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Gene Therapy for Parkinson's Disease

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1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disease, with a prevalence of around 250 per 100,000 population and becoming of growing importance in ageing populations. Patients become symptomatic when around 75% of striatal dopamine is lost, with ongoing yearly declines of 5-10% (Brooks 1998, Fearnley 1991). The use of levodopa (L-3,4-dihydroxyphenylalanine) as a symptomatic therapy was established nearly 50 years ago and continues to be an important approach in early and late disease (Fahn 2006). Although the majority of patients initially respond well to dopaminergic therapies, many eventually develop fluctuations in their therapeutic response, often with associated dyskinesias (Jenner 2000). Additional agents, including catechol O-methyltransferase inhibitors and/or monoamine oxidase inhibitors are often added in to existing medications, with a proportion of patients dependent on infused apomorphine. These therapies are symptomatic, with no effect on the underlying pathogenic processes, particularly the progressive loss of dopaminergic neurons. Novel therapies to either compliment existing approaches, or potentially alter the course of disease, are clearly desirable.

This neuronal loss in Parkinson's disease is associated with multiple functional abnormalities, including changes in excitatory glutamatergic and inhibitory GABAergic pathways controlling movement (Wichmann 2003). Disinhibited activity in the subthalamic nucleus (STN) correlates with increased activity in excitatory projections to the major nuclei of the basal ganglia - the internal globus pallidus (GPi) and substantia nigra pars reticularis (SNr). The resultant increased inhibitory outflow to the pallidal receiving areas or the thalamus and consequently reduced cortical activity are thought to be responsible for many of the motor features of Parkinson's disease (Brown 2001). The pathophysiological importance of this overactive subthalamic nucleus activity can be targeted using several approaches, including stereotactic lesioning (Alvarez 2001, Su 2003), high frequency deep brain stimulation (Benabid 1996, The Deep-Brain Stimulation for Parkinson's Disease Study Group 2001) and pharmacological silencing (Levy 2001), leading to marked improvement in motor function. More recently, these approaches and observations have prompted gene therapy trials to deliver therapeutic vectors into the striatum itself and these will be discussed later. Typically, gene therapy approaches have focused on the restoration or preservation of dopaminergic cell function within the striatum either with neurotrophic factors (Kordower 2000, Marks 2010) or the delivery of enzymes needed for dopamine synthesis (Eberling 2008, Azzouz 2002).

2. Gene delivery to the brain

Gene therapy as a therapeutic approach to central nervous system (CNS) disorders has been theoretically possible for over a decade now, and several recent technical advances have made this avenue increasingly attractive. The method of delivery of the synthetic nucleic acid will fall into one of two groups – nonviral and viral vectors. Nonviral vectors are usually forms of chemically synthesized particles, such as cationic lipids, mixed with recombinant DNA, typically delivered by direct injections. This method is technically relatively straightforward, but usually only produces transient gene expression. Viral vectors, in contrast, are derived from DNA or RNA viruses and can potentially lead to sustained gene expression through genomic integration or the formation of episomes. The past five years have seen major advances in the range of available viral vectors, with individual vector systems having specific advantages and disadvantages for the delivery of therapeutic genes to the CNS. The properties of individual classes of vector are presented here, with a particular focus on adeno-associated virus (AAV), although adenovirus, herpes simplex and lentivirus systems are discussed. With the exception of lentiviruses, retroviral vectors (typically based on murine leukaemia viruses) are unable to infect the post-mitotic cells of the CNS, and are not considered in this review. An additional consideration relates to the method of vector delivery – peripheral systemic injection is clearly not appropriate unless the vector can cross the blood brain barrier with some degree of tissue specificity. Fortunately, deep brain surgical approaches are well developed in the field of movement disorders, making targeted viral injection relatively straightforward.

