

Stem Cell Therapy In Stroke

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Abstract

The ultimate aim of any therapeutic strategy is the maximum functional restoration possible and eventual complete normalcy of function. The non – regenerative capability of the injured adult brain has been challenged in recent years and neural plasticity has been observed experimentally in both global and focal brain ischemia in animal models. Whether neuro - genesis increases in response to brain lesions and whether stem cells can be used for transplantation are potential questions to be answered. Functional recovery may occur in a small or localized brain injury using rehabilitation measures, but for large ischaemic strokes, the restoration may require new synaptic connections within and away from the damaged tissue. In an infarcted area, the ischemic core may not respond to any pharmacological or rehabilitative intervention. For these reasons, the prospects of repairing the neuron system, using cell transplantation, seems promising and may offer a unique approach for brain repair and restoration of function. On-going animal and human trials have largely helped in burgeoning our hopes on this method of restorative therapy after stroke.

Key words: stem cells, stroke, neuroregeneration, brain plasticity, restorative therapies

Introduction:

"Physicians have always inspired two powerful sentiments in the contemporary society: the hope that they will create a healthier world than their forefathers without discarding fundamental values of professionalism and ethics, and the fear that they will make things worse by following pseudoscience which is misleading, wasteful, lacking in vigilant peer review, and advocated by drug and devices industry." These are the opening words by Prof. Bajaj in his preface of the Annual Medicine Update this year (1). There is no better way to aptly describe the hope and hype regarding stem cell therapy. The ultimate aim of any therapeutic strategy is the maximum restoration possible and eventual complete normalcy of function. The nervous system has little capacity for self repair. However, the non-regenerative capability of the injured adult brain has been challenged in recent years and neural plasticity has been observed experimentally in both global and focal brain ischemia in animal models. Neuroimaging studies in stroke patients indicate altered post stroke patterns suggesting functional reorganization. However, whether neurogenesis increases in response to brain lesions and whether same stem cells or progenitor cells present in brain

be used for transplantation are potential questions that need to be answered. Recent studies have shown in-vivo differentiation of progenitor cells into neurons in adult human dentate gyrus. Functional recovery may occur in a small or localized brain injury using rehabilitation measures, but for large ischaemic strokes, the restoration may require new synaptic connections within and away from the damaged tissue. Considering the relatively poor capabilities of neural self-regeneration, this seems quite impossible. In an infarcted area, the ischemic core may not respond to any pharmacological or rehabilitative intervention. For these reasons, the prospects of repairing the neuron system, using cell transplantation seem promising and may offer a unique approach for brain repair and restoration of function. Considering the fact that the neuronal circuitry is a complex array of neurons and connections and the prospects of this technique at first thought seem remote, yet, the growing evidence from animal models and small clinical trials has suggested the possibility of reconstruction of neuronal network, making the aspect of restorative medicine significantly promising.

Compared with neurodegenerative disease, stroke poses special conditions that impact the potential success of

transplantation to enhance neurological recovery, including the anatomy and time of stroke, the vascular supply, site of implantation, and type of patients enrolled in clinical trials. In contrast to a neurodegenerative disorder such as Parkinson's disease (PD), which destroys a relatively homogenous population of neurons, strokes affect multiple different neuronal phenotypes. An infarct might involve the thalamus, hippocampus, and striate visual cortex affecting 3 or more very different neuronal populations. Besides, oligodendrocytes, astrocytes and endothelial cells are also affected. Reconstitution of the complex and widespread neuronal-glial-endothelial interrelationships may require cells for transplant to initially remain immature and phenotypically plastic to differentiate into appropriate neural, glial and endothelial cell types depending on the ectopic site. If white matter is destroyed in a stroke, cell implants may not produce functional connections with axons that can penetrate through the scar tissue of a chronic infarct.

There is uncertainty about the mechanism(s) by which cell transplantation might improve stroke deficits. Transplanted cells would ideally replace cells that are damaged by ischemia and take over function of

these cellular elements. However, it is also possible that transplanted cells secrete trophic factors that help to maintain marginally surviving cells or otherwise enhance the local environment sufficiently to improve function. Transplantation might also conceivably produce a host reaction that could include sprouting of new axons and synapse formation.

In the understanding of the efficacy of different treatment strategies on human brain, appropriate animal models are imperative which mimic human disease. Two major types of ischaemic models are studied: a) global and b) regional or focal. The global ischaemic model (produced by ligating vertebral and carotid arteries for 5 – 15 min) mimics the effect of cardiac arrest or coronary occlusion in humans and focal models replicate the consequences of stroke. The effects of focal ischaemia are seen on discrete regional group of neurons, especially CA1 neurons of hippocampus and neural transplantation into this region has been studied with benefit in rat ischaemic models. Improvement in this model however requires replacement of dead CA1 neurons and graft – host connectivity. The focal ischaemia models are produced by MCA ligation techniques thereby producing focal brain infarctions, generally in region of

striatum or cortex. Fetal cortical grafts placed in infarcted regions have shown to develop afferent connections from cortex, thalamus, and sub cortical nuclei of the host, whereas efferent connections are sparse, seen to improve with the housing of animal in an enriched environment.

