CAN HEALTHY TRANSPLANTED TISSUE BE USED TO RESTORE MOTOR FUNCTION IN PATIENTS WITH PARKINSON'S DISEASE?

Aliza Erlbaum

Abstract:

Parkinson's Disease is a condition that disrupts the lives the many people. The disease is characterized by a loss of dopamine producing neurons in the pars compacta of the substantia nigra of the ventral midbrain, and symptoms include a lack of motor control and rigidity in motion. Currently, there are many treatments available to treat patients with Parkinson's disease. However, each treatment involves many adverse side effects that most wish to avoid. Science is discovering possible innovative, alternative options to treat Parkinson's disease such as the transplantation of healthy dopaminergic neurons directly into the striatum of the patient. Methods include using stem cells from original fetal sources, embryonic stem cells, induced pluripotent cells, or directly converting somatic cells into dopaminergic neurons. This study explores each possible treatment method along with the risks and advantages associated with each one, citing original experimental data and significant review articles. The results of this study do suggest potential in this new area of treatment for Parkinson's disease, yet much perfection of techniques and additional research must be completed before this idea can be used as a standardized treatment plan.

Introduction:

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a loss of dopamine producing neurons, primarily in the pars compacta of the substantia nigra in the ventral midbrain (Aguila et.al 2012). The substantia nigra is a part of a cohesive group of nuclei in the brain commonly referred to as the basal ganglia. Other nuclei of the basal ganglia include the caudate, putamen, globus pallidus, and the nucleus accumbens (Knierim 2012). In addition to the death of neurons in the substantia nigra, resulting in a loss of dopamine and melanin, this damage caused by PD can spread to other parts of the brain as well and affect other neurotransmitters and instigate other symptoms. Parkinson's disease can also be characterized by a collection of protein consisting bodies found in the brainstem of the patient commonly referred to as Lewy bodies. Although the presence of Lewy bodies is now considered an acceptable neurological disorder of PD, there is still no identifiable direct relationship between their existence and the physical symptoms of Parkinson's disease (Aguila et. al 2012). The nigostriatal pathway, the pathway which transports dopamine from the substantia nigra to the striatum, is largely associated with motor control. Therefore, some primary features of PD are rigidness, trouble with simple motor movement such as standing, and tremor. Over the years various treatments have been used to treat Parkinson's disease. Most of these treatments and medications involve either replacement of dopamine in the substantia nigra, or a chemical that can imitate the action of dopamine in the ventral midbrain. However, although most of these methods do temporarily relieve some symptoms of PD, the side effects of the medication, and the inability of the therapy to last permanently and alleviate all symptoms begs scientists to pursue other alternative treatment plans. One popular idea is to transplant dopaminergic

Aliza Erlbaum graduated in June of 2013 with a B.S. in Biology. She will be attending LIU College of Pharmacy.

neurons directly into the striatum of a patient with PD. Different options include using healthy tissue from fetal sources, embryonic stem cells (ESCs), or induced pluripotent stem cells (Hedlund 2009). Science is also discovering the possibility of deriving dopaminergic neurons from other somatic cells such as fibroblasts (Cummins and Barker 2012). Much research and experimentation has been done to determine whether such an idea as transplantation of healthy nervous tissue into the damaged area of the brain is valid and whether it can ever be a used as a widespread treatment for PD.

Methods:

This study was conducted through obtaining original experimental data on the subject matter. Acquiring this information was possible through the cross referencing of various articles, the matching of sources, and the reccomendation of articles by people knowledgable in this area of interest. Additionally, review articles by noteworthy professionals were also obtained and evaluated. The various pieces of data from different sources was then organized by category and verified for its authenticity and reliability. All the works cited in this study proved to be dependable and accurate sources.