2.1 Adeno-associated viruses

AAVs are simple, 4.7 kb single stranded DNA viruses (Srivastava 1983) of the *Parvoviridae* family and *Dependovirus* genus. Two genes (*cap* and *rep*) coding for capsid and viral replication proteins respectively, are flanked by inverted terminal repeats (ITRs). Additional genes are required for replication, potentially derived from either adenovirus or herpes simplex viruses (Atchison 1965, Hoggan 1966, Buller 1981). The AAV serotype 2 (AAV2) was the first to be sequenced, and is the most widely used AAV-based gene therapy vector. Subsequent research has isolated at least 100 AAV variants, with many having different tissue tropisms and apparent transduction efficiencies (Gao 2004). AAV usually persists as monomeric or concatameric episomes (Schnepp 2003), although viral integration can occur, at a defined site on human chromosome 19 (Muzyczka 1992), AAVs are particularly attractive as candidate vectors for gene therapy as they have a high theoretical level of safety (Monahan 2002, Tenenbaum 2003), as wild-type AAV is already replication defective and the virus has not been associated with any known human disease. The weak promoter activity of the terminal repeat sequence reduces the overall risk of insertional mutagenesis and oncogenic activation, so genomic integration is not likely to pose a major risk (McCarty 2004).

Early approaches to the production of recombinant AAVs involved the packing of foreign DNA into viral coats by infecting cells with wild-type AAV and helper adenovirus. This process left residual wild-type viruses contaminating preparations of recombinant AAV, with obvious problems for *in vivo* applications (Hermonat 1984). More recent systems use a two or three plasmid system (Samulski 1989), in which cells are co-transfected with a plasmid construct coding for the synthetic gene of interest flanked by the 125 base pair viral inverted terminal repeats (ITRs), in combination with further plasmids coding for *cap*, *rep* and appropriate adenoviral helper genes. This leads to the production of recombinant virus

without the presence of contaminants, further enhancing safety. These advantages mean that AAV has been the predominant vector used in clinical trials.

Some disadvantages with using AAV include the relatively small size of the virus which limits the size of the insert to a maximum of approximately 4 kilobases of foreign DNA. Pre-existing humoral immunity to AAV is found in 80% of the human population, which may be enhanced after vector administration, potentially limiting transgene expression (Peden 2004, Sanftner 2004). The significance of this phenomenon is not clear for CNS based therapies, a site of relative immune privilege, but should prompt further studies into the significance and standardization of neutralising antibody titres during clinical trials.

2.2 Adenovirus

The earlier generations of adenoviral constructs were generally based around the Ad serotype 5 and containing E1 and/or E3 gene region deletions. These found early uses for *in vitro* work, but were associated with significant *in vivo* host immune responses and associated toxicity, with cell death leading to transient transgene expression. The deletion of further viral genes in more recent third generation ("gutless") vectors has reduced these problems significantly (Schiedner 1998), producing long-term gene expression. This class of viruses has several technical advantages, including relative ease in the production of high-titer stocks and generally strong gene expression (Verma 2005). Broader use of this class of vectors is still hampered by immune reactions induced by viral capsid proteins, which are likely to remain a persistent issue (Kafri 1998).

2.3 Herpes simplex virus

Herpes simplex virus has several obvious advantages for CNS delivery, including a large genome size, with resultant high packaging capacity, neurotropism and long lived episomal latency. The virus genome consists of 150 kb of double-stranded DNA, encoding more than 80 genes. Two broad classes of vector systems have been derived – amplicons and recombinant vectors. Amplicon vectors contain only *cis*-acting sequences (*ori* and *pac*) and require a packaging system, usually supplied in *trans*, from a *pac* deficient cosmid encoded HSV-1 genome (Cunningham 1993). Vectors with specific deletions in the infected cell polypeptide (ICP)-0 (ICP0), ICP4, ICP22 and ICP47 intermediate early genes retain long lived persistence, apparently without significant cellular toxicity (Samaniego 1998). Some safety concerns remain, however, but this class of vectors looks particularly promising when the delivery of a large DNA construct is required (Lachmann 1999). The use of specific promoters, such as the tyrosine hydroxylase promoter, increases transduction specificity (Cao 2008).

