NEURAL TRANSPLANTATION: A REVIEW

Ashok K. Agrawal and Rajnish K. Chaturvedi

Introduction

Neural transplantation has been considered as a classic approach in neurobiology for replacement of lost neurons, supplementation of neurotransmitters, reconstruction of damaged neuronal pathway and recovery of normal movement and functioning in rodents, primates and humans (Tandon et al, 1992, Bjorklund, 2005). Traditionally neural transplantation is defined as "replacement of missing/ degenerating/dysfunctional neurons", that has been lost because of disease or damage. These diseases may be hereditary or sporadic and are characterized by gradual and progressive loss of neurons of the central/peripheral nervous system (CNS/PNS). As the regeneration of mammalian CNS and PNS is extremely limited, therefore such neuronal loss in turn causes severe biochemical changes, leading to specific clinical deficits and altered neurobehavioral functions. Fetal neural transplantation raised hopes that a new treatment was imminent and possibly emerged as an alternative potential clinical therapy of neurodegenerative diseases.

Neural transplantation in neurodegenerative disease raises a number of fundamental questions of nervous system growth and development, because it relates to neuronal cell division, differentiation, migration, axonal elongation, axon target interaction, trophic factor mechanism, neural plasticity and repair (Bartlett *et al*., 2004). This technique has been extensively used as an experimental tool for the study of degeneration and repair in neurodegenerative and neurological disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington disease (HD), head trauma, epilepsy and stroke (Barlett et al., 2004, Kirik *et al*., 2005,Chaturvedi et al., 2008). Neural transplantation has attracted great interest over the past two decades for its potential as replacement therapy for human CNS diseases (Agrawal et al, 2003).

Replacement of chemically defined neurons in animal model of neurodegenerative diseases indicates that grafted fetal neurons survive post transplantation, integrate with the host brain and produce a sustained improvement of motor function and memory performance (Gopinath et al 1987; Agrawal, et al., 2004; Chaturvedi, et al., 2008). This approach is not only restricted to animals but has been also found to be successful in clinical cases of neurodegenerative diseases, especially PD (Bjorklund, 2005). Since the advent of neural transplantation research, considerable attention has been focused on its potential use in the treatment of PD. PD is a well studied neurodegenerative disorder for neural transplantation studies and was the first CNS disorder for which neural transplantation was attempted in clinical cases (Drucker-Colin and Verdugo-Diaz, 2004; Bjorklund, 2005). The degenerative nature of PD involves loss of dopaminergic neural cells as a cause or consequence of the disease; therefore cellular

replacement therapies offer a rationale and attractive therapeutic solution (Lindvall and Bjorklund, 2004). Neural transplantation in PD is based on the idea that DA-producing cells implanted into the denervated striatum may be able to substitute for those mesencephalic dopaminergic neurons that have been lost as a consequence of the disease process. (Yu *et al.*, 2005)

History of Neural transplantation

The history of neural transplantation can be traced back to 100 years when W.G. Thompson (1890) attempted to transplant cat neocortex brain tissue into the brain of dog. The efforts were not successful and about after 80 years from the work of Das and Altman, (1971) and Bjorklund and Stenevi, (1979), it was realized that only fetal neurons survived in brain and are able to reconstitute neural circuitry. During the last decades, emphasis has mostly been on clinical application of fetal neural transplantation focused on PD. Several years of successful research on neural grafting in animal models of PD have led to several clinical trials worldwide. The first transplantation of fetal dopaminergic neural tissue in PD patients was carried out by Lindvall and colleagues with modest clinical improvement (Lindvall et al., 1989). Over 350 patients worldwide have received fetal transplants with moderate to significant improvement in disease conditions (Drucker-Colin and Verdugo-Diaz, 2004; Linazasoro, 2003).

Fetal Neural Transplantation

Fetal neural transplantation of dopaminergic neuron rich ventral mesencephalic cells (VMC) has attracted great interest in recent years for its potential as cell replacement therapy of degenerated dopaminergic neurons in PD patients (Shetty et al, 1991; Borlongon and Sanber, 2002; Bjorklund et al., 2003; Agrawal et al, 2004; Snyder and Olanow, 2005). The discovery that immature nerve cells can survive where those form the adult tail and provided significant hope and impetus for human studies (Lindval et al, 1989). Adult CNS has very limited capacity for regeneration and only fetal neural cell transplantation was thought for developing neuronal circuitry with host brain towards functional restoration. In support of this, transplanted fetal dopaminergic neurons have been shown to survive, differentiate, re-innervate the host brain, restore DA synthesis, reverse post-synaptic receptor abnormalities, reverse functional deficits such as rotational abnormality and form functional synaptic contacts with adult host brain neurons (Gopinath et al, 1987, Agrawal et al, 2004b). Moreover, the brain being "immunologically privileged" site to a significant extent, immune rejection of fetal brain tissue appeared mild or even absent when grafted in the CNS (Drucker-Colin and Verdugo-Diaz, 2004). Further brain lacks a major lymphatic system and the blood-brain-barrier (BBB) blocks the infiltration of immune cells to get into the brain. On the other hand fetal brain tissues used as donors, lack the cell surface antigens leading to immunological rejection since the major histocompatibility gene complex (MHC) are inactive in normal fetal brain tissues (Drucker-Colin and

Verdugo-Diaz, 2004;). For transplantation, fetal VMC rich in dopaminergic primodia is obtained from the ventral mesencephalon (VM) of midbrain substantia nigra region of embryonic day (ED) 13-14 rat fetuses and 5-8 weeks post conception in case of human fetuses, because during this period neurogenesis and proliferation of neurons is still progressing. Further at this stage of development, neurons become terminally differentiated, before they have formed extensive axonal connections (Dunnett and Bjorklund, 1994).

