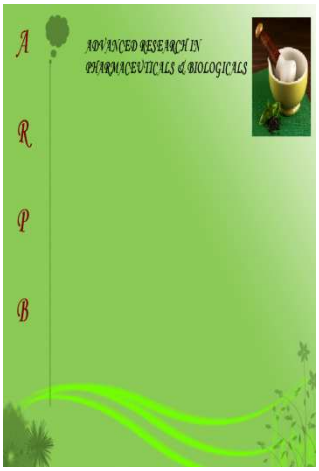
Advance Research in Pharmaceuticals and Biologicals
(A peer reviewed International journal for Pharmaceutical and allied research)

Vol -2 (1)
JAN- MARCH 2012



Received on 14/03/2012
Revised on 26/03/2012
Accepted on 31/03/2012

Corresponding author
Painter Stuti R.
Department of Pharmacology,
K. B. Institute of Pharmaceutical Education and Research,
Gh-6, Sector-23
Gandhinagar-382023
Gujarat, India.

STEM CELLS IN NEUROPHARMACOLOGY: AN OVERVIEW

*S. R. Painter, A. R. Parikh, V. R. Trivedi, and M. R. Chorawala

Department of Pharmacology, K. B. Institute of Pharmaceutical Education and Research,
Gandhinagar-382023
Gujarat, India.

ABSTRACT:

Stem cells have remarkable potential to develop into many different cell types in the body. Stem cells are the unspecialized cells capable of renewing themselves through cell division and under certain pathological or experimental conditions, they can be induced to become tissue or organ-specific cells with special functions. Stem cell–based approaches have received much hype as potential treatments for neurodegenerative disorders. In chronic cases, there is either a selective loss of specific cell population, such as dopaminergic neurons in Parkinson disease, inflammatory de-myelination of neurons in Multiple sclerosis, degeneration of motor neurons in ALS, widespread degeneration of many types of neuron, such as occurs in Alzheimer disease over a period of several years. Stem cell–based approaches could be used therapeutically to restore functions in neurodegenerative disorders. Transplantation of stem cells or their derivatives in animal models of neurodegenerative disorders can improve functions by replacing the lost neurons and glial cells, by mediating remyelination or trophic actions and modulation of inflammation. Endogenous neural stem cells are also potential therapeutic targets because they produce neurons and glial cells in response to injury and could be affected by the degenerative process.

Keywords: Stem cells, neurodegenerative disorders, glial cells, neural stem cells.

INTRODUCTION

Stem cells are the precursors of all cells in the human body. Stem Cells are the centrepieces of regenerative medicine-medicine that involves growing new cells, tissues and organs to replace or repair those damaged by injury, disease or aging. They have the ability to replicate themselves and to repair and replace other tissues in the human body. Unlike other cells in the human body, stem cells are undifferentiated, which means they do not have a fixed identity and function. Consequently, they possess an ability to be manipulated in the laboratory in ways that may change their identity and function. This ability to change and be manipulated makes them powerful tools for research and therapy.

Different sources of stem cells like:

Embryonic stem cells These stem cells come from embryos that are four to five days old. At this stage, an embryo is called a blastocyst and has about 150 cells. These are pluripotent stem cells i.e. they can divide into more stem cells or they can specialize and become any type of body cell. Because of this versatility, embryonic stem cells have the highest potential for use to regenerate or repair diseased tissue and organs in people.

Adult stem cells These stem cells are found in small numbers in most adult tissues such as bone marrow. They are also found in children and in placentas and umbilical cords. Until recently, it was believed that adult stem cells could only create similar types of cells like stem cells residing in the bone marrow could give rise only to blood cells. However, emerging evidence suggests that adult stem cells may be more versatile and able to create unrelated types of cells like the bone marrow stem cells may be able to create muscle cells.

