophins and growth factors [58].

Adipose-derived MSC (AD-MSC) are isolated from fat. They can multiply stably and have a low *in vitro* apoptosis rate [59]. In hemorrhagic stroke, it was shown that when rats were given AD-MSC to the lateral cerebral ventricle, cells differentiated around the hematoma into neuron and astrocyte-like cells. In ischemic stroke animal model, rats were given human adipose-derived SC [60]. Implanted cells did not proliferate or differentiate, however endogenous neurogenesis and inflammation-modulating effects were observed [60].

Another study analyzed the effects of human multipotent adult progenitor cells and human MSC graft in mice stroke models [61]. Authors observed (*i.a.*) reduction of brain tissue loss, neoangiogenesis, reduced inflammation and higher cellular proliferation in SVZ with increased subsistence of neoteric cells.

The schema of the main sources of the exogenous SC used in the studies on the treatment of stroke is presented in Fig. (**2**).

The therapeutic potentials of human stem and progenitor cells from other sources have been extensively studied, including bone marrow, muscle, skin mononuclear cells, umbilical cord blood CD34 + cells, and tooth pulp SC [52, 62]. Other potential sources of human NPC are those derived from reprogrammed cells, such as induced Pluripotent SC (iPSC) [63, 64]. This offers an autologous source of cells for transplantation. NPC are artificially obtained from non-pluripotent cells by forcing the expression of appropriate genes in them. These cells can be generated from various types of somatic cells (*e.g.* skin fibroblasts, keratinocytes, peripheral blood cells [65]) and can differentiate into neuroepithelium-like/neuroepithelioid SC and neural cells [50], and they can secrete neurotrophic factors. In contrast, Vonderwalde *et al.* [66] reported the beneficial therapeutic potential of human cell populations that were directly reprogrammed from somatic cells to NPC without passing through a pluripotent state during reprogramming.

Sadly, the survival rate of transplanted SC has been shown to be less than 5% *in vivo* [35]. Most of them die within 1 week after transplantation (defined as immediate death), while those which survived die within months (continuous death). Therefore, attempts have been made to modify SC to increase their lifetimes. Various strategies have been used to modify SC to improve their therapeutic potential as well. Korshunowa *et al.* [67] tried stimulating the prosurvival pathways of genetically modified human NSC to increase their survival. Four factors involved in the regulation of neuron survival during prenatal or postnatal neurogenesis were selected: Akt1, Hif-1α, Bcl2, and Bcl-xl. Each of them was found to inhibit caspase-3 dependent apoptotic pathway. Hif-1α expression delayed immediate cell death, while the expression of the other three factors protected transplanted cells from immediate and continuous death. The authors note that increasing NSC survival can improve the outcome of therapy as well as reduce the number of transplanted cells needed. In addition, by initiating cell differentiation directly in culture and removing growth factors from the medium, they reduced the proliferative potential of NCS to prevent uncontrolled growth and possible tumor formation.

