LECTURE

The use of foetal human brain tissue as brain implants: phenotype manipulation by genetic manipulation and biochemical induction

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Abstract. The use of dopaminergic mesencephalic (VM) human foetal brain tissue as implants to neurosurgically treat Parkinson's disease has been in progress since the 1980's. A major limitation in the use of VM tissue is the amount of tissue available from each human embryo. Usually tissue from about 7 embryos is required to treat each patient unilaterally. To overcome this we have developed various strategies. One is to convert embryonic cerebral cortex in human embryos into dopaminergic tissue which is stable, and which will secrete dopamine *in vivo* once implanted. The cerebral cortex is about 500 times larger than the VM and can therefore provide a lot more tissue for transplantation. This can be achieved by genetic manipulation of the embryonic cerebral cortex tissue, involving the lipo-transfection of multiple copies of the human tyrosine hydroxylase gene into both neurones and glial cells. In another approach we have biochemically manipulated the development of the cerebral cortex to direct the neurotransmitter phenotype towards the dopaminergic type, and away from other phenotypes. This tissue, too, is stable and will synthesise and secrete dopamine when transplanted. Our third approach has been to manipulate pluripotential neural cells which are yet to develop into neurones and glial cells. These cells can be expanded in number many-fold before treatment to direct their development into stable dopaminergic neurones in large numbers (70%), which synthesise and release dopamine. When used as transplants in animal models of Parkinson's disease, these various types of artificially induced dopaminergic tissue are very effective at reducing the Parkinsonian syndrome.
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Key words: Parkinson's disease, cell graft/transplantation, phenotype manipulation, dopamine, stem cells

Introduction

The use of dopaminergic mesencephalic (VM) human foetal brain tissue as implants to neurosurgically treat Parkinson's disease has been in progress since the 1980's.

A major limitation in the use of VM tissue is the amount of tissue available from each human embryo. Usually tissue from about 7 embryos is required to treat each patient unilaterally. To overcome this we have developed various strategies. One strategem has been to change the neurotransmitter phenotype of embryonic cerebral cortex in human embryos into a dopaminergic phenotype which is stable, and which provides neural tissue which will secrete dopamine *in vivo* once implanted. The cerebral cortex is about 500 times larger than the VM and can therefore provide a lot more tissue for transplantation.

This conversion of developing human and rat cerebral cortex into largely dopaminergic tissue in a stable fashion, and away from its genetically determined phenotype, has been achieved by two methods. One has been the genetic manipulation of the embryonic cerebral cortex tissue, involving the lipo-transfection of multiple copies of the human tyrosine hydroxylase gene into both neurones and glial cells simultaneously in culture.[1]

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In another approach we have biochemically manipulated the development of human and rat cerebral cortex to direct the neurotransmitter phenotype towards the dopaminergic type, and away from other phenotypes.[2–6] This tissue, too, is stable and will synthesise and secrete dopamine after transplantation into rat models of Parkinson's disease. Our third approach has been to manipulate pluripotential neural cells (once called ''neural stem cells'') which are yet to develop into neurones and glial cells. These cells can be expanded in number many-fold before treatment to direct their development into stable terminally fully differentiated dopaminergic neurones in large numbers (70%), which will synthesise and release dopamine.[7] When used as transplants in animal models of Parkinson's disease, these various types of artificially induced dopaminergic tissue are very effective at reducing the Parkinsonian syndrome.

The Use of Fresh Human Primary Ventral Mesencephalon

It was during the 1980's, that fresh human embryonic ventral mesencephalon, isolated by dissection, was first transplanted into the striatum of patients with Parkinson's disease. Some 1,000 patients have subsequently received this surgical treatment, mainly in Sweden, USA and the UK. The various techniques employed in this surgery, and the overall outcome for the patients, have been reviewed on various occasions in the last decade.[8–10]

The use of rodent, and other, animal models of Parkinson's disease to assess the effectiveness and mechanism of action of such dopaminergic tissue implants has been the rule. In the early 1990's, we published[11] the curing effect of implanting freshly isolated human and rat embryonic ventral mesencephalon tissue into the neostriatum of rat models of Parkinson's disease. The procedure was 100% effective. However, freshly isolated, but untreated, embryonic human cerebral cortex was completely ineffective, though it formed stable and very viable grafts, surviving at least 9 months (Fig. 1). Of course, these animal models of Parkinson's disease are too simple to give accurate predictions of the outcome of such dopaminergic tissue implants into patients. Moreover, the patients, allowed by Hospital Ethics committees to receive this essentially experimental treatment, are advanced cases with many many years progression into knock-on complications of Parkinson's disease. These include disease progression and the nervous system's accommodation to long-term drug treatment. Interlinked neurotransmitter systems (*e.g.*, cholinergic, serotonergic, aminoacidergic, peptidergic) develop changes in their responses to the declining dopaminergic input, and thereby alter their balance and

