# Trophic Factors and Stem Cells for Promoting Recovery in Stroke


## Abstract

*Background:* Stem cell therapy for stroke is in its initial stages as an option to restore lost neurological functions after stroke.

*Objective:* To provide a comprehensive review of studies involving stem cells in stroke treatment and to highlight new evidence from the ongoing clinical trials.

*Methodology:* We performed a systematic study of various published journals in online medical libraries using Pubmed, Sciencedirect, and hajournal. Evidence synthesis is done with specific search words of – stem cell therapy, stroke, trophic factor, neural progenitor cell, pathophysiology, mechanism of action, clinical trial and mesenchymal stem cell in various combinations. Emphasis was given to articles published in year 2000 and onwards.

*Results:* Current research on stem cell therapy for stroke focuses on transplantation and endogenous neurogenesis of stem cells in brain. The sub-ventricular zone in the adult brain is identified as an endogenous resource of neuronal precursors that can be recruited to adjacent lesioned areas. Several factors can increase adult neurogenesis by stimulating formation or improving survival of new neurons, such as FGF-2, EGF, stem cell factor, erythropoietin, BDNF, caspase inhibitors, and anti-inflammatory drugs. Much of the beneficial effects of stem cell in stroke models are related to secretion of trophic factors.

*Conclusion:* The complex pathophysiology involving various trophic factors, growth factor and gene modification in animal studies have showed promising result. Future research involving these trophic factors should open up new additional or clinically significant alternative for the treatment of stroke.


*Key Words:* trophic factors, clinical trials, neural stem/progenitor cells, stroke, transplantation


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## Background

Current research on stem cell therapy for stroke focuses on transplantation and endogenous neurogenesis of stem cells in brain. We provide a comprehensive review of studies involving stem cells in stroke treatment and the new evidence from the ongoing clinical trials in the following sections.

## Pathophysiology and role of growth factors in stroke

A better understanding of the pathophysiology of stoke has resulted in developing new strategies aimed at reducing inflammation, reducing the scar tissue and promting angiogenesis within the penumbra region. Cell death is secondary to catalytic pathways of apoptosis and necrosis lead to cellular debris. The pathway leading to apoptosis is mediated by caspases[74,75] and modulated by genes in the Bcl-2 family.[76] Over-expression of Bcl-2 reduces neuronal apoptosis in vitro and vivo.[77-80] Intravenous administration of bone marrow stromal cells increases survivin and Bcl-2 protein expression and improves sensorimotor function following ischemia in rats.[29] Bcl-2 is implicated in neural differentiation and maturation,[46, 47] and in the induction and maintenance of axonal growth.[48–51] High levels of Bcl-2 expression also correspond to the entire phase of axonal elongation.[52] Limiting the debris from decreasing the cell death by increasing the stem cell survival may prevent an additional burden to the post-ischemic brain already compromised by a cellular debris load.[81, 82] Gene modification to over-express Bcl-2 in Embryonic stem cell promotes functional recovery after transient cerebral ischemia[8] [Table-1].

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Table 1: Effects of trophic factors / gene modification in stem cell transplanted in animal models of stroke.