It was demonstrated that the grafted dopaminergic neurons display many of the morphological and functional characteristics of intrinsic dopaminergic neurons including differentiation, re-innervation of host brain and releasing DA and forming functional synaptic contacts with host brain neurons (Gopinath et al, 1987; Agrawal et al, 1995). Further, grafting of immature fetal neurons reverse post-synaptic receptor abnormalities, restore DA synthesis and transmission capacity to near normal levels, gain neuro-electrophysiological characteristics of mature dopaminergic neurons, reverse functional deficits such as rotation abnormalities (stereotypy) and restore spontaneous movement defects to some extent. Once grafted cells survive and develop to maturity with neurites, functional recovery is attained.

Neural Transplantation at All India Institute of Medical Sciences (AIIMS), New Delhi

Pioneer work of Prof. Tandon and Prof. Gopinath at Department of Neurosurgery and Anatomy at All India Institute of Medical Sciences (AIIMS), New Delhi, laid the foundation for neural transplantation studies in India. Prof. Tandon was inspired to establish neural transplantation research in India by the work of Prof Anders Bjorklund and Prof G.D. Das, presented at the First Congress of the International Brain Research Organization at Lausanne, Switzerland, 1982. Prof. Bjorklund and Prof. Das had shown that, after transplantation fetal neural tissue survive, make specific connections with host brain and restore the lost function. After the Congress, Prof. Das of Purdue University, USA, kindly agreed to extend his help in establishing neural transplantation in India and to teach this technique to neuroscientists and clinicians across the country. In 1985 Tandon and Gopinath arranged first national workshop with the help of Prof. Das, which was financially supported by Department of Science and Technology (DST) and Biotechnology Board of Department of Biotechnology, Govt of India. Later on, Prof Tandon established a national facility for Research and Training in Neural Transplantation supported by the DST, New Delhi. This led to establishment of a unit on neural transplantation in 1986 under the DST program of “Intensification of Research in High Priority Areas (IRHPA). This unit consisted multidisciplinary investigators to under take systematic studies on the morphological details and over all growth of the fetal neural transplants, the general cytoarchitecture, using Golgi and HRP labeling for neuronal tracings in rats and monkeys for upto a period of two years (Tandon, 1992; Tandon et al., 1994).

The first experimental neural transplantation in India was carried out in 1987 using rodent model of Parkinson's disease followed by assessment of morphological characteristics of the transplant at graft site. For standardization of the neural transplantation technique rat embryonic neocortical tissue has been transplanted into the different brain regions of adult rat (cerebellum, lateral ventricle, third ventricle, striatum, hippocampus and the anterior chamber of the eye). Neocortical tissue from gestational day 17 was transplanted into the caudate putamen region of adult rat to validate the development and integration of the transplanted neuronal cells in an area, which has intimate connections with the neocortex (Shetty et al., 1991; Tandon et al, 1992). Presence of surviving grafted neurons and increase of transplant volume after 2.5 months with no glial scar was observed with varied graft volume in different rats. However, no significant synapse formation between host and grafted tissue was evident, which could be possible due to lack of neurotrophic influence on grafted neocortex neurons in transplanted area. After successful transplantation studies in rodents this group standardized neural transplantation technique and morphological investigations of the transplants in primates. In 1987, the first neural transplantation was performed in sub-human primates in India. Studies using fetal neural transplantation in caudate putamen of monkeys revealed only 20-30% success of survival of transplanted fetal cells, possibly due to immunorejection (Gopinath et al., 1987). Quantitative morphology revealed a progressive reduction in the number of neurons at transplantation site and parallel glial cell density with passage of time (Shetty et al., 1991a). Tandon et al., 1990 demonstrated that in lesser-evolved sub-human primates such as Bonnet monkey, the survival of transplanted neurons was high as compared to Macaca rhesus. Significant survival of fetal neural transplant in monkey was obtained with fetal tissue cryopreserved for four days in culture media in comparison to the high failure rate of fresh fetal tissue transplant (Tandon et al., 1990). Shetty et al (1991b) and Gopinath et al (1991) transplanted rat fetal neural cells of substantia nigra in the lateral ventricle / striatum of adult rats. Grafted neurons matured and showed phenotypic characteristics comparable to that of normal nigral neurons in adult rats. However, with the passage of time grafted neurons showed age related changes in grafted neurons like dendritic thickening, membrane bound vacuoles and increase in lipofuscin granules in the cytoplasm. Further they have shown that interaction between host and grafted neurons are necessary for long term survival of transplanted neurons (Shetty et al., 1991). They have also carried out autograft of adrenal medullary tissue in anterior eye chamber, lateral ventricle and striatum of adult rats. They have reported that transplanted adrenal medulla chromaffin cells survived upto 360 days in anterior eye chamber as compared to lateral ventricle and striatum (Gopinath et al, 1996a). Further, Gopinath et al., (1996b) reported the maturity of fetal cell transplantation in adult host brain using two cell surface markers; neuronal cell adhesion molecule (NCAM) and L1 in intrastriatal transplanted fetal cells. They observed delayed maturity of the transplant in adult brain in comparison to fetal brain. In continuation they further pursued ultra structure study of transplanted neurons after 2 years post transplantation to evidence the

status of transplanted fetal substantia nigral tissue into the striatum of 3-4 months old rats and they found very few synapses and gliosis at the site of transplantation. However, they have reported the presence of normal neurons in transplants even after 2 years in the rat, which has life span of only 3-4 years. Sable et al (1997) demonstrated that fetal neural cells after transplantation survived in brain of kainic acid lesioned rats. Mohankumar (1998) first time demonstrated role of basal fore brain transplantation in thermoregulation in rodent model.

Neural transplantation at Indian Institute of Toxicology Research (IITR)-Lucknow

IITR has a strong Neurotoxicology and Neuropharmacology group under Developmental Toxicology Laboratory which developed in the leadership of Dr. P.K. Seth. The group took advantage of national facility of neural transplantation developed at AIIMS, New Delhi, where Dr. Agrawal and late Dr. Roshan Hussain obtained initial training on neural transplantation technique, which helped in establishing the technique at IITR in 1992. During last 15 years, the thrust was to develop and modify neural transplantation techniques, which help long term survival and functional viability of transplanted cells.