Current Treatment:

Currently, there are many treatments used to relieve symptoms of Parkinson's disease. The most common and effective method is the administration of levodopa (L-Dopa), a chemical which can convert to dopamine once injected into the brain. For patients of PD, L-Dopa is useful as it alleviates rigidity and slowness of movement often associated with the disease. However, only 1-5% of levodopa actually enters the dopamine neurons, and the rest is broken down into dopamine elsewhere in the brain causing a number of adverse side effects. The main side effect, as observed in more than 50% of PD patients, is a loss of muscle control or dyskinesia (Hedlund and Perlmann 2009). It has also been found that the effects of levodopa only last for approximately ten years. Specifically in one study, 50% of patients exhibited signs of decreased motor ability after being on levodopa for five years and 80% of patients displayed similar symptoms after ten years. Also, levodopa only targets the deficiency of dopamine neurons whereas to completely relieve PD patients of symptoms the restoration of other neurotransmitters may also be important (Hickey and Stacey 2009). Other side effects include low blood pressure, nausea, gastrointestinal bleeding, and disturbances in breathing. Sometimes levodopa may be combined with other drugs, such as carbidopa, in order to decrease side effects. Carbidopa decreases some side effects of levodopa by preventing the conversion of L-dopa to dopamine before reaching the brain (Rao et al.2006). However, the adverse side effects can never be completely avoided and therefore the use of levodopa is generally avoided if possible.

Often to temporarily defer treatment of levodopa, a dopamine agonist can be used alternatively. A dopamine agonist is a chemical that imitates the actions of dopamine. Unlike levodopa, a dopamine agonist does not convert to dopamine itself, but rather behaves like the neurotransmitter and stimulates the dopaminergic receptors of the brain. Yet, although the risk of developing dyskinesia is less while using dopamine agonists, this method is altogether less effective and can cause other issues such as hallucination, addiction, and drowsiness (Hudlund and Perlmann 2009).

Another treatment for PD is the use of monoamine oxidase-B inhibitors. Monoamine oxidase is an enzyme responsible for the breakdown of dopamine in the basal ganglia. By inhibiting this enzyme using drugs such as selegiline and rasagiline, symptoms of PD can be mildly relieved (Rao et al. 2006).

If a patient does not significantly respond to any medication, or if the side effects are too severe, deep brain stimulation therapy may also be used. In brief, deep brain stimulation is a surgical procedure which involves implanting a device to transmit high frequency electrical currents and block abnormal impulses of the brain. Deep brain stimulation is effective in treating symptoms of PD involving motor dysfunction. However, like any other form of brain surgery, patients who undergo deep brain stimulation therapy run the risk of internal bleeding and infection (University of Maryland 2009).

Transplantation

Methodology:

This involves transplanting healthy dopamine neurons directly into the striatum to replace the dysfunctional tissue and restore motor function in PD patients. However, although this suggestion does look promising, there are several potential complications that must be taken into consideration. First of all, it is crucial that the cells survive the transplantation process itself (Hedlund and Perlmann 2009). Some measures taken to increase survival of the cells have been to incubate the cells together with growth factors and substances that reduce the risk of cell death. One common substance used is glial cell line derived neurotrophic factor (GDNF). Another idea is to first increase the number of dopamine cells by growing them in vitro prior to grafting them into the patient. This growth process not only proves beneficial by increasing the number of dopamine producing cells, but it also allows for increased cell differentiation of the graft as this way more dopamine precursor cells are enabled to reach maturity. In fact, in a study which used dopamine neurons derived in vitro, the chances of the graft survival were increased based on the cultured growth of the cells before transplantation. In this particular study, the growth factors used were brain-derived neurotrophic factor (BDNF) together with ascorbic acid and later GDNF was added amongst other substances such as FGF2, Wnt5a, and FGF20. Results of the experiment revealed a direct correlation between the growth of cells with growth factors in vitro the graft survival in vivo (Sanchez-Pernaute et al. 2008). In addition, during the actual transplantation process, care must be taken to inject the dopamine neurons into strategic multiple locations in the striatum. This is to ensure that all necessary parts of the brain are innervated and that there is maximal axonal coverage (Hedlund and Perlmann 2009).