2.4 Lentivirus

Lentiviral vectors are usually considered separately from other retroviruses, as they can efficiently infect both dividing and non-dividing cells, potentially leading to long-term gene expression following chromosomal integration. Most lentiviruses are based on the human immunodeficiency virus (HIV) (Vigna 2000), with transgenes incorporated between elements known as long terminal repeats (LTR), sequences required for host genome integration. The *env* gene product is typically substituted for sequences from other RNA viruses (frequently the vesicular stomatitis virus glycoprotein VSV-G) to impart a wide cellular tropism, including neurons (Naldini 1996). Further specificity can be given through the use of the human glial fibrillary acidic promoter (hGFAP) or neuron-specific enolase

promoter (rNSE), giving glial or neuronal specificities respectively (Jakobsson 2006). This class of vectors certain several advantages, including a relatively large capacity for cloned genes (approximately nine kilobases) (Zhao 2007), but concerns relate to the possibility of recombination events, producing replication-competent virus. The use of two or three plasmid based transfection systems, in which the capsid assembly genes are genetically isolated has increased the safety profile of this class of vectors (Zufferey 1997) and this class looks particularly promising for future studies.

3. Clinical trials with gene therapy vectors

Gene therapy vectors have been used in clinical trials for patients since 1990, with most experience using retroviral vectors (Verma 1997). Initial enthusiasm was tempered, however, following the death of a patient receiving an adenoviral vector as replacement for the enzyme defect ornithine transcarbamylase deficiency (Somia 2000). The patient death - from systemic inflammation and multiorgan failure - led to a temporary suspension of trials in 1999. Since then, technical advances and tighter regulatory frameworks have led to an increase in registered clinical trials using gene therapy and particularly using AAV. Prompted by favourable results in preclinical studies using animals ranging from mice to nonhuman primates, over 40 clinical trials using AAV as a therapeutic vector are currently registered with the US Food and Drug Administration (Mueller 2008).

Early trials, such as the Phase I trial of AAV encoded human factor IX in patients with haemophilia B provided useful safety information (Kay 2000). Following intramuscular vector injection, small but detectable levels of secreted factor IX were produced. No toxicity or chromosomal integration was seen during the time of the study. Unfortunately, cell mediated immunity to AAV was observed, which led to hepatocyte damage and eventual loss of therapeutic gene expression (Manno 2006). Phase I and II studies examined outcomes following intranasal or endobronchial administration of AAV encoded cystic fibrosis transmembrane conductance regulator. Antibody responses to AAV were observed, with limited biological effects. The safety profile appeared good, with no adverse events seen in the 120 patients treated (Mueller 2008).

In addition to the trials for PD there are several other ongoing clinical trials examining AAV mediated gene therapy to the CNS. These include ocular delivery of the RPE65 gene in Leber's congenital amaurosis, with no reported toxicity in a small number of treated patients despite a transient rise in neutralising antibodies (Simonelli 2009). Also in progress is a phase II trial using AAV to deliver nerve growth factor in patients with Alzheimer's Disease (Mandel 2010). A recent phase I trial of AAV mediated delivery of aspartoacylase for Canavan disease reported good safety, with detectable antibodies to AAV2 observed in the serum of a minority of patients. In this case, the vector was infused intracranially via burr holes, which may explain the absence of neutralising antibodies in the cerebrospinal fluid of patients and lack of CNS inflammation (McPhee 2006).

4. Gene therapy for Parkinson's disease

Clearly, several important issues need to be addressed when attempting therapeutic cellular transduction (gene delivery and expression) for Parkinson's disease. Most obvious is deciding on the therapeutic target - the pathological process underlying PD is widespread and involves multiple brain structures and their relevant cell signaling pathways. Great care

needs to be taken with the specific design of the therapeutic gene, including relevant promoters, particularly if the construct is to be constitutively active.