Initial studies conducted in this laboratory have demonstrated a significant functional restoration with fetal VMC of ED 12-14 days fetus using neurobehavioral, neurochemical and electrophysiological parameters in neurotoxin induced rodent model of Parkinson's disease. During the last few years this laboratory has been perusing the studies related to restorative effect of fetal neural transplants, using standard macrotransplantation techniques in restoring the dopaminergic and cholinergic deficits induced by specific neurotoxins like 6-hydroxydopamine (6-OHDA), colchicine and environmental neurotoxin trimethyltin and acrylamide (Roy et al., 1998; Adhami et al., 2000). Studies using acrylamide and tri methyl tin, well known neurotoxins affecting central and peripheral neuropathy cause hind limb paralysis on sub acute treatment exhibited significant neurobehavioral deficits assessed using rotarod performance, learning ability and grip strength (Husain et al., 1992). They have demonstrated that fetal cerebellar transplantation in acryalamide exposed animal exhibit significant functional recovery using neurobehavioral and neurochemical parameters 9 weeks post transplantation. Further survival of significant number of neurons at transplantation site has been confirmed by detail neuroanatomical studies (Husain et al., 1994).

Rat model of PD was developed by unilateral stereotaxic injection of classical neurotoxin, 6-hydroxydopamine, into the caudate putamen (striatum) of rats (Agrawal et al., 1995) leading to specific degeneration of dopaminergic neurons exhibiting significant alteration in neurobehavior and neurochemical parameters. Fetal neural transplantation of VMC in the caudate putamen region of 6-OHDA lesioned rats resulted in significant functional restoration in spontaneous and drug induced motor activity and dopamine receptor 8 weeks post transplantation. The

morphological and electrophysiological studies in transplanted rats showed significant recovery in neuronal firing rate of neurons transplanted in caudate putamen revealed presence of functionally viable neurons at transplantation site. Further, electron microscopic assessment of transplanted area indicating presence of viable neurons and cytoplasmic organelles at transplanted site (Agrawal et al., 1995). Restoration of cholinergic deficit has been observed in trimethyltin exposed rats following intra hippocampal transplants. Further, transplantation of fetal cortical neuronal cells considered serotonergic neuron rich cells restored both cholinergic and serotonergic deficits (Roy et al., 1998). Adhami et. al. (2000) demonstrated that lead induced deficits in locomotor activity and learning ability is restored by intrahippocampal cholinergic neurons transplantation. Although significant functional restoration was observed in early post transplantation weeks (4-6 weeks) it could not persist beyond 10-12 weeks post transplantation.

Multiple Microtransplantation

Conventional macrotransplantation approach in which large number of fetal neural cells have transplanted at one site of lesioned brain region, has been demonstrated to cause significant trauma, inflammation and free radicals mediated degeneration of transplanted fetal cells in first few weeks post transplantation (more than 90%). Hence only partial functional restoration is possible and does not persist for long duration. Collaboration with the Prof. G. Nikkhah, well-known neurosurgeon, of Hannover medical school, Germany, was established to develop multiple microtransplantation technique at IITR. This technique helps in distributing same number of fetal cells around lesioned site resulting in significant long term functional restoration assessed by neurochemical, neurobehavioral and immune histopathological parameters (Agrawal et al., 2003). We could demonstrate long term functional restoration, upto 24 weeks post transplantation, using multiple micro transplantation approach in rodent model of dementia developed by colchicine, when compared with macro transplanted animals.

Although the early results of fetal neural transplantation in clinical trials were reported promising worldwide, but due to certain limitations in application to clinical cases, fetal transplantation approach could not be used routinely as a therapeutic measure in neurodegenerative disorders. Most studies reported degeneration of transplanted fetal dopaminergic neurons in rodents as well as in humans ranging from 5-20% within the first 7-10 days post-transplantation. This low survival makes it difficult to obtain sufficient human fetus (6-8) for grafting in Parkinsonian patients. Although the underlying reason for the poor survival rate of transplanted human dopaminergic neurons is not well defined and attributed to free radical generation, apoptosis, energy deprivation by mitochondrial dysfunction, glutamate excitotoxicity, hypoxia, disturbances in calcium homeostasis, lack of neurotrophic factor support (Chaturvedi *et al.*, 2003,) and inadequate vascularization at transplant site. These factors, along with mismatch condition (fetal cells transplantation to adult brain) have been shown playing major role in early

degeneration (more than 90%) of transplanted cells. The poor survival of grafted dopaminergic neurons, ethical issues in using human tissue, in combination with a limited supply of human fetal donor tissue, constitute major limitations that prevent possible widespread application of the fetal neural transplantation in patients with PD.

Extensive research was carried out to minimize the amount of fetal tissue required by improving the survival of grafted fetal tissue or on finding alternative fetal neural tissue. Attempts were made in our laboratory to explore the possibilities for improving the post transplantation survival of grafted cells by using antioxidants (Agrawal *et al*., 2004b,) antiapoptotic agents and neurotrophic factors support (Chaturvedi *et al*., 2003, 2005).

Co transplantation of fetal neuronal cells with antioxidants

Free radical mediated damage has been reported to contribute significantly towards low survival (5-10%) of grafted dopaminergic neurons, attempts were made to use co-transplantation approach using fetal VMC with two major antioxidants, ascorbic acid (AA) and glutathione (GSH), in rat model of Parkinson's disease (Agrawal et al., 2004). Use of antioxidants exhibits significant increased protection of transplanted cells as evident from the functional restoration using neurobehavioral, neurochemical and immunohistochemical observations (Chaturvedi et al., 2003, 2004).

Role of Neurotrophic Factors

It is well established that poor cell survival is also due to lack of appropriate neurotrophic factor (NTFs) support at transplantation site. NTFs are family of proteins, which promote neuronal survival, stimulate axonal growth and influence axonal target finding to establish synaptic contacts. NTFs not only promote the differentiation and growth of developing neurons and phenotypic maintenance and survival of adult mature neurons but also represent a potential means of modifying neuronal dysfunction and modulate neuronal plasticity under pathological conditions.