Adult cells altered to have properties of embryonic stem cells (induced pluripotent stem cells) Regular adult cells can be transformed into stem cells by using a technique called nuclear reprogramming. By altering the genes in the adult cells, it can be reprogrammed to act similarly to embryonic stem cells. This new technique avoids the controversies of the embryonic stem cells, and prevents immune system rejection of the new stem cells[1]. The application of the described stem cells in neurodegenerative disease is discussed here.

MULTIPLE SCLEROSIS (MS)

Multiple sclerosis (MS) is widely believed to be an autoimmune condition. The body's immune system mistakenly attacks and damages the 'myelin sheath'

which protects the nerve cells in the brain and spinal cord. This inflammation-induced destruction of the myelin sheath surrounding the axons, leads to the conduction deficits and a variety of neurological symptoms. This damage causes messages to and from the brain to be slowed, distorted or stopped altogether. This is what leads to the symptoms of MS[2, 3].

Treatments in MS

Immunomodulation and remyelination are the potential treatments for MS**.** Immunomodulation is preventing the immune damage to the nervous system and remyelination repairs the myelin sheath that has already been damaged. These are both considered 'neuroprotective' therapies. There are several different types of stem cell that have shown potential benefit.

HSCs (hematopoietic stem cells): These are adult stem cells found in bone marrow and blood. They are capable of producing all of the cells that make the blood and the immune system. HSCs are being trialled in highly active forms of MS, where it is thought they may help prevent damage to myelin by altering how the immune system functions ('immunomodulation').

MSCs (mesenchymal stem cells) These are adult stem cells, found in several places in the body including the bone marrow, skin and fat tissue. They produce cells which help other stem cells function properly. MSCs are being trialled for MS. It is thought they may have a positive effect through 'immunomodulation' and might also promote the nervous system's own repair mechanisms to repair damaged myelin ('remyelination').

NSCs (neural stem cells) These are the cells responsible for repairing myelin in the brain, but when someone has MS, their NSCs don't seem to function properly as they don't 'turn on' to repair the damage that has occurred. NSCs occur naturally in the brain, but because of the difficulty in harvesting cells from the brain, fetal stem cells are used in clinical trials.

ESCs (embryonic stem cells) and iPSCs (induced pluripotent stem cells) ESCs can naturally produce every type of cell in the body. iPSCs are engineered to do the same. This is still a controversial and uncertain area of research as both ESCs and iPSCs have the potential to develop into tumors. However, it is widely accepted that ESCs and iPSCs will be extremely useful in the laboratory to identify and test potential drugs before they are tested in clinical trials[3].

Oligodendrocyte is responsible for producing the myelin sheath around the axon in the adult CNS. Thus myelin-

producing oligodendrocyte progenitor cells (OPCs) which are abundant in the adult human brain can be used for the treatment of MS. Spontaneous remyelination occurs to varying degrees in the early stages of MS, and OPCs are also present in chronic demyelinated MS lesions. An important area of research is focused on finding ways to enhance remyelination from these cells and identifying the factors that lead to a failure of cells to produce myelin[4]. It is recently showed that astrocyte-derived hyaluronan accumulated in demyelinated lesions from MS patients and prevented the maturation of endogenous OPCs[5]. The transplantation of remyelinating cells represents another approach for treating myelin loss in MS. However, the inflammatory environment could destroy the grafted OPCs and inhibit their maturation. So, immunosuppressive and anti-inflammatory treatments might be necessary. Another problem is that the demyelinated MS lesions are distributed across multiple locations throughout the CNS. An effective therapy will require the implanted OPCs to migrate to these sites. Interestingly, after systemic administration in mice, NS cells migrated to inflammatory demyelinating lesions, where some became OPCs and remyelinated axons. Most cells remained undifferentiated and suppressed pro-inflammatory mechanisms[6,7]. Amnion epithelial cells (AECs) - a novel stem cell source and are gaining attention in the treatment of MS. The amniotic membrane is the innermost membrane that surrounds the fetus. It provides structural support from external insult and is made up of two distinct layers, the amniotic epithelium and the amniotic mesoderm, separated by a basement membrane. Two stem-like cells are located in the amniotic membrane; amniotic epithelial cells are found in the amniotic epithelium and amniotic mesenchymal cells are located in the amniotic mesoderm. At day 8 during embryo formation, differentiation of AECs takes place. At this point the cells are pluripotent and will develop into all the tissues of the body. AECs cause reduction in pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), monocyte chemotactic protein-1 (MCP-1) and interleukin 6 (IL-6), resulting in a decrease in inflammatory cells infiltration and an increase in the anti-inflammatory cytokine, interleukin 10 (IL-10). In a study, it was found that intraperitoneal injection of 1 million AECs on day 8 can significantly reduce clinical symptoms and decrease CNS inflammation, demyelination and axonal degeneration, in stained sections of the spinal cord and brain. This suggests a