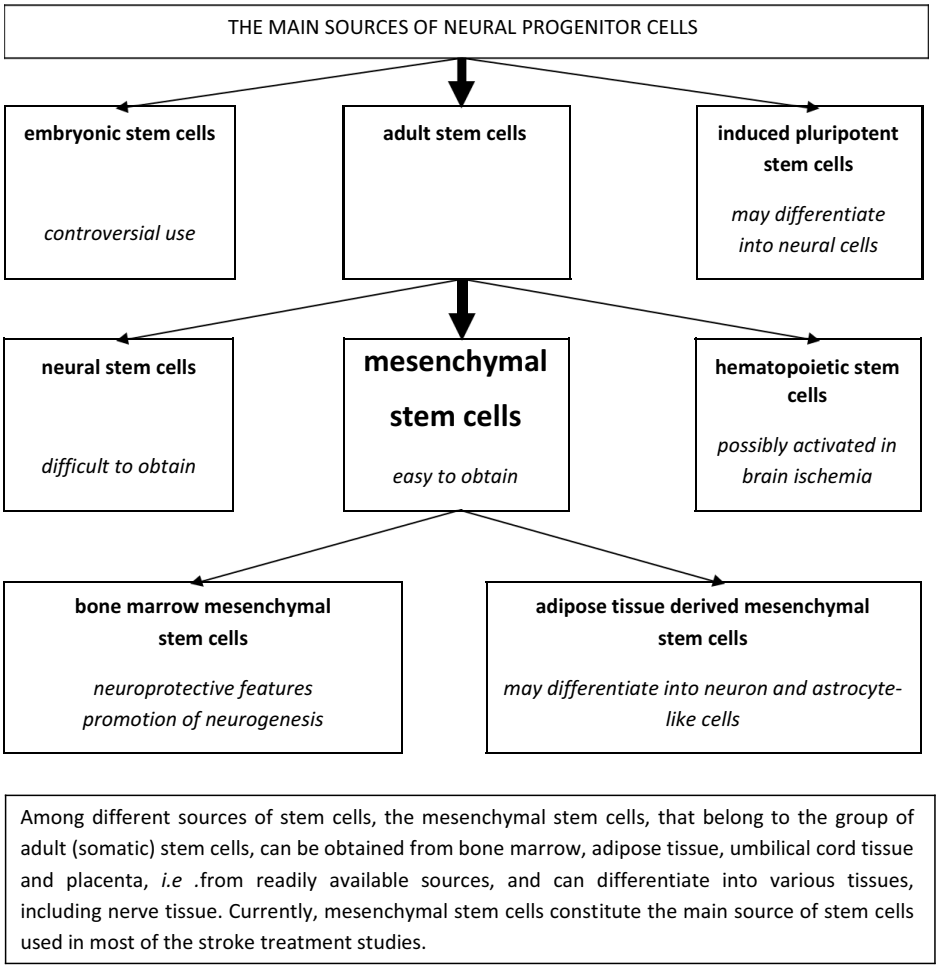

Fig. (2). Exogenous stem cells in the treatment of stroke.

To increase NPC survival, transduction of cells with TAT-thermal shock 70 protein prior to transplantation was used [68]. Studies have shown that the therapeutic functions of exogenous NCS could be enhanced by combining them with some neurotrophins, such as BDNF, VEGF or NGF [35]. Human NSC overexpressing BDNF [69] as well as overexpressing VEGF [70] transplanted to the cerebral cortex damaged by hemorrhagic stroke induced behavioral improvement in animals and increased cell survival 2-3 times after 2 weeks and 8 weeks post transplantation. They stimulated renewal of angiogenesis in brain of the host. These effects were observed in both hemorrhage and ischemic stroke [71]. Improved neuroprotective effects in a rat hemorrhage model were observed after administration of BM-MSC overexpressing GDNF [72]. Co-administration of NSC and low dose of pro-inflammatory IFN-γ cytokine, which affects SC neurogenesis, improved therapeutic results in the ischemic stroke model in rats [73].

The combination of both treatments has been found to significantly increase neurogenesis *in vivo* and have a beneficial neurological effect compared to NSC transplantation alone. Tobin *et al.* [1] compared the effects of interferon-γ-activated MSC (aMSCγ) with native MSC (nMSC) in a model of acute stroke in animals. Administration of MSC three hours after MCA closure significantly restored the functions of experimental animals' brains and reduced the volume of infarction and penumbra, as assessed by MRI. Researchers found that MSC reversed the inflammatory changes caused by hypoxia and ischemia in microglia and transform its pro-inflammatory phenotype into a pro-regenerative phenotype. In animals treated with MSC, microglia morphology was much more consistent with non-hypoxic tissues than untreated. In contrast, microglia grown in media containing nMSC and aMSCγ reduced the secretion of pro-inflammatory cytokines IL-6 and TNF-α. Treatment of aMSCγ after stroke resulted in a strong pro-oligodendrogenic response, a significant increase in the number of oligodendrocyte progenitor cells in SVZ with further differentiation, and an increase in myelin.

The process involved proteins, such as bone morphogenetic protein 1, which promotes the generation of mature myelinating oligodendrocytes *in vivo*, chondroitin sulfate proteoglycan, which is an important component of the neurovascular unit, and collagen α-2, which is protective in stressful conditions. Researchers observed a reduction in the level of the immunomodulator SEMA7A (semaforin 7A) involved in the inflammatory response. According to published reports, aMSCγ is a better therapeutic option than nMSC because of its effect on the inflammatory process; aMSCγ administration causes increase in anti-inflammatory

secretion of microglia with a corresponding decrease in pro-inflammatory cytokines, it is also more effective in induction of *in vivo* oligodendrocyte differentiation and myelination. Chen *et al.* [74] in an animal model compared the effect of transplantation of native NSC and NSC with modified strong neurotrophic genes derived from glial cell lines (GDNF/NSC). It was observed that the total volume of ischemic lesions was lower in the NSC and GDNF/NSC groups as compared with the control group (without treatment). They showed that administration of GDNF/NSC resulted in significant neurological improvement, an increase in BDNF protein, higher expression of synaptophysin and postsynaptic elements, but reduced the number of positive caspase-3 cells.

3.3. Extracellular Vesicles in Stroke Therapy

Exosomes are membrane-limited extracellular vesicles that are secreted following production in the endosomal compartment of the majority of eukaryotic cells. Examples of membrane-bound vesicles include apoptosomes (1-10 μm), microvesicles (200-1000 nm), and exosomes (30-200 nm) [75]. These vesicles can be used to transport proteins, lipids, and nucleic acids.

