Stem Cell Therapy for CNS diseases - Therapeutic Role, Current Status, Challenges and Perspectives

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Abstract

Many of the human central nervous system (CNS) diseases are mimicked in animal models to evaluate the efficacy of stem cell therapy. The therapeutic role of stem cell in replacement of diseased neurons, oligodendrocytes, astrocytes, secretion of neurotrophic factors and delivery of therapeutics/genes has been reported. Scaffolds can be used for sustained delivery of stem cells and may play role in promoting their neuronal differentiation. Though many of the animal studies have progressed to clinical trials, stem cell therapy in clinical setting is still facing so many challenges. This review discusses the therapeutic role of stem cells, use of scaffolds in stem cell therapy, clinical status, challenges and future perspectives of stem cell therapy in central nervous system diseases.

Keywords: stem cell therapy, CNS diseases, cell replacement, gene therapy, challenges, scaffold.

Introduction

Treatment of CNS injury had always been a challenge for medical and veterinary clinicians. It was in the naive stage until early 20th century ⁹⁰,¹⁴,¹⁵ as the recovery in CNS is limited by the insufficient self-repair and regeneration of the brain tissues ²⁰,²¹,²³,²⁸. The main challenges in the treatment of brain diseases included blood brain barrier (bbb) with tight intracellular junctions and absence of fenestrations ²⁹,¹¹,⁹ which prevents the uptake of majority of therapeutics ¹⁸,¹⁷,¹⁰, active drug efflux pumps in the bbb (Golden and Pollack, 2003), which pumps out the drugs from the brain, high intercellular fluid pressure due to space occupying lesions that limits diffusion ⁵,⁷,¹⁷ and the sensitivity of brain tissue that emphasizes the need for appropriate and precise dosing of chemotherapeutic agents ¹⁵-¹⁶. Unrelenting reports on therapeutic uses of stem cells for CNS diseases have led to their wider acceptance and importance in the present scenario. Though according to the early school of thoughts, neurons of adult CNS of mammals have limited regeneration capacity, later studies have confirmed that subgranular zone and dentate gyrus of hippocampus and lateral ventricles of forebrain are regions of potential neurogenesis in adult mammalian brain. The endogenous regeneration potential of CNS could be stimulated to aid the repair of damaged brain tissue ³¹,¹⁴,³⁰,³². Even from the areas of adult CNS where neurogenesis is not apparent, stem cells and their progenitors can be extracted, expanded and differentiated into neurons and glia in culture ³³,³⁴. Innumerable diseases including stroke, brain tumors, epilepsy, Parkinson’s Disease (PD), Huntington’s disease (HD), Alzheimer's disease (AD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and spinal cord injuries could benefit from stem cell therapy ¹¹,⁵⁰,⁵⁶,⁵¹,⁹,⁴⁰,⁴¹,⁴⁴. The term stem cell is a broader concept. The stem cells which are used for treating CNS diseases include adult
neural precursor cells, bone marrow derived mesenchymal stem cells, adipose derived mesenchymal stem cells, umbilical cord blood mesenchymal stem cells, embryonic/foetal mesenchymal stem cells and induced pluripotent stem cells. Induced pluripotent stem lines derived from reprogramming adult somatic cells to an embryonic stem cell state are a potential autologous source of stem cells according to Hu et al. (2010), and they could be differentiated even to neurons. The therapeutic role of stem cells in treatment of CNS diseases include cell replacement, delivery of genetically engineered genes and drugs via stem cells, release of neurotrophic factors that provide neuroprotection and reduction of glial scarring and release of vasoactive factors like anti-inflammatory cytokines which also provide neuroprotection.

