Stem cell therapy for patients suffering from ischemic stroke – current perspective

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DOI: http://dx.doi.org/10.17486/gyr.3.1028

SUMMARY: Stroke is the leading cause of disability in the U.S. and is responsible for 4.4 million deaths per year worldwide. Only 10% of stroke victims are able to recover almost completely while the vast majority either die or are left with severe impairments requiring assisted living. The fixed neurological deficits are difficult to treat and because of this, even slight improvements are considered valuable. Studies have shown that stem cell therapy can provide trophic support or cell replacement in the infracted areas and improve the recovery and quality of life for patients. This article describes the possible candidates for stem cell repair in ischemic strokes and its mechanism of action. It discusses the advantages and disadvantages of each candidate, including the health hazards involved. Even though the use of stem cells to treat strokes in humans shows flourishing promise, there are still some obstacles and theoretical reasoning that need to be assessed in further detail in order to make it more effective. With an increased attention in using stem cells to treat ischemic strokes over the past 5 years, the process is soon to be incorporated into standard medical procedures.

KEYWORDS: clinical application of stem cells, embryonic stem cells, ischemic stroke, mesenchymal stem cells, neuronal stem cells, stem cell

The current treatments available for stroke recovery are limited, such as one method using tissue plasminogen activator based therapy[2] which is only effective if given within the first few hours of incident. In order to improve the somatosensory cognition and motor function of stroke ischemia in the brain, researchers are now focusing on utilizing stem cells (SC) as a novel new pathway to recovery. However, the mechanisms for utilization of these types of treatments are largely mysterious and needs to be elucidated further in order to start clinical trials on humans instead of animal test subjects. It has not yet been demonstrated if the implanted neurons or the endogenic neurons themselves repair the old connections or make arbitrary ones. Additionally, the capacity of self-renewal is not great enough for full neuroregeneration[3]. It is essential that not only neurons, but also oligiodendrocytes, astrocytes are formed. An intrinsic advantage enables asymmetric division of SC by allowing creation of new differentiating cells while simultaneously retaining additional progenitor cells for continued proliferation.

General Information

The basis of the mechanism can be determined by the observation of how the brain tries to compensate for the loss of function in the injured area. The are two large zones in the brain where the majority of neural stem cells can be found, the subventricular zone (SVZ) lining the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus.[4] In the healthy human brain these neural stem cells (NSC) can move via the rostral migratory stream (RMS) toward the olfactory bulb (OB). However, during a stroke, the cells utilize different migration methods in order to transport themselves to the site of injury and assist in the rejuvenation of the damaged area. One of the largest obstacles to overcome in this repair system is circumventing glial scar formation. The glial scars can exert this effect by physically forming a matrix that blocks NSC movement to injury site, it also releases chemical to debilitate the NSC migration and it transmutes promigratory cues into inhibitory cues. This thus slows down the rate of recovery and makes it impossible to recover totally.[4] The second great problem is that, human neuroblasts do not travel in a column of cells like in rodents, instead they travel individually because of longer axons. Thus, the results might not translate well from non-human to human experiments.

Neurogenesis and Migration

The mechanism of action is that the injured area releases trophic factors which induce differentiation and migration of NSC towards it. The movement of neuronal cells to the site of injury is influenced by a myriad of cytokines and chemokines such as GABA, VEGF, BDNF, PSA-NCAM, netrins, ephrins, β1 integrins.[4] The most important of them is stromal cell-derived factor 1 (SDF-1) that is expressed by migrating neuroblasts which functions in tandem with cysteine X cysteine chemokine receptor 4 (CXCR-4) receptor to reach its destination. SDF-1 changes the RMS-OB pathway of NSC towards the injured region. Figure 1 presents this schematically[4].