Exosomes may contain CD81, CD63 and CD9 and tissue/cell type specific proteins [76]. The specific proteins indicate the origin of exosomes. This feature, in the context of stem cell and mesenchymal stromal cell therapy, is interpreted as promising in the future therapy of strokes. The transferred factors form vectors which could be beneficial in obtaining therapeutic effect in stroke [77]. In one of the studies, the transfer of exosomes of MSC 24 hours after induced brain ischemia resulted in the stimulation of neurogenesis and caused an increase in the number of axons [78]. The study was based on the examination of rats. MicroRNA transported using exosome is associated with neurorestorative features among patients affected by stroke [79]. Exosomes induce inhibition of apoptotic and inflammatory reactions and stimulate growth and expression of trophic factors [79]. This results in facilitating brain parenchyma repair. Authors of the study additionally observed the introduction of exosome therapy could enable cell-free therapy. The used exosomes would be derived from SC [80]. Beneficial results were obtained in a study highlighting exosomes derived from adipose SC. Authors of the work stated that exosomes derived from the cells decreased the area of brain injury, which followed the infarction. The mechanism was based on the inhibition of autophagy and promoting M2 microphage/microglia polarization [81]. The studies based on exosomes derived from human umbilical cord blood showed positive effect in the treatment of brain injury, that is attenuation of the infarct size, however no improvement in clinical manifestation was observed [82]. A different work based on the examination of experimental models after induced MCA occlusion, revealed that exosome injection caused reduction of infarct volume. The exosomes were injected 2 hours after an induced stroke. Additionally, the neurological outcome was beneficial [83]. This study does not explain more beneficial clinical outcome in exosomes derived from endothelial cells than those derived from SC. Exosomes derived from MSC were administered among animal models with intracerebral hemorrhage. The significant improvement was observed in the context of functional recovery, lesion size, fiber tract integrity, axonal sprouting and white matter repair [84]. In mice model of Alzheimer's disease, administration of exosomes derived from MSC caused increased neurogenesis in SVZ and reduction of cognitive impairment [85]; what suggests possible use of cell-free therapy in Alzheimer's disease.

The remaining question concerns the exact mechanism of this beneficial role of exosomes derived from SC. The contemporary literature does not provide any definite explanation. The main doubt of exosome therapy is the questionable clinical outcome. Though the majority of studies show the positive role of exosome therapy in reducing the morphological consequences, the clinical manifestation is often not improving. Additionally the results of experimental models lead to a question on the possible correlation of morphological and clinical presentation in clinical trials.

3.4. Routes and Time of Stem Cells Administration

Many studies analyzed data that may indicate how the therapeutic properties of transplanted SC vary depending on the route of administration and how safe the method of administration is. The optimal choice of route of MSC administration may depend on individual factors. The ways of administration of SC to the brain may be different, including intracerebral, intraventricular, intranasal, intravenous, intrathecal, subarachnoid, intraarterial and intraperitoneal routes [49, 59, 86-92]. In clinical trials, intravenous or intraarterial SC routes were mainly used, mostly due to the greater safety and lower technical requirements of these methods compared to intracerebral transplantation [57].

Intravenous methods have been widely accepted for their ease and non-invasiveness [93]. However, systemic intravenous transplantation may reduce the number of SC in the brain because many cells are trapped in peripheral organs, especially in the lungs [94]. They can modulate trophic factors secretion and immune responses from these organs [93]. It has been suggested that systemically administered cells, later found in internal organs, might have a peripheral therapeutic effect by reducing the levels of inflammatory mediators and the number of activated macrophages [95]. This process may be argued by the fact that BM-MSC collected one day after stroke and given intra-arterially, showed greater therapeutic efficacy and smaller size of brain ischemia compared to BM-MSC collected one day before the stroke. This benefit has been attributed to elevated levels of cytokines with anti-apoptotic, proangiogenic, pro-neurogenic and immunomodulatory agents found in BM-MSC collected after stroke [96].

After intravenous administration, only a small number of cells reach the brain. Despite this, there is evidence that intravenous SC injection is able to promote their relocation to CNS and survival by stabilizing BBB, simultaneously with reduced activation of matrix 9 metalloprotease and reduced formation of ROS [68, 97]. During the first 72 hours after intravenous administration, SC were found in both brain hemispheres [98]. Over the next seven days, these cells disappeared in the hemisphere opposite to damage, while in the hemisphere with the ischemic area, their number increased steadily around the border of damage. This phenomenon,

together with the presence of the Ki67 proliferation marker, was explained by local proliferation of transplanted cells. In addition, intravenous and intra-arterial administration of BM-MSC and MSC proved to be more promising because their activities are largely mediated by neuroprotective mechanisms [57]. The therapeutic effect of the intravenous method has been shown to be independent of the number of cells reaching the brain, as opposed to local administration, which is closely related to the amount of SC at the site of injury [11].