The simplest approach is to restore dopaminergic levels in the basal ganglia, usually through the introduction of genes coding for enzymes important in dopamine production (Azzouz 2002, Hadaczek 2010) or relevant cell signaling proteins (Kaplitt 1994). One early series of experiments examined AAV mediated gene transfer into the CNS, with injection of a virus coding for tyrosine hydroxylase into 6-hydroxydopamine-lesioned rats, finding sustained transgene expression, with no cytopathic effects and no reactive gliosis (Kaplitt 1994). Other strategies aim to slow dopaminergic cell death, usually through the localized production of trophic factors such as brain-derived neurotrophic factor (BDNF) (Hyman 1991, Klein 1999), glial cell line-derived neurotrophic factor (GDNF) (Kordower 2000, Bjorklund 2000) or neurturin (Marks 2008) to promote cell survival and function. Finally, an alternative strategy is targeted at the abnormal activities of the basal ganglia, particularly the subthalamic nucleus and the internal and external segments of the globus pallidus.

4.1 Glutamate decarboxylase

It has been consistently observed that PD is associated with decreased inhibitory activity of the nigrostriatal projections, resulting in overactivity of the subthalamic nucleus and overinhibition of the thalamus. It was therefore hypothesized that, by increasing levels of locally produced γ -aminobutyric acid (GABA) in the subthalamic nucleus, these pathways could be restored to equilibrium and improve patient function. The synthetic pathway for GABA involves the catalytic action of glutamate decarboxylase (GAD) on glutamate, an enzyme found as two genetically distinct isoforms - GAD65 and GAD67 (Erlander 1991, Bu 1992). These have differing enzymatic properties, functional requirements and intracellular distributions. The first experimental study used recombinant AAV (rAAV) encoding the GAD65 and GAD67 isoforms of glutamic acid decarboxylase, with function initially characterized *in vitro*. Two AAV2 based constructs, AAV/rGAD65 and AAV/rGAD67 were able to productively infect cell lines, with both genes transcribed, leading to the production of enzymatically active GAD65 and GAD67 (Mi 1999).

The cDNAs were then used in a series of *in vivo* experiments, in which GAD65 or GAD67 were produced by rAAV with bicistronically encoded green fluorescent protein (GFP). Subsequent stereotactic injection of either vector into the STN resulted in prolonged transgene expression (monitored up to five months), with no inflammatory response seen. The cellular distribution of GAD was as expected for each isoform - with membrane restricted GAD65 and cytosolic GAD67. Stimulating electrodes were inserted into the STN, with microdialysis probes inserted into the SNr finding significantly increased release of GABA following STN stimulation in the GAD65 gene treated rats (Luo 2002). Parkinsonian 6-hydroxydopamine (6-OHDA)-lesioned rats were then injected with the viral vectors, leading to a four-fold increase in GABA release following STN stimulation. This was associated with a marked increase in the ratio of inhibitory to excitatory SNr responses. Importantly, controls (injected with GFP or saline) had unchanged responses, indicating that these results were not primarily due to local lesioning effects. GAD67 treated rats, in contrast, had a predominantly excitatory response. Additional effects and outcomes were examined, in particular potential neuroprotective effects of GAD gene administration. By pre-treating with the GAD65 containing construct prior to 6-OHDA lesioning, several

functional outcomes (limb use and apomorphine-induced rotations) were significantly improved as compared to control infusions. A corresponding increased survival of tyrosine-hydroxylase positive cells was also seen in the GAD65 treated group, again suggesting a neuroprotective effect.

Subsequent studies examined the properties of the vector when injected in hemiparkinsonian macaques (Emborg 2007). In this model, MPTP is injected into the carotid artery, with subsequent injections of AAV-GAD or GFP control into the ipsilateral STN. Over the course of a 56 week period, 13 macaques (seven on active treatment, six control) were monitored, finding sustained transgene expression, improvement in clinical parameters (bradykinesia, tremor, motor skills) in GAD-treated animals and increased ipsilateral ¹⁸F-fluorodeoxyglucose (FDG) PET motor cortical glucose activity. All animals survived to the 1 year end point without significant adverse events.