There are many studies being done to determine the optimum cellular composition and proportional makeup of the graft. Typically, grafted tissue contains a mix of cells including dopamine cells, a majority of glial cells, GABA and serotonin. Many experimental studies have proven that high concentrations of dopamine producing cells within the graft are most beneficial for the patient's recovery. One particular experiment published in The Journal of Neuroscience yielded results which support this hypothesis. In animals injected with 6-hydroxydopamine (6-OHDA), an organic synthetic compound used by researchers to target and destroy dopaminergic neurons in the brain, the ones which received a graft with the highest amount of dopamine neurons had the highest rate of recovery with the least side effects. This was in contrast to the animals that were transplanted with tissues of higher concentrations of serotonin. This group exhibited the most side effects, the main one being graft induced

dyskinesia. It should be noted that the experiment used control groups to be sure that the success rates of dopamine were independent of the concentrations of serotonin and vice versa (Carlsonn et. al 2007). Furthermore, contained within dopaminergic neurons are two subtypes of cells, the A9 neurons, primarily found in the substantia nigra, and the A10 neurons of the ventral tegmental area, (located on the floor of the midbrain). Experimental data tends to favor the significance and functionality of the A9 neurons as pertains to PD over the A10 neurons. Yet, there are no techniques that specialize in purposefully differentiating between the A9 and A10 neurons and therefore their proportional composition within a graft will vary, resulting in an increased variability of experimental results (Lindvall and Bjorklund 2012).

It should also be noted that formulation of the cell graft as it is injected into the patient is significant. Different studies have proven that the method of transplantation, (i.e. suspension of cells, graft of solid tissue, or pieces), can greatly impact the results of the experiment. In one specific study, 90% of the patients who received grafts through suspension of the cells did not exhibit nearly as many side effects as those who received transplanted tissue through other methods (Mendez et.al 2005).

Sources of Cells

Fetal Tissue:

The dopamine producing cells grafted into the patient used can be derived from several sources each with their own advantages and setbacks. Most logically, healthy mesencephalic tissue can be obtained from aborted fetal embryos. Although this tissue is ideal for its authenticity, the experimental results from using fetal tissue are extremely varied. One experiment was performed using aborted fetal embryos taken 6-9 weeks after conception. Tissue from 3-5 donors was transplanted in each patient into each putamen. According to the UPDRS (Unified Parkinson's Disease Rating Scale) scores, symptoms of PD were reduced in these trials by 30-40% and the postoperative need of L-dopa was decreased by 16-45% (Lindvall and Bjorkland 2012). In another trial, similar positive results were also produced. Embryos of seven weeks old were aborted and the tissue was cultured in preparation for the graft. In this experiment, however, the patients were divided into two groups. In the first group, tissue was transplanted unilaterally into both the putamen and caudate, (most of the time the tissue is transplanted into the putamen since that is the area of the brain sustaining the greatest loss of Dopamine neurons in PD). In the second experimental group, tissue was transplanted bilaterally into just the putamen. Although in the end both groups exhibited improvement in motor function, the relief of the groups receiving the grafts bilaterally was slower to come. In the four to six weeks following the surgery, the symptoms of these patients actually worsened. The reason for this phenomenon is unclear. To test the results of the study, positron emission topography was used (PET scan). The PET scan indicated an increased uptake of fluorodopa. Fluorodopa is an organic compound that is often used as a tool to test dopamine function and activity. Patients with PD have a low fluorodopa uptake and in this particular study, all the patients displayed an increase in fluorodopa uptake (Freed et al.2011). This experiment is especially valid as it tested the effects of transplantation on seven humans each with different health backgrounds, and symptoms of PD were relieved in each individual case.