In order to further potentiate the post transplantation survival of fetal cells attempts at IITR have been made to use neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in rodent model of PD. Our studies suggest that co-transplantation of fetal VMC with either alone or mixture of NTFs significantly increased the survival of transplanted fetal VMC as compared to VMC transplanted alone assessed at 6 and 12 weeks post transplantation (Chaturvedi et al., 2003a; 2006a). Further we have shown that NTFs not only increase the post transplantation cell survival but also rescue the stressed dopaminergic neurons undergoing apoptosis. Our studies have further shown that these NTFs increase the transplanted cells survival more significantly when used as a mixture of NTFs as they act co-operatively and increase the activity of each other (Chaturvedi et al.,

2005). Single infusion of these NTFs only provides short term functional restoration studied upto 12 weeks post transplantation, while to achieve long term functional restoration continued NTFs support has been considered necessary. Long term and continuous NTFs support has been achieved by peristaltic infusion pump, genetically modified cells capable of producing NTFs and viral vectors. However, these techniques at first hand involve severe surgical trauma and raise safety issues, hence are not recommended suitable as therapeutic candidate in clinical cases (Chaturvedi et al, 2006a).

In order to provide sustained neurotrophic factors to transplant fetal VMC attempts have been made to co-transplant fetal VMC with continuously GDNF releasing genetically modified BHK-GDNF cells to provide continuous NTFs support to grafted cells in 6-OHDA lesioned rat model of PD and compared with single infusion of GDNF. Functional restoration was assessed using neurobehavioral, neurochemical, immunohistochemical and molecular indices at 6 and 24 weeks post transplantation. VMC+GDNF co-transplanted and VMC+BHK-GDNF cells co-transplanted group exhibited more significant restoration of neurochemical deficits at 6 weeks, persisting significantly upto 24 weeks post transplantation only in VMC+BHK-GDNF cells co-transplanted group (Chaturvedi, 2006b; Seth et al 2002).

Paraneural Cell Transplantation

Considering the usefulness of NTFs in neuroregeneration and neuroprotection, led us to develop newer approach using transplantation of endogenous paraneural cells obtained from olfactory bulb, which could serve as neural cell substitute for DA and also provide trophic support for better survival of transplanted fetal neurons. Paraneural cells are nerve-supporting cells of PNS, which naturally synthesize multiple NTFs and have been shown to exhibit significant neuroregenerative properties in animal models PD as well as other CNS disorders. Therefore paraneural cells have emerged as an attractive alternative in cell transplantation approaches in PD as well as other neurodegenerative diseases as demonstrated by us (Agrawal *et al*., 2004 a; Shukla *et al*., 2004).

Our group has explored the use of different paraneural cells viz. olfactory ensheathing cells, carotid body cells and Zuckerkandl's organ in Parkinsonian rats.

1. Olfactory Ensheathing Cell (OEC) Transplantation

The OEC is a specialized glial cell that associates with the olfactory nerve, surrounding the axons of olfactory receptor neurons (ORNs). Their ability to support neuronal regeneration, both within the olfactory system and elsewhere in the CNS has made them an attractive cellular model for transplantation studies. OECs share properties with both Schwann cells (glia from the PNS) and astrocytes (glial cell type from the CNS). OECs have been shown to secrete various NTFs such as NGF, GDNF, BDNF, NT-4/5, NTN, CNTF, FGF2, and their receptors (Fairless and

Barnett, 2005). OECs also express cell-adhesion molecules such as L1 and NCAM, important for growth cone extension and axonal elongation as well as extracellular matrix molecule, laminin which has been demonstrated to substantially enhance neurite outgrowth, axonal re growth and the migration and of OECs.

Keeping in view the characteristics of OEC, our laboratory for the first time co-transplanted fetal dopaminergic cells (VMC) with cultured OEC (derived from adult rat olfactory bulb) in CNS to support the grafted as well as remaining host dopaminergic neurons and demonstrated long-term survival of fetal cells reflected from significant functional restoration in the rat model of PD (Shukla et al., 2005).

2. Carotid Body (CB) cell Transplantation

CB is a sensory chemoreceptor organ located in the vicinity of bifurcation of common carotid artery, which monitors changes in blood levels of O_2 and CO_2 (Toledo-Aral *et al*., 2002). Chromaffin-like glomus cells are physiological arterial oxygen sensors and contain chemoreceptor that release large amounts of DA in response to hypoxia, similar to conditions prevailing at the transplantation site. CB cells also express high levels of GDNF, NGF, BDNF and NT-3 (Shukla et al., 2005). This extraordinary characteristics of CB cells encouraged us for the first time to use cultured CB cells as co-transplantation with fetal dopaminergic cells (VMC) in rat model of PD. Significant functional restoration was observed at 12 weeks post transplantation when compared with the animals transplanted only VMC. This significant effect of CB could be due to the multiple NTF support of CB to the grafted/host dopaminergic neurons at trans plant site. Moreover the property of CB cells to thrive in hypoxic conditions resulted in long-term survival of grafted cells (Shukla *et al*., 2003, and 2004).

3. Zuckerkandl's organ

Zuckerkandl's organ (ZKO), is an extra adrenal paraganglion tissue, located on abdominal sympathetic region having ability to synthesize and release mixture of NTFs (GDNF, FGF and TGF nor-epinephrine and minute amount of DA. ZKO cells also express cell adhesion molecules such as NCAM, L1 and GAP-43 & chromogranins which helps in providing cues to growing axons to their targets and are important for growth cone extension and axonal elongation. Another characteristics is that the ZKO cells can be cultured easily for appropriate differentiation (Espejo et al., 2001, 2005).