There are several studies suggesting that local (intracerebral, intraventricular) NPC administration has the most significant impact on endogenous neurogenesis [11]. This route of cells administration allows the accumulation of a large number of them in the brain, thereby achieving a high level of locally available tissue trophic factors that can better induce a strong endogenous neurogenic response [99].

Methods of intracranial SC administration by stereotactic device have been shown to be safe and feasible in humans [62], however, the neurosurgical procedure may be difficult to accept for some patients with recent stroke. Intranasal administration of cells is less invasive than their injection into the brain or into the ventricles of the brain and may be a more acceptable and practical way of therapy [92]. In hemorrhagic stroke, BM-MSC migration to sites around the hematoma was observed after intranasal administration [90].

According to the meta-analysis of Satani *et al.* [93] no significant differences in the effect of treatment were found depending on the route of administration, dose, fresh *vs*. frozen preparations. There was no predominance of fresh cells, whereas BM-MSC from passage 4 or higher produced significantly better motor and sensomotor results in animals compared to cells from passages 2-4.

Guidelines for choosing the optimal time window for cell therapy and its doses are still under discussion [100]. Transplanting a bigger number of NSC did not result in a longer cell lifetime or increased neuronal differentiation [101]. The mean intravenous MSC dose was four times higher than by intracerebral administration [90]. Janowski *et al.* [102] reported frequent strokes in rats due to microembolism when injecting cells at a higher dose (2×10^6 cells), but not at a lower dose (1×10^6 cells).

Pre-clinical and clinical results suggest that the window of intervention for stroke stem cell treatment, although potentially longer, may be limited [57]. NPC survival was strikingly reduced after delayed cell administration [101]. Transplant shortly after stroke (48 hours) resulted in better cell survival than transplant 6 weeks after stroke. However, delayed transplantation did not affect the size of migration, neuron differentiation and cell proliferation in transplants. Chen *et al.* [100] suggested that for best functional results, homologous cells should be used and administered within the first 24 hours after stroke or for no longer than 72 hours. However, consistent results confirming the difference in treatment based on time windows from acute periods (0-6 hours) compared to later periods (2-7 days) were not obtained [93]. MSC have been shown to reduce infarction volume even when treatment was applied 1 day or week after ischemia [97]. However, the reduction in the size of the destructed area was greatest when MSC was given as early as possible, *i.e.* 0 to 8 hours after the onset of stroke. It was shown that MSC given ≥24 hours after stroke had a very good effect on behavioral results. The inclusion of therapy on days 7 to 30, considered to be the subacute and chronic stroke phase, may still bring benefits, although to a lesser extent [93].

3.5. Post-stroke Stem Cell Therapy in Humans

Promising results obtained from studies conducted on animal models considering the possible effectiveness of SC in promoting regeneration of CNS after stroke, lead to trials conducted on humans. First clinical trials date to the early 21st century used human embryonic cell line derived from a primitive teratocarcinoma induced to differentiate into neurons by retinoic acid implanted with stereotactic methods [103, 104]. There were no cell-related side effects and both studies reported clinical improvement. Savitz *et al.* [105] performed neurotransplantation of fetal porcine cells in patients with basal ganglia stroke, however, 3 out of 5 participants did not improve and two patients worsened after the procedure – the authors noted seizures and temporary motor deterioration. The literature describes results of Pilot Investigation of Stem Cells in Stroke (PISCES) phase-I trial, with the use of CTX-DP – a drug product developed from CTX0E03 – an immortalized human neural stem-cell line derived from human fetal cortex and modified [106]. This study confirmed the safety of SC therapy in humans. Authors demonstrated that single intracerebral administration of NSC may cause a minor improvement of neurological function in post-ischemic stroke patients with no drug-caused side effects. This cellular population is transient and possibly has a trophic impact on CNS. PISCES was continued in phase II (PISCES II [http://www.reneuron.com/ clinical-trials/phase-ii-clinical-trial-in-stroke-disability-pisces-ii/]) and IIb (PISCES III [http://www.reneuron.com/ clinical-trials/phase-iib-clinical-trial-in-stroke-disability-pisces-iii/]) – however, due to the current epidemiological situation (COVID-19 pandemic), the project was temporally suspended. It was reported that the neurogenic potential of adult NPC decreases with age [107], however, the ischemic environment can be successfully modified to restore deficiencies in the neurogenic response in the aging brain [108].