**Stem cells for cell replacement**

Neural stem cells (NSCs) are the most logical stem cell type to be used in neural tissue engineering as they have the ability of self-renewal and can be differentiated into neurons, astrocytes, and oligodendrocytes. NSCs are mostly harvested from SVZ region of brain. If meant for generation of dopaminergic neurons, NSCs are harvested from the ventral midbrain. Amyotrophic Lateral Sclerosis is a neurodegenerative disease of the CNS causing abnormal function and degeneration of motor neurons in human spinal cord, cerebral cortex and brainstem resulting in rapidly progressing muscle weakness and death due to respiratory failure in a few years time period. Multiple Sclerosis (MS) is a chronic inflammatory disease of CNS in which there is demyelination of axons. Oligodendrocytes are the glial cells in CNS concerned with myelination of axons and hence stem cell derived oligodendrocytes could be a promising therapeutic option for MS. Research had shown that NSCs upon differentiation into oligodendrocytes can remyelinate axons in a model for multiple sclerosis. NSCs could differentiate into cholinergic neurons, astrocytes, and oligodendrocytes and helped in amelioration of the learning/memory deficits in animal models of Alzheimer’s disease. Parkinson's disease (PD) which is a progressive, idiopathic neurodegenerative disorder of the CNS where there is dysfunction and loss of dopamine secreting neurons in the substantia nigra, leading to the characteristic symptom of debilitating motor impairments. Stem cell therapy could aid in its cure by serving as a source for dopaminergic neurons. When dopaminergic neurons generated from stem cells were transplanted into primate models of Parkinson’s disease it diminished symptoms observed in this neurodegenerative disorder. Stem cells have found a role in successful treatment of stroke as there are reports on migration of neural progenitor cells towards the lesion with formation of new neurons and reestablishment of neural connections with functional recovery. The finding that human embryonic stem cell derived oligodendrocyte progenitors and motor neuron progenitors, when transplanted into the transected spinal cord of adult rats immediately after the injury, could differentiate into oligodendrocytes, astrocytes and neurons in addition to improving locomotor functions without teratoma formation shows the role of stem cells in cell replacement in spinal cord injury. Though the bone marrow stem cell could give rise to a lesser proportion of neuron like cells in comparison to brain derived neural stem cells, they could be promising therapy for CNS injury and neurodegenerative diseases.

**Gene therapy using stem cells**

Gene therapy is the concept and procedure for transfer of therapeutic genetic material into the cells to cure disease. Using stem cells ex-vivo gene therapy is performed, which means that the genetic material is transferred into the cultured cells prior to transplantation.
embryonic stem cells or neural stem cells are used owing to their expanding capabilities and differentiation potential to various types of neural cells. However, they have drawbacks because of limited ability to get incorporated into the brain and to generate the desired neural phenotype. Genetically engineered stem cells have proved useful in Parkinsonism; Huntington’s disease and gliomas. IFN – β + CD + ; IL-4; IL-12; IL-23; TRAIL; PEX; endostatin; aaTSP 1; oncolytic adenovirus transgene modified neural stem cells in animal models of glioma intratumorally, intravenously or intracranially showed reduction or inhibition of tumor and higher survival times. Genetic modification of NSCs with NT-3 has been reported to promote myelination and to reduce astroglial scarring after transplantation in rodents with spinal cord injury or ischemic brain injury. GDNF (glial cell line derived neurotrophic factor) – over expressing neural stem/precursor cells were shown to increase the survival of neuronal cells for up to 3 months post transplantation in the striatum of presymptomatic transgenic mouse model of Huntington’s disease and to delay the degeneration of motor neurons in the spinal cord of rat model of ALS. Genetically engineered stem cells expressing cytokines have reported promising results in glioma models following intracranial administration. Transplantation of genetically modified embryonic stem cell derived cells overexpressing neuroprotective factors results in functional recovery in animal models of ischemia 12.15.81.59.74.72.60. Huntington’s disease is caused by mutation of gene coding for protein mHTT (mutant huntingtin protein) resulting in cellular toxicity. Research in several HD animal models has shown that neuronal survival could be prolonged by enhancing the degradation/clearance of this protein from affected neurons. Patient derived iPSCs could be used for studying gene manipulation strategies for achieving this. Genome editing approaches directly targeting DNA for reducing this protein has shown success in patient iPSC-derived neuronal models and needs validation in in vivo. Small interfering RNAs can reduce mHTT and studies regarding safety and efficacy of siRNA delivery system using human MSCs are underway.