In view of the special characteristics of ZKO, first time our laboratory has made an attempt to use ZKO transplantation in brain as co-transplantation with fetal VMC (dopaminergic neurons) to study long-term functional restoration in Parkinsonian rats at twelve weeks post transplantation. Long-term functional recovery was assessed using neurobehavioral, neurochemical, immunohistochemical and molecular approaches. Significant potentiation in survival

of transplanted cells and functional restoration in rat model of PD co-transplanted with VMC+ZKO as compared to animals without ZKO (Chaturvedi et al., 2008).

4. Neural stem cells (NSC) transplantation in Parkinsonian rats

Due to ethical and legal issues and limited availability of same age fetuses associated with use of fetal tissue for transplantation in clinical cases, use of neural stem cells (NSCs) has emerged as an appropriate alternative cell source for clinical application. It has offered a promising future for replacement of dysfunctional and degenerated cell types in the neuro degenerative diseases. NSCs have been defined as multipotent cells; mitotically active, self-renewing and can give rise to cells of all the three lineages of nervous system; neuronal, oligodendrocytes and astrocytes. These cells can be cryopreserved, expanded in culture, and are useful for generation of stem/progenitor cell in cryopreserved condition. Further, being isolated from adult brain as well, NSCs can be used for auto-transplantation, thus minimizing the chances of immune rejection (Takagi et al 2005).

These cells can be potentially manipulated to expand *in vitro* in response to neurotrophic and mitogenic factors (NTFs) such as bFGF and EGF and further expanded to express the dopaminergic phenotype during progenitor cell differentiation (Chaturvedi, et al., 2008). The use of such expanded progenitor cells could overcome the limited availability of primary fetal VM tissue for transplantation in PD and furthermore, cryopreservation of these cells could provide a readily available store to be utilized when needed. NPCs under influence of mitogenic factors proliferate in culture and after addition of trophic factors and cytokines transform into specific neuron type (dopaminergic, or cholinergic). Thus DA neurons derived from stem/progenitor cells constitute one of the most promising tools for cell replacement therapy in PD.

Despite several advantages of NPCs, transplantation of these cells in PD has not reached to a level to become a routine therapy in clinical cases. Like other cells (fetal neural cells, adrenal medulla cells, and sertoli cells), survival of NPCs differentiated to specific neuronal type also require neuro trophic factor support. Without optimum conditions, reported survival is less than 10% at transplantation site (Chaturvedi et al., 2006b). This low survival could be due to the oxidative stress, apoptosis, altered host microenvironment, release of toxic factors from host brain, absence of appropriate developmental cues and lack of NTFs support (Shukla *et al.*, 2006). It has been established by us and others that continuous NTFs support is required for long term survival of transplanted cells. These NTF support helps in better integration with host cells and also cell-to-cell communication. Therefore attempt has been made to use co transplantation of NPC with continuous NTF supporting paraneural cells transplanted in parkinsonian rats this may help in long term survival leading to functional restoration.

Neural progenitor cell culture could be established at IITR and characterized using specific markers. NPC transformed to specific neuronal type (dopaminergic and cholinergic) neuron with the help of specific mitogenic and neurotrophic factors. Characterized dopaminergic type cells were then transplanted into lesioned brain region (caudate putamen) of rat model of PD to assess the viability of these cells towards functional restoration. Attempts were made in our laboratory to use OEC and/or ZKO as co transplant along with NPCs to assess the long term survival of transplanted NPCs. NTFs support to transplanted cells leads to long term functional restoration upto 24 weeks post transplantation (Shukla et al., 2006; Chaturvedi et al., 2008).

In summary, transplantation of NPC has been considered a better cell replacement therapy in rat model of neurodegenerative disease as it avoids fresh fetal neuronal cells, which is a strong limitation in clinical application of this technique. Further more co-transplantation with paraneural cells gives advantage for long-term survival of NPC providing constant NTF support. Application of NTFs supporting paraneural cells in co-transplantation approach with NPCs may prove to be a better approach in protecting transplanted cells.

5. Cryopreservation/ hibernation of fetal neural tissue

Due to low survival of transplanted fetal VMC, 6-8 fetuses are necessary for survival of optimum number of cells for sufficient clinical improvement following transplantation in one PD patient. For clinical use safe and reliable ways to ensure graft tissue availability on the day of transplantation, the ability to accumulate fetal tissue from several donors over time, the transport of tissue over long distances and microbiological and immunological testing all require impracticably long time interval between the actual dissection of the fetal donor tissue and its implantation in the host brain. This has led to an increased interest in donor tissue storage methods without affecting the cell survival, which would allow for the accumulation of sufficient amount of tissue until the day of transplantation. In many clinical transplantation programs, it would be a great advantage if human fetal nigral donor tissue could be stored for at least 3-5 days. To achieve this, attempts were made to hibernate (cold storage) the rat fetal brain without losing cell viability. In order to prevent cell death during cool storage (hibernation) we preserved fetal tissue at 4^0C in hibernation medium (HM) supplemented with, GDNF which has been shown to possess antioxidant and antiapoptotic activity. Functional viability of transplanted VMC and functional restoration was studied 4 weeks post-transplantation in rat model of PD. We have shown that fetal brain tissue remains more viable when hibernated as tissue pieces as compared to single cell suspension and intact brain (Chaturvedi et al., 2006a). Further, the supplementation of GDNF in hibernation medium upto 5 days increased the functional viability of stored cells and improved functional restoration in rat model of PD. These findings have clinical significance by enabling longer storage periods of graft material. Thus establishment of a donor

tissue bank can be envisaged. This may facilitate multicentre studies and stimulate more rapid advances in the field of clinical neural transplantation.