Another cell population used in trials focused on stroke therapy is MSC. Multipotent cells have been found in most organs, including the brain [109]. Some trials with fat-/bone marrow-derived allogeneic MSC injected intravenously can be found in the literature [110, 111]. Some papers describe clinical improvement [112], however other works suggest this approach is inefficient [http://www.fiercebiotech.com/ story/ athersys-tanks-itsstem-cell-therapy-flunks-phase-ii-stroke-trial/2015-04-17; 113, 114]. Although animal stroke models showed improvement after intravenous or intraarterial BMSC administration, the same results have not yet been observed in humans [57]. Differences between animal models and patient results may be due to the differences in the time of BMSC administration. In experimental studies, BMSC were usually given through intravenous or intraarterial routes within three days of stroke. In clinical studies, collection and infusion time autologous BMSC varied from study to study. Although no significant neurological improvement was achieved in the patients groups, in the studies

that resulted in some improvement the treatment started within seven days after stroke [115-117]. The mechanism of MSC action is not a simple cell replacement as it was incipiently considered, as very small number of injected cells are able to reach the lesion and prevail on a long-term basis [118]. Currently, there is increasing evidence that the MSC has so called paracrine bystander effect leading to post-stroke improvement. MSC secrete many different cytokines, extracellular vesicles and growth factors inclusively called the secretome. Various MSC populations have different secretomes, for example, MSC derived from adipose tissue have higher expression of VEGF-D, IGF-1 and IL-8, dermal-derived MSC population has more CCL2 and leptin compared to other MSC subtypes [119]. Therefore MSC's secretome could be preconditioned and used to enhance its regenerative potential in patients with stroke [120].

3.6. Stem Cells Therapy in Intracerebral Hemorrhage

According to numerous studies, SC therapy supports neuroregeneration and neuroprotection not only in ischemic stroke but in intracerebral hemorrhage (ICH) [62, 90, 121, 122], including subarachnoid hemorrhage [123, 124].

Brain lesions in ICH can be divided into primary and secondary [50]. The primary type is the effect of the hematoma's direct action, which mechanically damages adjacent tissues. The secondary type includes various molecular, cellular and biochemical responses caused by primary damage. Blood and decomposing components of blood cells (*e.g*. enzymes, hemoglobin, iron ions) are toxic. The expression of inflammatory mediators intensifies the damage and participates in the formation of brain edema around the hematoma. BBB damage, programmed death of neurons and glial cells by apoptosis, lipid peroxidation, free radical damage and glutamate excitotoxicity are involved in the process. The mechanism of SC action in ICH is considered to be the same as in ischemic stroke [50, 90]. For example, BM-MSC treatment has been shown to reduce the levels of pro-inflammatory cytokines IL-1β, IL-2, IL-4, IL-6, TNF-α, and IFN-γ, and BM -MSC and UC-MSC increased the levels of anti-inflammatory cytokines IL-10, TGF-β1, IL-1α and IL-1β [90].

MSC, NSC, ESC, HSC and iPSC are the most common types of SC that are used in experiments on ICH treatment [50]. In clinical studies, MSC derived from bone marrow or umbilical cord were mainly used, less often – combination cell transplantation of Olfactory Ensheathing Cells (OEC), NPC, and Schwann cells [90]. Better effects of treatment were obtained by modifying cells. Implantation of NSC overexpressing VEGF or BDNF into the brain near sites of hemorrhage damage provided better differentiation and survival of transplanted cells, increased angiogenesis in the brain, and led to more pronounced functional improvement in the mouse model of intracerebral hemorrhage [69, 70]. The therapeutic methods used in various preclinical studies differed significantly [50] and, as in ischemic stroke, no definitive guidelines have been established yet. There is still a need to determine what type and number of cells should be given, by what route and in what time window since the beginning of the hemorrhage, whether to give the treatment once or several times [122].

The analysis of preclinical studies on intracerebral hemorrhage conducted by Turnbull *et al.* [90] showed the beneficial effects of MSC therapy in the vast majority of studies. MSC administration alleviated experimentally induced sensory-motor dysfunction, improved cognitive function, reduced hematoma volume, and loss of gray and white matter while increasing the density of blood vessels around the hematoma, indicating angiogenesis. A 1-10% reduction in brain edema has been reported in treated animals compared to untreated animals. In addition, BM-MSC therapy prevented the development of hemorrhagic hydrocephalus. The improved structural integrity of brain tissue and BBB was found in electron microscopy.

In clinical studies, 9 patients with severe neurological disability, one year after intracerebral hemorrhage, received autologous MSC from the bone marrow intravenously [62]. Despite the significant time lapse after the hemorrhage, cell therapy facilitated the recovery of neurological functions, as demonstrated by comparing the condition of patients receiving MSC with those patients who were given placebo. In addition, the results obtained suggested that the treatment was safe. Li *et al.* [125] examined 100 patients with intracerebral hemorrhage and 60 of them were given their own bone marrow mononuclear cells in the hematoma area in the basal ganglia. No adverse effects were observed. Patients' condition was assessed by the National Institute Stroke Scale (NIHSS) and Barthel index before treatment and after 6 months. Neurological and functional improvement was observed in 52 (86.7%) treated patients and in 17 (42.5%) untreated patients ($p = 0.001$). Patients who received bone marrow cells after 6 months had lower NIHSS ($p < 0.01$) and higher Barthel scores ($p < 0.01$).

