Current Trends in Treatment of Parkinson`s Disease using Gene Therapy

Tamene Milkessa[a], Tileye Feyissa[a]

[a]*Microbial Cellular and Molecular Biology Department, College of Natural Science, Addis Ababa University, Addis Ababa, Ethiopia.*

Corresponding author: Tamene Milkessa

Abstract

Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons that produce a neurotransmitter called dopamine in the substantia nigra. Various therapeutic methods have been used and still in use for the treatment of PD. The therapeutic approaches that target the subthalamic nucleus or globus pallidus have been used to improve motor function in PD. Earliest therapeutic techniques include stereotactic lesioning, high frequency deep brain stimulation, pharmacological silencing and transplantation or restorative surgery. These methods were replaced by therapeutic drugs such as levodopa. Patients respond well to levodopa treatment initially, however, with long term treatment, the response to dopamine replacement fluctuates and disabling dyskinesias develops. The problems with long-term levodopa treatment have led to the search for new therapeutic drugs for PD. These include dopamine agonists, amantadine, anticholinergics, selegiline etc. All therapies described above aim to improve the symptoms of PD but none are proven to preserve or restore dopaminergic neurons lost during the disease progress. All the above mentioned limitation led scientists to search for a better alternative therapeutic for PD: gene therapy. The types of gene therapy strategies for the treatment of PD can be enzymatic [AADC, GAD or tricistronic (TH, AADC and GCH-1)] or neurotrophic (GDNF or NTN).These are delivered into the part of brain affected by PD using vectors.

Keywords: Dopamine, Gene therapy, Levodopa, Parkinson`s disease, Vectors

1. INTRODUCTION

PD is the second most common progressive neurodegenerative disorder of the central nervous system next to Alzheimer's disease, affecting at least 2% of the population aged 65 and older [1]. PD was first described in 1817 by James Parkinson in his famous monograph "An Essay on the Shaking Palsy" [2]. Clinical features of PD consist of severe motor defects produced by resting muscle tremor, shaking, muscle rigidity, bradykinesia and postural instability [3]. As described by Braak and Del Tredici [4] a range of cognitive and psychiatric dysfunctions often co-manifest. In recent years, it

has become increasingly recognized that a range of other non-motor features, including olfactory dysfunction, dysautonomia, as well as mood and sleep disturbances are seen [5].

The progressive loss of dopamine neurons in the substantia nigra (SN) represents one of the most noticeable and core neuropathological hallmarks of PD. The SN is located in the midbrain, halfway between the cerebral cortex and the spinal cord. In healthy individuals, the SN contains certain nerve cells, called nigral cells, which produce the neurotransmitter dopamine. Dopamine travels along nerve cell pathways from the SN to central area of the brain, called the striatum. In other words, information comes to the striatum, which works with the SN to send impulses back and forth from the spinal cord to the brain. The basal ganglia and the cerebellum are responsible for that movement is carried out in a smooth and fluid manner [6]. In people with PD, nigral neurons degenerate and die at a faster rate, and the loss of these cells reduces the supply of dopamine to the striatum. When 80% of the dopamine is lost, symptoms of PD which are mentioned above will appear [7]. In addition to the loss of nigral neurons by PD, cholinergic, serotonergic and noradrenergic neurons also being adversely affected [8].

Naturally impulses pass from neuron to neuron moving quickly from brain to spinal cord and finally to muscles. When dopamine receptors in the striatum are not adequately stimulated, parts of the basal ganglia are either under stimulated or over stimulated [6]. Particularly the subtalamic nucleus (STN) becomes overactive and acts as a brake over the globus pallidus interna (Gpi) causing shutdown of motion and rigidity. When GPi is overstimulated,it poses an over inhibitory effect on the thalamus, which in turn increases thalamus output causing tremor. The general diagram of the part of the brain affected by PD is shown in figure 1.