Cell types and sources of intracerebral grafting (2-6)

The most important issue in any cell transplantation technique would be the availability of appropriate cell type having the ability to proliferate *in vivo*. The therapeutic effects of implanted neurons or neuronal precursors are likely to be successful if they structurally and functionally integrate into the brain. It is important that cellular elements used in transplantation are immature i.e., at a developmental stage where they are terminally differentiated but have not formed connections and are phenotypically plastic so as to differentiate into different cell types i.e., neural or glial depending upon the site of implant. Although, the ability to develop neuronal connectivity is maximum during the fetal period, nevertheless, the adult brain has the capacity to establish functionally active connections, especially during the periods of injury thereby suggesting reactivation of regulatory mechanisms

during degeneration or insult. Following cell types have been studied as potential candidates for neural repair in ischaemic stroke.

a) Embryonic/ Fetal cells (7-14)

Fetal tissue has been the major source of cells for transplantation in animal models of stroke. As mentioned above, the likelihood of survival is best for immature cells, before they have arborized their axonal network. Transplanted fetal cells have shown to survive, integrate and improve deficits in animal models of neurodegenerative disease, as well as PD in humans. The gestation age of 14 – 20 days is generally used in animal models. For fetal hippocampal and cortical donor cells, days 18 – 20 and for fetal striated cells, less than 16 days have been generally used in majority of studies. Since there are major ethical and legal issues governing the use of “human fetal embryonic tissue”, other cell sources are being seriously considered and investigated, as studies have shown that multiple donor or first trimester tissues are not prerequisites to generate long term surviving human brain grafts. Owing to the short supply of human donor material and ethical concerns about the human embryos as donors, animal tissues have been considered as an alternative source with advantage of being genetically modifiable. Pigs are

useful as donors as they are non – endangered species and produce large litters as opposed to non – human primates. Transplantation of fetal cells from primordial striatum of porcine origin, known as lateral ganglionic eminence (LGE) was shown to improve function in rat ischaemic models. In small scale trials, porcine tissue has been used to treat patients with PD, HD, focal epilepsy and stroke with partial success. However, some issues regarding use of this cell source necessitate pondering. Studies have shown that the cells tend to retain original phenotype of striated origin even if implanted into non – striatal regions making use of these cells into stroke regions outside striatum questionable. The likelihood of graft rejection in humans is of potential concern and strategies need to be devised to overcome this. The risk of host contamination of viruses is of immense concern. It has been reported that porcine endogenous retrovirus particles (PERV) could be released from the porcine cell lines and can infect human cell lines. Since then a debate on PERV infection from xenotransplantation or its integration into human retrovirus, with resultant novel mutations has been ongoing. Guidelines call for regular monitoring of patients undergoing xeno-transplantation.

b) Immortalised cell lines (15,16)

In view of the ethical difficulties in transplanting embryonic cells and technical problems in xenotransplantation, alternative sources of graft cells have been devised. One of these cell lines, called “immortalized cell lines” have been an important technical advance in the field of neurotransplantation. These cell lines are derived by infecting neuroepithelial precursor cells from predefined CNS regions before their terminal mitosis, with a retrovirus encoding an immortalizing oncogene. Individual clones are then isolated and characterized e.g., MHP36, MK31, Ht9–C7 etc. The tissue sources have been variable i.e., mouse hippocampus, hypothalamus, mesencephalon, mouse striatum and others. The transforming protein is generally expressed, even if the cell is being pharmacologically induced to differentiate, making it of potential concern both *in vitro* and *in vivo*. Data from studies suggest that the neural precursor cell lines are plastic, and have ability to differentiate into multiple lineages *in vitro* and can respond to local microenvironmental cues. However, these cell lines do not behave similarly either *in vitro* or *in vivo*. Probably, each cell line responds to a set of effector molecules *in vivo*. The advantage of establishing an

immortalized cell line is in providing an unlimited number of identical cells from a single cell propagated in culture, higher level of neurotransmitter production using genetic manipulation, better pooling and sorting of viable cells, screening for infectious diseases and efficient planning of surgical procedure.

c) Spontaneously arising neural cell lines or neuron like cells (17-20)

Neuroblastomas and glioblastomas are the chief spontaneously arising neural cell lines. These contain cells of mixed population which are often undefined. Embryonal carcinoma (EC) cells are derived from spontaneously occurring testicular germ cell tumors and can differentiate into both neural and non – neural cells. In response to therapy with retinoic acid, the mouse derived EC cell line (P19), differentiates into neurons, astrocytes and oligodendrocytes. However, neural transplantation studies on rat striatum showed that these cells tend to retain their original characteristics established *in vitro* and have phenotypic plasticity *in vivo*. Although tumorigenicity has not been observed, the risk is potential once transplanted.

