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Stem Cell Therapy for Stroke, Recent Advances, Controversies and Literature Review

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Authors' contributions

This work was carried out in collaboration between all authors. Author PE managed literature search, analyzed the data and wrote the first draft of the manuscript. Author LR co-managed the literature search. Author MA supervised the literature search as well as the drafting of the manuscript.

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Review Article

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ABSTRACT

Stem cells based therapy has shown to improve stroke recovery in multiple animal models. Translating these findings into clinical research can result in a potential therapeutic option for stroke. At this moment multiple animal models, different stem cells type and administration route have been used for research purposes. So far ideal timing, type of stem cells and administration routes has not yet been determined. This review summarizes current approaches and progresses in stroke stem cell therapy.

Keywords: Stroke; stem cells; penumbra; neuromodulation; neuro- plasticity.

1. INTRODUCTION

Stroke is the second leading cause of mortality worldwide, the third one in the United States and the leading cause of serious, long-term disability in the United States [1-4]. From epidemiologic studies it has been reported that ischemic stroke represent as high as 73% to 86% of all stroke cases [5].

The mean expenses per person for a stroke case in the United States in 2007 were estimated at

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$7657 and the mean lifetime cost of ischemic stroke is estimated at $140048 [6].

At this moment stroke therapy is based on vessel recanalization, medical management and installation of hypothermia when indicated. In the last years research activity has been focusing on stem cells as a source of potential functional recovery [7]. Therefore stem cell transplantation has become a promising therapy in stroke management [8].

The adult central nervous system has a very limited capacity of self repair [9]. Stem cells have the ability to migrate long distances to injured brain areas and differentiate into new neurons. Different chemokines and growth factors are involved in these migration and differentiation process including stromal cell-derived factor-1 and vascular endothelial growth factor [10].

In many stroke cases there is a central area of necrosis surrounded by a partially injured area called penumbra. Neurons seem to be affected the most by ischemic lesions; therefore it is possible that they might be completely lost in the penumbra area while glia and vasculature appears to remain intact [11]. Regenerative strategies are nowadays targeted towards neuron and or glial cell replacement.

Many preclinical studies have shown that stem cell transplantation improved stroke recovery [11-14]. Stem cell therapy can be divided into two main categories. One is based on transplantation of exogenous cells while the other consists in the migration proliferation and differentiation of endogenous stem cells [14].

2. EXOGENOUS STEM CELL THERAPY

Different stem cells type has been used in stroke models, including embryonic stem cells, adult-derived neural stem cell, bone marrow or mesenchymal stem cells and umbilical cord blood stem cells (UCBCs) [14].

2.1 Animal Stem Cells

2.1.1 Embryonic stem cells

Undifferentiated embryonic stem cells have not been widely used in stroke animal models since they have been reported to produce teratomas containing derivatives of all three embryonic germ layers when grafted into SCID mice [15].

Erdö et al. investigated in two rodent stroke models the therapeutic effects of transplanting undifferentiated and pre differentiated murine (mouse) embryonic stem cells. As a result, they found that in rats, xenotransplanted undifferentiated embryonic stem cells where able to migrate along the corpus callosum towards the damaged tissue and differentiate into neurons in the border zone of the lesion. But in the homologous mouse, the same murine embryonic stem cells not only did not migrate but at the same time they produced highly malignant teratocarcinomas at the site of implantation, no matter whether they were pre differentiated in vitro to neural progenitor cells. These results raised safety concerns regarding the use of Embryonic Stem cells for clinical purposes [16]. Despite these results they could not exclude the presence of impurities in their embryonic stem cells and they suggested that in homologous stem cell transplantation even minor contaminations of undifferentiated embryonic stem cells can promote tumorigenesis [16].

2.1.2 Adult stem cells

Adult derived neural stem cells (NSCs) are obtained from adult CNS found in tissues of fetus, neonate, young, and adult animals. They are able to renew themselves and to differentiate into different types of cells within the CNS [14]. In 1992, Reynolds et al reported that cells from the adult mouse striatum were able to proliferate and differentiate into neurons and astrocytes when exposed to different growth factors such as epidermal growth factor (EGF) [17].

Toda et al in 2001introduced neural stem cells (NSCs) into the hippocampus of rats exposed to transient global ischemia which resulted in 90-95% loss of pyramidal neurons in the hippocampus CA1 region. As a result they found that NSCs when transplanted to the injured brain were able to differentiate into neurons and improved impaired spatial recognition [18].