Gene therapy is an experimental technique that uses genes to treat or prevent disease. It derives its name from the idea that DNA can be used to supplement or alter genes within an individual's cells as a therapy to treat disease [10]. The most common form of gene therapy involves using DNA that encodes a functional, therapeutic gene to replace a mutated gene [11]. Other forms involve directly correcting a mutation, or using DNA that encodes a therapeutic protein drug to provide treatment. In gene therapy, DNA that encodes a therapeutic protein is packaged within vehicle called a vector, which is used to get the DNA inside cells within the body. Once inside, the DNA becomes expressed by the cell machinery, resulting in the production of therapeutic protein, which in turn treats the patient's disease.
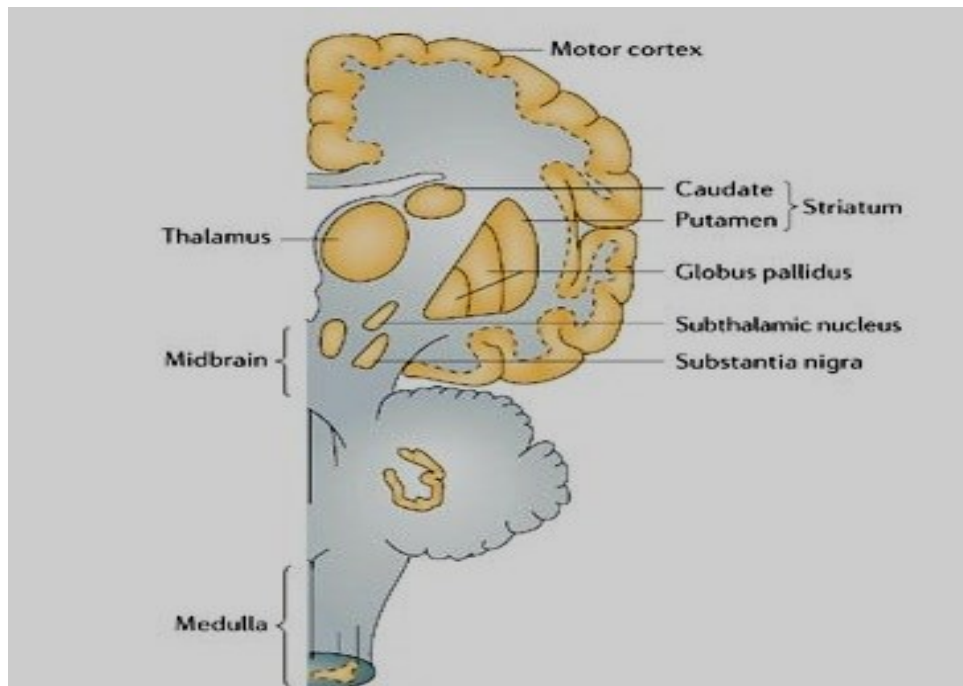
Figure 1. A lateral section through a human brain with the anterior to the left. The yellow shading indicates regions of the brain that are affected in Parkinson disease [9].

There are two types of gene therapy: somatic gene therapy and germ line gene therapy [11]. In somatic gene therapy, the therapeutic genes are transferred into the somatic cells or body of a patient. Any modifications and effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring or later generations [12]. Somatic gene therapy represents the mainstream line of current basic and clinical research, where the therapeutic DNA transgene (either integrated in the genome or as an external episome or plasmid) is used to treat a disease in an individual [11].

There are two categories of somatic gene therapy. These *ex vivo* (*in vitro*) and *in vivo* somatic gene therapy [11]. In *ex vivo* therapy, cells are modified outside the body for later transplantation back into the body whereas, in *in vivo* gene therapy, genes are changed in cells still in the body.

In germ line gene therapy, functional genes are inserted into the reproductive cells, i.e., eggs or sperm cells [13] or possibly into embryos [11] to treat diseases that could be passed on to latter generations. This is the more controversial of the two forms. Many jurisdictions prohibit this for application in human beings, at least for the present, for a variety of technical and ethical reasons

2. HISTORICAL DEVELOPMENTS INTO GENE THERAPY FOR PARKINSON`S DISEASE

Historically various therapeutic methods have been used and still in use for the treatment of PD. The therapeutic approaches that target the STN or globus pallidus have been used to improve motor function in PD patients. Earliest therapeutic techniques include stereotactic lesioning [14], high frequency deep brain stimulation [15], pharmacological silencing and transplantation or restorative surgery [16]. Neurosurgeons were innovated in the 1900`s and often based on animal studies [17]. Deep brain stimulation is still in use when medications are not enough to control symptoms [18]. These methods were replaced by therapeutic drugs. The main families of drugs useful for treating motor symptoms were/are levodopa, dopamine agonists and MAO-B inhibitors. Levodopa as dopamine replacement therapy was discovered by Cotzias and his associates in 1960s [19] and still constitutes the mainstay of treatment for PD [20].