Yet, a separate experiment displayed an increased variability of results. In this study, four patients received unilateral transplants into the caudate nucleus of the brain. After six months following

the surgery, only three of the patients exhibited signs of improvement while the symptoms of one patient worsened. Also, any improvement was to a small extent as no drastic recovery was recorded in any of the four patients tested. Interestingly enough, in this experiment there was a lack of tissue rejection after the transplant. It could be that using tissue from fetal sources provides the advantage that there is a decreased risk of immune system rejecting the graft. In this case, it is possible that the mild improvement can be related to the fact that all the patients were in an advanced state of PD or that not enough tissue was transplanted due to the lack of availability of fetal sources (Spencer et al. 1992). In general though, this fluctuation of results caused by using fetal cell sources can be attributed to the de-standardization of cells in fetal samples. Additionally, tissue from fetal sources is not available in large enough quantities to be used as a standard procedure as tissue from more than one donor is needed to treat each patient (Lindvall and Bjorklund 2012). Also, aside from the technical difficulties with using fetal tissue sources, there are also many ethical issues involved with obtaining the fetal embryonic tissue (Cummins and Barker 2012). Many believe that the life of a fetal embryo should be considered as much as the life of an unborn fully developed baby.

Embryonic Stem Cells:

An alternative to the use of fetal tissue, the use of embryonic stem cells (ESCs) seems a more promising prospect. ESCs are a valuable source since they can be culturally harvested in large quantities in an undifferentiated state, providing unlimited access to many cell types (Kim et. al 2002). Nevertheless, this process of inducing ESCs into dopaminergic neurons can be complicated. Many groups of neurons in the human brain are marked by Tyrosine Hydroxylase. Tyrosine Hydroxylase is a rate-limiting enzyme involved in the production of dopamine which in turn gives rise to other catecholamines. Many tyrosine hydroxylase neurons can be derived from ESCs and some even produce dopamine. However, it is unclear whether those dopaminergic neurons are compatible with the ones of the substantia nigra lost in PD, and as to whether they can serve as an effective replacement (Sanchez-Pernaute et al. 2008). Yet, one study did prove successful in improving symptoms of PD through the process of transplanting ESCs into rats subjected to PD symptoms and conditions. In this experiment, ESCs were obtained through the process of parthenogenesis, the development of an embryo without fertilization. This was to avoid many ethical problems typically associated with the use of embryonic stem cells. The ESCs were first culturally harvested using a variety of growth factors including brain-derived neurotrophic factor and ascorbic acid. Later on the cells were differentiated by removing certain proteins involved in organogenesis and stem cell division, such as sonic hedgehog hemelog, and by adding other specific growth factors and proteins, such as glial cell-derived neurotrophic factor and dibutyryl cAMP. Finally, the cells were suspended and prepared for the actual transplantation procedure. The rats were immunosuppressed using cyclosporine A and the grafts were injected into the right striatum at two locations. To assess the effectiveness of the graft, throughout the transplantation process the rats were subjected to different behavioral tests specialized to check motor asymmetry and coordination. Before being grafted with the embryonic stem cells, rats exhibited severe motor deficits, and after the ESC transplant rats displayed an increased motor ability. To contrast, the experimental control group did not exhibit any significant change in behavior over time (Sanchez-Pernaute et. al 2008). This experiment provides hope that the use of ESCs can eventually

become an acceptable treatment for PD. However, this study cannot determine what the effects of such a type graft would have on a human. Additionally, it is possible that the experimental evidence would have differed with a more diverse group of rats each lesioned with 6-OHDA at a different time. (There is experimental evidence that the effectiveness of the treatment is related to the amount of time the patient had been suffering from the disease).

Besides the benefits though, there are also a number of potential problems posed through the use of embryonic stem cells. There is a risk of the graft containing residual undifferentiated embryonic stem cells which can lead to unwanted growths and tumors. Specifically, this problem was obvious in one experiment where the amount of dopaminergic neurons in vivo seemed to decrease. This could possibly be due to the proliferation of residual undifferentiated neurons, as graft overgrowth was observed in the sample. Further information derived from the study proved that this overgrowth of cells did not result from pluripotent cells within the body, rather from induced pluripotent stem cells cells that had failed to differentiate in cultures before the transplant itself. It should be noted that this particular experiment used the same growth factors and induction strategies as the studies that proved successful, (such as GDNF, SHH, FGF8 etc.) and no major differences in methodology between other experiments and this one were apparent. The results of this study encourage extra precautionary measures to be taken while dealing with the transplantation of ES cells. Safer methods of isolating and restricting composition of the graft to nerve cell progenitors or mature dopaminergic cells are still being developed. Additionally, scientists should also be concerned that the new implanted differentiated cells maintain their appropriate phenotype and survive once inside the CNS (Roy et al. 2006). Sometimes, to prevent or detect this potential danger, a chemical such as BrdU is used to detect cell division. BrdU is also helpful as it distinguishes the cells derived in vitro from the cells in the transplant inside the brain (Sanchez-Pernaute et al. 2008).