6) Neural transplantation at other Institutes in India

Badgaiyan and Gupta (1994) from Behavioral Brain Research Laboratory, Institute of Medical Sciences, Varanasi studied influence of intracranial transplantation of fetal noradrenergic cells on selected age related alterations of the behavioral parameters and demonstrated significant functional restoration at various period. Neural transplantation was also carried out at Postgraduate Institute of Basic Medical Sciences, Chennai by Muthuswamy, and associates, who have demonstrated that transplanted embryonic neocortical tissue from monkey and human fetuses into the cerebellum and visual cortex of adult bonnet monkey exhibited survival and maturity and viability of transplanted neurons (Muthuswamy and Krishnamurti 1987; Muthuswamy et al., 1987, 1988). The results support the observation that both human and monkey neocortical tissue survived for long time, showed good growth potential and made synapses with host neurons of adult and juvenile monkeys as well. Murthy and Desiraju of National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, transplanted fetal hippocampal neurons into the neocortex of neonatal rats to investigate the growth characteristics of transplanted neurons and reported that grafted neurons manifested significant deviation in morphology and growth as compared to normal native hippocampal neurons. (Murthy and Desiraju, 1989).

Kayalvizhi (2003) from Department of Anatomy, University of Madras (Chennai), transplanted human embryonic cortical tissue (HECT) in adult Bonnet Monkey (M*acaca radiata*). Solid HECT when transplanted in superfund neuroblastic cells in early period (18hrs-120 days) were seen in clusters Most of the grafter cells were viable and exhibited mitotic activity. They have reported that solid fragments exhibits survival when transplanted in devascularised cortical area of bonnet monkey.

Mishra (1992) highlighted the grafting of genetically modified cells for intracerebral implantation. He has further emphasized the usefulness of genetically modified cells transplantation as therapeutic use in P.D. and Huntington's diseases where the disease phenotype corrected by introducing functional genes in mutant cells.

Mohanakumar and research team at Indian Institute of Chemical Biology (IICB) Kolkata, have investigated dopaminergic functional recovery following controlled release of dopamine from biodegradable polymer matrices implanted in the lesioned striatum in a hemiparkinsonian animal model. Significant dopamine depletion in the striatum ipsilateral to the side of infusion was observed in animals unilaterally infused with 6-hydroxydopamine (6-OHDA) in the substantia nigra. These animals displayed apomorphine induced contralateral rotational behavior, when examined on the 16th day. Implantation of a controlled release delivery system

(hydrogel obtained by mixing dextran dialdehyde cross-linked with gelatin) containing dopamine in the denervated striatum on the 1st day or the 18th day significantly abolished the apomorphine-induced contra lateral rotational behavior in these animals. The recovery was visible for about 17 days thereafter the behavioral bias reappeared. (Senthilkumar et al., 2007).

Future Research

These studies, along with the others carried out globally, provided valuable information not only about issues related to developmental neurobiology but also the therapeutic potentials of the neural transplantation previously thought to be impossible. In the meanwhile some new discoveries, i) the persistence of neurogenesis throughout life, at least in some regions of the brain, ii) the isolation of totipotent cells from human embryos at blastocyst stage with potentials to differentiate into any type of adult cells including neurons, and more recently, iii) the existence of stem cells or progenitor cells in adults, as also, iv) the ability to manipulate adult somatic cells to function as stem cells has opened new vistas to overcome the problems associated with fetal neural transplants. No doubt in the present stage of knowledge we still have to go to a long way before the current laboratory research can reach the clinic (Tandon, 2001). But fortunately, owing to the efforts of the Department of Biotechnology, a number of groups around the country are currently actively engaged in pursuing this field of research.

References

Adhami VM, Husain R, Agarwal AK, Seth PK: Intrahippocampal cholinergic-rich transplants restore lead-induced deficits: a preliminary study in rats. Neurotoxicol Teratol. (2000) 22,41-53.

Agrawal AK, Husain R, Raghubir R, Kumar A, Seth PK: Neurobehavioral, neurochemical and electrophysiological studies in 6-hydroxydopamine lesioned and neural transplanted rats. Int J Dev Neurosci. (1995) 13, 105-11.

Agrawal AK, Roy A, Seth K, Raghubir R, Seth PK: Restorative potential of cholinergic rich transplants in colchicine induced lesioned rats: a comparative study of single and multiple micro-transplantation approach. Int J Dev Neurosci. (2003) 4, 191-98.

Agrawal AK, Chaturvedi RK, Shukla S, Seth K, Chauhan S, Ahmad A, Seth PK: Restorative potential of dopaminergic grafts in presence of antioxidants in rat model of Parkinson's disease. J Chem Neuroanat. (2004) 28, 253-264.

Agrawal AK, Shukla S, Chaturvedi RK, Seth K, Sinha C, Ahmad A, Seth PK: Olfactory Ensheathing Cell transplantation restores functional deficits in rat model of PD: A co-transplantation approach with fetal ventral mesencephalic cells. Neurobiology Dis. (2004a) 16, 516-526.

Agrawal AK, Chaturvedi RK, Seth PK: Co-transplantation of fetal ventral mesencephalic cells with antioxidants (Ascorbic acid and Glutathione) ameliorates functional deficits in rat model of Parkinson's disease. Ann of Neurosci. (2004b) 11, 9-16.

Badgaiyan RD, Gupta S: 5th International symposium on neural transplantation. France. (1994) 25-29.

Barlett LE, Sadi D, Lewington M, Mendez I: Functional improvement with low dose dopaminergic grafts in hemiparkinsonian rats. Neurosurgery. (2004) 55, 405-12.

Bjorklund A, Stenevi U: Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. Brain Res. (1979) 177, 555-60.

Borlongan CV, Sanberg PR: Neural transplantation for treatment of Parkinson's disease. Drug Discov Today. (2002) 7, 674-82.

Bjorklund A, Dunnett SB, Brundin P, Stoessl AJ, Freed CR, Breeze RE, Levivier M, Peschanski M, Studer L, Barker R: Neural transplantation for the treatment of Parkinson's disease. Lancet Neurol. (2003) 2, 437-45.

Bjorklund A: Cell therapy for Parkinson's disease: problems and prospects. Novartis Found Symp. (2005) 265,174-86.