N-Tera-2 Cells were derived from human testicular germ cell tumor, years ago. Also called LBS – neurons (after

Layton Bioscience Inc. Ath. Cal), the credit of development and patenting of the process to cleverly transform this rapidly dividing cell line into fully differentiated non – dividing neurons goes to researchers at University of Pittsburgh, Pennsylvania. Upon several weeks treatment with retinoic acid (an agent known to produce maturation of cancer cells into their normal looking non – cancerous equivalents) and mitotic inhibitors, an enriched population of post – mitotic differentiated neurons known as NT2N or HNI cells, showing an exclusive commitment to neural lineage were produced. They have been seen to closely resemble neural precursor cells, and express cell surface markers and cytoskeletal proteins unique to neural stem cells. They represent a well characterized and unlimited source of human neurons for transplantation that can be reproducibly generated. NT2N cells, are “Neuron Like Cells” as they have a symmetrical morphology, elaborate an extended axon and elongated dendrite. These cells can express neurotransmitters, functional glutamate receptors, calcium channels and proteins capable of secretory activity and synaptogenesis. The ready constant availability of cryopreserved pure neurons has made the NT2N cells an attractive graft source and trials in

animal studies and initial results in ongoing clinical trials in humans are encouraging. The use of these neurons obviates the need for fetal cells, which has raised ethical concerns especially regarding elective abortions. Spontaneous abortions are rare and unpredictable events, so harvesting tissues from these fetuses would be impractical. Further, cells from spontaneously aborting fetuses are likely to harbor genetic defects. Another feature of the LBS – Neurons is that they can be frozen and transported to clinical centre for transplantation and thawed without damage before use.

d) Stem cells (21-23)

The discovery of adult tissue specific stem cells, such as hematopoietic stem cells, having ability to differentiate into other tissues has generated immense interest among cell biologists and transplant clinicians. Stem cells are cells capable of proliferation, self maintenance and production of differentiated functional progeny that are characteristic of the organ from which they are derived. In adult animals stem cells are present in organs like bone marrow, skeletal muscle, intestine, liver, peripheral nervous system and retina etc.

The long standing dogma of adult mammalian brain lacking neurogenesis

and evidence of progenitor cells, has been recently challenged by studies showing continuous neurogenesis in olfactory bulb, hippocampus and dentate gyrus, from the neural stem cells (NSCs). These NSCs are defined as undifferentiated cells that are able to self – review as well as generate the three major cell types that constitute the CNS: neurons, astrocytes and oligodendrocytes, signifying their pluripotent nature.

These features have lead to many studies aimed at characterizing, isolating, expanding and transplanting these fascinating cells. Whether neural stem cells meet all these criteria is still unresolved. It is likely that cell lineages generated from NSCs differ among stages and regions of the CNS, e.g. in the cortex there may be selective progenitor cells giving rise to neurons (neural progenitor cell) or astrocytes and oligodendrocytes (glial progenitor cell). The NSCs can be expanded to a particular clone using free floating “neurosphere” cultures and the lineage potential can be assayed using clonal monolayer cultures. The identification process and isolation of NSCs is tedious and difficult. There are selective marker molecules for NSCs e.g., *mushashil* (RNA binding protein), *nestin* (intermediate filament) and the members of SOX family, but are not cell

surface antigens unlike haematopoietic cells.

What is the localization of neural precursor or stem cells? In the embryonic stage, they have long been believed to be located in the ventricular zone of the neural tube after neurulation and this is consistent with immuno chemical staining analysis using mushashil. In the adult rodents, neurogenesis is seen to occur in dentate gyrus and olfactory bulb and has been experimentally shown even in adult humans. The sub ventricular zone (SVZ) and ependymal layer also corresponds to neurogenesis site in adult brain. Regeneration may also be made effective by stimulating the endogenous neural precursor cells or stem cells by injury, as has been shown in studies, either spontaneously or by using exogenous stimuli like neurotrophic factors (BDNF) administered intraventricularly or by infusion. Thus, it is likely that adult brain parenchyma may recruit and/ or generate new neurons, which could replace the lost neurons.

e) Bone Marrow Stromal Cells (24-28)

Over the years, tissue specific stem cells have been shown to give rise to cells, not normally found in the organ or tissue of residence. The plasticity of

adult stem cells opens up possibility of using autogenous adult stem cells to treat various disorders (38). The bone marrow stromal cells (BMSCs) provide structural and functional support for the generation of blood cell lineages from haematopoietic stem cells e.g., fibroblast reticular cells, adipocytes, macrophages etc, and under specific conditions differentiates into a variety of tissue, e.g. bone, cartilage, muscle, glia and neurons. When exposed to epidermal growth factor or neurotrophic factors like BDNF *in vitro*, or cultured with neural cells, human BMSCs differentiate into cells, expressing neural precursor cells (NPC) markers. Sanchez – Ramos *et al* used retinoic acid (RA) in combination with growth factors to induce differentiation of BMSC to neural phenotypes whereas others have used β -mercaptoethanol for inducing rapid differentiation into neuron-like cells. The advantages with these cell lines seem many. Obtaining marrow cells would be easy and expanding them in culture would not be that difficult. Using patient's own BMSC would theoretically eliminate the risk of rejection. However, differentiation mechanism for these cells is poorly understood. Whether these cells truly produce neuronal synaptic network with plasticity or produce trophic factors alone is questionable and speculative. Issues like long-term

survival, safety, plasticity and behavior of BMSCs need further evaluation before clinical use.