**Release of trophic factors by stem cells and other paracrine effects**

MSCs and Neural/progenitor cells secrete immune modulatory or neurotrophic paracrine factors which may have therapeutic benefits in treating experimentally induced CNS diseases in animal models Lavoie and Rosu-Myles, (2013), have reviewed that beneficial effects of MSCs may be attributed to the immunomodulation and paracrine activity rather than the stem cell plasticity. In Parkinson's disease, NPC transplants secreting glial-derived neurotrophic factor (GDNF) and vascular-endothelial growth factor (VEGF) have shown beneficial results in experimental studies and are being assessed in pre-clinical trials for the treatment of the disease. Upregulation of SDF-1, VEGF, and TGF β was noted in MSC transplanted spinal cord injury models of beagle dogs. Many recent studies focus on the use of paracrine effects of stem cells in therapy of CNS disease, where instead of going for implanting stem cells, the biologics secreted by stem cells termed as ‘secretome’ are used for repairing injured brain. Human umbilical cord blood-derived mesenchymal stem cells delivered intracranially, in a mouse model of Alzheimer’s disease, improved spatial learning and memory decline possibly by neuroprotective effect by inducing a feed-forward loop by alternative activation of microglial neuroinflammation. Inconsistent finding was reported where single intracerebral injection of only neuron-like cells, differentiated from human umbilical cord derived mesenchymal stem cells (hUMSC) but not the undifferentiated hUMSC, ameliorated memory deficits in mouse model of Alzheimer’s disease possibly by alternative activation of microglia cells leading to modulation of neuroinflammation. The inconsistent findings with hUMSC might have resulted from single injection verse multiple injections of mesenchymal stem cells given intracerebrally 16.17.45.). Elevated amyloid β-peptide deposition is the key pathogenic factor for neuronal
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loss in Alzheimer’s disease (AD). It was found that transplantation of hUMSC in transgenic mouse model of AD reduced amyloid plaques by secretion of a soluble intercellular adhesion molecule-1(sICAM-1) that induce Aβ degrading enzyme. This outlines the paracrine mode of action of hUMSC.

**Stem cells as vector for drug delivery**

Stem cell therapy combined with nanotechnology could be a promising tool to efficiently deliver drugs to brain tumors. Glioblastoma, is a lethal malignant tumor where the standard protocols like surgical resection followed by fractionated radiotherapy with concomitant chemotherapy can only prolong the life span by near about one year. Advances in the field of nanotechnology have led to the development of chemotherapeutic loaded nanoparticles, where the therapeutic agent is entrapped in, adsorbed or chemically coupled onto the nanoparticle surface. By this technique the therapeutic agents are protected from enzymatic and chemical degradation, thereby ensuring its sustained and controlled release. The stem cells can be used to carry the drug bound nanoparticles to the lesion site. Neural stem cells, owing to their tropism towards glioma cells and ability to cross bbb makes them excellent carriers for cytokines, viral particles and prodrugs. Mesenchymal stem cells, owing to their ability to migrate and localize around glioma cells by mechanisms mediated by epidermal growth factor, stromal-derived factor-1 platelet-derived growth factor, matrix metalloproteinase-1, and macrophage chemoattractant protein-1, could be used in glioma treatment. Once the chemotherapeutic loaded nanoparticles are incorporated in the stem cells in vitro via passive or active endocytosis or spontaneously, the stem cells can be injected intracranially into the tumor mass which could localize in the border between tumor cells and normal brain parenchyma. This concept has been proved by Roger et al. (2011), using MIAMI (Marrow-Isolated Adult Multilineage Inducible) cells, a subpopulation of human MSCs. Increased survivability and inhibition of tumor growth and proliferation have been reported when NSC mediated delivery of secreted soluble variant of TRAIL has been combined with therapeutics like proteasome inhibitor bortezomib and kinase inhibitor PI-103.

Stem cells also have a role in treatment of epilepsy by delivery of adenosine which is a purine ribonucleoside with neuromodulator and neurotransmitter functions. Antiseizure and neuroprotective potentials of adenosine are known for long. The role of adenosine in seizure results from its binding to the presynaptic A1 receptors which inhibits the release of excitatory neurotransmitters like glutamate. Systemic use of adenosine has severe side effects like decreased blood pressure and heart rate which emphasizes the need for its local delivery into the brain via stem cells. According to Dycke et al. (2010), astrocytes derived from neuroprogenitor cells (NPCs) and undifferentiated NPCs of adenosine kinase deficient mice released amounts of adenosine which could be of therapeutic relevance under in vitro conditions. In case of brain tumors therapeutic delivery may be needed for a short time duration only whereas in epilepsy, which is a chronic disorder that needs lifelong local delivery of therapeutics, cell or gene therapy sounds theoretically a successful strategy as long term release can be ensured without replacement or refilling.