Chaturvedi R.K, Agrawal A.K, Seth K, Shukla S, Sinha C. and. Seth P.K: Co-transplantation of fetal neural cell with GDNF and BDNF ameliorates cellular and behavioural deficits in 6-OHDA lesioned rat model of Parkinson's disease. Ann Neurosci. (2003) 10, 32-37.

Chaturvedi RK, Agrawal AK, Seth K, Shukla S, Chauhan S, Shukla Y, Sinha C and Seth PK: Effect of glial cell line-derived neurotrophic factor (GDNF) co-transplantation with fetal ventral mesencephalic cells (VMC) on long term functional restoration in 6-hydroxy dopamine (6-OHDA) lesioned rat model of Parkinson's disease: Neurobehavioral, neurochemical and immunohistochemical studies. Int. J. Devl. Neurosci. (2003a) 21, 391-400.

Chaturvedi RK, Agrawal AK, Seth PK: Restorative potential of dopaminergic grafts in presence of antioxidants in 6-OHDA lesioned rat model of Parkinson's disease. Ann Neurosci. (2004) 11, 9-16.

Chaturvedi RK, Shukla S, Seth K, Agrawal AK: Co-transplantation of Zuckerkandl's organ cells with ventral mesencephalic cells (VMC) in rat model of Parkinson's disease: Assessment of functional restoration. J. Neurochem. (2005) 94, 116.

Chaturvedi RK, Shukla S, Seth K, Agrawal AK: Glial Cell Line Derived Neurotrophic Factor (GDNF) increases the survival and function of hibernated fetal dopaminergic cells transplanted in rat model of Parkinson's disease. Ann Neurosci. (2006a) 13, 56-64.

Chaturvedi RK, Shukla S, Seth K, Agrawal AK: Nerve growth factor increases survival of dopaminergic graft, rescue nigral dopaminergic neurons and restores functional deficits in rat model of Parkinson's disease. Neurosci Lett. (2006b) 398, 44-49.

Chaturvedi RK, Shukla S, Seth K, Agrawal AK: Zuckerkandl's organ improves long-term survival and function of neural stem cell derived dopaminergic neurons in parkinsonian rats. Experimental Neurol. (2008) 210, 608-23.

Das GD, Altman J: Transplanted precursors of nerve cells: their fate in the cerebellums of young rats. Science. (1971) 173, 637-638.

Drucker-Colin R, Verdugo-Diaz L: Cell transplantation for Parkinson's disease: present status. Cell Mol Neurobiol. (2004) 24, 301-316.

Dunnett SB, Bjorklund A : Functional neural transplantation. Raven Press, New York. (1994).

Espejo EF, Gonzalez-Albo MC, Moraes JP, El Banoua F, Flores JA, Caraballo I: Functional regeneration in a rat Parkinson's model after intrastriatal grafts of glial cell line-derived neurotrophic factor and transforming growth factor beta1-expressing extra-adrenal chromaffin cells of the Zuckerkandl's organ. J Neurosci. (2001) 21, 9888-9895.

Espejo EF, Armengol JA, Flores JA, Galan-Rodriguez B, Ramiro S: Cells of the sympathoadrenal lineage, biological properties as donor tissue for cell-replacement therapies for Parkinson's disease. Brain Res Brain Res Rev. (2005) 49, 343-54.

Fairless R, Barnett SC: Olfactory ensheathing cells: their role in central nervous system repair. Int. J Biochem Cell Biol. (2005) 37, 693-699.

Gopinath G, Mahapatra AK, Nayar U, Tandon PN: Transplantation of the embryonic neocortex in the caudate-putamen of adult rat. Indian J Med Res. (1987) 86,246-52.

Gopinath G, Shetty AK, Tandon PN: Ageing changes in the transplants of fetal substantia nigra grafted to striatum of adult rat. Neuroscience. (1991) 40,429-43.

Gopinath G, Sable V, Sailaja K, Tandon PN: Cell surface molecules (NCAM and L1) in intrastriatal transplants of embryonic mesencephalon in rats. Neuroscience. (1996a) 73,161-169.

Gopinath G, Sailaja K, Tandon PN: Long-term nigral transplants in rat striatum: an electron microscopic study. Int J Dev Neurosci. (1996b) 14, 453-460.

Husain R, Agrawal AK, Hasan M, Seth PK: Functional recovery following fetal medullary transplants in spinal cord of rats with acrylamide induced spino cerebellar lesion. Restorative Neurol. Neurosci. (1992) 4,184-88.

Husain R, Agrawal AK, Hasan M, Seth PK: Modulation of acrylamide-induced neurochemical and behavioral deficits by cerebellar transplants in rats. Pharmacol Biochem Behav. (1994) 49, 443-448.

Kayalvizhi, I: Experimental Transplantation of Human Embryonic Cortical Tissue After Vascular Lesion in the Motor Cortex of Bonnet Monkey (Macaca radiata) J Anat. Soc. India. (2003) 52, 38-41.

Kirik D, Breysse N, Bjorklund T, Besret L, Hantraye P: Imaging in cell-based therapy for neurodegenerative diseases. Eur J Nucl Med Mol Imaging. (2005) 32, 417-434.

Linazasoro G: Stem cells: solution to the problem of transplants in Parknson's disease? Neurologia. (2003) 18, 74-100.

Lindvall O, Rehncrona S, Brundin P, Gustavii B, Astedt B, Widner H, Lindholm T, Bjorklund A, Leenders KL, Rothwell JC: Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6 –month follow-up. Arch Neurol. (1989) 46, 615-631.

Lindvall O, Bjorklund A: Cell therapy in Parkinson's disease. Neurox. (2004) 1, 382-393.

Mishra BK: Trends in experimental neural transplantation. Current Science. (1992) 63, 163-166.

Mohan Kumar V, Verma S, Gopinath G, Sharma R, Tandon PK: Preliminary studies on basal forebrain transplantation and thermoregulation. Indian J Physiol Pharmacol. (1998) 32, 31-32.