Prompted by these promising results, an open label phase I trial of unilateral subthalamic viral vector injection, using an AAV2 serotype encoding human GAD65 or GAD67 under the control of a CMV promoter was performed (Kaplitt 2007). Patients recruited for the trial - the first use of gene therapy for an adult neurodegenerative disorder - were reasonably typical for idiopathic Parkinson's disease, with a disease duration ranging from 6 to 13 years. Exclusion criteria included significant cognitive or psychiatric illness.

The protocol involved a stereotactic frame and MRI guided STN injection of a 50 µl solution of a 1 (low dose) to 10 (high dose) $\times 10^{11}$ genomes/ml solution. Unilateral injections were performed into the most symptomatic hemisphere, leaving the contralateral side untreated. Several outcomes were examined, including safety, tolerability, Parkinson's disease symptoms as rated by the Unified Parkinson's Disease Rating Scale (UPDRS), and ¹⁸F-fluorodeoxyglucose (FDG) PET imaging was performed in a blinded manner, at baseline and 12 months post surgery. The procedure was well tolerated, with no deaths or unexpected neurological complications during the study period. Post procedure assessments found an improvement in the UPDRS motor scores from 3 months, sustained through to 12 months post surgery. The change was located primarily to the body side contralateral to the procedure. PET imaging found a significant decline in thalamic metabolism, ipsilateral to the injection. No changes in anti-AAV and GAD65/67 antibodies were seen after surgery (Kaplitt 2007).

Although the study was small and not blinded, the procedure appeared safe and clinical outcomes were encouraging. Some definite benefits over traditional deep brain stimulation procedures include the lack of implanted hardware and theoretical benefits could include a more physiological approach to restoring motor network function through activity dependent GABA release. Findings suggest that GABA release may be subject to autoregulatory pathways involving GABA_A receptors of the STN. The full conclusions of the Phase II trial were published recently (LeWitt 2011), confirming a significant improvement in the UPDRS score six months post procedure for the AAV-GAD treated group (decreasing by 8.1 points $p < 0.0001$), although sham treated patients also improved (decreasing by 4.7 points, $p < 0.003$). The improvement seen in AAV-GAD treated subjects was significantly greater than in sham surgery subjects ($p = 0.04$). No additional safety issues were observed. This important trial highlighted not just the importance of including significant patient numbers (22 and 23 in the treatment and control groups respectively) to provide a significant study difference, but also the need for meticulous study design, not least in the placebo group (which included a detailed sham surgery protocol). Several important questions and issues remain to be answered, in particular (i) whether the beneficial effects will be maintained in the medium to long-

term; (ii) whether there will be longer term side effects of this therapeutic approach, particularly important in a gene therapy vector in which the agent is constitutively active and; (iii) how this approach compares to more traditional 'advanced' therapeutic options, such as deep brain surgery (DBS).

4.2 Glial cell line-derived neurotrophic factor

Glial cell line-derived neurotrophic factor (GDNF) was characterized as a selective neurotrophic factor for dopaminergic neurons from its ability to increase dopamine uptake in midbrain cultures without effect on serotonin or gamma amino butyric acid (GABA) uptake (Lin 1993). In addition, GDNF promoted dopaminergic neuron survival, dopamine uptake, cell body size and neurite outgrowth (Lin 1993), and was therefore identified a potential therapeutic agent in PD.

Early experiments in which GDNF was stereotactically injected into MPTP treated mice (i.e. remove comma after mice) found that injection of GDNF into the striatum prior to MPTP treatment was associated with preservation of dopamine levels in the substantia nigra and striatum and preserved striatal TH immunoreactive cells. Treatment with GDNF at any time point was shown to improve the motor function as assessed by locomotion, motility and rearing compared to controls (Tomac 1995).