possible immunomodulatory mechanism by which AECs can suppress the development of MS[8].

PARKINSON'S DISEASE (PD)

The pathological hallmark of Parkinson's disease (PD) is a gradual loss of nigrostriatal dopamine-containing neurons. The main symptoms are rigidity, movement disorder (bradykinesia), tremor and postural instability. Current therapies centre on the oral administration of l-dopa and dopamine receptor agonists. These treatments are effective for some symptoms, but are associated with side effects and do not stop the progression of the disease. To be clinically competitive, a stem-cell-based therapy must lead to long-lasting, significant improvement in mobility or counteract disease progression[3, 9].

Treatment in PD

Stem cell–based approaches could be used to provide therapeutic benefits in two ways: first, by implanting stem cells modified to release growth factors, which would protect existing neurons and/or neurons derived from other stem cell treatments. Second, by transplanting stem cell derived DA neuron precursors/neuroblasts into the putamen, where they would generate new neurons to ameliorate disease-induced motor impairments. Clinical trials with intrastriatal transplantation of human embryonic mesencephalic tissue, which is rich in postmitotic DA neuroblasts, have provided proof that neuronal replacement can work in PD patients[10, 11]. The DA neurons that form from the transplanted tissue reinnervate the denervated striatum and become functionally integrated, restoring striatal DA release and giving rise to clear symptomatic relief in some patients. However, there is a strong need for other sources of DA neurons, because availability of human embryonic mesencephalic tissue is limited and variability of functional outcome after transplantation is high. Poor standardization of the transplanted cell material contributes to the high variability. But above problems could be solved if large numbers of standardized DA neuroblasts were generated from stem cells. After transplantation of stem cell–derived DA neuron precursors or neuroblasts and subsequent maturation, the resultant cells must exhibit the properties of substantia nigra neurons in order to induce substantial benefit in PD[12,13]. DA neuroblasts for preclinical transplantation have been generated in vitro from stem cells from several different sources and species, including humans. For example, they have been derived from ES cells, therapeutically

cloned ES cells, NSCs and progenitors of embryonic ventral mesencephalon, adult NSCs from the subventricular zone (SVZ), bone marrow stem cells and fibroblast-derived iPS cells[14]. Human stem cell–derived DA neuron precursors/neuroblasts, which will be required for patient application, can survive in animal models of PD and, after maturation, exert functional effects. But, it has not been shown that they can substantially reinnervate striatum, restore DA release in vivo, and markedly improve deficits resembling the symptoms experienced by patients with PD. A major concern when transplanting ES cell–derived DA neuroblasts is the risk for tumor formation, which has been observed in animal models[15]. The use of patient-specific DA neuroblasts made from iPS cells for transplantation would eliminate ethical concerns associated with ES cells and their progeny and would avoid immune reactions. Importantly, PD is a multisystem disorder, and symptoms that are caused by pathology in nondopaminergic systems will not be improved by intrastriatal DA grafts[16]. Finally, for long-term functional restoration in PD patients, DA cell replacement has to be combined with a neuroprotective therapy to hinder disease progression[17]. One possible strategy could be concomitant transplantation of stem cells genetically modified to secrete a trophic factor such as glial cell line–derived neurotrophic factor (GDNF) into the striatum and substantia nigra[18].