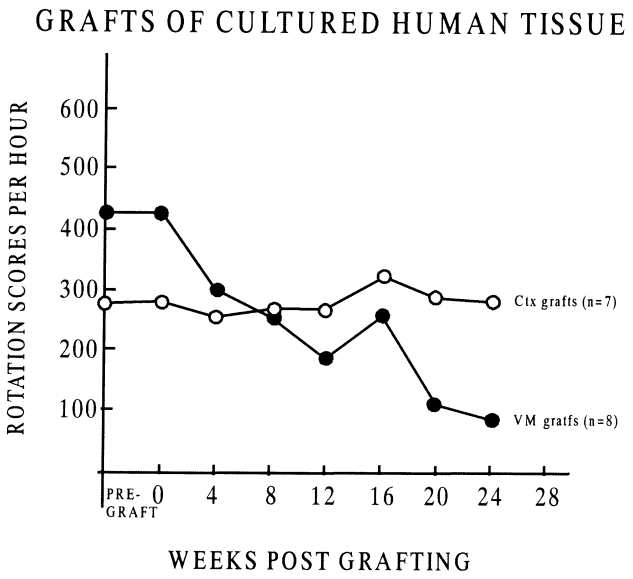

Fig. 1 The effect of grafts of cultured human foetal tissue in preventing metamphetamine-induced rotational, circular, movement in rats whose nigrostiatal pathways had been unilaterally removed by unilateral 6-hydroxydopamine injection into the brain. Rotation was almost entirely towards the lesioned side of the rat where the graft tissue was injected, and was counted by an automatic computer controlled system.[11] (Reproduced from Walters AM, *et al*: Neurochem Res 1992; 17: 893–900, used with permission)

equilibrium within the complex cross-talk of the basal ganglia and other brain structures.

All this weighs against a high level of positive outcome in the patients selected to receive the tissue implants. Also, these considerations highlight the inadequacies of the animal models as predictors of the extent of positive clinical outcome in these patients. The animal models are tested within a relatively short period (6 weeks) after unilateral degeneration of their dopaminergic nigrostriatal pathways, and before the development of any linked permanent changes in other neurotransmitter systems. Moreover, rat basal ganglia are at a far less complex stage of evolution compared with the equivalent human system, as well as being 200× smaller.

Tissue Source, Condition and Supply

In practice, the ventral mesencephalon from some 7 to 8 human embryos is required to unilaterally treat each patient. It is clearly difficult to coordinate the availability of such relatively large numbers of human embryos of the correct age (7–9 weeks), dissect out the VM, test for disease agents such as AIDS virus, and have fresh tissue ready for implantation. So the dissected tissue has often been stored frozen. Unfortunately, 60% of the cells often die on thawing. So the viability of the implanted cells is severely compromised.

This large number of human embryos is required because they supply the required number of dopaminergic neurones for the human neostriatum. Thus, there are about half a million dopaminergic neurones in the human substantia nigra, and therefore this is the number which surgeons have tried to implant into the putamen on each side, though only half of these project to the putamen. Actually, only about 5% of these neurones survive transplantation and produce the required relieving action on the disease. Fresh VM tissue dissected out and stored in culture has only occasionally been used.[8–10]

Another problem is that since 15 different human embryo donors are required to treat the putamen on each side of the patient's brain, this will present an immunorejection challenge.

In conclusion, these and many other challenges are barriers to the future development of the use of multiple batches of fresh human embryonic VM tissue from different donors to neurosurgically treat one human Parkinson's disease patient.

The Use of Human Embryonic Dopaminergic Tissue Produced from Cerebral Cortex

In 1994 we published[2,3] a method which allows the biochemical induction of large numbers of neurones (7%) expressing the full dopaminergic phenotype in embryonic rat cerebral cortex which, under the normal pattern of rat genotype expression, develops very few dopaminergic neurones (Fig. 2). Following this, in 1998, we published[4,5] a procedure for the successful conversion of neurones (1%) in the human foetal cerebral cortex into the dopaminergic transmitter phenotype and away from whatever terminal transmitter phenotype was prescribed in the natural human genotype (Table 1). Although these percentage conversions are relatively low, dopaminergic neurones develop in sufficient numbers to make the cerebral cortex effective as neostriatal implants to cure the Parkinsonian symptoms seen in rat models of the disease. In contrast, untreated foetal cerebral cortex of the same age and from the same source was ineffective in this respect. Thus the asymmetric rotation of the animals was reduced, by 40 times, to below 7 turns per minute by the converted cerebral cortex. Moreover, this conversion to dopaminergic phenotype was stable over at least nine months.