Induced Pluripotent Cells:

Another possible source for obtaining dopamine neurons is through the use of pluripotent stem cells, or stem cells that have the potential to differentiate into any cell type. Induced pluripotent stem cells (IPs) can be cultured in large quantities and can be accessed easily from patient donor tissue, characteristics that make IPs cells seem ideal for use. IPs cells are also useful since they avoid any ethical issues that can potentially arise through the use of embryonic stem cells or primary fetal tissue. An additional advantage is that since IPs cell lines are obtained from the patient donor all the genetic information is matched and no immunosuppressants are needed after transplantation (Hargus et al 2010). However, similar to the ES cells, induced pluripotent cells require special attention to ensure that they mature into their desired cell form. Because of this, researchers are developing specific markers and intrinsic and extrinsic factors in order to identify and induce precursor cells into their appropriate cell type (Aguila et al.2012). Some examples of proteins used in experimental studies to guide these undifferentiated stem cells include sonic hedgehog hemelog, and fibroblast growth factor 8. Sonic hedgehog hemelog is more commonly used by the body to regulate human organogenesis and fibroblast growth factor 8 also typically plays a key role in regulating biological processes and embryonic development (Aguila et al. 2012).

One specific experimental study using IPs cell lines yielded satisfactory results. First, IP cell lines were obtained from a PD patient. Interestingly enough, the IP cells of a PD patient did not significantly differ from cells of a healthy person. Then, to test the ability of these stem cells to survive in vivo, differentiated IP cells were grafted into healthy rats, unlesioned by OHDA-6. All IP cell lines survived and integrated into the striatum with no evidence of tumor formation or graft overgrowth even after twelve weeks after transplantation. Finally, grafts derived from IP cell lines were transplanted into the striatum of a lesioned Parkisonian rat. The grafts contained a large number of dopaminergic neurons distributed evenly throughout. After the procedure, significant outgrowth and branching of the transplanted dompaminergic neurons was recorded. The grafted dopamine neurons were tested for markers such as Girk2 and calbindin and came out positive for most of the neurons. Additionally, no tumor formation or graft overgrowth was observed in the transplanted cells (Hargus et al.2010). Although this experiment does seem to promise a future for the use of IP cell lines in treating PD, the information obtained must be verified by repeated experimentation with identical or similar results. It is possible that if left for more time the grafts may exhibit signs of overgrowth or tumor formation. Also, because IP cell lines were originally derived from the patients themselves, there is a risk that the dopaminergic neurons may lose some function or may display symptoms of PD, (such as the appearance of Lewy bodies within the graft), given a significant number of years after the initial procedure.

One experiment, in fact, came out with the idea that dopaminergic neurons derived from IP cells of PD patients are inferior to Da neurons derived from IP cells of a healthy person. Dopaminergic neurons were taken from IP cells of both a healthy person and a person with PD. The two sets of Da neurons were then cultured under identical conditions and were subjected to careful analytical watch. In the end, after a lengthy period of time, the dopaminergic neurons from the PD patient exhibited signs of neurodegeneration, an increase in apoptosis (cell death), and a decrease in neural branching and integration. This is an important discovery as it indicates that symptoms of PD are encoded in the genetic makeup of all the cells of the patient and the disease is not a result of environmental factors (Sanchez-Danes et al. 2012). Therefore, some researchers suggest that the use of IP cells be restricted to studying the pathology of PD through cellular modeling and to experimentation in laboratories (Cummins and Barker 2012).