| Trophic Factor/ Gene modified vector | Animal/procedure detail | Route of Delivery/ Time of delivery after stroke | Effect on lesion size | Survival/Number of cells | Functional Recovery | Actions |
|---|---|---|---|---|---|---|
| TGF-Alpha[109] | Female 4–6 week old C57B mice | Inta-parenchymal/2 weeks after stroke | 90 days poststroke: 50% reduction compared to control | Not done | Not done | Neurogenesis, angiogenesis, cell proliferation, |
| Angiopoietin-1 gene and the VEGF gene modified human mesenchymal stem cell[111] | Inta-parenchymal / 2 weeks after stroke | Intravenously infused 6 h later | Reduction in lesion volume. | Not done | MRI and behavioral analyses; greatest structural and improved functional recovery | Angiogenesis |
| Bcl-2 in ES cells[8] | Adult male Wistar rats(MCAO)/Gene modification | Intraparenchymal transplantation/ after one week | Not done. | Increased survival | ES cells overexpressing Bcl-2 recovered more fully than animals transplanted with wild-type ES | Reduced cell death within the transplant, enhanced differentiation into neuron-like cells, and increase in functional benefits |
| Erythropoetin[28] Survival / Number of cells | MCAO/tMCAO/ MCAO with reperfusion | Intraperitonial/ iv/intra-cerebro-ventricular | Not done | Not done | Improved functional recovery. | Anti-apoptosis, neuroregeneration and anti-inflammation |
| GCSF(11) | Adult male Wistar rats/tMCAO | Subcutaneous route daily /3 days after reperfusion | Not done | At 7 days, NeuN double-positive cells increased by 43.3% in the G-CSF-treated group, and endothelial cells were increased 2.29 times in the G-CSF-treated group, to a level as high as that in the vehicle-treated group ($P < 0.01$), in the periischemic area | Not done | Neurogenesis and neuroprotection |
| GDNF gene transferedNSCs cells[102] | (MCAO) MCAO with reperfusion/ Adult male Wistar rats(tMCAO) | Intravenously infused 3 days after reperfusion | Significant reduction of total lesion volume | GDNF/NSCs group were significantly more than those in the NSCs group. | Significantly improves functional outcome(mNSS scores decreased), and decreases lesion volume compared with control group. | Neurogenesis, neuroprotection |

## Angiogenesis factors

Angiogenesis and remodeling after stroke is mediated by several factors–bFGF, VEGF, angiopoietin-1 (Angpo-1), and angiopoietin-2 (Angpo-2), which are essential for the survival of transplanted cells. Basic FGF is a biologically active polypeptide with mitogenic, angiogenic, and neurotrophic properties. Basic FGF protects against hypoxia-ischemic insult in vitro[38] and in vivo[39] and enhances recovery of rat behavior following traumatic brain injury.[40] VEGF has a direct effect on neural cells and may be involved in neuroprotection as well as angiogenesis. Intra-cerebro-ventricular[57] VEGF administration 1 day after reperfusion reduces infarct size, improves neurological performance, enhances the survival of newborn neurons in the dentate gyrus and subventricular proliferative zone (SVZ), and stimulates angiogenesis.

## Angpo family

Angpo-1 is the ligand for the Tie-2 receptor on the endothelial cell surface[58] and stimulates phosphorylation of Tie-2 receptors in endothelial cells, Angpo-2 blocks the binding of Angpo-1 to Tie-2 receptors, suggesting that Angpo-2 may antagonize Angpo-1 in its activation of the Tie-2 receptor. A decreasing Angpo-1/Angpo-2 mRNA ratio may reflect sprouting of capillaries due to remodeling activity or low vascular remodeling activity, whereas a higher Angpo-1/Angpo-2 mRNA ratio is crucial for remodeling within the large vessels. Therapeutic benefit angiogenetic gene-modified[111] human mesenchymal stem cells after cerebral ischemia is discussed in the table.[1]

## Tumor Necrosis Factor ( TNF)

TNF levels can be increased in human brain with ischemia and other insults.[86–89] TNF-modulates the expression of several growth factors, such as VEGF and bFGF.[90] TNF exerts its neuro-protective action via activation of NF-B.[83,84] NF-B acts as a mediator in the TNF-α signaling pathway. NF-B may also play a role in the anti-apoptotic actions of Bcl-2.[85] The anti-apoptotic action of TNF can be reproduced by treatment with IB antisense oligonucleotides, which stimulates NF-B activation.[91] Treatment of neurons with NF-B decoy DNA,which selectively blocks NF-B activity, and abolishes the cyto-protective effect of TNF.[92] NF-B decoy DNA also increase kainite-induced neuronal death within the CA1 and CA3 regions of the hippocampus.[93]

