Ann Natl Acad Med Sci (India), **34(1):** 49-56, 1998

Neural Transplantation

*PN Tandon and Gomathy Gopinath**

Department of Neurosurgery and Anatomy*,
All India Institute of Medical Sciences, New Delhi-110 029.

INTRODUCTION

In recent years nothing in the field of surgery has captured the imagination of common man more than the subject of organ transplant. Ever since Christian Bernard successfully transplanted a human heart, not only the lay public but even the members of the scientific community have often asked us. "When will a brain transplant be done?" A decade or so ago, the simple answer would be "Never". However, much has happened during the past decade to make us a bit more circumspect in giving a categorical answer. Thousands of neuroscientists all around the world, including a group in our own Institute, have been currently working hard to explore various possibilities leading to such a goal. A large number of publications both in scientific and lay press refer to the tantalising possibilities of at least replacing part of the damaged or diseased brain, by viable neural tissue, with capabilities to grow, differentiate, develop connections with the host brain, produce chemicals responsible for transmission of nerve impulses and ultimately compensate for the lost function. It is therefore timely to take stock of the current status and future perspectives.

Why the Whole Brain cannot be Transplanted

It is natural to question that if heart, liver, lungs, kidneys can be transplanted as an 'organ' why brain cannot be transplanted in the same manner. Heart, liver and kidney are connected with the body primarily through blood vessels, which can be divided and sutured to the vessels of the donor organ. Similarly the ureter or bile duct which have to be divided while removing a kidney or liver, can be easily stitched to the appropriate tissue of the donor. These structures i.e., blood vessels, ureter or bile duct are simply mechanical conduits which can be sutured easily and successfully. In contrast, in addition to the blood vessels, the brain is connected to the body by the spinal cord. The donor brain will thus be required to be anastomosed with this

*Correspondence : Prof. P.N. Tandon, Emeritus Professor, Department of Neurosurgery, C.N. Centre, All India Institute of Medical Sciences, New Delhi 110 029.

structure, which is not easy to be sutured. Even if it is sutured, it has no capacity to regenerate, or, to develop functional connection with the donor brain. In addition, the brain is connected to the body by twelve pairs of cranial nerves which would have to be cut if brain is to be removed. Some of these, like the optic nerves connecting the eyes to the brain, even if connected to the nerve of the donor brain, somehow fail to become functional.

The next best thing to full brain replacement is therefore replacement, if possible, of a part of the brain which is diseased or damaged. This is what is currently being tried.

Historical Background

Historically, attempts at mammalian neural transplantation started nearly one hundred years ago. Thompson in 1890 attempted exchange of large pieces of brain tissue between adult cats and dogs. Saltykow in 1905 reported the survival of transplanted mammalian cortical tissue. Nevertheless, it soon became obvious that adult neural tissue seldom survived and that too for a very short duration. Dunn in 1971 demonstrated that immature neural tissue had longer survival. However, it was only in 1940 that Le Gros Clark successfully transplanted embryonic brain tissue in the cerebral ventricle of a six week old rabbit. Four weeks after transplantation, he demonstrated well differentiated neurons attempting to reproduce the laminar arrangements of adult cortical neurons. Sporadic efforts by a series of workers (May 1954, Greene and Arnold 1945 : Royo and Quay 1959) reconfirmed the capability of embryonic neural tissue to grow when transplanted in anterior chamber of the eye in adult host. On the other hand, Willis (1935) and Glees (1955) could not confirm the ability of embryonic neural transplants to survive. However, concerted efforts in this direction started only in early seventies, when the work of Das and Altman from USA and Bjorklund and his colleagues from Sweden in a series of publications unequivocally demonstrated growth, differentiation, integration and even production of appropriate neurotransmitters following embryonic neural tissue transplanted in various regions of the brain in adult rats (Das 1983). These successes have resulted in a virtual explosion of researches in this field so that thousands of neuroscientists all over the world are currently engaged in this field of study. According to Morrison (1987), in 1983 alone, as many papers were published on neuronal grafting in mammals as in the whole century preceding 1970.