2.1.3 Bone marrow or mesenchymal stem cells

Bone marrow derived stem cells transplantation has showed controversial result [19]. In 2001 Chen et al concluded that bone marrow stem cells transplanted into the peripheral ischemic stroke areas survived, differentiated and improved functional recovery in adult rats after MCA stroke [20]. They reported that in stroked rats, the intracerebrally transplanted bone

marrow stem cells were able to migrate longer distances towards the lesion area when compared to the control ones [21]. They also tested intravenous infusion of human bone marrow stromal cells and concluded that when intravenously injected into rats 24 hours after middle cerebral artery occlusion, bone marrow stem cells enhanced angiogenesis mediated by VEGF and VEGFR2 and resulted in significant recovery of somatosensory behavior and Neurological Severity Score [22,23].

Different mechanisms have been proposed to explain functional improvement after bone marrow stem cell transplantation. Among them was promotion of endogenous cell proliferation, enhancement of axonal remodeling, cell differentiation, diminishing apoptosis and inducing angiogenesis [20-29].

On the other hand Coyne et al investigated in 2006 long term survival and plasticity of bone marrow stem cells transplanted into normal brain and found that surgery induced inflammatory response resulted in rejection of the transplanted bone marrow stem cells [30]. In their opinion bone marrow stem cells do not have the plasticity identified in many other studies [30].

2.2 Human Stem Cells

Although many human derived stem cells have been tested in stroke models they all fall within 3 different categories. (1) Neural stem cells (NPCs) cultured from fetal tissue. (2) Immortalized neural cell lines and (3) Hematopoietic/endothelial progenitors and stromal cells isolated from bone marrow, umbilical cord blood, peripheral blood, or adipose tissue [31].

2.2.1 Neural Stem Cells

Human neural stem cells (NSCs) can be transplanted in rodent stroke models [32-33]. Either transplanted intracerebrally around the lesion or delivered intravenously they were able to survive, differentiate and enhanced functional recovery [31-33]. In 2004 Kelly et al. [34] characterized the migration differentiation and survival of human CNS-stem cells isolated by flow cytometry from fetal (16–20 wk) brain tissue transplanted to ischemic rat models.

Rats received three deposits of hNSC, 7 days after MCA occlusion and 4 weeks later, brains were stained to identify the transplanted cells.

They reported that stem cells survival was influenced by the proximity of the graft to the stroke lesion and it negatively correlated to the number of IB4-positive inflammatory cells. They found that graft placement was critical for cell survival. Cells deposited at the edge of the lesion did not survive well compared to those placed further medial suggesting that to improve survival cells should be transplanted into non-ischemic tissue.

They also described targeted migration towards the lesion, with many stem cells migrating as much as 1.2 mm, while migrating shorter distances, radial diffusion-like, of 0.2 mm when transplanted to naïve rats [34]. Theoretically this targeted migration could have been due to a loss of repulsive stimulus normally found in the parenchyma or mediated by attracting injury-induced signals such as cytokines [34]. Not all but, some cells at the border of the lesion and some migrating cells expressed the chemokine receptor CXCR4, an alpha-chemokine receptor specific for stromal-derived-factor-1 (SDF-1) [34].

Cells profile depended on their location relative to the lesion. 49,5% of human stem cells near the edge of the lesion expressed tubulin III, an immature neuronal marker, a smaller proportion 14,7% expressed GFAP an astrocyte marker and none of the cells were positive for oliigodendrocyte markers. They finally concluded that the microenvironment as well as the cells final location relative to the lesion influenced in the migration and differentiation process [34].

2.2.2 Immortalized neural cell lines

Human neural stem cells will eventually undergo growth arrest and senescence after a number of cell divisions in culture. Oncogene-mediated immortalization of human neural stem cells was developed as an alternative source of human neuro stem cells with unlimited expandability in an attempt to overcome those limitations [35].

Immortalized neural cell lines (NT2N) are postmytotic immature human neurons generated from immortalized pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2 [31]. Pluripotent embryonal carcinoma clones derived from Tera-2 are in vitro cultured human embryonal carcinoma cells, capable of differentiating into different somatic tissues [36].