Reprogramming Fibroblasts:

Recently, science has discovered that it could be possible for one somatic cell to transform into a completely different type of cell with a different function. Specifically, experimentation is being performed using fibroblasts (Cummins and Barker 2012). Fibroblasts are a type of cell in the body that produces the structural matrix outside the cells, supporting them and holding the cells in place. Fibroblasts are in charge of the production of collagen and are found in the largest quantity in most of the connective tissue of the body. If a method could be developed for transforming fibroblasts into dopaminergic neurons it could mean a whole new avenue of treatment for PD patients. An additional advantage is that if this procedure were possible, the cells could avoid a pluripotent state, eliminating the danger of graft overgrowth and the risk of tumors. Much experimentation has been done in this area and many have even proven successful. It should be noted that extra care must be granted to ensure that within the originating fibroblast material only fibroblast cells are present. If within the sample other cells

such as neural glia or neural crest cells are existent, they could expand once in the culture and serve as contaminating material for the remainder of the transplantation process (Pfisterer et al.2011). In one specific study, specific antibodies targeting neural progenitors were employed to rid the fibroblasts of any unwanted material. Then the fibroblasts were induced into neural cells through certain transcription factors, (Ascl1, Brn2, and Myt1l). Afterwards though, further specification using viruses was required to further differentiate the induced neuronal cells into dopaminergic neurons. For this to occur, each cell had to be exposed to six viruses, including A, B, M, Fuw, Lmx1a, and FoxA2. The percentage of induced neuronal cells converted into Da neurons turned out to be approximately 10%. This is a satisfactory achievement although with further study a greater turnout rate can be anticipated. Even more interesting, though, is the fact that within the original induced neuronal material, no expressions of tyrosine hydroxylase were found, yet dopaminergic neurons were still able to be induced from these cells (Pfisterer et al. 2011). It is important to note that a separate experiment performed by Vierbuchen et al., (2012), used the same four initial transcription factors to induce a pluripotent state from the fibroblasts and this also proved to be a successful procedure as induced neurons were produced. In both experiments these induced neuronal cells were deemed functional as they were able to conduct action potentials and form a performing synapse (Vierbuchen et al. 2012, Pfisterer et al.2011). Also, similar to IP cells, the use of fibroblast material dodges any ethical concerns typically raised in relation to stem cell discussions. Using induced neuronal cells for transplantation additionally provides the benefit that it poses no problems of rejection by the surrounding body tissue. One obstacle, however, with using this method of induced neuronal cells is that the number of derived Da neurons is solely dependent on the amount of original fibroblast cells in the starting material. So far, a limited number of Da neurons have been able to be successfully converted from the fibroblast cells. Further experimentation, though, should eventually determine precisely the ideal amount of fibroblast cell starting material (Pfisterer et al.2011).

Risks and Side Effects of Transplantation

Graft Induced Dyskinesia:

Although the idea of transplanting healthy nervous tissue into the striatum of PD patients does sound like a tempting alternative to the current available treatments for Parkinson's disease, there are still a number of remaining challenges and risk factors involved with this method that need to be resolved before the use of transplantation can become widespread (Hedlund and Perlmann 2009). The main concern associated with grafting of dopaminergic neurons is graft-induced dyskinesias (GID). It is suspected that a main cause for this condition is the presence of too much serotonin contained within the graft. The reason for this hypothesis is that in a healthy functional human brain, dopamine levels are regulated by the Da transporter and the D2 auto receptor feedback control mechanism. Serotonin is a particularly significant neurotransmitter since it has the power to convert L-dopa to dopamine, (Levodopa is always continuously administered to the patient during transplantation with the hopes of the dosage to be eventually being reduced after the patients show signs of improvement after surgery), store dopamine in vesicles for later use, and then release the neurotransmitter when seems necessary. However, when the striatum is damaged and there is a lack of regulatory feedback control, dopamine

can be released from the serotonin terminals in excessive quantities, possibly causing Graft Induced dyskinesia (GID) (Carlsson et al.2007).