Need for Neural Transplantation

Brain cells - the neurons - have no capacity to regenerate when they are damaged. In addition, groups of neurons and their interconnections constitute functional systems with specific functions. Damage to any component of a particular system leads to loss of that function. Brain has limited capacity to delegate or transfer this function to any other region. Just to give an example, if the area of the brain concerned with speech is damaged, the patient loses his speech. The remaining

healthy brain cannot take over this function. This is true for other specialized functions like seeing, hearing, voluntary movements, sensory perception etc. The individual units of a functional system communicate with each other through electrical signals which utilise specific chemicals called neurotransmitters. Some neurotransmitters are also specific for a particular functional system. Thus for maintenance of smooth motor functions, besides the motor cortex and cerebellum, the nigrostriatal system is essential. The specific neurotransmitter that subserves this system is dopamine. Deficiency of dopamine results in abnormal motor symptoms like poverty of movement (akinesia), stiffness or rigidity and involuntary movements (tremors) so characteristic of Parkinson's disease. No other neurotransmitter, of which there are a large number, can take over the function of dopamine. In contrast, in the case of liver or kidney, all the liver cells (hepatocytes) or kidney cells (nephrons) have identical functions. When diseased, remaining healthy cells can take over the function. Furthermore, upto a certain limit these organs have an inherent capacity to regenerate. Brain unfortuately does not possess any of these characters. Hence, once any specific part of it is damaged, it can only be replaced by those cells which have genetically been ascribed that particular function or which are able to produce the specific neurotransmitter involved in that function. To make matters more complicated, for some complex functions, large number of functional units are required to work in harmony and this may involve more than one transmitter. This is characteristically seen in respect to memory. Impairment of memory, so characteristic a feature of ageing, especially in cases of Alzheimer's disease, represents such a situation.

Theoretically speaking, sequelae of injury, infections, stroke and a large number of degenerative disorders of brain which have no medical or surgical therapy available for restoration of function, are all potential candidates for neural transplantation. Experimental neural transplantation in animal models of a host of such disorders has been shown to benefit to a varying degree the impaired function. Thus, neural tissues from a number of morphologically, biochemically and functionally identified regions have been "harvested" from foetal brain and successfully transplanted in the appropriate region of the adult brain with pre-existing mechanical, biochemical or pathological lesion. These grafts have been shown to develop organotypic maturation, produce appropriate neurotransmitters and result in varying degree of recovery of lost function. However the procedure has so far been utilized for treatment of Parkinson's disease only. The remaining portion of this article will predominantly deal with this disorder.

It is now well established that the dominant abnormality in patients with Parkinson's disease involves the nigrostriatal dopaminergic neurons. A variety of experimental models of this disorder have been developed in animals by destroying the dopaminergic neurons either chemically or mechanically. It is

possible to isolate dopaminergic neurons from either embryonic brain or adult adrenal medulla. These neurons have been transplanted in the lesioned animals. Morphological, Neurophysiological, biochemical, histochemical and behavioural studies have unequivocally demonstrated that such transplants are able to revert the lesioned animals towards normalcy (Bjorklund et al 1981, Schmidt et al 1982, Dunnett et al 1983). This knowledge has already been utilized to treat patients suffering from Parkinson's disease (see later).

Two decades of intensive research all over the world has established that the most suitable tissue for grafting in the brain and spinal cord is the fresh neural tissue obtained from a developing foetus. Atleast in lower mammals one can easily achieve a success rate of 80 to 85 percent. Most such studies have been carried out in rodents. These findings have also been confirmed in a host of other animal species including sub-human primates and even man. However, the work carried out in monkeys has been very limited (Ridley and Baker 1991) and the work carried out in rhesus monkey has been far from satisfactory (1992). It is therefore not surprising that several investigators have questioned the advisability of using this procedure in man on the basis of results obtained in rats (Fishman 1986, Tandon 1988, Sladek and Gash 1988, Sladek and Shoulson 1988, NMJ 1988, Lindvall 1991). Inspite of such reservations, it is surprising that more than 400 human patients have been subjected to such transplants, using either autologous adrenal medulla or foetal substania nigra. Such "experimental" surgery has no doubt demonstrated the feasibility of the procedure to succeed and atleast partially and temporarily alleviating some of the deficits resulting from Parkinson's disease. The developmental window during which the fetal cells need to be harvested is reasonably well established for rodents. The optimal donor age may vary depending upon the region of the brain selected for transplantation. On the other hand, while most persons believe that in the case of human foetuses, the desirable age to take the graft is from 6 to 12 weeks.