Sources of SC for clinical treatment

There are 3 sources of NSC that can be used for therapeutic use: embryonic stem cells (ESC), adult-derived neural stem cells (aNSC), bone marrow or mesenchymal stem cells (MSC). These 3 types are briefly presented in Figure 2[5].
ES are advantageous in regards that they are multipotent, self-renewing and a high rate of proliferation. On the other hand, the caveat of this method is the formation of teratomas and

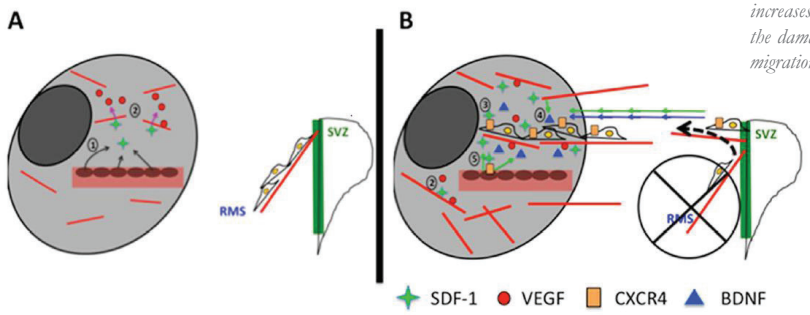

Fig 1:The production of SDF-1 functions as a positive feedback loop from the peri-infarct regions. SDF-1 increases the expression of VEGF which increases the angiogenesis, thus providing an increased nutritional supply to the damaged region. These extra vasculature structures allow for improved migration of NSC.(4)

ethical concerns considering the harvesting process. The risk of teratomas can be reduced by decreasing the density of donated ESC and using xenotransplants instead of homologous to prevent teratocarcinomas. The last solution of to predifferentiate ESC in vitro.

aNSC can be found SVZ and CGZ. These aNSC will proliferate when exposed to growth factors such as fibroblast growth factor (FGF) and epidermal growth factor (EGF). They form into either neuronal or glial cells. In different animal stroke models it has been shown that SVZ NCS were able to differentiate into neural and glial cell types in the hippocampus of ischemic stroke subjects. The recovery led to increased learning and memory function when compared to control test subjects. The genetic modification of NSC can augment the effect of regeneration process and allow the expression of a non-default phenotype[6]. The tumorgenic potential of this method is minimal compared to the ES method. A disadvantage for this type of SC is that they have a limited self-renewal capability compared to ESC. MSC studies have shown that when injected into an ischemic stroke region the neurons exhibit an increased regeneration, survival and function rate.[6] MSC are able to penetrate the blood brain barrier (BBB) allowing this therapy to be administered intravenously into the subject. Theoretically MSC activate supporting repair functions by activating neurotrophic factors rather than actually replacing the damaged cells. The transplantation efficiency and safety has not yet been well explored and is in need of further assessment before therapeutic usage.

Clinical Data

To determine the best course of treatment each cellular strategy must prove firstly by premedical models, followed by type I and II clinical trials and avoid hemorrhage inducement, viral and bacterial infection, tumorigenesis, inflammation, and seizures.

The greatest problem in these clinical studies has been the safety of patients because this technique is still in the nascent stages and the mechanisms are still convoluted. One of the safety precautions being that the induced cells were programmed in a way that apoptosis could be triggered if necessary. Stem cells are engineered with a suicide gene that can be switched on and prevent adverse effects like tumor formation. In one clinical trial, an intraparenchymal route was used to deliver the stem cells of primordial porcine striatum –type of ESC[7].

Friedrich et al. have conducted clinical trial involving intra-arterial infusion of MSC, which was taken from bone marrow, to patients with ischemic strokes of varying severity. The study graded the progress of therapy recovery based on National Institutes of Health Stroke Scale (NIHSS) where measurements are recorded using electroencephalogram (EEG). Improvement was defined as a change of 4 or more points on NIHSS. The success rate after 90 days can be noted as satisfactory with 6/20 of the patients claiming significant improvement, with 8/20 showing good improvement. The MCS were administered to the patients within hours of the stroke incident, this allowed for presumably maximum efficiency of treatment.

The clinical trial was executed with the intra-arterial method instead of the stereotactic implantation because it is less-invasive and contains a greater MSC cell count. While the actual mechanism is only theoretical, it is assumed that the added MSC stimulate the production of cytokines and trophic factors and do not replace endogenic cells at the ischemic site. Additionally these cells are thought to revascularize the ischemic tissues and increase angiogenesis and endothelium reconstruction. Thus allowing for a higher nutritional supply of the damaged tissue for enhanced effectiveness.