f) Umbilical cord blood cells

Human umbilical cord blood may also harbor cells (human umbilical cord blood cells HUCBs) capable of differentiation into neural lineages. When exposed to nerve growth factor and RA, the derived umbilical cells produce progeny that show positivity of neural and glial cell markers. However, biology of the cells is currently poorly understood, and it is likely that positive effects of these cells are related to their neurotrophic action, rather than actual neuronal circuitry formation. A better understanding of these cells is needed before clinical transplantation studies are undertaken, although experimental data in animal models of stroke have shown functional benefits.

How do transplanted cells work? (24, 29)

In most cases of neural transplantation, it is likely that therapeutic effects of the implanted neurons or their precursors would be dependent upon their functional and structural integration into the brain tissue. However, the question is whether establishment of neural circuitry the only means of improvement. It is likely that

transplanted cells release neurotransmitters or neurotrophic/neuroprotective factors which counteract degeneration or promote regeneration. Even transplanted glial cells have been used to modify response to injury and assist in structural repair and promote remyelination. Studies using bone marrow stromal cells or umbilical cord blood cells as potential donors have shown functional improvement in behavioral recovery in animal models within days of transplantation. This raises issues whether recovery observed in such short periods is related to release of trophic factors rather than engraftment and differentiation of transplanted cells into mature neurons and / or glia. The functional benefits after neural transplantation are likely to be mediated by one of the following mechanisms.

1. Neurotransmitters released from the graft tissue act on the afferent deprived limb of the post synaptic receptors.
2. Release of the neurotrophic / growth factors (brain derived neurotrophic factor [BDNF], glial derived neurotrophic factor [GDNF], nerve growth factor [NGF] etc) acting as local pumps to support cell function and to prevent cascade of apoptosis. Regenerating neuronal population

further prevents subsequent cell death.

3. Reestablishment of local interneuronal connections and synaptic connectivity between the host and graft.
4. Cell differentiation and integration.
5. Improvement of regional oxygen tension.
6. Limit glial reaction and prevent retrograde degeneration.

Possibly, the overall success of functional outcome is mediated by a combination of the above mentioned factors. Taking the model of PD, it is easy to understand the mechanisms operating for improvement as a specific cell function restoration is needed along the dopaminergic neurons. Ischaemic stroke, however, damages several neuronal circuits and not specific cholinergic or dopaminergic pathways, and therefore, neural transplantation is likely to be less effective. Restoring circuits, and enhancing cell survival can be achieved by cell transplantation. However, improvement might require reconstruction by long distance projection systems requiring multiple site implantation, and "bridging grafts" as explored in PD, may be applied for ischaemic lesions in future for bridging intact host tissue with ischaemic penumbra and core.

Cellular support for survival, integration and functional outcome

One of the most important factors deciding the fate of the graft is the trophic support offered by the intrinsic milieu, the host environment or administered *in vivo* postgrafting. Neurotrophic factors (NTFs) have been studied intensely as agents promising brain recovery in neurodegenerative disorders and acquired conditions like trauma or stroke. Neural differentiation of neuronal and glial cells and their maintenance are under the control of NTFs. In addition, an excessive administration of NTFs greatly protects sensitive brain tissue from injury. It is now thought that neural stem cells have the inherent potential to compensate and recover neural functions lost after ischaemic stroke as discussed previously. However, even the stem cells are under the control of NTFs to differentiate into a certain species of neural cells. In the present date, the number of growth factors available and likely to affect survival and development of progenitor cells is probably the largest. Astrocytes and endothelial cells surrounding or infiltrating the transplant are susceptible to the effects of platelet derived growth factor (PDGF) which is likely to mediate survival of graft through neovascularization. BDNF has

been closely related to graft survival of dopaminergic neurons or their precursors. It is now accepted that BDNF and its receptor trk B are widely distributed, both in developing and mature CNS. Currently, BDNF is most useful in cell transplantation to promote cell differentiation pre-implantation. It has been observed that under conditions like ischaemia and seizures, NTFs like BDNF, GDNF, NGF etc; are induced in brain cells. However, each NTF species shows a different effect on temporal and cellular differentiation. In ischaemic brain, BDNF, GDNF and vascular endothelial growth factor (VEGF) are seen to be upregulated within hours of stroke in experimental animal ischaemic models. Therefore, it possibly implies that neural transplantation may be made more successful with the concomitant use of these neurotrophic factors. Since development and plasticity of neural networks and connections are strongly influenced by the microenvironment of the host tissue, the conduciveness of this environment can be improved by either enhancing graft cell viability or improving host tissue responsiveness, achieved best by the use of trophic factors. In animal models of transplantation, housing conditions after the transplant affect outcome strongly. Whether the role of NTFs is confined to pre-implantation mechanism of differentiation of cells