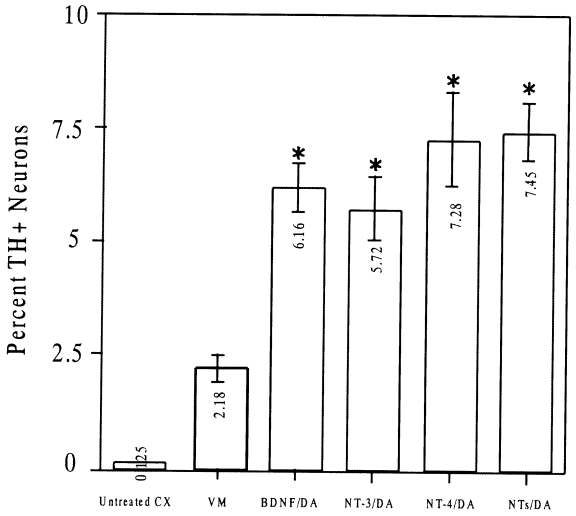

Fig. 2 Effects of combined actions of neurotrophins (BDNF, NT-3, NT-4) and dopamine on tyrosine hydroxylase (TH+) induction in foetal rat cortical neurones. The histobars show the percentage of TH+ neurones in cultures treated as shown 2 Indicated values represent mean + SEM for triplicate cultures. In two separate platings. NTs = BDNF + NT-3 + NT-4. Statistical significance is denoted by * ie $P < 0.05$, compared with untreated foetal cortex (CX), or with ventral mesencephalon (VM).[2] (Reproduced from Zhou J, *et al*: Eur J Neurosci 1996; 8: 2828–2339, used with permission)

Table 1 The effects of BDNF, Dopamine and Forskolin, alone and in combination, on the numbers of tyrosine hydroxylase-positive cells in cultures of human foetal cerebral cortex

Treatment	TH^+ cells/well	% TH^+ cells compared with control
Control(BDNF 50 ng/ml)	0.28 + 0.18	100
BDNF + dopamine	16 + 10*	1,600
BDNF + forskolin	78.51 + 4.90*	7,851
BDNF + dopamine + forskolin	219.51 + 15.85*	21,950

Human cortical neurones were grown in the presence of 50 ng/ml BDNF for 5 weeks. The cultures were treated 3 times each week from DIV 0 with 10 dopamine and 10 forskolin, either alone, or together, and were then stained for TH^+ cells. These numbers were expressed as percentage of the control. Data are mean + SEM for 4 to 6 separate experiments carried out in triplicate. Statistical difference from control group is indicated as * for $P < 0.001$.

The embryonic human cerebral cortex is about 500 times larger than the human VM. Moreover, this artificially induced dopaminergic tissue, derived from cerebral cortex by biochemical induction, is stable, in the sense that it is permanently induced. Most importantly it has been demonstrated to synthesise and secrete dopamine. Therefore, it should greatly ameliorate the problem presented by the need to provide sufficient volume and genetic uniformity of human foetal embryonic dopaminergic tissue for use as implants to treat Parkinson's disease.

All investigation has shown that the method we have employed induces the complete dopaminergic phenotype. This means that the enzymes tyrosine hydroxylase, dopa-decarboxyalase, monoamine oxidase, aldehyde dehydrogenase and catecholamine-O-methyltransferase are all induced in parallel in these neurones. In addition, we have demonstrated that the membrane proteins expressed to provide the critical dopaminergic

phenotype-marker, high-affinity dopamine uptake, are also expressed by these artificially induced dopamine cells.

There are ethical objections, in many countries, to the use, for medical treatments, of tissue from human embryos obtained as a result of pregnancy termination. Obviously, therefore, it is attractive to be able to provide 500 times more usable dopaminergic tissue per embryo through this route, which involves the high-jacking and redirection of the pathway of terminal differentiation of neurotransmitter phenotype.

The Use of Genetically Engineered Human Foetal Cerebral Cortex

In a parallel study designed to genetically modify human and rat embryonic cerebral cortex by inducing the ability to synthesise and release dopamine, we used lipofection as the transfection method of choice.[1] The reason for undertaking this research project was again to make use of the relatively large size of the cerebral cortex as a source of modifiable tissue to be used as brain implants, but to adopt a completely different method for conversion to the dopaminergic phenotype. The successful transfection, by lipofection, of human and rat embryonic cerebral cortex, in tissue culture, with multiple copies of cDNA for human tyrosine hydroxylase was published by our research group in 2001.[1] About 4% of the cultured cerebrocortical cells were transfected, the tissue actively secreted dopamine, and was successful in eliminating the asymmetric turning induced by amphetamine in animal models (Fig 3).