iPS cell derivation methods is used for generating cell populations for cell replacement therapy, disease modeling and drug discovery. iPS cell–based therapies may be particularly important for PD. iPS cell–derived dopamine neurons functionally integrated into adult brain in a rat model of PD, leading to an improvement of the phenotype. The concept of utilizing iPS cells to model a disease in a culture dish is based on the unique capacity of these cells to continuously self-renew and their potential to give rise to all cell types in the human body. Thus, pluripotent iPS cells could provide a limitless reservoir of cell types. Indeed, it was found in a study that differentiated dopaminergic neurons from iPS cells generated from individuals diagnosed with sporadic PD equally as efficiently as from those generated from healthy individuals and did not report any phenotypic differences. However, although it takes many years for the pathological features of this disease to become evident, it is possible that analysis of iPS cell–derived dopaminergic neurons might identify

more subtle early phenotypic changes PD[19].

AMYOTROPHIC LATERAL SCLEROSIS (ALS)

ALS or Lou Gehrig's disease is a progressive fatal neurodegenerative disease affecting mainly motor neurons in the spinal cord, brain stem and cortex. The most common clinical features of ALS are degeneration of motor neurons producing fasciculation, muscle wasting and hyperreflexia. Respiratory complications usually develop in patients with advanced disease and the cause of death is generally paralysis of the respiratory muscles and diaphragm. ALS is considered one of the most common motor neuron diseases. Although the exact pathophysiological mechanisms underlying neurodegeneration in ALS remain uncertain, the presence of a persistent inflammatory reaction prompted researchers to study the involvement of a non-cell-autonomous component in motor neuron death[20].

Treatment in ALS

ALS is dominantly inherited in 5–10% of patients (referred to as familial ALS, fALS), but in 90–95% of patients there is no apparent genetic linkage (referred to as sporadic ALS). Approximately 15–20% of fALS cases have been linked to mutations of superoxide dismutase 1 (SOD1). HESC (human embryonic stem cells) have been used for modeling both the autonomous and the non-cell-

autonomous effects of ALS in vitro by using a gene (SOD1) that is mutated in 20% of the familial cases. Recent studies in animal models as well as primary and embryonic stem cell models of ALS, utilizing overexpression of mutated forms of Cu/Zn superoxide dismutase 1, have shown that motor neuron degeneration in these models is in part a non cell-autonomous event and thus by providing genetically non-compromised supporting cells such as microglia or growth factor-excreting cells, onset can be delayed and survival increased. SOD1 is not required for development or survival of motor neurons, but is necessary for the maintenance of normal neuromuscular junctions (NMJs). Motor neuron death in ALS appears to involve non-cell autonomous as well as cell-autonomous events. Consequently, replacement of mutant SOD1-expressing cells with wild-type non-neuronal cells can substantially increase the life span in animal models of ALS. Initiation of disease appears to be dependent on over-expression of mutant SOD1 within motor neurons[20].

Motor neurons have also been generated in vitro from stem cells from various

sources, including mouse and human ES cells, NSCs derived from fetal rat spinal cord and human forebrain[4]. Stem cell–derived motor neuron precursors and neuroblasts establish functional synapses with muscle fibers in vitro; extend axons to ventral roots after transplantation into the spinal cord of adult rats with motor neuron injury. They form neuromuscular junctions with host muscle, and give rise to partial recovery from paralysis[21, 22].