Successful transplantation has been achieved using cell suspension or solid pieces of foetal tisue. Bjorklund et al (1980), Dunnett et al (1987) and many others advocate cell suspension to be better than solid pieces. Das et al (1979) and others including us, find the latter to grow as well. The size of the graft in animal experiments was not a critical parameter, but it acquires great importance when the technique is used for therapeutic purposes in human patients. Lindvall et al (1987) estimated that the human putamen and caudate nucleus (striatum) are normally innervated by about 60,000 dopaminergic neurons each. Grafting ventral mesencephalic tissue from one fetus into one of these structures might then be able to restore upto 30%-40% of the normal number of cells. Hence, they used tissue from four foetuses for each of their patients (Lindvall et al 1990), though Madrazo et al (1991) and Hitchcock (1991) claimed satisfactory results using cells from a single fetus.

It is now obvious that, for proper functional integration, the graft must be located as near the target as possible. However, the problem arises when one deals with such a large structure as striatum. With the restricted axonal growth into the host and limited diffusion of dopamine into the surrounding brain, it appears to be desirable to transplant at several sites and not at one (Perlow 1987). Madrazo et al 1990). Furthermore. It has been demonstrated that selected aspects of behavioural abnormalities are benefited by transplants at different sites in the caudate nucleus (Dunnett et al 1989, Perlow 1987). Efforts are, therefore, underway to evaluate the utility of transplants in only caudate nucleus or putamen or both unilaterally or bilaterally, at single or multiple sites in cases of Parkinson's disease (Freed et al 1990, Hitchcock et al 1989).

It must be stated at the outset that neural transplant has not been attempted in India so far inspite of the fact that we have extensive experience in achieving successful transplantation of foetal tissue in adult animals. The technique itself is simple, does not require any sophisticated equipment and can be performed with little, if any risk to life. There will be no dearth of patients, the cost of surgery itself would be affordable by a common man. The reasons for not attempting it is, however, based on its purely experimental nature as at present. In addition results of our animal studies as also a host of others entreat us to resolve some of the controversial and unanswered questions through experimentation in animals. Some of these are briefly described below.

Donor Tissue

As mentioned above, so far, the only suitable tissue necessary for successful transplantation is human foetal tissue, that too from a foetus of a particular age. To procure such tissue raises some practical and ethical issues notwithstanding the fact that abortion having been legalized in the country, thousands of such procedures carried out every day could easily provide the required donor tissue. The current practices of medical termination of pregnancy may need to be modified to procure the desired donor tissue in suitable condition. The donor tissue thus obtained will have to be transplanted promptly.

Preservation of Graft

Most investigators studying neural transplant used fresh donor tissue. For clinical use, specially keeping in mind the need for tissue from more than one foetus for a patient, it would be more practical to use stored tissue. Attempts have been made to use cryopreserved tissue (Brundin et al 1985, Redmond et al 1988, Gibbs et al 1986, Victorov and Lyjin 1990). We were able to successfully transplant cryopreserved foetal tissue in rhesus monkey (Tandon et al 1990). However, Gash and Sladek (1989) observed that survival of human foetal cells whether cryopreserved or dissociated in-vitro is still extremely low (5% to 15%). A proper protocol needs to be developed to achieve better survival. In addition one would have to ensure that tissue thus obtained is free from any infection, specially the human immunodeficiency

virus [HIV], and is not contaminated during collection, transportation and preservation. In view of these problems, efforts are being made to look for satisfactory alternatives (vide infra). However, before we do that we may have a look at what has already been achieved using human foetal tissue transplantation for Parkinson's disease, since this is the only disease for which this procedure has been utilized and very carefully studied.

