Embryonic stem cells and parkinson’s disease

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Abstract

Current treatments of Parkinson’s disease (PD) remain symptomatic, for example, Levodopa, and results in a number of negative side effects after a foreseen period of time. However, investigations into using human embryonic stem (ES) cells to generate dopaminergic neurons hold huge potential for future treatments. Results show several patients gain an increased percentage of dopaminergic neural mass in the substantia nigra following transplantations of neural cell grafts into the striatum. This suggests that grafts can work, although other studies show some patients fail to show improvements. There is also the risk of the formation of teratomas and teratocarcinoma in post-operative subjects, due to the self-renewal characteristics of the stem cells. Despite a number of studies proving that using human embryonic stem cells could be a reliable method of treatment, there are still a number of issues unresolved. Since the majority of experiments have taken place on primates, it is unreliable to assume that the same results would occur in human trials. There are also a number of ethical issues such as destroying the ‘ potential human life’ of an embryo which, in several cases, is limiting the research and progression of new methods of treatment.

Introduction

Parkinson’s disease is a progressive degenerative disease of the substantia nigra, resulting in the depletion of dopamine in the striatum and the formation of protein bundles known as Lewy bodies (1); such depletion of dopamine results in motor symptoms of which patients with PD present with,

including resting tremor (2, 3). At present, the most common form of treatment for PD is Levadopa; however, it is only given once symptomatic, and is thought that its effectiveness decreases over time (1). Investigations into the treatment of PD involving the use of embryonic stem cells to generate dopaminergic neurons for transplantation into the brain are currently in progress and this review is going to look at the prospects and issues that the use of human embryonic stem cells may bring about. (4).

What Are Human Embryonic Stem Cells?

Human ES cells are unspecialized and can differentiate into a range of cells via cell division, in this case, the most important being dopaminergic neurons (5). Human ES cells are generated from the pre-implantation stage of an embryo, a blastocyst, and can multiply rapidly, generating large numbers of cells (5, 6). This suggests there could be enough to provide sufficient amounts of DA neurons for clinical procedures. ES cells are taken from the eggs that have been fertilized in vitro, provided by consented donors (7, 8).

Human ES Cells for the Generation of Dopaminergic Neurons

ES cells are being used to produce tyrosine hydroxylase positive neurons by culturing, expansion, and differentiation (fig 1). ES cell colonies are selected individually and grown in agar plates allowing for embryoid bodies to develop. Embryoid bodies are then further cultured in a medium containing Fibroblast Growth Factor-2 (FGF-2) for seven days. A number of cell masses in rosette formations (figure 2b), resembling neural tube structures, were observed within the embryoid bodies (6).

Figure 1: Generating Dopaminergic neurons (12).

Neural precursors showed expression of ‘ nestin and musashi’, markers for neural progenitor cells (4): they act as a form of labeling identifying specific cells (9): Without FGF-2, no rosettes of orderly formation grew (6). When spherical neural masses formed after 14 days (figure 2a), it was noted that they could develop and multiply for over 120 days, providing evidence that there would be vast numbers of cells available for treatment (4). 14 days is a short period of time tells us how efficient this method of neuron generation is.

Figure 2: A – time inculture. B- ES cell colonies (10).

One study (10) found that the number of tyrosine hydroxylase-positive cells generated by neural masses was very small (5. 4% +/- 1. 8%) and to increase these figures, looked at the effects of growth factors and how they altered differentiation in culture. When FGF2 and FGF20 were present, percentages of TH+ cells increased by 18. 6%; however, neither growth factors alone had such an effect. Once cultured in a differentiation medium, neural masses showed physiological characteristics of neurons providing evidence that differentiation was in progress. (4).

Human ES Cells as a Treatment for Parkinson’s Disease

Several studies (4, 5, 11) quote that human ES cells are able to successfully produce dopaminergic neurons and decrease the symptoms of PD when grafted into the brain. One study (10) experimented on a primate model with an induced form of PD and investigated as to whether the isolated TH+

neurons were able to function as dopaminergic neurons. The neurospheres grown from ES cells were grafted into the putamen of monkeys, only of which started to show signs of PD and whose conditions had deteriorated beyond a period of 12 weeks. After transplantation, the behavior of the primates was observed and analyzed using MRI scanning. 10 weeks following, neurological scores of the primates increased significantly in comparison to the control models. Having control enables a clearer analysis to take place, and distinguish definite improvements in the primates’ behaviors.

A similar investigation (11) performed bilateral implants on 61 patients into the putamen. When looking at fluorodopa PET scans 12 months post-surgery, 85% of patients presented with neural mass growth and showed physiological improvements. However, one study (9) suggests that the outcome for each individual is not certain to gain the same result, because processes such as apoptosis are unable to be controlled, resulting in cell death within days after transplantation: this presents in scans as a lack of fluorodopa uptake, suggesting a range of cell survival. It is thought that if treated with neurotrophic factors before surgery, it could improve cell survival (11).

Comparing the two studies (10, 11), both results showed promising improvements, emphasizing that although one study (10) was on primates, and the second (11) involved humans, both experiments gained a similar positive result. However, this point could be argued as the primate was induced with PD rather than developing progressive disease naturally.

Analysis of more than one trial (11, 12) has indicated that the response to transplantation could show a positive correlation alongside the patients’ ability to successfully react to L-dopa prior to the operation, as well as the patients’ stage of the disease. One study (12) presented that if a patient scored lower than 50 on the unified Parkinson’s scale before surgery, then they were more likely to gain a positive result from the transplant. Some may say that for the treatment to be appreciated, it shouldn’t matter what stage the patient is at when receiving the transplant, and that it should work at all stages of the disease, although as proven with diseases such as cancer, this is not always possible.

