# PARKINSON'S GDNF THERAPY AND OXIDATIVE STRESS

By

PARVIN SINGH DHALIWAL

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Abstract: Parkinson's disease is a prevalent neurodegenerative disease that affects people all over the world. There is no cure; however, there are therapies that are intended to slow down the progression of the disease. Oxidative stress is a method that describes how the brain becomes toxic from free radical formation and other biological by products. This toxicity targets the substantia nigra of the brain, the area of dysfunction in Parkinson's disease. Glial cell line-derived neurotrophic factor (GDNF) therapy is paramount in finding ~~of~~ a cure. GDNF is an agent that causes both neuroprotective and neuroregenerative effects. Previous methods only focused on mitigating symptoms of Parkinson's but GDNF is unique because it aids in the formation of new neurons to replace damaged ones. This thesis will seek to find the correlation between the two topics, oxidative stress and GDNF therapy, and how the knowledge of one is critical to the understanding of the other. The mechanism of action of GDNF is still experimental and due to the recent suspension in clinical trials progress in this field may not resume.

Parkinson's disease is part of a group of neurodegenerative diseases that affect the motor and non-motors systems of the brain. Symptoms that are common in PD (Parkinson's disease) patients are tremors, rigidity, stiffness, and other movement impairment problems[2]. PD is the most common movement disorder affecting the elderly[1].With the onset of a new generation of baby boomers the prevalence of Parkinson's will see an increase due to the influx in the age group (70-80 years of age) most susceptible for Parkinson's. About half a million people suffer from Parkinson's disease in the United States with about 50,000 individuals freshly diagnosed each year. The etiology of Parkinson's is a topic that has been heavily researched and remains unclear. A mix of genetic and environmental factors can elicit PD although the real cause remains unclear. There is no laboratory test for Parkinson Disease. There are clinical signs including but not limiting to resting tremor, bradykinesia, rigidity and loss of postural reflexes[2]. Staging disease progression is done by the Unified Parkinson Disease Rating Scale (UPDRS). This test is broken down into three major parts. The first part is Mentation Behavior and Mood[3]. This category is further broken down into categories touching upon depression and motivation. The second part is Activities of Daily Living[3]. This category takes into consideration obstacles patients will have to encounter every day. Subcategories include swallowing, speech, salivation, and handwriting. The last category is the Motor Exam[3]. This section is divided into many physical functions that are affected by the disease itself. They include speech, tremor at rest, facial expressions, rigidity, posture, and gait[3]. This test has been standardized by researchers and clinical

doctors to stage the progression of patients suffering from Parkinson's disease. Although Parkinson's currently has no cure there have been treatments that have had limited success. L-3,4-dihydroxyphenylalanine[14] (L-dopa) has been used, but as the dose increases and the disease progresses treatment becomes less effective and severe side effects[14] ensue. Once this tolerance to L-dopa has set in the next step in treatment is surgery placing electrodes in the brain. Deep brain stimulation is the placement of electrodes to stimulate the subthalamic nucleus within the brain[12]. This therapy, although reversible, is very broad ranged in its effects. Unfortunately, it is not neuroprotective and would not stop the loss of dopamine neurons[11].

Parkinson's disease will begin to affect millions of people within the next 10 years. The need to find a cure is paramount. Although there are numerous theories, from oxidative stress to the proteinaopathy theory, no one theory has stood out from the rest. This thesis will go into detail to describe one of the modern theories of PD, oxidative stress. It will also analyze glial cell line-derived neurotrophic factor (GDNF) therapy and how it interrelates with oxidative stress. Oxidative stress is a major contributor to Parkinson's disease. Various factors that initiate oxidative stress cause damage to brain tissue in the areas related to the disease. GDNF is one of the most potent therapies in curing Parkinson's. Its neuroprotective and neuroregenerative properties have proved to be promising, although most of the experimentation has not reached the clinical level.