As stated by Parkinson Study Group [21], patients generally respond very well to levodopa treatment initially, however, with long term treatment, the response to dopamine replacement fluctuates and "wearing off" phenomena or troubling dyskinesias develop.

The problems with long-term levodopa treatment have led to the search for new therapeutic strategies for PD. Pharmacological agents such as dopamine agonists (bromocriptine, pergolide, pramipexole, ropinirole, piribedil, cabergoline, apomorphine and lisuride) were initially used for patients experiencing on-off fluctuations and dyskinesias as a complementary therapy to levodopa but they are now mainly used on their own as an initial therapy for motor symptoms with the aim of delaying motor complications [22].

All therapies described above aim to improve the symptoms of PD but none them are proven to preserve or restore dopaminergic neurons lost during the disease progress. Such limitation led scientists to search for a better alternative therapeutic for PD: gene therapy.

The idea of gene therapy for human genetic disease was proposed in the 1970`s [23]. The first approved gene therapy case in the United States took place on September 14, 1990, at the National Institute of Health. It was performed on a four year old girl named Ashanti DeSilva. It was a treatment for a genetic defect that left her with adenosine deaminase-deficiency (ADA

-SCID), a severe immune system deficiency [24]. Since then gene therapy has become successful in treating X-linked chronic granulomatous disease [25], color blindness [26], thalassaemia, cystic fibrosis, some cancers and sickle cell anemia [27], chronic lymphocytic leukemia [28], acute lymphatic leukemia [29] and chroideremia [30].

Clinical trials of gene therapy of PD on human begin in 2003 and are still going on [31]. Gene therapy has distinct potential advantages over conventional treatment modalities for PD as it could theoretically be used to preserve or restore dopaminergic neurons affected by PD through the action of neurotrophic factors [32,33] or alternatively increase the availability of enzymes required for dopamine synthesis [34,35]. They could potentially target the underlying pathophysiological imbalances and may result in much less fluctuation in response and a lower prevalence of dyskinesias than conventional pharmacotherapy for PD.

3. VECTORS (CARRIERS) FOR PARKINSON`S DISEASE GENE THERAPY

In gene therapy method DNA is transported into the targeted brain cells. Naturally cells of human bodies have developed a number of enzymes that breakdown unprotected DNA. In order to protect the target DNA from damage, most gene therapies use vectors as protective envelop. Vectors carry the genetic material and deliver the gene to targeted cells.

There are two types of vector systems used for gene therapeutic applications: viral and nonviral. The most commonly used viral vectors are derived from retrovirus, adenovirus, and adeno - associated virus [36]. Nonviral vector vector delivery systems include, the injection of naked DNA, electroporation, the gene gun, sonoporation, magnetofection and the use of oligonucleotides, lipoplexes, dendrimers and inorganic nanoparticles) for achieving high-efficiency gene transfer 11]. The latter have associated problems that still hinder their application to gene therapy, such as immunogenicity, pathology, targeting and/or the duration and level of gene expression. Nonviral vectors such as liposomes and DNA conjugates are nonpathogenic, but are less effective for gene transfer.

The preclinical gene therapy studies in PD are mainly concerned with the selection of a proper vector for gene delivery; the optimum delivery vector for crossing the blood-brain barrier; and the optimum delivery of gene within the target. The choice of a given viral vector depends on   its neuron specificity and the prevention of evoking of immunological response against the encoded transgene, its clinical safety, and its large scale

production by commercial entities. The different viral vectors used safely for the gene therapy of PD are adeno-associated virus, lentiviruses vectors, herpes simplex virus-1 vectors and adenoviruses [37] for their specific advantages. Other non-viral vectors have also been used such as liposomes as they are devoid of any immunological effect within the body compared to the viral vectors.