**Scaffolds for stem cell delivery in CNS diseases – a new arena for sustained release and survival**

Scaffolds usually assist cell proliferation and differentiation, as they allow diffusion of nutrients and exert mechanical and biological influences on the cell. Delivery and duration of action of stem cells could be prolonged by use of suitable scaffolds. They can also be used for sustained release of lineage-specific inductive factors or small interfering ribonucleic acids (siRNAs) as molecular mediators of differentiation. The efficacy of transplantation of NPCs can be improved in CNS injury by the co-
administration of biomaterial scaffolds. Biomaterials made of nanofibres, nanotubes and nanoparticles have been widely used in manipulating the fate of stem cells. Carbon nano tubes can provide support, direct the differentiation of stem cells to neural lineages and could promote signal transmission among neurons. A nano-biohybrid system created by NSC progeny and graphene showed that graphene films can not only support neural network without affecting its structure and function but also could enhance the network activity and efficacy of neural signals. In ICC construct, ie., inverted colloidal crystal scaffold comprising chitin, chitosan and gelatin, mouse iPS can remain viable. Also it can accelerate differentiation of iPS to neurons (Kuo and Lin, 2013). The knowledge of downregulation of RE 1 silencing transcription (REST) factor in embryonic stem cells and neural progenitor cells upon differentiation of neurons had been utilized to enhance in vitro neuronal differentiation of stem cells. Low et al. (2013), investigated the possibility of scaffold mediated gene silencing by delivering small interfering RNA/transfection agent complexes via Mussel-inspired polydopamine modified electrospun polycaprolactone nanofibre scaffolds and concluded that it could be a future promise for therapy as enhanced neuronal commitment of primary mouse neural progenitor cells and decreased glial cell differentiation was seen.

Clinical trials using stem cell therapy in various CNS diseases

Clinical utility of stem cell therapy is being evaluated for treatment of a large number of CNS diseases and it is in different phases. In Mutiple sclerosis, phase I clinical trials in human patients are in progress to evaluate the safety and proof of concept of using autologous bone marrow derived mesenchymal stem cells. In basal ganglia ischemic or haemorrhagic stroke, randomized phase II clinical trials in humans were conducted by giving neural stem cells by stereotactical surgery combined with cyclosporine a week before and six months after surgery. The study concluded that cell related adverse effects were absent and nearly half of the study group showed clinical improvement at six months. However, the procedure had comorbidities like a single seizure, an episode of syncope and subdural hematoma. In another study by Bhasin et al. (2013), conducted in 40 human patients following 3 months to 2 years of index event of stroke, it was concluded that autologous intravenous mesenchymal stem cell therapy is safe and feasible. According to Karussis et al. (2013), human NSCs when used in clinical trials of Parkinson’s disease were less promising owing to the lesser number of dopaminergic neurons (15%) produced following transplantation. Phase I clinical studies were conducted in 10 human Amyotrophic Lateral Sclerosis patients where in vitro expanded autologous bone marrow derived mesenchymal stem cells suspended in autologous cerebrospinal fluid were transplanted into the spinal cord at a high thoracic level by a surgical procedure. It showed that the therapy is safe but the vitality of transplanted stem cells could not be traced because of lack of post mortem specimens. In addition to those mentioned above, currently clinical trials with stem cells are going on in various neurological diseases like MS, brain tumors, spinal injury, and also genetic–metabolic diseases and leukodystrophies, Huntington's disease, Alzheimer's disease, multi-system atrophy, cerebral palsy, peripheral neuropathy, myasthenia-myopathies, epilepsy and systemic autoimmune diseases with neurological complications. Initial clinical studies showed promising results in terms of their therapeutic potential. The therapy using autologous stem cells was largely safe. Further studies using allogenic stem cells are under progress.