The discovery of pesticides and its involvement with Parkinson's introduced the idea that toxins may be a precursor step to the disease. MPTP, a neurotoxin, was accidentally found by Barry Kidston a graduate student in chemistry in Maryland[4]. He synthesized MPPP incorrectly and injected it into himself; his solution had traces of MPTP. The initial intent was to produce a synthetic drug that had similar properties to heroin. Within 3 days he began exhibiting PD symptoms. This tragedy has allowed Parkinson's research to mimic symptoms of the disease. In current research monkeys are given MPTP to mimic the symptoms of PD[4]. MPTP is not itself toxic however when it is metabolized into MPP+, within the glial cells, it begins to kill dopamine-producing neurons[17] as well as produce Lewy Bodies[5 16]. Other toxins that have been thought to induce Parkinson-like symptoms include carbon disulphide[5], carbon monoxide[5] (following acute poisoning), cyanide[5], n-hexane[5] and methanol[5]. Endotoxins should be an integral part of research as well as the mechanisms they employ to subvert the blood-brain barrier and harm the brain[17].

Oxidative stress is one of the key theories to the etiology of PD. Although, not entirely proven to be the root cause, it may be another part of the multi-hit hypothesis involving the disease. PD is described as a loss of catecholaminergic neurons within the brain[17]. The cause of the degeneration is unknown. PD was once thought to be caused from a neurotoxin, but it is unlikely due to the widespread nature of PD around the world[1]. Neurotoxins that are thought to induce PD are MPTP, tetrahydroisoquinolines, and beta-

carbolines[22]. The theory of oxidative stress suggests that the influx of free radical formation outside the normal homeostatic range can lead to neurological problems. Neuronal death within the substantia nigra leads to a decrease of dopamine relative to normal physiological levels[18]. MPTP is not itself a neurotoxin. The oxidation of MPTP to MPP+ by monoamine oxidase type B (MAO-B) causes excess free radical formation[17]. Animal models have showed that inhibition of MAO-B by MAO-B inhibitors such as deprenyl and paragyline prevent the symptoms of PD[18]. MPP+ is thought to inhibit complex I on the mitochondrial respiratoration chain and lead to superoxide generation[17]. Complex I has vital functions that can affect the biochemistry of the body. NADH is an electron source that provides 2 electrons to the electron transport chain[20]. Four protons are pumped into the inter-membrane space[20]. The electron acceptor in Complex I is ubiquinone[20]. Causing an interruption within complex I will inhibit any action further downstream in the mitochondria and lead to cell death[21].

Ubiquinone is an important nutrient in the human body. With increasing age ubiquinone levels are said to decrease. Humans above the age of 21 may benefit from supplementation [25]. Failure to add ubiquinone to the diet causes irreversible damage to the brain. In PD the affect of MPP+ may have some interaction with not only complex I but also with ubiquinone. This interaction may lead to neuronal death within the substantia nigra and dopamine producing cells. MPP+ is also a substrate for the dopamine transporter[17]. This causes decreased cellular adenosine triphosphate (ATP)

levels and altered calcium homeostasis within affected cells[17]. Complex I and III in the mitochondria are both capable of producing Reactive Oxygen Species[19].

Elevated levels of free iron cause increases in oxidative stress[19]. Free iron is toxic to cells and therefore a medium of retaining iron in the body is used indirectly through Ferritin. Ferritin is a 24 protein subunit iron storing protein found in both eukaryotes and prokaryotes[28]. The iron from the microglia exacerbates oxidative stress problems encouraging the formation of superoxide-dependent reductive release of ferritin iron[17]. This can cause oxidation of various substrates including lipids[17]. Nitric Oxide (NO) both reversibly and irreversibly inhibits complex I and IV of the mitochondria[16]. NO has shown to reduce levels of GSH which is important to the defense of damage induced by exposure to nitric oxide[20]. Increases in GSH and ascorbic acid have a protective effect on neurons[20]. The GSH protection mechanism detoxifies scavenging free radicals and electrophiles which converts GSH to GSSG[16]. GSH and ascorbic acid act as a redox coupler[24]. The goal of GSH is to rid neural tissue of peroxides and ROS and act as an antioxidant

Fats are susceptible to destruction by radical oxygen species. The double bond within a fat molecule present highly unstable electrons that lead to stress mechanisms such as lipid peroxidation[22]. Iron accumulates in astrocytes in the substantia nigra of old rats[16]. Aging nigral astroglia may be a factor that causes an alteration in the uptake and storage of

redox – active iron[16]. This is another predisposing factor of Parkinson's disease. Protection against lipid peroxidation increases the longevity of the animal. For example, lower rates of lipid destruction will decrease the passive flow of ions across the permeable membrane. Increases in fatty acid content within the brain and liver increases free radical damage of mitochondrial DNA (mtDNA)[22]. This positive feedback mechanism can be the target for future drugs that are aimed to reduce fatty acid break down within target areas of the brain. Two enzymes that are critical to the production of lipids are delta-5[23] and delta-6 desaturase[23]. These enzymes are limiting reagents in the synthesis of highly unsaturated fatty acids[23]. The degree of fatty acid unsaturation is an indicator of aging[19]. The goal of reducing lipid peroxidation rates within the brain is to slow down the rate of aging, which in turn slows down the progression of PD.