## Transforming Growth Factor (TGF)

TGF-α can induce angiogenesis, neurogenesis, and neuroprotection after stroke,[109] TGF-α is a pleiotropic cytokine that binds to the epidermal growth factor receptor (EGFR) to produce its downstream effects.[16, 18] TGF-α treatment caused a fourfold increase in the influx of GFP(astrocyte markers, glial fibrillary acidic protein) cells into the ischemic hemisphere in the brain compared with vehicle control. A 2.4-fold increase in the area covered by blood vessels surrounding the infarct was seen compared with vehicle controls. NSC marker nestin was significantly more abundant in animals treated with TGF-α. Both TGF-α and EGFR are present in the SVZ where they modulate the activity of NSC and NPC.[13] Notch signaling pathway mediates adult SVZ neural progenitor cell proliferation and differentiation after stroke. Exogenously applied TGF-α increases NSC number and survival and can induce differentiation to neural and glial cells.[16, 17] TGF-α also reduces the infarct size after ischemic injury; an effect that is also mediated by EGFR.[14] [Table-1]

## Glial Derived Neurotropic Factor(GDNF)

GDNF has a potent neuroprotective effect on a variety of neuronal damage both in vitro and in vivo.[93, 94, 95, 96] Exogenous GDNF gene transfer reduced the infarct size in rat middle cerebral artery occlusion (MCAO) model[97, 98] and promoted striatal neurogenesis after stroke.[99] The transient effects of GDNF needs repeated administration into intracerebral or intraventricular space. In addition, simple application of GDNF protein is difficult to administer in clinical situations because of the blood–brain barrier.[100] Topical application of GDNF decreased ischemic brain edema and number of TUNEL-positive neurons by suppressing apoptotic pathways such as caspases-1 and 3.[101] No significant differences in modified neurological severity scores (mNSS) scores were detected among the groups at 3 days after reperfusion (transplantation time point). The scores of mNSS at different times after reperfusion in the NSCs and GDNF/NSCs groups were significantly lower compared with the control group. From 1 to 7 weeks after reperfusion, the scores of mNSS in the GDNF/NSCs group were decreased compared with NSCs group, but significant decrease was observed only at 2 and 3 weeks after reperfusion. GDNF improves the functional recovery in the animal model of MCAO.[102]

## Granulocyte Colony Stimulating Factor (GCSF)

G-CSF is a 19.6-kDa glycoprotein that is a member of the cytokine family of growth factors, along with TNF-α and the interleukins. G-CSF[12] administration in Wistar rats has shown to potentiate neuroprotection and neurogenesis.[11] G-CSF has been used for 10 years for the treatment of neutropenia[54] bone-marrow reconstitution[53] and stem cell mobilization.[55] Above study demonstrated absence of G-CSF led to enlarged infarct volumes and impaired functional recovery and substitution significantly abolished detrimental effects of G-CSF deficiency, leading to a reduction of infarct volumes and improved functional recovery. G-CSF deficiency increased the MMP-9 response in the direct peri-ischemic area after MCAO, whereas substitution of G-CSF suppressed the increase of MMP-9 (Matrix metalloproteinase 9) expression. Endothelial cells secrete MMPs, which might in turn lead to neurovascular matrix degradation associated with increase in vascular permeability.[56, 57]

## Erythropoetin ( EPO)

Erythropoeitin[28] was tested in clinical trials as a possible treatment for adult stroke and found to be both safe and beneficial.[58] Protective effects by EPO presumably results from a decrease in apoptosis, an increase in neuroregeneration, and contributions to anti-inflammation and angiogenesis.[110] In vivo,

**Table 2:** Potential mechanism of action of stem cells in restoring neurological function after stroke

| Mechanism | Method of Action |
|---|---|
| Secretion of growth factor/ Trophic factor | Stimulation of plastic response, improved survival and function of host response. Reduction in host cell death. recruitment of endogenous progenitors. Neovaculatization. |
| Tissue damage | Attenuation of inflammation, interference with the host neural activity. |
| Correction of biochemical deficit | Release of missing transmitter(minipump) |
| Reconstruction of neural circuitries | Re-establishment of functional afferent and efferent connections |