into mature precursors or later after transplanting in improving further differentiation, organization and structural integration into the host tissue is speculative. Although current data has not supported strongly their role in acute ischaemic stroke as neuroprotectants, their role remains strong and essential for neural transplantation studies. Thus transplant therapy, rehabilitative medicine and other strategies need to be combined together for a clinically effective and functionally active outcome.

Immunosuppression and transplantation

It is believed that host parenchymal interaction to the graft tissue is an important factor mediating success of transplantation. Clinical trials have explored the need for immunosuppression and contribution of graft vs host response during cell grafting. It is generally considered that brain is relatively immunologically benign and the concept whether immunosuppression is really necessary has been challenged. Herderson *et al* showed long term benefit of neural transplanting in PD patients in absence of immunosuppression. However, the above therapeutic benefits have been with the use of human fetal tissue, which theoretically may have less

immunogenicity mediating tissue acceptance. The concept however, may not hold true for the xeno-transplantation methodologies. Several immunological issues need to be solved before a clinical trial of xenogeneic neural transplantation. No concrete evidence of NK cell (natural killer cell) and complement activation mechanisms mediating neural xenograft rejection have been reported. Human serum contains not only anti-Gal, but also natural antibodies against other epitopes present in porcine embryonic brain. Current immunosuppressive drugs are not efficient enough to protect neural xenografts from rejection, thereby necessitating development of alternative or supplementary strategies to reduce or abolish "T" cell response, a major element during xenograft rejection. Pretreatment strategies for donor host interactions might sound plausible but efficiency of pretreatment for reducing immunogenesis depends upon the donor-recipient combination used in evaluating them. For transplantation of porcine tissue in humans, optimal donor – tissue treatment is directed at both direct and indirect antigen arm of the T – cell response. Response of microglia towards the graft is one of the most intriguing aspects of host-graft reaction. Microglia act as antigen presenting cells, initiating immunological cascade, but

also mediate survival of graft and host-brain regeneration with increasing evidence suggesting their beneficial role in CNS. In vivo, nigrostriatal dopamine neurons develop neurites around the wound or graft correlating with active neurotrophic factor production by macrophages and microglia. It is thus difficult to understand the behaviour of microglial cells. Whether specific factors or signals promote undue inflammatory response, experimental data does support their beneficial and neurotrophic effects on the CNS.

Types of stroke lesions amenable to stem cell therapy:

Not all stroke lesions may be amenable to cell transplantation. Most preclinical studies involve intrastriatal implantation. Studies of the middle cerebral artery implantation rodent model have shown that the striatum is the primary site of damage and many believe that the resulting deficits in memory, learning and motor behavior are directly associated with striatal injury. Cortical lesions also may be accessible to transplantation, but infarcts involving white matter are more problematic. A proliferation of transplanted cells in the cortex may not necessarily repair underlying axonal damage. There is even rationale for neural transplantation in patients with

pure white matter infarcts, which require an entirely different therapeutic strategy. The size and extent of infarction involving major arterial territories will play a significant role in patient selection. In patients with widespread damage, the number of cells potentially needed to restore function may be daunting.

Timing of stem cell transplantation after stroke:

The appropriate time to transplant after a stroke is unknown. In the acute setting, release of excitotoxic neurotransmitters, free radicals, proinflammatory mediators might threaten new tissue introduced into the peri-infarct region. Also, cells may be dying by apoptosis in the penumbra for several weeks after stroke. Inflammation leading to microglial activation may inhibit endogenous neurogenesis and may thereby suppress the growth and survival of transplanted cells.

On the other hand, in the acute stage, local repair processes are active, including the release of neurotrophic factors from the intrinsic milieu and the host environment during the early phase to facilitate implant growth, survival, differentiation and /or integration. The ischemic environment also promotes the generation of new

neurons in periventricular regions and in the cerebral cortex. How transplantation will affect the on-going endogenous neurogenesis is unknown. There is accumulating evidence that stroke recovery involves plasticity of connections, which occur early after a stroke but may disappear months or years later. Transplantation might benefit from such plasticity and become maximally beneficial during this reorganization.

However, delaying the stem cell transplantation for several weeks after stroke must also contend with the disadvantage of formation of scar tissue which might adversely affect implanted cells. The choice of timing must also consider the natural course of recovery from stroke. Impairments have different courses of improvement depending on the type and severity. Many neurologists would therefore prefer to delay transplantation till the deficit plateaus. For these reasons and many others, some investigators have preferred to transplant at least a few months after a stroke. The two clinical trials have chosen to study disabled patients at least 6 months after a stroke. However, there are no corroborating animal models of chronic stroke. Few outcome measures exist for animals with chronic stroke infarcts. Most importantly, recovery in animals cannot

be easily equated across studies or related to humans.