Stem cells releasing neurotrophic molecules: Transplantation of stem cells to counteract motor neuron loss by releasing neurotrophic molecules or modifying the inflammatory environment, which probably plays a major role in disease progression, is a more realistic clinical goal for ALS. It is hoped they will exert a neuroprotective effect. First, derivatives of human embryonic germ cells (pluripotent cells derived from primordial germ cells in the gonadal ridge) delivered into the cerebrospinal fluid of rats with motor neuron injury have been found to migrate into the parenchyma and induce motor recovery through neuroprotection as a result of growth factor production[23]. Second, human fetal NSCs transplanted into the spinal cord in a rat model of ALS have been found to protect motor neurons and delay disease onset[24], probably as a result of their neuronal progeny (i.e., GABAergic interneurons synapsing on host motor neurons) releasing GDNF and brain-derived neurotrophic factor (BDNF), dampening excitotoxicity, or both[25]. However, when human mesenchymal stem cells (MSCs) engineered to secrete GDNF were transplanted into the muscles of rats with an ALS-like disease, motor function improved and disease progression was delayed[26]. Compared with direct gene transfer, an advantage of cell-based gene delivery is that production of the trophic factor continues even if the disease process destroys the endogenous cells.

ALZHEIMER DISEASE (AD)

As one of the most common causes of dementia. AD, known for its hallmarks of amyloid-β peptide (Aβ) plaques and neurofibrilary tangles, results in the death of several types of neuronal lineage cells within multiple regions of the brain, specifically cholinergic neurons. Both of these hallmarks lead to cognitive impairment and loss of memory[27].

Treatment in AD

Currently available drugs for the treatment of AD are purely for symptoms and among these drugs are the cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonist named memantine. Since the current treatments have only marginal effects and greatly vary in their effectiveness in

patients, the need for new treatments is great. Recently, in a study published in which NSCs is injected into the hippocampal regions of the brain of both a transgenic AD mouse model and an age-matched non-transgenic mouse model. Interestingly, the mice improved in cognitive function and there was no change to the existing Aβ plaques or neurofibrilary tangles. Instead, the authors discovered brain-derived neurotrophic factor, which is important for neuron outgrowth, and synapse formation increased, leading to improved cognition through increased synaptic density. This demonstrated cognition could be improved without the need for modifying the existing pathological conditions[28]. Neurons Directly from Human Skin: Researchers have come up with a recipe for making functional neurons directly from human skin cells, including those taken from patients with Alzheimer's disease. The new method may offer a critical short cut for generating neurons for replacement therapies of the future. In earlier approaches to generate neurons from skin cells, adult cells first had to be returned to an embryonic stem cell state. These cells, called induced pluripotent stem (iPS) cells, are hard to come by -- less than one percent of cells are typically reprogrammed successfully. In addition, the entire process is time-consuming, requiring months to coax cells into iPS cells and then stimulate them to become neurons. There is also an increasing concern about the stability of iPS cells. Their ability to grow and produce any cell type makes them a cancer risk. When studied in a dish, the neurons derived from healthy skin cells could fire and receive signals, just like normal neurons. When placed into the brains of developing mice, the converted cells were able to connect up to the existing circuitry. This method can also produce neurons from the skin cells of patients with a rare familial form of Alzheimer's disease (AD). The AD neurons superficially looked normal, but upon closer inspection, abnormalities in the processing of amyloid precursor protein were found. The neurons also showed more general differences in the way proteins inside the cell move around[29].

REFERENCES

1. Stem cells: What they are and what they do? Last updated 13/3/2012 *http://www.mayoclinic.com/health/stem-cells/CA00081*
2. Multiple Sclerosis. Last updated 2/6/2012*http://www.mssociety.org.uk*