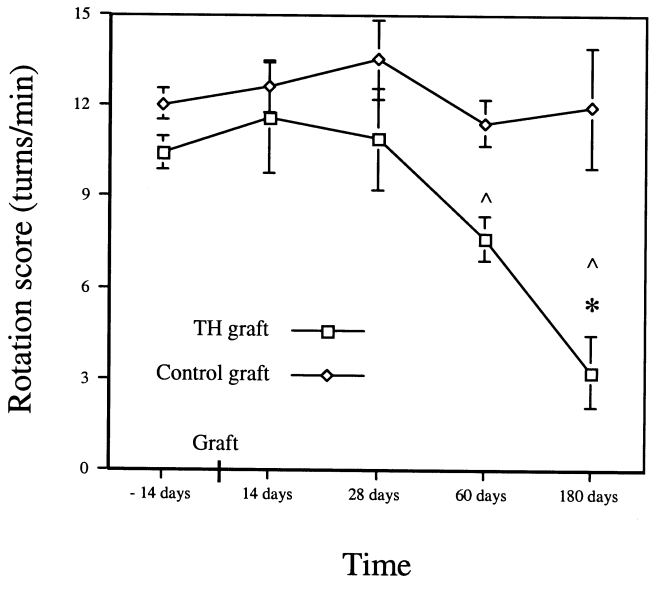

Fig. 3 The effects of grafts of human foetal tissue genetically engineered, by lipofection, to increase the content of human tyrosine hydroxylase cDNA which increased the grafts' ability to synthesise and secrete dopamine 1. The tissue grafts were injected into the neostriatum on the side of the brain lesioned by 6-hydroxydopamine to remove the nigrostriatal pathway. The data show the number of circular rotations towards the lesioned side, counted by an automated computerised system, (y-axis), on the days before and after grafting.[1] (Reproduced from Hurley M, *et al*: J Neural Transm 2000; 108 781–792, used with permission)

Thus, genetic manipulation may be used to produce a large quantity of human foetal cerebral cortex which produces and secretes dopamine, and could therefore be used for transplantation into the putamen of patients with Parkinson's disease. Indeed, the grafts of this genetically modified neural embryonic tissue are stable. The animal models of Parkinson's disease remain "cured" for at least 9 months, the longest period tested, and the grafts are seen to remain localised, and intact. Moreover, tyrosine hydroxylase persists in the neurones at this time, indicating that expression of the enzyme by TH-cDNA continues.[1]

The Use of Pluripotential Neural Progenitor Cells Isolated from the Ventral Mesencephalon

The advantage of using pluripotential neural progenitor cells (called brain "stem cells" in the past) is that a few cells isolated from human embryos (up to 8 weeks-old) can be allowed to multiply indefinitely by mitosis in an expansion medium, and thereby increase their numbers, and provide an indefinite supply of cells which are clone-like perfect neurospheres, which produce no neurites, and form clumps of many cells. A large proportion of these expanded neurospheres can subsequently be taken and treated with factors which will initiate their differentiation, first into neurones, and then into neurones of specific neurotransmitter phenotype (Figs 4 and 5).

Using this approach we have produced large numbers of cells terminally differentiated into dopaminergic neurones, which appear to be stable, and have been shown to produce and release copious amounts of dopamine.[7]

Conclusions

The future use of human embryonic cells as implants to treat the specific loss of dopaminergic neurones which occurs in Parkinson's disease currently seems assured. Freshly dissected, uncultured, unmodified, brain tissue from 6 to 8 different human foetuses has so far featured as the choice for these implants. These neurosurgical treatments have been judged to be successful in a sufficiently large proportion of patients to merit continuation.

Advances in the progress towards much greater success in these treatments will come from the changes we will soon see in the nature of the embryonic tissue