Initial approaches to delivering sustained in vivo GDNF used a replication deficient adenovirus containing human GDNF in the rat 6-hydroxydopamine (6-OHDA) model (Choi-Lundberg 1997). Adenovirus GDNF significantly increased substantia nigra dopaminergic cell survival compared to controls, although protein and mRNA levels were not sustained and all animals had host reactions around the needle site. Subsequent experiments used stereotactically injected GDNF encoding lentivirus in MPTP treated rhesus monkeys (Kordower 2000). Again, lentivirally injected substantia nigra displayed increased levels of TH immunoreactive neurons. In a follow-up series of experiments, animals displayed improved functional scores, with a corresponding improvement on FDG PET scanning. Importantly, and in contrast to adenoviral approaches, lentivirally encoded GDNF transgene expression was sustained for up to eight months and inflammatory responses were minimal. Possible idiosyncratic reactions were hinted at, but not detailed, which is an important omission as, for example, cerebellar toxicity has been mentioned as a limiting factor in some cases (Berry 2010). However, this approach shows promise, prompting Amsterdam Molecular Therapeutics to obtain a license to use the GDNF gene delivered via an adeno-associated virus platform.

Future trials of GDNF gene therapy will need to be performed with scrupulous attention to control subjects. Fortunately, there already exists considerable experience using continuous putaminal infusions of recombinant GDNF. Initial open-label trials were encouraging (Gill 2003, Patel 2005), finding significant improvements in UPDRS III OFF and ON scores and statistically improved FDG uptake. It was observed, however, in a separate study that the initial benefits seen at one year had returned to baseline following a one year period of treatment withdrawal (Slevin 2007). Unfortunately, the subsequent randomized control double-blinded phase I/II trial (Lang 2006) did not support the open label findings, finding only improved FDG PET appearances at 6 months compared to baseline and improved mental health as measured by the SF-36 scoring system (frequently used to rate quality of life parameters) in the actively treated group. The reasons for the differences between the results are not clear, but highlight the critical importance of study design and the use of double blinded placebo subjects, including sham operative procedures.

4.3 Neurturin

Neurturin (NTN) was first identified 15 years ago (Creedon 1997), finding sequence homology to GDNF, with similar *in vitro* neuroprotective properties. These were confirmed by subsequent *in vivo* experiments, promoting TH positive neuronal survival when injected into the substantia nigra of 6-OHDA treated animals (Horger 1998), with improvements in functional parameters. Stereotactic injection of an NTN encoding AAV vector into the caudate nucleus, putamen and substantia nigra of MPTP treated monkeys found similar benefits (Kordower 2006), with no significant side effects at three months and one year (Herzog 2008, Herzog 2009).

These promising findings led to a Phase I, open-label trial of AAV2-NTN in 2008 (Marks 2008), involving 12 patients (ages 35 to 75) with moderate to severe levodopa responsive PD. In this cohort the diagnosis was established for a minimum of 5 years, patients were on stable doses of antiparkinsonian medications, but without good control with at least 3 hours of "off" time per day. The participants were divided between low and high treatment groups and received stereotactic guided injections bilaterally throughout the putamen. Participants experienced a significant improvement in UPDRS Part III motor score in the practically defined "off" period as compared to baseline, on average an improvement of 36%. Other than a presumed air embolus, which did not lead to complications, there were no clinically important operative adverse events.

This was followed up by the first double blind randomised Phase 2 trial of gene therapy for Parkinson's disease, published in late 2010 (Marks 2010). The 58 participants met similar eligibility criteria as the open-label study, with groups were divided 2:1 to receive either AAV-NTN or sham surgery respectively, with assessments at baseline and 1, 3, 6, 9 and 12 months and every 3 months thereafter until the final patient had been seen at 12 months. At each visit the patients were assessed with the UPDRS in the practically defined "off" state and the best "on" state. Home diary and quality of life questionnaires were also employed. The primary outcome - UPDRS Part III score in the "off" state was not significantly improved at 12 months. A range of secondary outcomes, including the mental score (Part I) in the "off" state and activities of daily living score (Part II) in the "on" state were significantly improved.