Challenges and perspectives

The main limitations of stem cell therapy are with regard to the excessive cost of commercialization, and the anticipated difficulties of clinical translation and regulatory approval. Another major problem related to NSC mediated regeneration in trauma to CNS is due to the upregulation of
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inhibitory immune factors around the site of lesion resulting from the inflammatory process that ensues. Recent studies by Kyritsis et al. (2014) suggest that in mammals, acute inflammation is followed by chronic inflammation, which prevents functional recovery of brain tissue. Survival of the implanted differentiated neurons is to be ensured after transplantation for successful outcome. In ALS, though it had been shown that motor neurons derived from stem cells can be grafted safely without any rejection, the microenvironment remains hostile for their survival because of neuroinflammation, oxidative stress and glutamate excitotoxicity. As dysfunctional astrocytes also have a role in the survival of dying motor neurons in ALS, new studies are aimed at transplanting stem cell derived astrocytes for protecting the diseased motor neurons. The beneficial effects of NSC transplantation are limited by the unfriendly microenvironment at the site of CNS injury/degeneration. Hence stem cell transplantation together with enrichment of microenvironment with trophic factors is under investigation. In vivo tumorogenic potential of mesenchymal stem cell exosome, neural stem cells embryonic stem cells and induced pluripotent stem cell is another impeding factor in the progress. However, many studies have shown positive outcomes with the above stem cell sources without tumor formation. Presence of undifferentiated pluripotent cells as contaminants in neural committed transplants is the cause for teratomas and this stem cells incorporated with suicidal gene can halt the tumor transformation. The homing tendency to hypoxic regions around tumor margins and potent revascularization potency of MSCs though are advantageous for targeted delivery of chemotherapeutics in brain tumors, they when given alone have the flaw of supporting the survival of existing tumors, if any. So it is important to rule out any existing tumors by functional imaging modalities before stem cell infusion into brain or spinal cord. Studies have shown that though human embryonic stem cells elicit immune responses, short term immune dampening treatment could prevent post-transplantation rejection. Undifferentiated induced pluripotent stem cells are also immunogenic but they are seldom used in transplantation studies without differentiating to desired cell lines. Presently preclinical trials are being carried out in animal models of CNS diseases and predictive clinical outcome of such therapies seems to be promising. Large animal models and controlled clinical trials are needed to confirm the efficacy, safety and clinical feasibility of stem cell therapy as a therapeutic modality. Even in those CNS diseases where the clinical phase trials have started or culminated the diagnostic techniques for accurately predicting the cell integration and survival in live patients is underway. A solution to this has evolved from the finding that NSCs may be labelled with superparamagnetic iron oxide before administration and their distribution in the body can be noninvasively monitored by MRI. This is awaiting FDA approval for conducting clinical trials.

Certain issues need to be addressed in the future and one of them is survivability of transplanted stem cells. Studies have shown that the survival, growth and function of the neurons can be enhanced by trophic protein factors and hence combining differentiated neurons with trophic protein factors could be a topic for future research. The optimal dose of stem cells to be used, route of administration and sex of the donor/recipient vary with the stem cell type being used and future trials need to be aimed at standardizing this factors with regard to different stem cell types for various CNS diseases. When to give stem cell therapy after disease onset is yet another issue. Most of the CNS diseases that gained benefit from stem cell therapy had shown that an early intervention is needed for successful outcome. Though there are reports of improvement of locomotor function after stem cell therapy immediately after injury, optimal time period for stem cell transplantation following spinal cord injury is 1-2 weeks after injury as neither the immediate post-traumatic microenvironmnet supports the survival and differentiation of neural stem cell/progenitor cells nor the chronic stage as it inhibits the regeneration of neuronal axons due to glial scar formation at the site.
A recent progress in the field of cell therapy is the invention of technology for cell reprogramming and development of induced pluripotent stem cells. Induced pluripotent stem cell lines derived from patients suffering from PD, AD, Autism, Rett syndrome (a paediatric neural development disease) and Schizophrenia are now used as cell models for studying pathogenesis, and to develop assays for drug discovery. They may be used in future for accurate laboratory evaluation of drug-cell interactions, to develop drug for the management of CNS diseases and also as autologous stem cell sources for therapy with more controlled methods of reprogramming.

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