Back in 1993 Trojanowski et al. [37] studied the survival of these postmitotic human neurons

when transplanted into the rat's brain and found out that they survived as long as 8 weeks although most of them were rejected by week 4 or up to sacrifice (week 12) when cyclosporine was administered. None of them underwent neoplastic transformation.

In 1995 Kleppner et al. [38] grafted NT2N cells into an athymic mice. They found that these cells integrated and survived for a period longer than 1 year after implantation and that between 6 weeks and 4-6 months they acquired the molecular phenotype of fully mature neurons. But despite having dendrites and axons and form synapse like structures, the axons remained unmylinated [38]. Finally, none of the transplanted cells underwent neoplastic transformation [38].

Different authors have utilized ischemic rat models to investigate the beneficial effect of transplanted NT2N cells. In 1998, Borlongan et al. [39,40] reported that functional improvement was seen as early as one month after transplantation and that transplanted rats exhibited significant improvement in passive avoidance task as well as a normalization of asymmetrical motor behavior. Therefore, these cells were brought up as a possible alternative to human fetal cells for the treatment of ischemic stroke disease [41].

2.2.3 Bone marrow, umbilical cord blood, peripheral blood, and adipose tissue cells

Bone marrow and umbilical cord stem cells have surged as an alternative viable approach for treating neurological stroke disease [42]. These cells can exhibit neuronal or glial differentiation and have mediated therapeutic effects in several animal stroke models [42-45]. Nonetheless when analyzing post transplanted brains only a few transplanted cells were found, therefore it is unlikely that these cells replace damage tissue, it is more likely that neurologic benefits are mediated by increase in growth factors in the ischemic tissue, apoptosis reduction and proliferation of endogenous cells [31,46].

2.3 Endogenous Stem Cell Therapy

Arvidssoni et al. [47] published in 2002 that new neurons are continuously created in the adult brain in the subventricular zone and dentate gyrus and that brain insults which caused neuronal death exhibit increased neurogenesis in those areas. They used a stroke rat model to show that these new neurons do migrate to the damaged area and differentiate into the phenotype of most of the destroyed neurons [47]. How functional these neurons are is yet to be determined but this might be a natural repair mechanism that could potentially be enhanced by transplanted stem cells [31].

Alvarez-Buylla et al. [48] in 2002, reported that these cells could grow in culture enriched with epidermal growth factor (EGF) and or fibroblast growth factor (FGF) therefore they are an important reservoir of stem cells in the adult brain, which could potentially be used for neuroregeneratvie therapy.

3. ADMINISTRATION ROUTE

In 2004 Jin et al. [49] compared ischemia-directed migration and phenotypic differentiation of transplanted neural precursor cells after different routes of administration. For this purpose they transplanted neuronal precursors from embryonic cerebral cortex of green fluorescent protein (GFP)-expressing transgenic mice to rats, 24 h after induction of focal ischemia, via intrastriatal, intraventricular and intravenous [49]. Even though they did not conduct a comparison of the efficiency of cell transplantation by the 3 different routes, they found that the migration of these cells in the ischemic brain was perhaps most evident after intrastriatal delivery and that intravenous administration yielded the least number of cells in the striatum while the direct administration route the most [49]. On the other hand the administration route had little effect on the phenotype of cells that entered the brain [49].

In 2010 Pendharkar et al. [50], compared the biodistribution of neural stem cells after intravascular, (intra-arterial vs. intra-venous) transplantation for ischemic brain injury. They found that immediately after transplantation brain bioluminescence imaging was 12 times higher when cells were administered intra arterial compared to the intravenous route. They also found that after intra arterial injection, 69% percent of the luciferase activity came from the brain early after transplantation and they reported a 32% signal loss after 1 week [50]. On the other hand after intravenous injection, 94%of the signal was found in the lungs followed by a 94% signal loss after 1 week indicating a lack of survival of these cells outside of the brain [50]. They finally concluded that intra arterial administration of neural stem cells resulted in

superior delivery and sustained presence of transplanted cells in the ischemic brain when compared to intravenous infusion [50].

Intraarterial delivery although less invasive carries the risks of vascular occlusion and development of micro embolic strokes which could potentially contribute to stroke worsening and increased mortality [51,52].

Stereotactic transplantation appears to provide the best stem cell survival rate and precision regarding graft placement but tends to provide poorer cell distribution towards the lesion. Intravascular injections on the other hand are less invasive and provide wider cells distribution but can carry the risk of microembolic strokes [51,52].