For the small number of patients followed up until 18 months the UPDRS Part III "off" score was significantly improved. Histological analysis of two patients' brains in the treatment group revealed only limited expression of the neurturin protein in the putamen and even more modest expression in the substantia nigra. Three patients in the active treatment group developed tumours: one glioblastoma, one adenocarcinoma of the prostate and one oesophageal adenocarcinoma. Quantitative PCR of biopsied tissue was negative for AAV-NTN and retrospective reanalysis of pre-procedure MRI suggested that the glioblastoma predated the intervention. There were two deaths in the treatment group one from myocardial infarction and one from a pulmonary embolus. Headache, nausea, post-procedural pain, dyskinesia, insomnia and worsening of PD were the most commonly reported adverse events and occurred more frequently in the active treatment compared to sham surgery groups. Serious adverse events secondary to surgery occurred in both groups without subsequent lasting neurological sequelae. Neurturin protein and antibodies were not detected in patient sera.

Although the trial failed to show a significant difference in the primary endpoint, the data gathered from the study was generally informative. The reasons underlying the therapeutic failure are not clear, potentially relating to inadequate levels of neurturin at the site of pathology. Planned future work, currently recruiting, will also include direct injection into the substantia nigra as well as increased dosing (Clinical trials.gov identifier NCT00985517).

4.4 Aromatic-L-amino decarboxylase (AADC)

Production of dopamine from either endogenous or exogenous levodopa is dependent on aromatic-L-amino decarboxylase (AADC). As PD progresses, patients typically require increasing doses of L-dopa and are therefore at increased risk of medication induced side effects. It is postulated that AADC activity is depleted in PD and that therapeutic restoration of this activity may lead to clinical improvement and allow reduced doses of levodopa (Bankiewicz 2000).

In a series of *in vivo* experiments using MPTP-induced hemiparkinsonian rhesus monkeys, AAV-AADC was injected throughout the caudate and putamen (Bankiewicz 2000). This led to increased *in vivo* AADC tracer activity and immunohistochemical staining, with partial restoration of the ability to convert levodopa to dopamine in an *ex vivo* assay. Improvement on a range of functional scores was seen at 24 months and a subsequent imaging experiment found increased AADC levels up to 72 months (Bankiewicz 2006).

The work prompted an ongoing clinical trial in which five patients, with levodopa responsive Parkinson's disease and intractable motor fluctuations despite optimised medical therapy (Hoehn and Yahr stage III-IV), received bilateral putaminal infusions of 9×10^{10} vector genomes by stereotactic infusion. No adverse events were reported that were attributable to AAV-AADC infusions. FDG PET uptake was significantly increased at 6 months compared to baseline. The absence of controls in this safety study makes secondary clinical outcome interpretation difficult, although there were significant increases in 6 month total UPDRS scores on and off medication and 3 participants were able to take lower doses of levodopa (Eberling 2008).

This cohort was then compared with 5 patients who received a higher dose (3×10^{11} vector genomes) of AAV-AADC (Christine 2009). Of concern, three patients had intracranial haemorrhages, and four patients developed a transient increase in dyskinesias. In both cohorts the total UPDRS and UPDRS III scores improved at 6 months when assessed "off" medication, but no significant improvements were seen "on" medication in the UPDRS III score. This is odd, as the effect of AAV-AADC is thought to be dependent on levodopa therapy, although all patients in the high dose group were able to manage on lower doses of levodopa, hinting at efficacy. The study is ongoing and is expected to be completed in 2013 at which point 60 months of efficacy and safety data will be available.

4.5 Prosavin

Parkinson's disease potentially results from deficiencies in several steps of the dopaminergic synthetic pathway in which L-tyrosine is converted to levodopa by the enzyme tyrosine hydroxylase (TH). GTP cyclohydrolase 1 (CH1) is the rate-limiting enzyme for the generation of tetrahydrobiopterin which is a co-factor for TH. Levodopa is then converted to the biologically active dopamine by AADC. In an attempt to reconstitute these steps, genes coding for TH, CH1 and AADC were combined into a single lentiviral vector for administration to the striatum (Azzouz 2002).