Murthy SK, Desiraju T: Quantitative assessment of dendritic branching and spine densities of neurons of hippocampal embryonic tissue transplanted into juvenile neocortex. Dev Brain Res. (1989) 46, 33-45.

Muthuswamy R, Krishnamurti A: Neural transplantation in the spinal cord of bonnet monkeys after experimentsl traumatic lesion: a preliminary report. J Anatomi Soci India. (1987) 36, 61-65.

Muthuswamy R, Sheeladevi A, Namasivayam A, Sundarraj T, Sunderraj S, Balakrishnan K, Marimuthu KM, Govindarajulu P: Heterospecific transplantation of human embryonic cortical tissue into the cerebellum of bonnet monkey (Macaca radiata). Neuroscience. (1987) 22, 765.

Muthuswamy R, Sheeladevi A, Namasivayam A, Sundarraj T, Sunderraj S, Mudaliar AK: Transplantation of monkey embryonic cortical tissue into the brain of bonnet monkey (Macaca radiata). J Anatomi Soci India. (1988) 37, 27-25.

Roy A, Agrawal AK, Husain R, Dubey MP, Seth PK: Cholinergic and serotonergic alterations in the rat hippocampus following trimethyltin exposure and fetal neural transplantation. Neurosci Lett. (1998) 259, 1-4.

Sable V, Sailaja K, Gopinath G, Tandon PN: Fetal dopaminergic neurons transplanted to the normal striatum of neonatal or adult rats and to the denervated striatum of adult rats. J Neural Transplant Plast. (1997) 6, 73-81.

Senthilkumar, KS, Sarvana, KS, Chandra, Gautam Sindhu, KM, Jaykrishnan A, Mohanakumar, KP: Unilateral implantation of dopamine-loaded biodegradable hydrogel in the striatum attenuates motor abnormalities in the 6-hydroxydopamine model of hemi-Parkinsonism. Behavioural Brain Res. (2007) 184, 11-18.

Seth K, Agrawal AK, Date I, Seth PK: The role of dopamine in manganese-induced oxidative injury in rat pheochromocytoma cells. Human & Experiment Toxicol. (2002) 21, 165-170.

Shetty AK, Banerjee R, Gopinath G, Tandon PN: Fetal nigral grafts in the anterior eye chamber of adult rats: a long-term morphological study. Exp Neurol. (1991) 111, 106-114.

Shetty AK, Gopinath G, Tandon PN: Adrenal medullary autografts in anterior eye chamber, lateral ventricle and striatum of adult rats: a long-term study. J Neural Transplant Plast. (1991a) 3, 175-191.

Shetty AK, Gopinath G, Tandon PN: Fetal nigral transplants in the lateral ventricle of adult rats. A long-term morphological study. Dev Neurosci. (1991b) 13,1-10.

Shukla S, Agrawal A.K, Seth K., Chaturvedi R.K., Sinha C., Seth P.K: Role of Antioxidants supplementation in fetal ventral mesencephalic cell (VMC) and carotid body induced functional deficits in rat model of Parkinson's disease. Ann Neurosci. (2003) 10, 33-38.

Shukla S, Agrawal AK, Chaturvedi RK, Khanna VK, Sinha C. Srivastava N., Seth PK: Co-transplantation of carotid body (CB) and ventral mesencephalic cells (VMC) as an alternative approach towards functional restoration in 6-OHDA lesioned rats: implications for Parkinson's Disease. J Neurochem. (2004) 91,274-284.

Shukla S, Chaturvedi RK, Seth K., Agrawal AK: Co-transplantation of carotid body and ventral mesencephalic cells as an alternative approach towards functional restoration in rat model of Parkinson's disease. J Neurochem. (2005) 94, 119.

Shukla S, Chaturvedi RK, Seth K, Agrawal AK: Co-transplantation of neural progenitor cell with olfactory ensheathing cell restores functional deficits in rat model of Parkinson's disease. J Neurochem. (2006) 98, 44.

Snyder BJ, Olanow CW: Stem Cell treatment of Parkinson's disease: an update for 2005. Curr Opin Neurol. (2005) 18, 376-85.

Takagi Y, Takahashi J, Saiki H, Morizane A, Hayashi T, Kishi Y, Fukuda H, Okamoto Y, Koyanagi M, Ideguchi M, Hayashi H, Imazato T, Kawasaki H, Suemori H, Omachi S, Iida H, Itoh N, Nakatsuji N, Sasai Y, Hashimoto N: Dopaminergic neurons generated from monkey embryonic stem cells function in a Parkinson primate model. J Clin Invest. (2005) 115, 102-109.

Tandon PN, Gopinath G, Mahapatra AK, Shetty AK: Neural transplantation in mammals: our experience. Proc Indian Nat Sci Acad. (1990) 56, 51-58.

Tandon PN: Neural transplantation. Promises and Problems. Proc Indian Nat Sci Acad. (1992) 58, 1-16.

Tandon PN, Kumar VM, Verma S, Gopinath G, Shetty AK: Fetal brain transplantation in kainic acid lesioned caudate nucleus of adult rats. J Neural Transplant Plast. (1992) 2,125-133.

Tandon PN, Gopinath G: Clinical use of neural transplant. The unresolved problems. In Neural Transplantation, CNS Neuronal Injury and Regulation. Recent Advances eds. J Marwah, H Teitelbaum and K.N. Prasad. CRC Press, Boca Raton, Florida. (1994) I-16.

Tandon PN: Neural stem cell research: A revolution in the making. Current Science. (2001) 80, 507-514.

Thompson WG: Successful brain grafting. NY Med J. (1890) 51, 701-702.

Toledo-Aral JJ, Méndez-Ferrer S, Pardal R, López-Barneo J: Dopaminergic cells of the carotid body: physiological significance and possible therapeutic applications in Parkinson's disease. Brain Res Bull. (2002) 57, 847-853.

Yu G, Fournier C, Hess DC, Borlongan CV: Transplantation of carotid body cells in the treatment of neurological disorders Neuroscience Biobehav Rev. (2005) 28, 803-810.