Improvement of motor function and reduction of muscle spasticity after autologous bone marrow mononuclear cell transplantation was observed in 4 children who underwent intracerebral hemorrhage in the neonatal period [121]. However, significant changes in MRI of the brain were observed in only one case in which ventricular dilation decreased after transplantation. Children were given 1-4 doses of intrathecally infused cells at intervals of several months, between 14 and 74 months of age. No adverse effects were observed. In addition, children were chronically rehabilitated.

It has been shown that a combination of minimally invasive hematoma aspiration with a human umbilical cord MSC transplant can increase efficacy in reducing damage and improving neurological functions [126].

3.7. Meta-Analysis of Research of Stem Cell Therapy

Numerous preclinical and clinical studies on SC therapy for ischemic stroke have many discrepancies *e.g.* the type of transplanted cells (including their species, the use of fresh or cultured cells, their earlier passage), cell dose, route and time of their administration, in addition, different methodologies for measuring and analyzing the obtained data were used in different laboratories. These differences made it difficult to compare the results and assess the quality of the tests. Nevertheless, in subsequent years, attempts were made to systematize the knowledge obtained and assess progress. Published works were subject to meta-analysis.

Lees *et al.* [127] analyzed 117 publications. In 187 experiments involving 2332 animals, changes in the structural result were described, and in 192 experiments with 2704 animals - changes in the functional result after treatment were observed. It was shown that there was a dose-response relationship for the structural result and that each day of delay in treatment reduced its effectiveness by 1, 5%. However, the functional result was independent of the time of cell administration.

Clinical trials were reviewed in 2012 to assess the safety of MSC administration [128]: 36 studies were analyzed involving a total of 1012 healthy volunteers and adults and children who were diagnosed with ischemic stroke, Crohn disease, cardiomyopathy, myocardial infarction, and graft versus host disease. No relationship was found between MSC treatment and the development of acute infusion toxicity, organ complications, infection, death, or *de novo* tumor formation. However, a significant relationship was found between MSC administration and transient fever. This fever was not associated with long-term sequelae or increased susceptibility to infection. In addition, although mismatched allogeneic MSC were used in 13 studies, no acute infusion toxicity was observed, which was explained by the low MSC expression of MHC proteins and co-stimulatory molecules [129].

A re-meta-analysis on the safety and efficacy of MSC therapy in ischemic stroke in both clinical and preclinical settings was performed in 2019 [130]. The evaluation included 10 clinical and 76 preclinical studies. In preclinical trials, MSC therapy had a beneficial effect on many neurological and motor tests. In clinical studies, MSC therapy seemed promising in the early stages of research, but the most stringent work carried out so far has not shown efficacy. The earlier opinion was confirmed that MSC therapy seems to be generally safe without any noticeable increase in mortality in clinical trials, except for the increased risk of fever immediately after injection. The analyzed preclinical studies focused on the administration of cells in the acute period of stroke, but there has been little experimental work on the chronic phase of stroke (*e.g.*, 30 days after stroke). Problems with low viability of cryopreserved MSC have been demonstrated, which was associated with poor clinical efficacy of therapy. The significant methodological heterogeneity of clinical trials and the lack of data prevented final evaluation.

Vu *et al.* [97] analyzed 46 studies of animal models of stroke, finding that 44 showed significant improvement after MSC treatment. In the authors' opinion, the beneficial effects of MSC observed in preclinical studies on ischemic stroke can be classified as very high, including both early and later stage of stroke. The magnitude of the result varied significantly depending on the route of administration; with intravenous administration, the obtained effect was very large. Therapies used in the early post-stroke period usually were aimed at reducing the volume of damage, while therapies started a few days or weeks after the stroke aimed to improve function. A meta-analysis of preclinical studies on NSC therapy showed that NSC transplanted after stroke resulted in significant functional and structural improvement [100]. The size of the treatment effects was related strongly to the time of NSC administration in relation to the onset of stroke and the type of NSC source. However, dose and route of NSC administration were not found to be significant. In most studies, the transplant was performed intracerebrally using stereotactic techniques.

An analysis of the results of 78 preclinical studies and 8 clinical trials covering MSC therapy in 2008-2017 found that preclinical and clinical studies showed statistically significant effects, but clinical results were not as promising as experimental studies [131]. About 5% of the studies used animals with comorbidities, which indicates that animal stroke models did not mimic human stroke models (strokes in old age, often with comorbidities such as hypertension, dyslipidemia or diabetes). Eighty-nine (89) patients participated in human studies. The most commonly reported adverse events were headache (19/89, 21.3%), fever (7/89, 7.8%) and convulsions (2/89, 2%). Nausea, vomiting, depression, fatigue, local pain, and drowsiness were mentioned.