Serial reevaluations were screened via CT and MRI. The results showed no greater anatomical or structural inauspicious effects nor did any additional medical problem arise from the treatment itself[8].

Another clinical study aimed to discern the health safety and efficacy of intravenous autologous mesenchymal stem cell transplantation was performed by Jin Soo Lee et al. The known secretion of cytokines, growth factors, and trophic factors causing neurogenesis, angiogenesis and synaptogenesis by MSC were the basis of the experimental treatment. Another premise was to discover if the quantity of SDF-1α and CXCL 12 protein expression from the infarcted brain region has an effect on the quality of recovery. The study also wanted to determine if the usage of fetal calf serum or fetal bovine serum to incubate the stem cell culture would cause to any adverse effects from the xenogeneic compounds.

For the experimental patient group, 5mL of bone marrow was placed into the posterior iliac crest, which contained approximately 5 x 107 autologous MSC. This procedure was again repeated 2 weeks afterwards.

The group used the modified Rankin Scale (mRS), a method of quantitatively measuring the disability of patients with a neurological disability. The scale ranges from 0 (no symptoms) to 6 (dead). A mRS observer-blinded diagnosis was performed on the control and experimental MSC groups, 2.7-

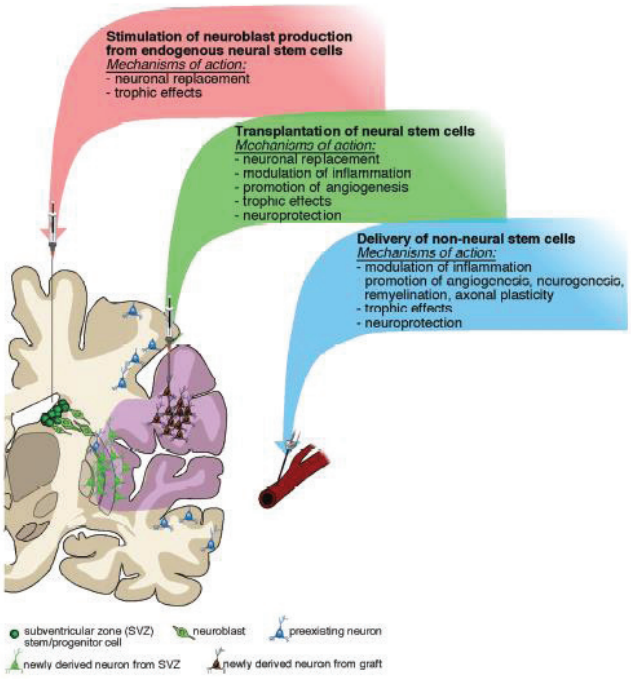

Fig 2. The theoretical benefits and mechanisms of action for different possible approaches in therapy.[5]

4.9 years after stroke incident. In the control group, 13/36 patients received a positive mRS score, indicating alleviation of symptoms. In the experimental MSC group, 11/16 patients received a positive mRS score. Figure 3 graphically outlines results.

Results found that the levels of SDF-1α at the time of MSC treatment positively correlated to a better mRS score after 1 year. This could mean that SDF-1αcould serve as a marker to determine if MSC treatment is a vital course of action. None of the experimental or control group experienced myoclonus, dementia, ataxia or prion diseases. However, it is believed that the risk of zoonoses from using fetal bovine serum could lead to a higher risk of an immunological reaction against the injected cells, or even an autoimmune reaction against the patient's own stem cells. Due to the decreased mortality rate in the experimental group and lack of directly contributable adverse effects, the group's data concluded that MSC therapy is an effective and safe method based on 5 years of follow up.[9]

A different method attempted by Sprigg et al., consisted of using granulocyte-colony-stimulating factor (G-CSF) to stimulate the growth of hematopoietic stem (CD34+) cells in order to regulate neutrophil progenitor proliferation and differentiation. Preliminary data in mice showed that it was effecting in bringing more neural progenitor cells to the ischemic site, reducing cerebral edema, improving survival and increasing sensory and motor recovery. It was shown that 5 daily doses of 10μg/kg G-CSF provided the largest increase in blood CD34+ levels, and provided some degree of clonal expansion and transdifferentiation of these cells into neurons, glia and vascular cells. Exact values using mRS were not measured, as the study focused on the safety of administering this method.[10]