3.1. Adeno-associated virus

The adeno-associated virus (AAV), is a member of the nonpathogenic *Parvoviridae* family and *Dependovirus* genus, and has been used to deliver gene therapy constructs of approximately five kilobases[38]. Most of these viruses contain single-stranded DNA and require a helper virus, such as adenovirus or herpes-simplex virus, for replication. They comprise two genes encoding capsid (*cap*) and viral replication (*rep*) proteins and inverted terminal repeat sequences, but require additional genes from other viruses (e.g., adenovirus) for replication. AAVs are well suited to gene therapy for PD as they are capable of inducing long term gene expression, usually via episome formation [39]. The AAV serotype 2 (AAV2) was the first to be sequenced, and is the most widely used AAV-based gene therapy [40]. Since then, more than 80 AAV gene therapy clinical trials have been initiated worldwide [40], without evidence of major immunological reactivity or direct pathogenesis. AAV2-derived vectors are the best characterized and most frequently utilized serotype in PD gene therapy studies. One advantage of AAV2, when administered locally, is that it transduces only neurons within the central nervous system and is particularly efficient in brain regions known to be involved in the pathophysiology of PD, such as the globus pallidus and SN[42]. Researchers have realized that, AAVs have the ability to integrate into a specific site at chromosome 19 of the human genome raising potential concerns regarding insertional mutagenesis, although the frequency with which integration occurs in vivo remains unclear [42].

Recent research additionally suggests that AAV-1, -5, and -8 are also able to transfect basal ganglia neurons in a highly efficient and specific manner in nonhuman primates and therefore these serotypes could be used in future gene therapy trials [43].

3.2. Lentivirus

Lentiviruses (LVs) are retroviruses that can efficiently infect both dividing and non dividing cells, potentially leading to long-term gene expression

following chromosomal integration [42]. LVs are derived from a group of pathogenic retroviruses that include HIV, which has been studied extensively, and most lentiviral vectors are consequently based on HIV [44]. HIV-1-derived vectors incorporate a transgene between the long terminal repeats (LTRs) required for integration into the host genome. The HIV-1 *env* gene product largely restricts the tropism of wild-type virus to CD4 containing cells. By substituting *env* for gene encoding other viral glycoproteins, such as the vesicular stomatitis virus glycoprotein, the cellular tropism can be broadened or made more specific to neurons [45].

The capacity of these vectors have relatively larger capacity for cloned genes (approximately 9 kb), makes them a very attractive option for future gene therapy research[46], with additional adaptations allowing improved packaging effectiveness[47]. However there are concerns related to the possibility of recombination events, producing replication-competent virus [46].

3.3. Adenovirus

Adenovirus (Ad) was one of the first viral vectors used successfully in animal models of PD and contains a 36 kb genome with linear double-stranded DNA [41]. It has the capacity to carry up to 30 kilobases of exogenous genetic constructs [48]. Ad vector was the most commonly used viral delivery vehicle in human clinical gene therapy trials with its frequency of use peaking before 2000 [41]. As reported by Lynch et al. [49], wild-type adenovirus is frequently associated with mild respiratory tract, gastrointestinal, and eye especially conjunctival infections in humans. Early adenoviral vectors were created using E1 or E3/ E4 gene region deletions, but these proved unsatisfactory due to the host inflammatory response and associated toxicity that occurred in vivo when the remaining wild-type genes were expressed in the host. These vectors are   the most efficient gene transfer systems in a variety of tissues [50].These classes of viruses have the ability to ease in the production of high titer stocks and generally strong gene expression [45].

3.4. Herpes simplex virus

Herpes simplex virus(HSV) is in a *Herpesviridae* family having 150 kb double -stranded DNA, that encodes 70-80 genes and is the largest and most complex viral vector being developed for gene therapy, packaging capacity up to 40 kilobases of transgenic DNA[48]. This large capacity is useful for

consisting of multiple therapeutic genes. These viral vectors are neurotropic and have long lived episomal latency. Like adenoviruses, HSV can infect a wide variety of cell types, including muscle, tumors, lung, liver and pancreatic islets.