3. O. Lindvall, Z. Kokaia. Stem cells for the treatment of neurological disease, Nature Med. 441: 1-2 (2006).
4. M. Windrem, *et al.* Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain, Nature Med. 10: 93–97 (2004).
5. S. Back, *et al.* Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation, Nature Med. 11: 966–972 (2005).
6. S. Pluchino, *et al.* Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis, Nature 422: 688–694 (2003).
7. S. Pluchino, *et al*. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism, Nature 436: 266–271 (2005).
8. C. McDonald, C. Siatskas. The emergence of amnion epithelial stem cells for the treatment of Multiple Sclerosis, Review of inflammation and regeneration 31 (3): 256-262 (2011).
9. S. Goldman. Stem and progenitor cell-based therapy of the human central nervous system, Review in Nat. Biotechnol. 23 (7): 863-865 (2005).
10. S. Pluchino, *et al*. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism, Nature. 436 (7048): 266–271 (2005).
11. O. Lindvall, A. Björklund. Cell therapy in Parkinson's disease, NeuroRx. 1 (4): 382–393 (2004).
12. O. Isacson, L. M. Björklund, J. M. Schumacher. Toward full restoration of synaptic and terminal function of the dopaminergic system in Parkinson's disease by stems cells, Ann Neurol. 253 (suppl 3): S135– S146; discussion S146–S138 (2003).
13. I. Mendez, *et al*. Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease, Brain. 128(7): 1498–1510 (2005).
14. O. Lindvall, Z. Kokaia. Stem cells in human neurodegenerative disorders-time for clinical translation? J Clin Invest. 120 (1): 30-31 (2010).
15. N. S. Roy, C. Cleren, S. K. Singh, L. Yang, M. F. Beal, S. A. Goldman. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes, Nat Med. 12 (11): 1259–1268 (2006).
16. A. E. Lang, J. A. Obeso. Challenges in Parkinson's disease: restoration of the nigrostriatal dopamine system is not enough, Lancet Neurol. 3 (5): 309–316 (2004).
17. C. W. Olanow, K. Kieburtz, A. H. Schapira. Why have we failed to achieve neuroprotection in Parkinson's

disease? Ann Neurol. 64 (2): S101–S110 (2008).

18. S. Behrstock, *et al*. Human neural progenitors deliver glial cell line-derived neurotrophic factor to parkinsonian rodents and aged primates, Gene Ther. 13 (5): 379–388 (2006).
19. I. Park, *et al*. Disease-specific induced pluripotent stem (iPS) cells, Cell 134(5): 877–886 (2008).
20. E. Hedlund, M. P. Hefferan, M. Marsala and O. Isacson. Cell therapy and stem cells in animal models of motor neuron disorders, Eur. J. Neurosci., 26: 1721-1737 (2007).
21. J. M. Harper, *et al*. Axonal growth of embryonic stem cell-derived motoneurons in vitro and in motoneuron- injured adult rats, Proc Natl Acad Sci U S A. 101 (18): 7123–7128 (2004).
22. D. M. Deshpande, *et al*. Recovery from paralysis in adult rats using embryonic stem cells, Ann Neurol. 60 (1): 32–44 (2006).
23. D. A. Kerr, *et al*. Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury, J Neurosci. 23 (12): 5131–5140 (2003).
24. L. Xu, *et al*. Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats, Transplantation. 82 (7): 865–875 (2006).
25. L. Xu, D. K. Ryugo, T. Pongstaporn, K. Johe, V. E. Koliatsos. Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the segmental motor circuitry, J Comp Neurol. 514 (4): 297–309 (2009).
26. M. Suzuki, et al. Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS, Mol Ther. 16 (12): 2002–2010 (2008).
27. H. P. Rang, M. M. Dale, J. M. Ritter, R. J. Flower. Rang and dale's Pharmacology, Neurodegenerative disease, Elsevier, U.K, 2007, pp. 514-515.
28. E. Dantuma, S. Merchant, K. Sugaya. Stem cells for the treatment of neurodegenerative diseases, Stem Cell Research & Therapy 1 (37): 2010
29. L. Qiang, R. Fujita, T. Yamashita, S. Angulo, H. Rhinn, D. Rhee, C. Doege, L. Chau, L. Aubry, B. William, et al. Directed Conversion of Alzheimer's Disease Patient Skin Fibroblasts Into Functional Neurons**,** Cell 5 146 (3): 359 – 371 (2011).