This hypothesis was proven by Carlsonn et al.(2007) in an experimental study using rats. The animals were first injected unilaterally with 6-OHDA, transforming them into a model of a human PD patient. After the lesion the rats were treated with levodopa every day to model dyskinesias in a PD patient. Twelve weeks later, the rats were divided into four groups. The first group was transplanted with cells from the anterior portion of the ventral midbrain containing high concentrations of dopaminergic neuroblasts and low concentrations of serotonergic neuroblasts. The second group received a graft containing a wider portion of Ventral Midbrain tissue and a larger number of serotonergic neurons. The third group was grafted with tissue from the dorsal pontine raphe region of the lower pons. This type of tissue contains high concentrations of serotonergic neurons and very few, if any at all dopaminergic neurons. Finally, the fourth group was set aside as a control group. In addition, the experiment included another control group of rats who were lesioned with 6-OHDA but who received neither the grafts, nor administrations of levodopa. After twenty eight weeks, the rats were euthanized and their brains were examined. One test detected the presence of TH positivity, a marker for the outgrowth of dopamine neurons, in each of the samples. The results of this test are displayed in the picture below. The brains which received the grafts from the ventral midbrain exhibited significant neuronal outgrowth and integration. Interestingly enough, the graft with a narrower portion from the VM displayed a greater density of neuronal outgrowth than the sample that contained a wider portion of the ventral mesencephalon. As predicted, the graft containing tissue from the dorsal pontine raphe region displayed significant serotonergic neuronal outgrowth but very little TH positivity was detected. The control groups displayed neither dopaminergic outgrowth nor a presence of serotonin (Carlsonn et al. 2007). After eight weeks, the rats were subjected to behavioral testing to determine whether the transplant led to any functional and observable improvement. Significant progress was noticed in the rats who received transplants rich in dopaminergic neurons from the VM whereas little improvement was observed in the rats who were grafted with tissue from the lower pons. In addition, while on a continued dosage of L-dopa, the rats who received grafts from the VM exhibited signs of reduced dyskinesias, whereas the rats who were transplanted with serotonergic tissue displayed worse symptoms of dyskinesias than prior to the surgery (Carlsson et al.2007).

The results of this experiment clearly indicate the risks of the graft containing serotoninergic neurons in large amounts. However, it is possible that if the serotonergic neurons were combined in the graft with high concentrations of dopaminergic neurons then the adverse effects would not prove to be as severe. This study may have been more productive had they included another experimental group of rats receiving grafts that were both dopaminergic and serotonergic rich. However, the results of this study can be relied upon since the results are consistent with numerous other experiments done to evaluate similar concerns (Carta et al.2010). If methods of transplantation should ever become a widespread treatment for PD, care must be taken to ensure that the graft contain only the type of cells that will benefit the patient and not cause graft induced dyskinesia (Hedlund and Perlmann 2009).

Suggested methods for differentiating between desired and undesired cell types include flourescence activated cell sorting and Pitx-3 enhanced green flourescent protein (Hedlund et al).

Graft Overgrowth, Tetratoma Formation:

Stem cells (ESCs or IPs) differentiated in vitro with the purpose of being transplanted into a PD patient at a later time can present many risk factors. The primary concern regarding these cells are the possibility for residual undifferentiated cells to be grafted into the brain of the patient and then undergo rapid proliferation (Hedlund and Perlmann 2009). A plausible solution to this potential problem is to use imaging and filtering techniques to efficiently differentiate and removed the unwanted cells from the mature desired cells prior to transplantation (Hedlund and Perlmann 2009). One particular study used biotechniques such Pitx-3 enhanced green flourescent protein and fluorescence-activated cell sorting to distinguish between cells and isolate mature dopaminergic neurons from less desirable cell types. In this experiment, it was observed that this method of cell differentiatin is possible and proved benefical in rat models of PD. Grafts composed of this enhanced cell culture survived longer in vivo than transplants containing a larger variety of cells with different pluripotencies. In addition, rats who received these grafts that underwent screening before transplantation experienced greater symptomatic relief (Hedlund et al.2009). If this filtering method could be employed as successfully as it was in the experiment by Hedlund et al., then immature cells can be separated from preferred cell types thus decreasing the risk of tumor formation and graft overgrowth. Of course, however, further repeated testing is required before such a treatment plan can become accepted. Another possible way to avoid this problem of tetratoma formation is to use chemicals before transplantation that inhibit the cells' ability to replicate (Hedlund and Perlmann 2009). Specifically in one study, the use of mitomycin C, (a chemical commonly used in chemotherapy), before culturing the cells in vitro eliminated the chances of these cells proliferating unnecassarily in vivo (Sanchez-Pernaute et al.2008). Even if a chemical is not used to isolate the desired cells, certain poisons can also rid the culture of any harmful cells before being transplanted into the PD patient (Hedlund and Perlmann 2009).