EPO administration reduced TNF-α, IL-6 and monocyte chemo-attractant-1 production in adult rats and reduced microglial activation and cerebral leukocyte influx in neonatal rats subjected to MCAO.[59, 60] Anti-inflammatory activities of EPO are likely mediated via reduced neuronal cell death thereby attenuating the cerebral attraction of inflammatory cells. Endogenously or exogenously applied EPO may stimulate the EPOR to induce phosphorylation of JAK2.[21, 22, 23] JAK2-phosphorylation in turn activates PI3K, induces the translocation and subsequent activation of NFκB and/or stimulates STAT5 homodimerization thereby initiating a number of downstream molecular cascades.[28] In vivo studies showed that inhibition of JAK2 or PI3K abolished the neuroprotective effects of EPO.[41, 42] Prolonged hypoxia induces cell death[61] and the detrimental consequences can be partly counteracted by an increase in endogenous EPO production from astrocytes.[62, 63, 64] Especially astrocytes, and also oligodendrocytes, endothelial cells, neurons and microglia can produce EPO.[64, 65, 66, 67, 69, 70] The homodimeric EPO receptor has been demonstrated on neurons, astrocytes, endothelial cells and microglia[66, 68, 71, 72] EPO treatment enhanced revascularization after stroke and Hypoxic Ischemic insult in adult and neonatal animals.[43, 44] Further more, in adult mice after focal cerebral ischemia, EPO was found to improve cerebral blood flow.[45] Protein levels of the angiogenic factors Tie-2, Angiopoietin-2 and VEGF were also increased by EPO treatment.[45] Chronic EPO usage has been associated with red cell aplasia,[46] hypertension and increased risk of thrombosis. Exogenous EPO (carbamylated EPO) may hold great promise for future treatment of focal and global cerebral injury, and further research involving the development and safety-profile of non-erythropoietic EPO alternatives should be encouraged. Better understanding of mechanism of neuroprotection is needed, It remains to be clarified the best time for EPO treatment after brain damage with respect to its anti-apoptotic and anti-inflammatory effects, its effects on the vascular system, and its effects on the regeneration of neuronal progenitor cells.

## Stem cells

The beneficial effects of stem cells are summarized in Table-2. Different types of cells used are 1. Neural Stem/Progenitor Cells, 2. hNT Neurons, 3. Bone Marrow, Umbilical Cord Blood, Peripheral Blood, and Adipose Tissue Cells. Now Nurr1-positive neuronal stem cells are generated from human wisdom teeth (tNSC) and the methodology is simple and can lead to new effective potential ways of stem cell transplantation for stroke[103] These NSC when injected into the brains of Sprague-Dawley (SD) rats affected by ischemia induced by MCAO treatment facilitated functional recovery. Easy availability of tNSC provides prospective neuronal stem cells for autologous and allogeneic transplantation. tNSC cells express MSC-specific markers,

**Table 3:** Non-invasive cellular imaging modalities.

| | | |
|---|---|---|
| 1. 19F MR hot spot MR imaging | 2. CEST MR imaging<br>3. (Chemical Exchange Saturation Transfer) | 4. X-ray/CT imaging |
| 5. Ultrasound imaging | 6. Bioluminescent imaging | 7. Near-infrared imaging |
| 8. PET imaging | 9. SPECT/radionuclide | 10. Electroencephalography |
| 11. Optical Imaging (Green Flourescent Protein, intravital microscopy). | 12. 1H MR imaging using metal-based (SPIO) contrast agents | |

**Table 4:** Summary of results of clinical trials:

| Clinical trial | No. of patients | Time of transplantation (after stroke) | Disease | Cell type | Route of transplantation | Therapeutic intervention | |
|---|---|---|---|---|---|---|---|
| | | | | | | Cell replacement | Fuctional recovery |
| Phase I[104] | 12 | Mean: 27 months (range: 7–55) | Basal ganglia infarcts | Human NT2 / D1 teratocarcinoma-derived NPCs | Intraparenchymal | (+) | Some improvement |
| Phase I[106] | 5 | Mean 5 years | Basal ganglia infarcts | Fetal porcine cells | Intraparenchymal | Not tested | No improvement |
| Phase I/II[107] | 30 | 4–9 weeks | MCA infarcts | Autologous mesenchymal precursorcells | Intravenous | Not tested | Some improvement |
| Phase II[105] | 18 | Mean: 3.5 years (range: 1–5) | Ischemic/ hemorrhagic infarcts | Human NT2/D1 teratocarcinoma-derived NPCs | Intraparenchymal | Not tested | Some improvement |

neuronal-specific marker , a migratory potential, and also retain the pluripotent plasticity of embryonic stem cells. The growing body of research focus on which cell to be used is in a stage of conclusion, and autologous tNSC and MSC can be that choice.