The wild-type virus is associated with encephalitis, as well as cold sores and corneal ulceration [51]. HSV vectors can be divided further into recombinant viral and amplicon vector systems. Recombinant viral systems retain most of the wild-type genome, using homologous recombination to insert the required foreign gene. This system allows for very large genes to be inserted if all the wild-type genes were removed [42]. On the other hand, the amplicon vector contains only a *cis* acting viral origin of replication and packaging signal, with the genes required for replication and virus production are supplied in *trans* by a separate helper virus. The *trans* is usually supplied from a *pac* deficient cosmid encoded HSV-1 genome [52]. Animal studies suggest that long lasting transgene expression in neurons can be achieved using HSV amplicons and the use of specific promoters, for example, those for tyrosine hydroxylase, can increase transduction specificity [53, 54].

4. APPROACHES FOR   GENE THERAPY IN PARKINSON`S DISEASE

4.1. Enzyme replacement strategies

4.1.1. Aromatic Amino acid Decarboxylase

Aromatic amino acid decarboxylase (AADC) whose locus is located on human chromosome 7 [55], is an enzyme responsible for the production of dopamine from endogenous or exogenous levodopa. Patients with PD require increasing oral doses of exogenous levodopa to control their symptoms in later stages of the disease and it has been suggested that AADC activity may be reduced in PD patients [56]. The loss of therapeutic efficacy of levodopa in later stages of PD is associated with significant drug-induced side effects and a direct result of loss of this enzyme activity within the striatum [37]. Researchers have attempted that augmenting the activity of AADC using gene therapy may reduce both the symptoms of PD and the amount of levodopa required to control them, it might alleviate the side effects of prolonged levodopa therapy [37].

Gene therapy experiments in rodents [57] and rhesus monkeys [58] with hemiparkinsonism induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) showed that basal ganglia injection of AAV-

AADC induced increased AADC activity *in vivo,* improved conversion of dopamine to levodopa and have brought behavioral enhancement.

 A phase I human clinical trial in which AADC (AAV2-hAADC) was injected into the putamen(where dopamine normally released) of PD patients has been completed at two different doses. This gene therapy trial investigated the safety and tolerability of administering AAV2-hAADC in 10 patients with moderate to advanced Parkinson's disease [59, 60]. Their clinical status was measured by subject diaries of motor function, clinical assessment and the daily requirement of levodopa. AADC gene expression level was monitored via postoperative positron emission tomography with 6-[^{18}F]-fluoro-L-*m*-tyrosine (FMT-PET) imaging [61]. FMT PET scans showed an increase of AADC expression (30% in low dose cohort, 75% in high dose cohort) after 6 months and well-tolerated. Higher AAV2-AADC vector doses have been correlated with increases in striatal PET uptake and improved behavioral responses.

4.1.2. Glutamic acid decarboxylase

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme for the synthesis of major inhibitory neurotransmitter gamma amino butyric acid (GABA) from excitatory glutamic acid in the mammalian central nervous system. GAD65 and GAD67 are the two genetically distinct isoforms [62]. The two differ in their gene loci, anatomical and subcellular distributions, functions, regulatory and enzymatic properties [62, 63]. GAD65 is concentrated in the nerve terminals and synthesizes GABA for neurotransmission, and therefore is only necessary at nerve terminals and synapses, whereas GAD67 is spread evenly throughout the cell and associated with non-neurotransmission functions such as synaptogenesis and protection from neural injury [64].

 As discussed by Fiandaca et al. [37], PD has been associated with disrupted levels of GABA neurotransmission, the cardinal symptoms and signs result from disinhibition of the striatum associated with dopamine depletion due to the nigrostriatal degeneration. Loss of inhibitory dopamine tone on the striatum leads to relative hyperactivity of the STN output to the SN pars reticulata, with resultant alteration in motor tone and function [65]. According to Fiandaca et al. [37] descriptions STN removal, electrical blockade and pharmacological suppression have all been utilized to improve PD motor function. This correlation of anatomy and pathophysiology led to the development of GAD gene therapy within the

STN for GABA production and suppression of STN excitatory efferents to SN pars reticulata in an animal model of PD such as rats [66].