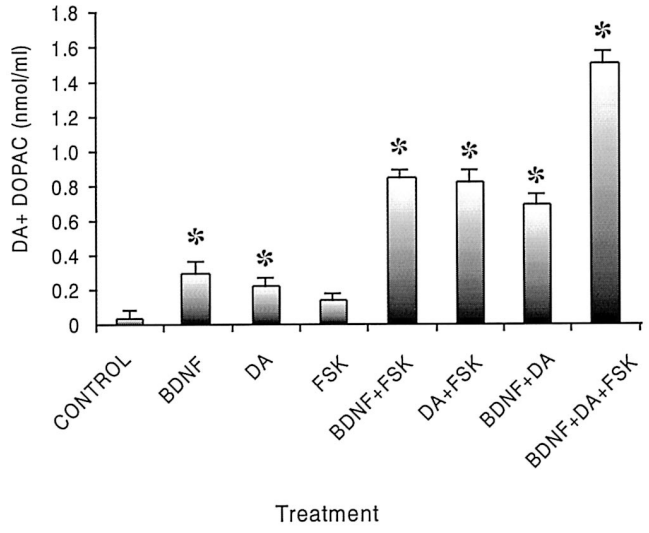

Fig. 4 Effects of BDNF, dopamine (DA), and forskolin (FSK) on the induction of the dopaminergic phenotype in pluripotential neural precursor cells differentiated by withdrawal of bFGF. Cells were treated for 3 weeks with different combinations of of the inducers as shown. The dopaminergic phenotype was measured as the synthesis and secretion of dopamine. The levels of dopamine and its primary metabolite DOPAC are shown (y-axis). Results are shown as mean + SEM from 5 separate harvests.[7] Statistical significance from control, untreated, cultures is denoted by * with $P < 0.05$. (Reproduced from Riaz SS, *et al*: Dev Brain Res 2001; 136: 127–134, used with permission)

employed as cell implants into the putamen. First will be the switch to using foetal tissue, of 8 to 9 weeks postconception age, which has been cultured to demonstrate its capacity to produce dopamine. Secondly, the cultured tissue will be modified either genetically or biochemically to induce, or increase, its capacity to produce dopamine. This could include the use of foetal human cerebral cortex because of its very large tissue volume, allowing the treatment of several patients from a single embryo. Thirdly, human foetal brain tissue at much earlier stages of development will be used as implants. These pluripotential neural cells can be expanded, possibly indefinitely, in number, and can then be induced to develop into largely (*e.g.*, 70%) dopaminergic neurones by *ex vivo* techniques.[7] The number of patients treated from a single human embryo could be even larger than is possible from modified foetal cerebral cortex. Such pluripotential progenitor cells can be immortalised and used in a variety of ways for CNS gene transfer and repair.[12]

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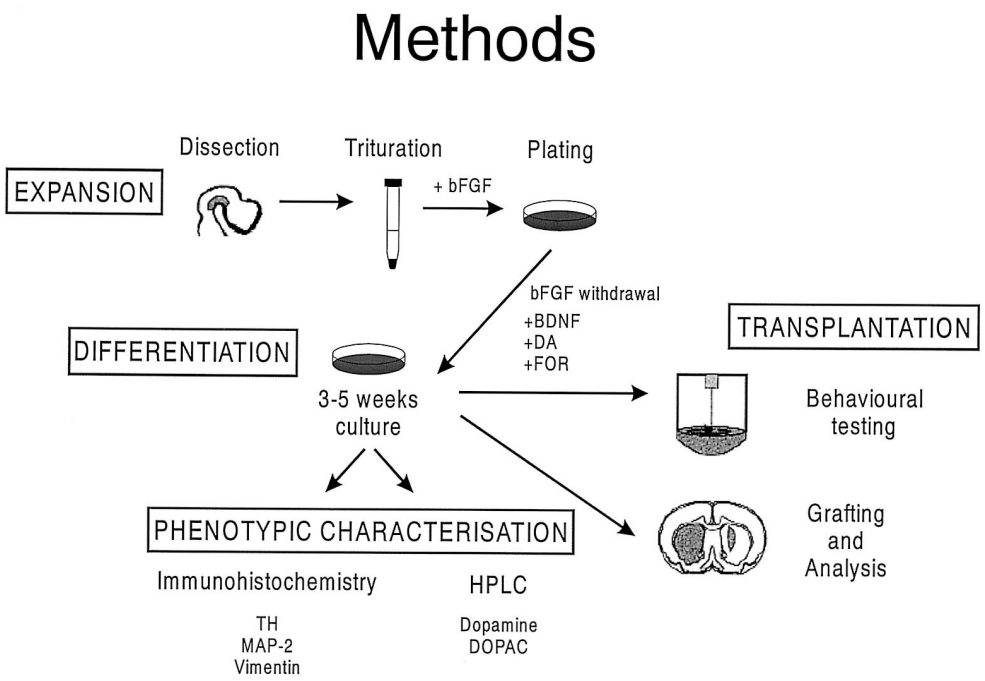

Fig. 5 Methods used for the expansion and differentiation of pluripotential neural progenitor cells.[7] (Reproduced from Riaz SS, *et al*: Dev Brain Res 2001; 136: 127–134, used with permission)

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