Stem cell transplantation coupled with endothelial cells increased the survival, proliferation and accelerated neuronal differentiation of ischemic induced neural stem/progenitor cells compared with transplantation of neural precursors alone in the subventricular zone.[1] NSCs deposited on synthetic extracellular matrix and were implanted into the ischemia-damaged area generated new vascularized parenchyma comprising of neurons and glia.[15] Combining stem cell therapy with endothelial cells in a seeded extracellular matrix might yield better result. Compensating the loss of matrix with the PLGA (plasma polymerised allylamine (ppAAm)-treated poly (d,l lactic-acid-co-glycolic acid) scaffold particles can act as a structural support for neural stem cells injected directly through a needle into the lesion cavity using magnetic resonance imaging-derived co-ordinates.[9] These neuro-scaffolds integrated efficiently within host tissue forming a primitive neural tissue.

The ongoing research will tell us whether stem cell alone are sufficient for regeneration or stem cell needs exogenous trophic factor/transinfection with a gene for the optimization of neurogenesis of the ischemic tissue. Adult derived mesenchymal stem cells (MSCs) exert their trophic action by secreting bioactive factors suppress the local immune system, inhibit fibrosis (scar formation) and apoptosis, enhance angiogenesis, and stimulate mitosis and differentiation of tissue-intrinsic reparative or stem cells.[2] Nevertheless the avalanche of data regarding which cell type is best suited has generated understanding of the potential cell characteristics. NSCs may be minimally immunogenic Modo et al.,[113] whereas marrow stromal cells (MSCs) may provoke a robust inflammatory response leading to rapid acute rejection Coyne et al.[114]. Immunosuppressive drugs such as cyclosporine A may also promote sprouting of host neural cells, potentially leading to functional improvement independent of the grafted cells. Challenges of serious side effects with the importance of immunosuppressioon in stem cell-based therapies will assume significant importance as the research move closer to clinical trials.

## Monitoring of stem cell activity in vivo

Different methodology to track stem cells fate and migration post transplantation are currently available (see Table 3). The superparamagnetic iron oxide (SPIO) nanoparticles used to label various cells for monitoring their temporal and spatial migration in vivo by magnetic resonance imaging (MRI) is emerging as good monitoring modality. Microgel Iron Oxide Nanoparticles For Tracking Human Fetal Mesenchymal Stem Cells through MRI did not affect either cellular proliferation or tri-lineage differentiation.[5] MRI of grafted adult as well as ESCs labeled with iron oxide nanoparticles is another method in evaluating cellular migration toward a lesion site.[6] Different combination of cellular imaging modalities can be made from the available choices. Application of HSVtk suicide gene to spio-labelled cells is another option however no evidence is found in current research.

## Route of administration of stem cells

Different routes of stem cell delivery are stereotactic parenchymal injection,[19,20,23] intravenous infusion,[34] intraventricular injection,[22] targeted intra-arterial infusion.[32] Although intra-arterial infusion targets the entire ischemic lesion, limitation include compromising the cerebral blood flow.[7] Intravenous route is safe but requires repeated dosing, causes initial random dispersion, and trapping of cells in the filtering organs.[25,26] Parenchymal injection is precise needs multiple injections which can enhance brain injury and can lead to non uniform cell distribution with

the risk of graft malfunction.[37] The route of delivery may influence recovery: intracerebral delivery of MHP 36 cells enhanced sensorimotor function compared to intra-cerebroventricular delivery only affected learning and memory.[30]