Blood supply:

Transplantation is unlikely to succeed if there is a severe arterial occlusion without collateral circulation; inadequate blood supply would not support graft survival. In contrast, transplantation efforts in progressive degenerative disorders are not necessarily concerned with arterial patency and inflammation.

Site of Implant:

From a mechanical point of view, injection of cells into the fluid-filled cavity of a chronic infarct facilitates the migration of transplanted cells. Without a definable cavitated area, transplantation requires more direct pressure to inject risking damage to normal tissue. However, cavity fluid can dilute the concentration of donor cells.

In the acute setting, it may be appropriate to inject cells in the salvageable penumbra but grafts might still be exposed to the detrimental effects of spreading depression and excitatory neurotransmitters. Fetal cortical grafts to the ischemic brain have been shown to survive in the penumbra but not in the core lesion. However, in chronic infarcts, glial scarring might impede the delivery of cells to the penumbral areas.

Some investigators believe that grafts could be more effective if the poorly vascularized inflammatory environment of the ischemic region is avoided altogether and suggest the plausibility of transplantation to distant regions, even to the contra-lateral side.

Patient selection:

Patients selected for stem cell transplantation for stroke should have measurable deficits, impairments and handicaps. The neuroanatomical relationship between image-defined infarct and deficits should be well established. Co-morbidities and the need for extensive follow-up also play a strong role in determining which patients are good candidates for experimental therapies.

Cell types for stroke neuro-transplantation:

A range of different cell types under investigation for transplantation in experimental and clinical stroke trials are; NT2N, LGE, BMSC, HUCBC, NSC, and Adipose tissue cells. (Table-1).

Animal Studies : (30-51)

Stem/precursor cells from different sources have been tested for their ability to reconstruct the forebrain and improve function after transplantation in animals subjected to stroke. (Table-2)

Table-1 : Various Cell types under investigation for transplantation in experimental and clinical stroke trials

Cell type	Description	Advantages	Disadvantages
NT2N	Immortalized Cell line	Unlimited supply	Lineage restricted long-term safety
LGE	Fetal pig	Abundant supply	Lineage restricted Pig infections
BMSC	Bone marrow	autologous	Painful extraction IV approach may affect Other organs
HUCBC	Umbilical Cord blood cells	alternate source	Incomplete studies
Adipose	Stromal Stem cells	Abundant source	Incomplete studies
NSC	Neural Stem cells	Extraction from patient	Incomplete studies

Table-2 : Properties of Stem/Precursor cells grafted in animal models of stroke

Cell Source & transplantation approach	Effect on behavioral deficit
Rat SVZ precursors in rat cisterna magna	Improved sensorimotor function
Mouse neuroepithelial stem cell line in rat Cortex, striatum or ventricle	Improved sensorimotor function or spatial memory
Human fetal teratomacarcinoma cell line (NT2) in rat striatum	Improved passive avoidance task and symmetric motor behavior
Immortalized mouse cerebellar precursors	?
On polymer scaffold in mouse cortex	
Rat bone marrow stromal cells systemically	Improved sensorimotor function and Neurological Severity Score
Or in penumbra zone in rat striatum	
Human bone marrow stromal cells	Improved sensorimotor function
Systemically in rats	Neurological Severity Score
Human umbilical cord blood cells	Improved sensorimotor function
Systemically in rats	Neurological Severity Score

The transplants, including a mouse neuroepithelial stem cell line, the human NTera-2 cell line, and human bone marrow cells, have been reported to partly reverse some behavioral deficits. However, in most cases, the underlying mechanisms are unclear and there is little evidence for neuronal replacement. Only few grafted cells have survived and they have not exhibited the phenotype of the dead neurons. Moreover, it is unknown if the observed grafted cells are functional neurons and establish connections with host neurons.

Despite the poor evidence for significant neuronal replacement in these studies, improvement of various stroke-induced behavioral deficits has been observed. Stem cell transplantation probably can lead to clinically valuable improvements through several mechanisms. First, the tissue damage per se can stimulate plastic responses or interfere with neural activity in the host. Second, the transplants can act as biological minipumps and release a missing transmitter or secrete growth factors. These factors can stimulate plastic responses and improve the survival and function of host neurons. Third, the grafts can restore synaptic transmitter release by providing a local re-innervation. Fourth, and this is true

neuronal replacement, the grafts can become integrated into existing neural and synaptic networks, and re-establish functional afferent and efferent connections.