Caloric restriction attempts to limit the production of Reactive Oxygen Species in the mitochondria. The idea behind this is to reduce the amount of calories expended by certain cells. By limiting ROS production caloric restriction can enhance neuroprotective effects, oppose the development of deficits in psychomotor and spatial memory tasks, and reduce dendritic spine loss[16]. The targets of caloric restriction are in the brain, liver, and heart. These areas show decreases in mitochondrial ROS generation in experimental studies of rats[22]. As stated previously ROS generation in the mitochondria occurs at complexes I and III, caloric restriction halts production of ROS at complex I due to substrate utilization at that complex[22]. The difference between limiting the mitochondria

of oxygen consumption and caloric restriction resides in the fact that caloric restriction maintains oxygen consumption but the percentage electron flow directed to ROS generation is decreased[22]. Ultimately the goal of caloric restriction in the mitochondria is to make the generation of ROS more efficient and less harmful to the cell.

With the advent of stem cell research combined with advancing technology in molecular and cellular biology, researchers have found new ways to combat the destruction of the substantia nigra in PD. Glial cell line-derived neurotrophic factor (GDNF), a protein, is a new form of therapy for diseases affecting the central nervous system. GDNF is also hopeful in treating other neural disorders ranging from spinal cord injury, neurodegenerative diseases, and amyotrophic lateral sclerosis (ALS). GDNF allows for the proliferation and survival of dopaminergic neurons[8]. GDNF has also been proved experimentally to have protective and restorative properties *in vivo*[9]. Intrastriatal and intraputaminal GDNF administration[7] are mediums of delivery because the protein does not pass the blood-brain barrier[7]. This form of injection has been found effective but newer methods have proven to ameliorate results. Alternatively, encapsulated GDNF implanted into the striatum or substantia nigra has shown to have increased dopaminergic fiber growth[7].

Injection efficiency is limited to the diffusion properties of central nervous tissue as well as chronic delivery of GDNF[10]. Viral vectors are another method administration[10]. If the

genes can be introduced into the affected cells then DNA recombination is possible. These target cells can begin to produce the chemical of need. There are four important advantages of GDNF therapy using encapsulated cell transplantation. 1.) Neurotransmitters can be produced continuously from encapsulated cells[6]. 2.) The immunological response is limited by the stiff envelope that houses the cells of importance[6]. 3.) Tumorigenesis is suppressed because donor's cells stay within the capsule[6]. 4.) In the event of adverse reaction to the treatment of encapsulated GDNF the cells can be removed from the transplanted brain[6]. The encapsulated cells are semi-permeable membranes that allow the exchange of oxygen and nutrients as well as allow the continuous flow of growth factors and neutrophic factors. This membrane keeps out antibodies and other immuno-competent cells that may destroy the capsule.

There are three main types of viral vectors. Adenoviral vectors are not ideal for GDNF therapy because they cause inflammation and elicit an immune response[10].These vectors are not ideal but do have some positive effects when injected with GDNF into the striatum to prevent dopaminergic degeneration[10].

Adeno-associated viral vectors (AAV) are ideal because they are able to infect non-dividing cells with high efficacy and are ideal for transfecting neurons. This type of vector has been the experimental basis for many rodent experiments involving repair of lesions induced by 6-OHDA. Intrastriatal injections were the most effective and showed regrowth of dopaminergic fibers as well as improvement in motor behavior[10]. The main

disadvantage of AAV's is that it takes 2-3 weeks for these vectors to take effect once injected.

Lentiviral vectors on the other hand bring immediate results[10]. This vector integrates into non dividing cells and is a good target for neurons. Injection usually takes place in the striatum or the substantia nigra and allows for behavioral recovery and protection of dopaminergic neurons. Lentiviral vectors have shown the most promise in the lab[10]. Rhesus monkeys were injected, intranigrally and intrastriatally, 1 week after induced lesions. Dopaminergic neurons were found intact in addition to an improvement in motor skills[10]. The use of non-primate models have shown great success and have been the basis of demonstration of long term results using the lentiviral vectors as a form of neuroprotective delivery medium.