The first operation, using autologous adrenal medullary cells was performed by Backlund and his colleagues in March 1982. A second operation was performed in May 1983. A suspension of chromaffin cells of the patient's own adrenal medulla which secrete a precursor of dopamine was stereotactically implanted into the caudate nucleus. The transitory and equivocal improvement observed in these patients, while establishing the validity of such an approach, dictated a review of the strategy (Backlund et al 1987). Madrazo and his colleagues in Mexico, modified this operation and claimed dramatic results. They carried out an open operation, exposed the caudate nucleus in the lateral ventricle, made a cavity in it and implanted adrenal medulla into the cavity. Few others could verify their dramatic results and soon the operation fell into disrepute. This promoted Hitchcock et al (1989), Lindvall et al (1989), Madrazo et al (1990) to use foetal substantia nigra neurons (ventral mesencephalon of embryo of 8 to 10 weeks gestation) for transplantation. Till date, this is the only transplant which has provided symptomatic relief lasting atleast for more than 2 years in selected patients.

The beneficial effects of nigral transplants may be summarised as follows :

- Some general improvement of motor symptoms has been reported by all. However, the degree of improvement is rated as mild to moderate. In no case there has been a full reversal of Parkinsonian syndrome (Lindvall et al 1990, 1992).
- The most consistent improvement has been in rigidity and bradykinesia. There is little improvement in tremors.
- Positron emission tomography [PET] scan studies have established long term survival of the graft (Lindvall et al 1990, 1992).
- Restoration of dopamine synthesis and storage in the grafted striatum has been established using an isotope, fluorodopa [6 FD PET] uptake (Sawle et al 1992). Vingerhoets et al (1994) summarising the current information based on 6 FD PET studies concluded that some of the relevant reports currently available are highly encouraging but *"there are also inconsistent, paradoxical and controversial findings"*.

Future Directions

To overcome the drawbacks and limitations of foetal tissue transplant, efforts are being made to look for

satisfactory alternatives. Cells from superior cervical sympathetic ganglia, glomus cells of the carotid body and pheochromocytoma cell line [PC 12] encapsulated in semipermeable membrane have been shown to provide variable beneficial effects in rat, guinea pig and monkey models (Tresco et al 1992). A variety of other genetically manipulated cell lines have been developed to provide a source of the appropriate neurotransmitter, dopamine or acetylcholine. It may be possible to do so for other transmitters and trophic factors as well. Wolff et al (1989) genetically modified fibroblasts to provide L-dopa and demonstrated its utility for relieving symptoms in a rat model of Parkinson's disease. Immortalized cell lines have been generated by retrovirus-mediated V-myc transfer into murine cerebellar progenitor cells, by culturing cells from human megalencephaly, and by somatic-cell fusion.

Cells capable of secreting a specific neurotransmitter can be encapsulated in a nonbiodegradable polymer. This prevents immunological destruction. These encapsulated cells have been shown to survive for long time and compensate for lesion induced deficit (Tresco et al 1992). It is still too early to say whether these efforts will succeed in providing a reliable source of transplants for therapeutic purposes in humans. Continued studies are no doubt essential.

In conclusion, at the current stage of our knowledge and the experience gained from limited human trials it is obvious that neural transplantations holds lot of promise for relief to a large number of neurologically disabled individuals, who have no other hope. It is ethically permissible, not as a routine treatment but as a measure of last resort carried out by well qualified teams, under controlled conditions, in institutions with capabilities to scientifically monitor such patients for long periods. Meanwhile efforts should continue in experimental laboratories to resolve the uncertainities still associated with the procedure.

This review is dedicated to the memory of Prof. Baldev Singh who was the inspiration behind several experimental studies done by the author along with Prof. G.S. Chhina and Prof. V. Mohan Kumar, which added to our current concepts of neural transplantation. Prof. Baldev Singh was associated with pioneering advances in India in this field.

This review is dedicated to the memory of Prof. Baldev Singh who was the inspiration behind several experimental studies done by the author along with Prof. G.S. Chhina and Prof. V. Mohan Kumar, which added to our current concepts of neural transplantation. Prof. Baldev Singh was associated with pioneering advances in India in this field.