In an attempt to modulate the STN neuronal phenotype, AAV2 used to deliver GAD to the STN of rats essentially converting glutamatergic neurons to GABA producing cells [66]. The neurons transduced with GAD synthesize and release GABA in an activity-dependent manner. The modified neurotransmitter pattern in STN projections leads to an inhibitory effect in the innervated SN and regulates the firing rates of basal ganglia nuclei in 6-OHDA-lesioned rats [67]. In addition it protected neurons from neurotoxicant- induced degeneration. As a result of the function of GAD expression levels, the biochemical and electrophysiological properties of the STN to SN projections improve the 6-OHDA-induced motor deficits in rats [67]. In a similar fashion, these findings were later confirmed in MPTP - treated rhesus monkeys, however, no effect was observed in the MPTP-treated monkeys [68]. This neuronal phenotype modulation therapy has moved to Phase I and II human clinical trials [69]. The Phase I trial established safety and tolerability. A second randomized, double blind, placebo controlled trial (Phase II) is underway and enrolling patients that have idiopathic PD (for at least 5 years).

4.1.3. Tricistronic Gene Therapy (TH/GCH-1/AADC)

Dopamine is chemically synthesized *in vivo* from L-tyrosine, which is provided either by the nutrition or through the conversion of phenylalanine into tyrosine by the enzyme phenylalanine hydroxylase. Tyrosine is next hydroxylated into levodopa by tyrosine hydroxylase (TH) and finally, levodopa is converted into dopamine by AADC. As described by Coune et al. [70] TH activity is the limiting factor in dopamine biosynthesis, and requires a cofactor, tetrahydrobiopterine (BH4), the synthesis of which requires the enzyme GTP-cyclohydrolase-1 (GCH-1). In other words, GCH-1 is a rate limiting enzyme in the synthesis of a cofactor, BH4, which is required for TH activity

It is realized that in patients with PD dopamine synthesis nigrostriatal pathway defected at several different points and replacement of a single enzyme may not be sufficient to achieve a clinical response single enzyme may not be sufficient to achieve a clinical response. Using TH alone [71, 72], only partial neurochemical and functional deficits were restored. Then a combination of TH and GCH-1[73, 74] was delivered. Although behavior was not ameliorated, neurochemical improvement was noted in a

parkinsonian rat model. At last, the use of triple AAV transduction of TH, AADC and GCH-1 to medium spiny neurons in the striatum in a parkinsonian rodent model showed improved dopamine production and corrected motor deficits [75, 76]. A subsequent study of MPTP-induced parkinsonian rhesus monkeys using tricistronic lentiviral vector coding for AADC, GCH-1, and TH restored extracellular restored extracellular dopamine within the striatum and also corrected functional motor disturbances without inducing dyskinesia when compared to conventional levodopa-therapy [77].

The safety, efficacy and dose evaluation Prosavin (lentiviral vector containing genes for TH, GCH-1 and AADC) are currently under evaluation in a phase I/II human clinical trial.

4.2. Neuroprotection/Growth factors

4.2.1. GDNF

In PD patients, protection of substantial loss of SN dopamine neurons has to be the primary objective before the appearance of PD motoric symptoms. As described by Feng and Maguire-Zeiss [69], neuroprotection has been achieved in animal models of dopaminergic neuron death following treatment with a prototypical neurotrophic or growth factors. Neurotrophic factors are secreted brain proteins responsible for the differentiation, growth, and survival of neuronal cells. They are also useful for the maintenance of mature neurons. From the many neurotrophic factors that have been identified over the years, the glial cell line-derived neurotrophic factor (GDNF), a member of the transforming growth factor (TGF)-β superfamily, has emerged as a powerful factor to protect the dopaminergic neuronal function [77]. GDNF has regenerative properties for brain cells and showed potential as treatment for PD. GDNF was discovered in 1991[79]. Lin et al.[80] characterized GDNF as a neurotrophic factor for embryonic rodent midbrain dopaminergic neurons, promoting their survival *in vitro* and increasing dopamine uptake in TH-positive neurons without altering uptake of serotonin or GABA.