## Evidence from clinical trial

Initial trials were designed to test safety and identity. Adverse events observed in these trials were transient and reversible (see Table 4). Kondziolka et al, published the results of a Phase I study of 12 patients with completed stroke involving the basal ganglia treated with human neuronal cells derived from an immortalized tumor cell line[104] (LBS cells). Phase II study[105] from Kondiolka et al with randomization of 18 patients to either implantation of 5 or 10 million cells and rehabilitation (14 patients) or rehabilitation alone (4 patients). Savitz et al reported the results of transplantation of fetal porcine cells implanted stereotactically in 5 patients with basal ganglia stroke 3 months to 10 years after onset.[106] The study was discontinued due to adverse events in 2 patients. One patient had transient weakness resolving after 10 days due to cortical vein occlusion. Another patient had seizures in the setting of hyperglycemia and a ring-enhancing lesion on MRI remote from the transplant site. The lesion resolved in 3 months. The relationship between these 2 complications and the transplants is uncertain and there is no clear indication that the cells were responsible. Bang et al, reported upon autologous mesenchymal stem cells given intravenously to 5 patients with middle cerebral artery territory strokes 4 to 5 weeks and again at 7 to 9 weeks after onset.[107] An additional 25 patients served as control subjects. All patients were followed for functional outcome and adverse events.

In the phase I LBS study, 8 patients had infarcts restricted to the basal ganglia and in 4 the cortex was involved. The phase II LBS trial included 9 ischemic strokes and 9 hemorrhages. Six of the 7 patients receiving 10 million cells had hemorrhagic strokes, whereas 5 of 7 strokes in the 5 million cell group were ischemic. A dose–response was not observed in this study with 4 of 7 patients in the 5 million cell group and 2 of 7 in the 10 million cell group improving at 6 months. The imbalance in hemorrhagic strokes and lack of improvement in the patients receiving higher numbers of cells suggest hemorrhagic and ischemic stroke may respond differently to cell therapy.

One patient in the phase I LBS trials suffered a seizure at 6 months and in one patient a brainstem stroke occurred 6 months after implantation. Two patients died of unrelated causes. In the phase II trial, a seizure occurred in one patient postoperatively and another was found to have a subdural hematoma 1 month after surgery requiring surgical drainage. In the study of intravenous mesenchymal stem cells, no procedure-related adverse events or complications related to the cells were observed up to 1 year after treatment.

In the phase I LBS study, 7 of 12 patients improved on the European Stroke Scale at 2 years. In the phase II study, 6 of 14 patients improved at 6 months, no difference in the mean change in European Stroke Scale motor scores between treated patients and control subjects. Four of 7 patients with nondominant hemisphere strokes showed improvements on tests of visuospatial ability and nonverbal memory.[108] In both studies[104, 105] and also in Savitz et al[106] study, several patients reported subjective changes, including improved walking, reduced stiffness, and improved memory. Improvement in gross movement measured by the Action Research Arm Test was observed in treated patients compared with control subjects and between pretreatment and posttreatment evaluations Improvement in Barthel Index was observed in patients treated with mesenchymal stem cells compared with control subjects after 1 year. Patients receiving mesenchymal stem cells had less peri-infarct atrophy and less ventricular dilation but no difference in infarct volume.

In a recent study, Bone marrow stem cells (BMSC) transplanted into perilesional area in stroke patient were safe with excellent tolerance and without complications.[3] Although some improvements were noticed in patients but the patient population was too small to make any statement. Current ongoing arterials are using autologous BMC transplantation through intra-arterial route in patients with Ischemic Stroke. Neurologic benefit resulting from hMSC treatment of stroke in rats may result from the increase of growth factors in the ischemic tissue, the reduction of apoptosis in the penumbral zone of the lesion, and the proliferation of endogenous cells in the SVZ .

## Conclusion

Fundamental questions related to the optimal candidate (including the patient age, etiology, anatomic location and size of the infarct, and medical history), the best cell type, the number and concentration of cells, the timing of surgery/procedure, the route and site of delivery, and the need for immunosuppression remain to be answered. Various vascular growth factors and trophic factor play a significant role in postischemic angiogenesis, neurogenesis and reduction in the infarcted volume—which are vital to the functional improvement.

Many of the actions of stem cell in recovery from stroke have been through secretion of trophic factor and biological amines. The percentage of functioning cells after transplantation is very low (6-8%) and devising strategy to optimize the functions of implanted cells may prove beneficial. Gene modification and growth factor replacement might play a crucial role in increasing the percentage of cells. Clinical trial evidence has shown that higher percentage of living transplanted cell correlated with functional improvement in patients monitored by positron emission tomography scans.[112] It is imperative to explore the induction of these factors with stem cell transplantion in future research and clinical trial.

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