As well as increased F-dopa uptake, studies on animals (13, 14) observed rotational behavior in response to amphetamine before and after surgery. Results showed a decrease in the scores of the animals given the graft, whilst sham controls showed no improvement (figure 3). One study (13) was repeated on 4 occasions across a 9 week period and continued to show a gradual reduction. This provides evidence that the graft was beginning to improve dopaminergic neuron function, as motor functions were showing significant improvements (14).

Figure 3: Rotational behavior scores (13)

Another study (12) says that neurons generated from the cells of early aborted embryos are able to survive when transplanted into the striatum and recover the loss of function by 10-40%, by restoring dopamine production in the grafted area (15). However, such results may be unreliable withrespectto human transplants as these studies were performed on rats and monkeys, of

which the disease was induced by creating selective lesions. Such lesions are, therefore, in a different condition compared to natural lesions found in the brain of a patient with progressive PD (9).

Transplants are thought to have decreased the occurrence of dyskinesias, a side-effect of levodopa; however, this has mostly only been proven in rats and monkeys. In addition, a number of patients have been able to stop using levodopa completely, although this is not a regular occurrence. On the contrary, a number of subjects have been noted having developed off dyskinesias: 7-15% of grafted patients (5). It is thought that this is down to the fact that the neurons are unable to function properly; this could be due to the overproduction of dopamine in the striatum (12). If there are consequences after transplantation, of the same severity as previous to the transplant, is it worth performing the operation?

Generated Neuron Transplantation Into the Patient

Grafts are injected into the brain whilst under anesthesia (16) and, as with all surgery, poses risks such as hemorrhage and infection (15). To minimize this, the number of needle passes is kept to a minimum. This was demonstrated in more than one case (9, 11, 15) where the needles were passed through the front of the head, through to the putamen bilaterally using a total of 4 needles (figure 4). In one study (16), 8 needle passes were used, however, this had no detriment to the outcome of the transplants, both achieving regeneration. ES cells themselves also pose risks. With characteristics such as pluripotency and self-renewal, stem cells have the potential to form teratomas and teratocarcinoma which, at present, is one of

the greatest risks; without knowing that they won’t form, transplantation of human ES cells will cease to be promising (17).

Figure 4: MRI and PET scans showing F-DOPA uptake and needle passes (8).

In order for this method to be successful, such characteristics of the cells need to be able to be controlled, and by doing so, will reduce the likelihood of teratomas forming. Nonetheless, human ES cells maintain a normal karyotype and, therefore, these growths are not thought to be of the malignant variety (5). Despite this, there is evidence to suggest that teratomas formed from the transplantation of early or unspecified neurons into the brain, can lead to incomplete motor benefit in models of PD (18).

In the majority of studies, patients were given immunosuppressive therapy before surgery to prevent rejection. The drug Cyclosporin was given, however, the course of the treatment varied, fluctuating between 2 days to 2 weeks (8, 11, 19). In such cases, when the immunosuppressive medication was withdrawn post-operatively, there was no evidence of a reduction in f-dopa uptake, suggesting that the graft was functioning properly (19). One study (9) claims that immunosuppressive treatment is not needed when the graft is kept within the same species.

Ethical Issues of Using Human ES Cells

One question that will continue to be brought up in regards to embryos is ‘ at what stage is the embryo a human life?’ One book (20)suggests that for research on an embryo to commence, informed consent must be given as many people believe that the blastocyst has the potential to be ahuman

being. However, because an embryo is incompetent of doing so, then the research cannot take place and, if it did, then it is thought that it would be restricting the embryos right or potential to life. Despite this, the use of stem cells derived from blastocysts continues to take place (20). Opinions vary and many of those who object to the use of embryos are subject to religious views: it is not necessarily a personal opinion but a matter of principle as to who has the right to determine life or death.

One way of presenting the idea of using ES cells to those ethically opposed is by carrying out a benefit to risk ratio, weighing up the pros and cons of the situation. One argument (21) emphasizes the potential relief of symptoms and the withdrawal of pharmacological treatment. It could not only benefit the subject but also influence those affected indirectly by PD. Opposing this is the risks of tumor formations and infection both during and after surgery, along with rejection by the immune system.

With ethical issues in mind, Parthenogenesis has provided an alternative way of deriving pluripotent stem cells without damaging embryos, preventing the destruction of potential life. Asexual reproduction of sex cells could be the route to generating vast amounts of pluripotent stem cells appropriate for transplantation, without the ethical issues that we face today (18).

Discussion

Looking at the evidence provided, it is viable to conclude that the use of human ES cells to produce dopaminergic neurons for transplantation into the brain has the potential for future treatments. However, at present, studies are unable to provide valid evidence that this alternative treatment is

guaranteed to work on a worldwide basis, as there is yet to be a steady correlation of improved brain function post-operative. Alongside this, there will continue to be a number of ethical arguments against the idea with respect to using human embryos as the source of stem cells, although, as discussed previously, other methods of generating stem cells such as parthenogenesis could be the answer to this. Greater knowledge of how the cells are differentiating and having the ability to gain control of this would provide a much better prospect for the future pioneers of embryonic dopamine cell transplantation. With greater research and more promising results, the use of human ES cells to generate dopaminergic neurons could provide an effective method of treatment in the near future, which could lead to the successful restoration of normal brain function.

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