Degeneration of Transplanted Tissue:

A major problem often encountered with transplanting healthy dopaminergic tissue into the striatum of a PD patient is the tendency for the graft to revert back to the previous PD state. This is often manifested in the observation of Lewy bodies, (a diagnostic characteristic of PD), within the graft. In most cases the presence of these bodies did not begin to appear until ten years after the transplantation took place (Hedlund and Perlmann 2009). According to Li et al. (2008), this difficulty poses the possibility that the disease can be transferred from the cells of the patient into the newly grafted tissue. One study compared the brain of a PD patient who died fourteen years after receiving a graft, with the brains of two PD patients who died four years after undergoing the transplantation process. In the brain of the fourteen year old graft there existed diagnostic features of Parkinson's Disease such as Lewy bodies and abnormal protein clusters. In the brains taken four years after the transplant, diagnostic features of PD did exist yet not at the same level of progression as the fourteen year old grafted brain. The results of this experiment suggest that the mechanism for the progression of PD is an ongoing process and can continue to affect even newly grafted tissue in a patient (Kordower et al.2008). However, it should be noted that in this particular study, only primary fetal tissue was used in the

patients' grafts and it is possible that the experiment would have yielded different results if stem cell tissue would have been used. Additionally, it is unknown whether the results of this study are universal. Different results could have been possible if a larger experimental sample was used (Kordower et al.2008). Interestingly enough, in a separate experiment preformed by Mendez et al. (2005), three subjects were grafted with healthy dopaminergic tissue using very specific methods and techniques, (graft composition etc.), and the grafts survived without any pathological features for fourteen years.

Another option is that the inclusion of Lewy bodies, and other pathological features of PD such as neuroinflammation, in grafted tissue can be a result of cellular stress from the surgical grafting procedure itself (Hedlund and Perlmann 2009). It has been tested and revealed that solid grafts containing blood vessels are more likely to cause cellular stress and induce immunoreactivity in the brain than grafts that do not contain blood vessels (Hedlund and Perlmann 2009). It is noteworthy that the experiment by Mendez et al.2005,in which the grafts did not exhibit signs of degeneration, the transplanted tissue did not contain any blood vessels. However, this idea can only explain the presence of Lewy bodies in relatively young brains and does not give reason for the degeneration of tissue in older samples.

Discussion:

Parkinson's disease is a condition characterized by a loss of dopaminergic neurons in the pars compacta of the substancia nigra in the human midbrain. Symptoms of PD include rigidity, and a loss of motor ability and coordination. Currently, there are many treatments available for patients of Parkinson's disease. However, due to the many side effects and imperfections associated with the existing treatments, science is now researching alternative options. Primarily, many studies have been done on the subject of transplanting healthy dopaminergic tissue directly into the striatum of a PD patient. Various options include the use of primary fetal tissue, embryonic stem cells, or induced pluripotent cells. Additionally, science has recently proposed the idea of reprogramming fibroblasts directly into nervous tissue. However, the results of each of these methods are highly varied and the methodology must be perfected before the use of this treatment can become widespread. Also, there are many risks and potential problems that must be resolved before the use of transplantation can be available as a standard treatment for PD. Such side effects include graft induced diskinesia, graft overgrowth, and the degeneration of the grafted tissue. Although there is still much to be improved and perfected in this area of treatment for PD, hopefully, with the appropriate dedication to the field and further testing and experimentation, patients with Parkinson's disease can fully recover from their symptoms and experience risk-free relief.

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