Recent findings in rodents suggest an alternative approach to cell therapy in stroke based on self-repair. Stroke leads to increased generation of neurons from NSCs in the SVZ, lining the lateral ventricles. These immature neurons migrate into the damaged striatum, where they express markers of striatal medium spiny projection neurons. Thus, the new neurons seem to differentiate into the phenotype of most neurons destroyed by the ischemic lesion. However, because more than 80% of the new neurons die during the first weeks after stroke, they only replace a small fraction (about 0.2%) of the mature striatal neurons which have died.

Clinical Trials: (52-55)

NT2 Neuron cell trials:

Immortalized cell line NT2 are derived from a human testicular germ cell tumor more than 20 years ago. Unlike other teratocarcinoma cell lines, the NT2 cells show an exclusive commitment to a neural lineage when exposed to retinoic acid. Several studies have shown that NT2 cells resemble neural stem cells. They express cell surface markers and cytoskeletal

proteins unique to neural stem cells. Treatment with retinoic acid and mitotic inhibitors for several weeks ultimately results in the production of postmitotic, NT2N, which expresses neurotransmitters, functional neurofilament and cytoskeletal proteins, and other proteins indicative of secretory activity and synaptogenesis. Transplanted cells also release neurotransmitters and elaborate typical neuronal proteins.

Phase I:

Seven years ago, a clinical trial began to assess the safety of intrastriatal NT2N (produced by Layton Bioscience Inc. and known as LBS neurons for human use) transplantation in patients with basal ganglia infarcts and stable motor deficits 6 months to 6 years before transplantation. Twelve patients were treated with NT2N cell transplants and immunosuppressed using cyclosporine for 9 weeks. Based on preclinical safety data, doses of 2 and 6 million cells were considered appropriate. Four years after the study began, there have been no adverse events related to the implants. Two patients died of unrelated medical illnesses. On autopsy examination of one of these patients, who did not show clinical improvement and died of myocardial infarction, the graft site showed no signs of inflammation,

neoplasia or infectious disease 27 months after implantation. Because NT2N cells are polyploidy for chromosome 21, grafted neurons were identified at the injection site with fluorescent in situ hybridization and DNA probes specific to this distinctive chromosomal feature. Positron emission tomography (PET) scanning at 6 months showed greater than 15% relative uptake of F-18 flourodeoxyglucose at the transplant site in six patients. This may reflect surviving and functioning implanted cells, enhanced host cell activity or an inflammatory response.

Phase 2:

A randomized open-label trial with observer blinded neurological evaluations was undertaken to test the effectiveness of neuronal cell transplantation in patients with substantial functional motor deficits following basal ganglia infarction. Fourteen patients were randomized to receive 5 or 10 million implanted cells followed by rehabilitation, compared with 4 patients who only underwent physiotherapy. Patients had stable motor deficits 1-6 years after the onset of stroke. Half the patients had an ischemic stroke, and the other half had a hemorrhage. The author tested the hypothesis that implantation of neuronal cells would be safe, feasible and improve motor neurologic deficits.

One patient had a single seizure and another had a subdural hematoma evacuated one month after transplantation. There were no cell-associated adverse events.

Functional outcomes were assessed by the National Institutes of Health Stroke Scale (NIHSS), European Stroke Scale Score, Stroke Impact Scale, Fugel-Meyer Score, and Action Research Arm testing. Cognition was also tested before treatment and after 6 months. Transplant patients showed a trend toward improvement in functional outcomes on several scales compared with baseline measurements before transplantation, but there were no statistically significant trends compared with the four controls. With such small numbers however, the significance of the findings is unclear. A third clinical trial will evaluate cell implantation for patients with stable cortical strokes.

Diacrin trial:

Phase I: A pilot safety and feasibility study was started in 1998. The original goal was to enroll 12 patients with chronic, stable, moderate-sized basal ganglia infarcts who would receive intrastriatal implantation of fetal cells from the porcine, primordial striatum, also called the LGE of porcine embryonic tissue and pretreated in culture with an anti-major

histocompatibility complex class I antibody, thus obviating the need for immunosuppression after transplantation. Five patients underwent transplantation. Their strokes occurred on an average five years earlier. Computed tomography at the completion of surgery showed no evidence of hemorrhage in any patient. The patients developed no new neurological deficits in the acute setting. One patient developed cortical vein occlusion thought to be related to the surgery, but the Food and Drug Administration terminated the study. At 2 years one of the patients showed improvement on the modified Rankin Scale.

The Korean University trial: (56)

This study was completed in 2005. It was a randomized controlled phase I/II trial. Cell transplantation improved recovery from ischemic stroke in 30 patients with intravenous autologous mesenchymal stem cells infusion. They prospectively and randomly allocated 30 patients with cerebral infarcts with middle cerebral artery territory and with severe neurological deficits into two treatment groups: the MSC group (n=5) received intravenous infusion of 1×10^8 autologous MSCs whereas the control group (n=25) did not receive MSCs. MSC treated patients received 5×10^7 cells twice: 4 to 5 (first boosting)

and 7 to 9 weeks (second boosting) over 15 – 20 minutes. Neurological deficits and improvements in function were compared between the groups for 1 year after symptom onset. Neuroimaging was performed serially in five patients from each group. Outcomes improved with the MSC treated patients compared with the control group.