A positive correlation has been observed between the number of CD34+ cell levels and ischemic stroke models of mice due to its ability to produce better neurological improvements. It increases β-nerve growth factor which is essential for increasing brain plasticity, axonal sprouting, synaptogenesis, neurogenesis and angiogenesis. Due to this information, Moniche et al. performed a single-blind clinical trial in which ten patients were treated with intra-arterially injected 1.59 X10 8 bone marrow mononuclear cells versus ten control patients. The mRS scores improved by less than or equal to 2. Again, this study focused on the feasibility and safety of future clinical procedures rather than on the efficacy, so mRS scores were the main way to grade the effectiveness.[11]

Conclusion

From the researcher's analysis of the stem cell treatment and biological background it can be surmised that while treating ischemic strokes by this method does seem promising, much more clinical and application work still needs to be done, especially on a larger scale. Also, the underlying mechanism on how the stem cells bring about a beneficial effect to the injury should be further deduced. The conflict between whether implanted cells merely stimulate the environment or actually renew the old cell neuronal circuits is still up to debate. Never the less, results from animal trials and early clinical trials have shown optimistic results for further study. Further analysis of the types of progenitor cells should be researched for determination for which type of cell line could prove to be most effective for clinical therapy. There are still many barriers considering the amount of stem cells needed to be injected into the injury as well as the time window in which therapy needs to be started in order to see beneficial results.

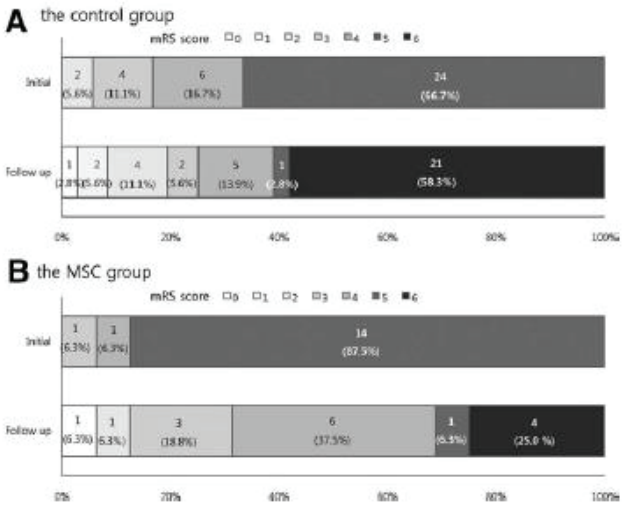

Fig 3: The results of the follow up observations for the control and MSC group.[9]

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Terapija matičnim stanicama kod pacijenata koji boluju od moždanog udara – dosadašnja dosezi

SAŽETAK: Moždani udar je vodeći čimbenik invalidnosti u SAD-u te godišnje uzrokuje 4,4 milijuna smrti u svijetu. Samo 10% ljudi koji su doživjeli moždani udar doživi skoro potpunu rehabilitaciju dok velika većina stradalih ima smrtni ishod ili trajne motoričke i kognitivne poremećaje koji zahtijevanju svakodnevnu skrb. Stagnirane neurološke defekte je veoma teško liječiti i najmanje poboljšanje stanja je smatrano dragocjenim. Studije su pokazale da terapija matičnim stanicama može trofički poduprijeti regeneraciju ili čak nadomjestiti odumrle stanice oštećenog predjela mozga i tako poboljšati oporavak i kvalitetu života pacijenata. Ovaj članak opisuje moguće kandidate za terapiju matičnim stanicama pacijenata akutne ishemije mozga. Raspravlja o prednostima i manama potencijalnih kandidata za tu vrstu terapije te mogućnosti smanjenja opasnosti za pacijenta. Još uvijek postoje prepreke kojima je potrebno posvetiti dodatna istraživanja. Potrebno je više kliničkih ispitivanja na većoj populaciji i dodatnih teorijskih objašnjenja za ovu perspektivnu terapiju.

KLJUČNE RIJEČI: akutna ishemija mozga, embriološke matične stanice, klinička aplikacija matičnih stanica, matične stanice, matične stanice mezenhima, živčane matične stanice