So far the optimal delivery route has not yet been determined [51].

4. POTENTIAL MECHANISMS MEDIATING RECOVERY

Many different mechanisms have been so far proposed to explain functional recovery after stem cells transplantation. Functional integration into host circuit, replacement of lost circuits, neuroprotection, reduced apoptosis, improvement of endogenous angiogenesis, decreasing inflammatory damage by immunomodulation, neuroplasticity, intravascular secretion of neuroprotective and trophic factors, increased neovascularization in the penumbra area and recruitment of endogenous progenitors have been all described to enhance functional recovery [31,51]. Additionally in 2011 Andres et al. [53] described the increase in dendritic plasticity, axonal transportation, sprouting and rewiring as some novel stem cell potential mechanisms and found that thrombospondins 1 and 2, VEGF and Slit, were important for dendritic and axonal outgrowth.

5. CLINICAL TRIALS

5.1 Mesenchymal Stem Cells (MSCs)

Jin Soo Lee et al. [54] conducted a randomized, observer blinded long-term safety and efficacy trial for intra venous transplantation of MSCs in patients with ischemic stroke. They included patients between 30 and 75 years old who had MCA territory lesions and had persistent neurological deficit after 7 days of onset [54]. Sixteen patients randomized to the MSCs group, underwent bone marrow aspiration 1 week after randomization and cells transplantation after 4 weeks. Primary outcome measure was long-term safety.

No significant side effects were reported, none of the patients developed a malignant tumor and co morbidities did not differ between groups. Regarding functional outcome 11 of 16 patients from the MSCs group had an improved functional outcome. In their opinion the MSCs group tended to have more patients with improved clinical outcomes when compared with the control group [54].

In 2011 Honmou et al. [55] reported a non-blinded study to assess safety and feasibility of autologous human MSC infusion. They included 12 patients with ischemic stroke disease, with both cortical and subcortical lesions, transplanted with autologous human mesenchymal stem cells expanded in autologous human serum delivered intravenously 36-133 days post-stroke [55].

They did not observe any significant adverse effects, any tumor formation or neurological deterioration. They measured mean lesion volume by magnetic resonance imaging and found it was reduced by 20% at week 1 post MSCs infusion [55]. Although this was not a blinded randomized trial, and did not assess overall functional outcomes their observations demonstrated the feasibility and safety of administering autologous human MSCs for ischemic stroke disease [55].

5.2 Human Neuronal Cells (LBS-Neurons; Layton BioScience, Inc.)

Kondziolka, Steinberg et al. [56] conducted an open-label, observer-blinded randomized trial for patients with stroke who received stereotactic implantation of human neuronal cells. They showed that although some patients did show improvement the study did not find evidence of a significant benefit in motor function as determined by the primary outcome measure. It did though indicate the safety and feasibility of neuron transplantation for patients with stroke [56].

6. FUTURE DIRECTIONS

6.1 Enhancing Intrinsic Neuro Repair Mechanisms

There are 3 main processes involved in brain recovery after stroke, development of new brain

pathways, activation of silent functional pathways and development of new dendritic sprouts in surviving neurons [57]. Angiogenesis, neurogenesis and synaptic plasticity are intrinsic neuro repair mechanisms that occur naturally after a brain insult and play a key role during the recovery period. These mechanisms can potentially be stimulated with exogenous treatments [57].

To date multiple pharmacological strategies have been tried but no definite conclusions were reached [58]. This is unfortunate since this kind of therapy would be an excellent alternative to more expensive and invasive treatments, especially in under developed countries.

6.2 The use of Biomaterials in Combination with Stem Cell Therapy for Stroke Treatment

From the neural stem cell perspective, these cells can be genetically modified to secrete different stimulating factors and therefore enhance above mentioned mechanisms [59]. Additionally transplanting scaffolds containing stem cells into an infarction cavity can provide the surface for new brain parenchyma regeneration as show in mice's stroke studies [60].

7. CONCLUSION

In summary, we have so far demonstrated that transplanted stem cells improved stroke recovery in multiple animal models, regardless of the administration route or the type of stem cells.

Further studies are needed to assess the optimal type of stem cells, timing for transplantation administration route and translate these findings into clinical trials.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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