Data obtained after analyzing 141 articles from the years 2000-2018 indicated a significant beneficial effect of BM-MSC treatment in the experimental model of stroke [93]. Functional improvement of comprehensive, motor, and sensorimotor results was obtained in treated animals compared to control groups. Administration of BM-MSC during the period from 2 to 7 days leads to the greatest benefits, while administration of BM-MSC during the period from 0 to 6 hours led to the most significant improvement in sensorimotor results. Greater improvement was obtained in animals without comorbid diseases compared to animals with comorbidities. After BM-MSC treatment, female animals achieved better results in sensorimotor evaluation compared to males.

The summarization of the effects of stem cell therapy in animals and humans is presented in the Table **1**.

3.8. Possible Mechanisms of Stem Cell Therapy Effectiveness in Stroke

Despite numerous preclinical and clinical studies, potential cellular and molecular mechanisms through which various types of implanted SC help restore lost brain function after stroke are still widely discussed [65, 66, 80, 132-134]. Among the main effects of exogenous neurogenesis are the reduction in the volume of vascular damage, palliation of inflammatory changes around ischemia, neuroplastic remodeling of the brain, and improvement of sensorimotor and cognitive functions in individuals after stroke.

The main NSC treatment strategies include reducing neuronal apoptosis, compensating for endogenous cell deficiency, replacing damaged cells, preventing secondary neuronal degeneration, limiting glial scar formation, reducing oxidative stress, stabilizing BBB integrity, improving the inflammatory microenvironment around the ischemic area, and enhancing endogenous repair processes [35, 100]. Studies have shown that in animals with cerebral infarction, SC therapy improved the results of functional tests and reduced the size of cerebral ischemia [67], and reduced delayed neuronal degeneration that is related closely to inflammatory and glial reactions [135]. Limitation of neuronal degeneration was found both within the ischemic lesion as well as in distal areas of the brain, which was demonstrated by significantly thicker *corpus callosum* 30 days after transplantation in

Table 1. Effects of stem cell therapy in animals and humans.

Stem Cell Type	Effects in Animals	Effects in Humans
Murine ESC	Histological and behavioral improvement with restored damaged synaptic connections	-
BM-MSC	Regulation of neurotrophins and growth factors production, prevention of hemorrhagic hydrocephalus, improvement of brain tissue structural integrity	Recovery of neurological functions after intracerebral hemorrhage, improvement of motor function and reduction of muscle spasticity after intracerebral hemorrhage
AD-MSC	Differentiation into neuron and astrocyte-like cells	-
BM-MSC overexpressing GDNF	Neuroprotective effects	-
NSC + IFN-γ cytokine	Improvement after ischemic stroke	-
Human NSC overexpressing BDNF	Behavioral improvement	-
Human NSC overexpressing VEGF	2-3 times increased cell survival, stimulation of angiogenesis	-
MSC	Reduction of infarction and penumbra/hematoma volume, restoration of brain functions, promotion of pro-regenerative microglial phenotype	-
aMSCγ	Reduction of pro-inflammatory microglial phenotype, prooligodendrogenic response, increase in myelin	-
NSC + GDNF/NSC	Increase in BDNF protein, higher synaptophysin expression and postsynaptic 95 support, reduced number of positive caspase-3 cells	-
Human embryonic cell line derived from teratocarcinoma cells induced to differentiate into neurons	-	Clinical improvement
Fetal porcine cells	-	No improvement / worsening
CTX-DP	-	Minor improvement
Fat-/bone marrow-derived allogenic MSC	Improvement	Ambiguous results, paracrine effect

NPC-treated mice compared to controls. Tissue survival was associated with decreases in inflammatory markers, glial scarring, and apoptotic neurons at both mRNA and protein levels. One of the transplant's therapeutic mechanisms is to replace damaged neurons, which involves the ability of the implanted cells to migrate, survive, proliferate, and differentiate into different types of cells that are needed [133]. However, the implantation of cells is limited and some of the cells remain undifferentiated. Usually, exogenous SC do not functionally integrate but differentiate into glia or electrophysiologically inert neurons. Still, they can affect clinical improvement, probably through metabolic, regulatory, or anatomic support.

These points notwithstanding, some experimental studies have shown that part of the transplanted cells became functionally active, exhibited a presynaptic vesicle marker, and connected to host neurons [65, 92]. It has been reported that post-stroke transplanted induced human neural progenitor cells could be functionally incorporated into the damaged rat cortex [132]. *In vivo* electrophysiological records from corti-

cal implants have shown that skin stimulation of the nose and paws of the rat could induce spontaneous activity in implanted neurons, what proves the existence of functional connections with the recipient's nervous system. There are suggestions that improvement in post-stroke function may be achieved without the cells being implanted [66].