Functional improvements were seen in stereotactically injected 6-OHDA lesioned rats, despite an apparently relatively modest increase in dopamine levels (Azzouz 2002). Subsequent *in vivo* microdialysis measurement in the MPTP macaque model demonstrated a greater increase in extracellular levels, so the therapeutic efficacy may have been greater than first thought (Jarraya 2009). Treated macaques exhibited restoration of the firing rate and pattern of neurons within the basal ganglia and reduced metabolic activity within the subthalamic nucleus, coupled with functional improvements. No safety issues were noted.

Although peer reviewed data has not yet been published, preliminary results of an ongoing Phase I/II clinical trial of Prosavin have been announced. Nine patients have now received Prosavin in three cohorts of 1x dose, 2x dose and 2x dose with an improved delivery method. The first cohort has had a 20% functional motor improvement at 24 months and the second cohort a 29% improvement at 12 months. The third cohort has had a 26% improvement at 3 months. All cohorts have had improved "ON" time, stable or improved quality of life assessments and stable or reduced levodopa dosing. Whilst these are preliminary announcements the results are encouraging and have prompted the initiation of a 5x dose cohort commencing early in 2011.

5. Conclusions

Despite decades of research, Parkinson's disease is a chronic progressive neurodegenerative condition of unknown aetiology and the underlying pathogenesis remains unclear. Despite understandable reservations about using a gene therapy approach for the condition, several Phase I and II clinical trials have now reported their clinical findings, providing a wealth of experience and data. The use of adeno-associated vectors for gene therapy, with trials including several hundred patients, appears to be generally safe, with little procedure related morbidity.

Although there are definite concerns relating to the development of immunity to systemically administered AAV, leading to destruction of transduced tissues, this phenomenon has not been observed with CNS administration, suggesting a degree of immune privilege which may lead to more sustained therapeutic effects. Ongoing concerns relate primarily to the specific choice of therapeutic agent, site of action and levels of production. The use of non selective (particularly constitutively 'on') mammalian promoters such as CMV, do not permit adjustment of therapeutic effects. Current models suggest that autoregulation may occur through homeostatic feedback pathways, but this has not been demonstrated formally and long term effects on cellular phenotypes are unknown.

Ongoing monitoring of patients recruited into Phase I and II studies will be essential to establish whether initially observed benefits are sustained and to look for long term complications. Further randomized double blinded studies, with appropriate longitudinal follow-up will be necessary to properly evaluate the therapeutic effectiveness of this novel class of agents.

Further technological developments are likely, particularly involving the use of more sophisticated AAV-based viral vectors. These could use more neuron-specific promoters (eg neuron specific enolase or synapsin 1 gene promoters) and regulate protein production through the use of inducible systems (eg ecdysone-based) to control therapeutic activity in a more directed fashion. Although still in its infancy, this technology still shows great promise as a novel therapeutic approach for this devastating disease.

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Towards New Therapies for Parkinson's Disease

Edited by Prof. David Finkelstein

ISBN 978-953-307-463-4

Hard cover, 396 pages

Publisher InTech

Published online 02, November, 2011

Published in print edition November, 2011

Parkinson's disease (PD) is characterised clinically by various non-motor and progressive motor symptoms, pathologically by loss of dopamine producing cells and intraneuronal cytoplasmic inclusions composed primarily of α -synuclein. By the time a patient first presents with symptoms of Parkinson's disease at the clinic, a significant proportion of the cells in the substantia nigra have already been destroyed. This degeneration progresses despite the current therapies until the cell loss is so great that the quality of normal life is compromised. The dopamine precursor levodopa is the most valuable drug currently available for the treatment of PD. However for most PD patients, the optimal clinical benefit from levodopa decreases around five to six years of treatment. The aim of the chapters of this book is to work towards an understanding in the mechanisms of degeneration and to develop disease modifying therapies.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Michael Douglas and Jonathan Hazlehurst (2011). Gene Therapy for Parkinson's Disease, Towards New Therapies for Parkinson's Disease, Prof. David Finkelstein (Ed.), ISBN: 978-953-307-463-4, InTech, Available from: <http://www.intechopen.com/books/towards-new-therapies-for-parkinson-s-disease/gene-therapy-for-parkinson-s-disease>

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