GDNF is capable of suppressing oxidative stress within the brain by neutralizing protein carbonyls. Experimentally it is possible to detect increases in oxidative stress before signs of neuronal death[9]. In an experiment done utilizing a rodent model of PD, a group of rats were given 6-OHDA (6-hydroxydopamine) prior to GDNF infusion. 6-OHDA, a neurotoxin, leads to the death of dopaminergic and noradrenergic neurons. This chemical depletes the availability of dopamine within the substantia nigra and striatum. The mechanism of action is thought to be apoptosis; however, the increase in apoptotic markers does not correlate with a change in cellular morphology[9]. Protein carbonyls and

4-hydroxynonenal are two chemicals widely used to measure oxidative stress *in vivo*[9]. Ultimately, the results of the study proved that when GDNF was administered 3 weeks after 6-OHDA there was a reduction in the loss of dopamine in both the striatum and the substantia nigra[9]. The mechanism of action remains unclear; however, the presence of oxidative stress markers, protein carbonyls and 4-hydroxynonenal, hint that GDNF may play a downstream role in protection[9]. This experiment did not take into consideration ~~of~~ other chemicals associated with oxidative stress (hydrogen peroxide and nitric oxide) and only focused on protein carbonyls and 4-hydroxynonenal. Further experimentation focusing on a broader range of oxidative stress markers will be necessary.

The mechanism of the etiology of Parkinson's disease is complex. There are multiple paths that can degenerate the substantia nigra, most importantly by oxidative stress. Limiting the effects of oxidative stress has had positive effects in the lab. Reduction in the levels of free radicals and other toxins in the brain is the first step. The more we are able to learn about the myriad of biological processes that lead to dysfunction in the brain the easier it will be able to target therapies to rectify them.

GDNF therapy has shown to be effective in restoring motor and cognitive issues associated with the disease. In experimental rodent models GDNF therapy has shown great promise. Not only are neurological impairments subsiding but neurons that were damaged have been repaired. Before techniques such as L-dopa attempted to only replace

what was lost due to dysfunction. This technology allows for both neuroprotection and neuroregeneration. However, recently clinical treatment has halted due to safety concerns. This has been a setback in the clinical realm of experimentation. For example, the most efficient method of delivery of GDNF is still debatable but until clinical trials are continued it will remain unclear which method yields better results.

GDNF therapy can oppose oxidative stress. GDNF therapy can reduce toxic levels of reactive oxygen species, peroxides, and beta-carbolines. The mechanism is unknown but it has seen convincing results in rodent models. Oxidative stress strains the electron transport chain of cells. Dysfunction in the component of cells that produces ATP (adenosine triphospate) leads to premature apoptosis. High levels of toxic materials lead to neuronal death. This is the reasoning behind dopaminergic cell death in the substantia nigra. Therapies sought to alleviate symptoms of Parkinson's target this problem and are a desirable fix due to the replacement and growth of new target neurons.

More research needs to be done integrating what is known about oxidative stress and GDNF therapy. GDNF therapy may not be the only answer but simply the genomic step in the right direction. GDNF theoretically may cause oxidative stress because it does create byproducts from synthesizing replacement neurons. Although this effect is thought to be minimal it is still significant due to the sensitivity of substantia nigra and the brain.

Understanding these principles it may be possible to target different neurodegenerative diseases that focus on other brain systems such as memory and speech.

This thesis focused on relating the two subjects that there is a plethora of knowledge on, oxidative stress and GDNF therapy. Combing through information on each individual topic I attempted to find relationships between the two. As much information that we have it still remains unclear if there is a link between GDNF therapy and oxidative stress. By understanding the fundamentals of GDNF therapy and learning what it targets we will be able to begin to develop cellular modifications that can cure the symptoms exhibited by Parkinson's. Oxidative stress is a very broad theory but it gives clear information on what toxins are most harmful to neurological function. Integrating the two ideas and developing cellular machinery that can be integrated in the area of dysfunction we will be able to combat neurotoxins and improve the quality of life of millions of patients. Being able to modulate neuro-biological processes and maintain their equilibrium is the key component of this research.

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