Researchers realized its potential application in the treatment of PD and a subsequent *in vivo* study in a mouse model of PD found that direct injection of GDNF into the SN or striatum resulted in a relative increase in dopaminergic nerve fiber density and improvements in motor behavior

regardless of whether the GDNF was administered before or after the MPTP used to induce parkinsonism [81].

Related experiments used a replication defective Ad vector to deliver the gene encoding human GDNF as a direct injection near to rat SN in vivo, prior to lesioning with 6-hydroxydopamine (6-OHDA)[82]. Survival of dopaminergic neurons was significantly increased in those rats treated with the Ad/GDNF vector compared to controls; however, transgene expression for both GDNF and the LacZ promoter (used as a transgene in a control group) was reduced over the four week follow-up period of the study, raising doubts about longer-term efficacy of transgene expression. In addition, all animals treated with the adenovirus vector had localized reactions at the injection site and this effect was observed in both the Ad/GDNF and the Ad/LacZ groups, suggesting it relates to the vector or injection method rather than choice of transgene. Other studies used a lentivirus vector to deliver the GDNF gene by stereotactic striatal and SN injection in vivo in rhesus monkeys one week prior to MPTP treatment [32]. This resulted in an increase in the number of TH-positive dopaminergic neurons in comparison to controls. In addition, the rhesus monkeys treated with the lentiviral vector encoding GDNF performed better in behavioral outcome measures than controls and demonstrated increased and more symmetrical fluorodopa uptake in the striatum in FDG PET scans. Transgene expression of GDNF using this lentiviral approach was sustained at 8 months and there were no issues with host inflammatory responses.

Future human trials of gene therapy using GDNF for PD will need to take into account the experience of clinical trials of direct recombinant GDNF infusions in PD [56].

4.2.2. Neurturin (NTN)

Another GDNF family member that supports dopaminergic neurons, (NTN), was shown to effectively protect dopaminergic neurons in rodent and rhesus monkey models of PD without the development of neutralizing anti-NTN antibodies or cerebellar degeneration [83]. Both NTN and GDNF share similar neuroprotection and neuroregeneration profiles to chemical lesioning of the nigrostriatal pathway in rats [84] and rhesus monkeys [32, 85] and hence promote the survival of dopaminergic neurons. NTN gene therapy was the first family of ligands (GFL) gene therapy to be

commercially developed in combination with an AAV delivery vector (CERE-120, AAV2-NTN) [86].

Horger et al. [87] stated that NTN into the SN increased the survival of dopaminergic neurons in 6-OHDA lesioned rats. Subsequent experiments in MPTP-lesioned young, middle, and old aged rhesus monkeys using stereotactically delivered AAV2 vector coding for NTN (CERE-120, AAV2-NTN), increased survival of nigral neurons and an 80–90% reduction in motor impairment(compared to controls) was noted at 4 months following gene therapy [84]. With no adverse effects, the NTN expression sustained for up to 12 months post-treatment [83].

These promising results led to phase I and II clinical trials. These clinical trials were completed by Ceregene, Inc. and involved intraputaminal injections of CERE-120, a AAV2- NTN vector [69]. 12 patients with PD underwent bilateral stereotactic intraputaminal injections of AAV2-NTN) in this phase I study an open-label phase I study [88]. During the procedure and the 1-year follow-up period no serious adverse events were noted, however, one patient developed a procedure-related air embolus. Furthermore, there were more frequent complications, such as headache occurring in eight patients, three patients experienced dyskinesias and one patient developed hallucinations.

A Phase II double-blind, multicenter, randomized controlled trial of intraputaminal stereotactic AAV2-NTN injection versus sham surgery was initiated in 58 patients with advanced bilateral idiopathic PD. Unfortunately, there was no significant difference in the primary outcome measure, the reduction in United Parkinson's Disease Rating Scale (UPDRS) - medication motor score at 12 months, between the control subjects and patients treated with CERE-120 [33]. However, the neurosurgery and gene therapy were well tolerated with no apparent adverse effects in these advanced-stage patients. In addition, thirty patients were clinically followed in a double-blind fashion for an additional 18 months at which time Ceregene officials reported a modest but statistically significant effect on the UPDRS-motor off score as well as on several secondary measures of motor function. This perhaps suggests that longer follow-up periods may be required to discern efficacy of AAV2- NTN in the treatment group.