Studies have shown that the use of SC after stroke induced neurological regeneration through indirect mechanisms of paracrine signaling [62, 91, 93]. Various signaling molecules released by the transplant take part: cytokines, chemokines, growth factors, neurotrophic factors, immunomodulators. These substances ultimately stimulate endogenous neurogenesis, angiogenesis, and synaptogenesis. Bacigaluppi *et al.* [135] have demonstrated that transplanted NPC located in the perischemical and ischemic regions promote plasticity and brain regeneration by increasing the expression of the glial glutamate transporter 1 on astrocytes and reduce perischemical extracellular glutamate. These processes involve VEGF secreted by NPC, which promotes long-term strengthening and plasticity of neurons in the mice's hippocampi. Increased synaptophysin expression in transplanted brains suggests that SC promote neuroplasticity through enhanced synaptogenesis [66]. It was found that post-stroke transplant changed the synaptic transmission by causing reduction of presynaptic glutamate release and enhancing NMDA mediated transmission [135]. This process can restore local and global electric stimulating-inhibiting balance.

Pericytes, which are specialized cells that play a key role in vascular homeostasis, participate in the repair processes [136]. These cells have mesenchymal stem cell features. They can differentiate into different types of cells, including nerve cells. They can act neuroprotectively by affecting the regeneration of neurons in the area of ischemia. They secrete neurotrophin-3 (NT-3), which can stimulate astrocytes to produce nerve growth factor. Platelet derived growth factor B/platelet derived growth factor β receptor signaling pathway has been shown to participate in the reconstruction of the ischemia area by recruiting pericytes and stimulating their migration towards new microvessels.

CONCLUSION

SC therapy for stroke has been a topic of research for years. The results obtained are promising and give hope for broader applicability. Additionally, this treatment would not be as time-limited as usual treatments are today. Understanding the mechanisms underlying endogenous and exogenous neurogenesis is very important for developing an effective brain repair strategy. Various lines of evidence obtained in animal models of SC find there is cell migration, survival, and differentiation, as well as the ability to modulate inflammation, trophic secretion, increased angiogenesis, and neuroplasticity processes. However, the potential mechanisms of therapy are not fully understood. Therefore, there is a need for further extensive research using well-designed trials. Their purpose will be to choose the type and source of SC, to specify the most effective routes of administration and the most favorable time frame for transplantation (SC may have different treatment effects at different time points after stroke, from acute to subacute and chronic), to determine the phenotypic transformation of microglia, and to demonstrate the safety. In addition, the possible identification of biomarkers for the recovery of damaged brain function is indicated. The optimization of preclinical trials is a key to increasing the chances of success in human clinical trials, and to the further development and progression of stem cell therapy to benefit immediate recovery and long-term functioning of stroke victims.

LIST OF ABBREVIATIONS

AD-MSC	=	Adipose-Derived Mesenchymal Stem Cells
ASC	=	Adult-Derived Stem Cells
BBB	=	Blood-Brain Barrier
BDNF	=	Brain Derived Neurotrophic Factor
bFGF	=	Basic Fibroblast Growth Factor
BM-MSC	=	Bone Marrow Derived Mesenchymal Stem Cells
BMP	=	Bone Morphogenic Protein
EGF	=	Epidermal Growth Factor
EPO	=	Erythropoietin
ESC	=	Embryonic Stem Cells
GDNF	=	Glial Cell Line-derived Neurotrophic Factor
HAMC	=	Hyaluronic Acid and Methyl Cellulose
HSC	=	Hematopoietic Stem Cells
ICH	=	Intracerebral Haemorrhage
IFN-γ	=	Interferon Gamma
IGF	=	Insulin-like Growth Factor 1
IL	=	Interleukin
iPSC	=	Induced Pluripotent Stem Cells
lncRNA	=	Long Non-Coding RNA
MCA	=	Middle Cerebral Artery
MMP	=	Metalloprotease Matrix
MSC	=	Mesenchymal SC
NCAM	=	Neural Cell Adhesion Molecule
NGF	=	Nerve Growth Factor
NIHSS	=	National Institute Stroke Scale
nMSC	=	Native Mesenchymal Stem Cells
NPC	=	Neural Progenitor Cells
NSC	=	Neural Stem Cells
PPARγ	=	Peroxisome Proliferator-Activated Receptor γ
PSA-NCAM	=	Polysialylated Neural Cell Adhesion Molecule
PSiNP	=	Porous Silicon Nanoparticles
RA	=	Retinoic Acid
rIL-6	=	Recombinant IL-6

ROS = Reactive Oxygen Species
SGZ = Subgranular Zone
SHH = Sonic Hedgehog
SSC = Somatic Stem Cells
SVZ = Subventricular Zone
TGF = Transforming Growth Factor
TNF-α = Tumor Necrosis Factor α
V/SVZ = Ventricular-Subventricular Zone
VEGF = Vascular Endothelial Growth Factor

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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