Indian Scenario: Current Status

Pilot study conducted at All India Institute of Medical Sciences (AIIMS), in patients with acute ischemic stroke (randomized within one month of acute stroke) who have been injected intravenously with autologous bone marrow derived mononuclear cells (BMMNCs), has proven safety of the procedure. The study was not designed for efficacy. Based on the results of the pilot study, a multicentric study co-ordinated by AIIMS and funded by Department of Biotechnology (DBT) is currently underway.

A department of science and technology (DST) funded study in patients with chronic hypoxic-ischemic injury to the brain including cerebral palsy with autologous intra-arterial injection of BMMNCs is also currently ongoing at AIIMS.

The National Brain Research Center (NBRC) at Manesar involved in research in neurosciences, is conducting

basic work on understanding how the stem cells might work in neuronal plasticity and recovery.

Center for stem cell research has come up in CMC Vellore under the aegis of DBT and several others are in the pipeline for a bench to bed side research on stem cell treatments in various diseases.

There are reports of unmonitored, outside experimental protocol “treatments” with various varieties of “stem cells” for a variety of “incurable” diseases being given by “self-proclaimed” stem cell specialists in India and elsewhere reporting “unparalleled” success stories in media. These reports have triggered an avalanche of patients and care givers of patients with degenerative disorders and as yet incurable diseases making a bee line to these unproven interventions by these unscrupulous practitioners who are making a fortune on public gullibility. Currently there seems to be no law to curtail these unscientific activities.

Adult Stem Cell Therapy in Stroke

Adult stem cell therapy for stroke can be divided in an endogenous and exogenous approach. The aim of the endogenous stem cell therapy is to exploit the population of adult stem cells already physiologically present either in

the CNS or hematopoietic system derived adult stem or precursor cells are administered locally or systemically after purification and propagation in culture.

Over the past decades, convincing evidence emerged that neurogenesis in the adult CNS is a continuous physiological process. Neurogenesis is present in two regions: the SVZ and the subgranular zone of the dentate gyrus. Additionally, recent studies also indicated the existence of NSCs in other regions of the CNS, namely the striatum, spinal cord and neocortex. External global stimulants such as enriched environment, physical activity and stress, or application of defined molecules such as fibroblast growth factor-2, VEGF, BDNF and erythropoietin differentially modulate adult neurogenesis and have been tried in experimental models of stroke.

Interestingly, acute cerebral ischemia in human subjects leads spontaneously to a threefold increase in CD34+ cell count in the peripheral blood. Considering this change as an insufficient self-repair mechanism, it is a logical consequence to further promote CD34+ cell mobilization pharmacologically by the administration of granulocyte colony stimulating factor (G-CSF). In addition, G-CSF has been described to exert neuroprotective effects following cerebral ischemia. A

recent preclinical study found functional improvement in rats with focal G-CSF.

Both global and forebrain ischemia, or middle cerebral artery occlusion (MCA-O), have been shown to induce neurogenesis in the dentate gyrus in adult mammals. The main questions are whether newborns in these conditions are able to replace lost neurons at the lesion site in a specifically appropriate manner and whether neuronal replacement through endogenous neurogenesis can be correlated with functional recovery.

There are several concerns regarding stem cell therapy in stroke. First, what is the intended strategy for implantation? Transplanted cells may play a role as neurotrophin "pumps" rather than as grafted tissue restoring segmental connections. The mechanism of recovery might impact the choice of cells to implant. If a trophic response is desired, then a cell line genetically engineered to supply trophic support may be a better choice. If reconstitution of a neural network is desired, then access to a broader array of lineage species may be necessary to reconstruct the complex and widespread neuronal, glial and endothelial damage in stroke. It is crucial to better understand the basic biological mechanisms of individual cell types as implant sources for transplantation in stroke patients.

Such properties that need further clarification are proliferation, differentiation, potential lineage restrictions and extent of integration into host structures.

The major concern is safety. Long-term studies on the biological behavior of cell grafts are mandatory to better understand the effects and safety of cell transplantation in stroke. Just as transplanted cells in the heart could be arrhythmogenic, transplanted cells in the brain may prove to be epileptogenic. There remains virtually no information regarding the long-term effects on organ physiology and tumor formation from parenteral delivery of transplanted cells. The number of cells needed to promote recovery is a matter of debate.

This issue impacts delivery methods given the volumes needed for adequate cell number. Lastly, the question regarding modulation of the transplanted tissue of the various responses after stroke, such as endogenous neurogenesis, synaptogenesis, angiogenesis and inflammation remains to be explored. Induction of endogenous neurogenesis also remains unexplored and is potentially very attractive.

In conclusion, basic and clinical research in stroke neurotransplantation remains in a nascent stage. Much more work is needed to further characterize the biology of different implant sources both *in vitro* and *in vivo*.

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