5. RESEARCH FOCUSES ON GENE THERAPY FOR PARKINSON`S DISEASE TREATMENT

Medical and surgical treatments of PD have lots of limitations, yet they are useful. Enzymes or neurotrophic factors may be the key to the cure for PD. This has been known by scientists for over two decades. As Bartus et al. [86] discussed in their literature for example rather than focusing on conventional methods of neurotrophic factor delivery, researchers in the US (Ceregene company) have turned to gene therapy. Conventional methods are extremely difficult and might have undesirable side effects. Instead of delivering the restorative protein to the targeted sites in the brain, the Ceregene researchers for example, have developed a way to deliver only the gene for the protein. Once integrated into, the gene induces local cells to make the protein on site [86].

According to Bartus et al.[86] CERE-120 is designed to deliver the gene for NTN to targeted neurons, and subsequently program these neurons to provide continuous, long-term, predictable NTN expression in selective, stereotactically-targeted regions of the brain.

During ten years of testing, which initially involved rats and monkeys, before working with human patients with PD, the researchers performed exhaustive experiments on the therapy's safety, efficacy and long term positive effect. One of the major issues considered is the expression of enzymes [AADC, GAD or tricistronic (TH/GCH-1/AADC)] or neurotrophic factors (NTN or GDNF) throughout the targeted region, while avoiding exposure to other areas of the central nervous system, and inducing positive responses in the brain.

This year researchers in the Oxford BioMedica attempted to assess the safety, tolerability, and efficacy of bilateral, intrastriatal delivery of ProSavin, a lentiviral vector-based gene therapy aimed at restoring local and continuous dopamine production in patients with advanced Parkinson's disease [89]. According to their report, the treatment has been safe, with no serious adverse effects. The patients' scores on movement tests have improved on average by 30 percent, and they also report having a better quality of life. PET scans confirm that dopamine is being produced in the brain where it wasn't before.

Another research direction that investigators are now focusing is to alleviate the problem of delivery of trophic factors across the blood brain barrier [90]. To solve drug delivery problems, bone marrow hematopoietic

stem cell-based gene therapy has emerged as a promising tool. Myeloid cells can cross the BBB and are recruited in large numbers to sites of neurodegeneration, where they become activated microglia that can secrete trophic factors. Bijua et al. [90] tested the efficacy of bone marrow-derived microglial delivery of NTN in protecting dopaminergic neurons against neurotoxin-induced death in mice (induced with MPTP). Microglia-mediated NTN delivery dramatically ameliorated MPTP-induced degeneration and restored MPTP-induced decline in general activity. Thus, bone marrow-derived microglia can serve as cellular vehicles for sustained delivery of neurotrophic factors capable of mitigating dopaminergic injury. Neurolucida is a protocol for best accuracy to perform neuron reconstruction and is the most effective method for studying neuron morphology [91]. To determine proper dosage, researchers have created a 3D model of the brain with Neurolucida, MRI images, and histological slides [86]. The 3D computer model have helped the scientists visualize the target brain region so they could determine how much of the enzymes [AADC, TH or tricistronic (TH/GCH-1/AADC)] or neurotrophic factors (GDNF or NTN) gene should be administered and where exactly it should be directed to provide the greatest coverage, with the fewest needle tracts, while avoiding protein expression outside its boundaries.

6. CONCLUSION

Investigators have realized that the use of gene therapy has distinct potential advantages over conventional treatment modalities (medical and surgical treatments) as it used to preserve or restore dopaminergic neurons affected by PD. This is done through the action of neurotrophic factors (GDNF or NTN) or alternatively by augmenting the availability of enzymes [AADC, GAD or tricistronic enzymes (TH, AADC and GCH-1)] required for dopamine synthesis. They could potentially target the underlying pathophysiological imbalances and may result in much less fluctuation in response and a lower prevalence of dyskinesias than conventional pharmacotherapy drugs such as levodopa for PD. Future research direction is the gene therapy's safety, tolerability, efficacy and long term positive effect.

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