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CONSIDERATION OF GENE THERAPY FOR PEDIATRIC NEUROTRANSMITTER DISEASES

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Abstract

The Pediatric Neurotransmitter Diseases (PND) are a group of inborn errors of metabolism characterized by abnormalities of neurotransmitter synthesis or metabolism. Although some children may react favorably to neurotransmitter augmentation treatment, optimal response is not universal and other modes of treatment should be sought. The genes involved in many of the currently known monoamine PNDs have been utilized in pre-clinical and in phase I clinical trials in Parkinson's disease (PD) and the basic principles could be applied to the therapy of PNDs with some modifications regarding the targeting and distribution of vectors. However, issues that go beyond neurotransmitter replacement are important considerations in PD and even more so in PND. Understanding the pathophysiology of PNDs including abnormal development resulting from the neurotransmitter deficiency will be critical for rational therapeutic approaches. Better animal models of PNDs are necessary to test gene therapy before clinical trials can be attempted.

1. Introduction

The Pediatric Neurotransmitter Diseases (PND) are a group of inborn errors of metabolism characterized by abnormalities of neurotransmitter synthesis or metabolism (1) (Pons, this issue). The inborn molecular genetic abnormalities result in a deficiency of the enzymatic metabolic pathways important to the synthesis or degradation of the biogenic amine neurotransmitters dopamine, serotonin, and/or noradrenaline. These enzymes include tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase (AADC) and other enzymes of the tetrahydrobiopterin (BH₄) cofactor pathway including guanine triphosphate cyclohydrolase I (GTPCH-I), 6-pyruvoyl-tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR) (Figure 1). The phenotypes in these diseases are variable, from a fairly isolated motor dysfunction that can be well controlled by L-3, 4-dihydroxyphenylalanine (L-DOPA) to a severe neurodevelopmental encephalopathy attributable to the wide-spread central nervous system involvement as well as autonomic dysfunction resulting from catecholamine deficiency (1) (Pons, this issue). Treatment of these diseases includes neurotransmitter augmentation by using dopamine and serotonin precursors and agonists as

well as inhibiting degradation of monoamines with catechol-O-methyl transferase and monoamine oxidase inhibitors. Although some children may react favorably to treatment, optimal response is not universal and other modes of treatment should be sought.

Gene therapy is a term used to describe the genetic modification of cells either *in vitro*, with the subsequent implantation of these cells into a recipient (termed *ex vivo* gene therapy) or *in vivo*, by delivering genes directly to endogenous cells and intervening directly with endogenous intracellular processes (2). Possible applications of gene therapy include the expression of molecules that substitute for defective or missing proteins, as seen in inborn errors of metabolism.

Gene therapy has been extensively studied over the last two decades for its potential to provide novel treatments for otherwise incurable diseases such as inborn errors of metabolism (3). The possible clinical applications of gene therapy for inborn errors of metabolism continue to proliferate, with our understanding of the genetics and molecular pathogenesis of these diseases. To date over 30 metabolic diseases have been investigated in pre-clinical trials with promising results, and some have reached the clinical trial phase including Canavan disease (4) and late infantile neuronal ceroid lipofuscinosis (5) targeting the central nervous system of children.

It would be safe to say that the monoamine PNDs could be one of the most extensively researched inborn errors of metabolism in regard to the possibility of gene therapy application. This has happened inadvertently, as among central nervous system disorders, gene therapy research has been most actively applied to Parkinson's disease (PD) (6–8). PD is a neurodegenerative disorder in which dopaminergic nigrostriatal neurons undergo degeneration, resulting in a dopamine deficient state. Research to replenish this neurotransmitter deficiency as well as secondary biogenic amine abnormalities has focused on the various monoamine biosynthetic pathways that are involved directly in the pathogenesis of PNDs, and have resulted in exciting initial reports. The basic principles from PD gene therapy could be applied to the therapy of various PNDs, but these will have to be modified according to the pathogenesis and clinical setting presenting in each PND. In this report, we will review current gene therapy status in the field of PD, while considering implications that these results have for possible treatment of PNDs. We will limit our review to PNDs involving monoamine system and other PNDs such as succinic semialdehyde dehydrogenase deficiency are covered in other chapters in this issue.

2. Gene therapy in PD

PD results in a relatively selective degeneration of the nigrostriatal pathway, comprising of dopaminergic neurons. This results in a deficiency of dopamine in the striatum, which is responsible for most, but not all, of the motor symptoms such as rest tremor, bradykinesia, rigidity of muscle tones, and gait and balance problems. Although the pharmacological replacement of dopamine is initially effective, significant medication-induced side effects develop over time (9). These include motor symptom fluctuations such as dyskinesias and wearing-off phenomena as well as non-motor symptoms such as cognitive changes including hallucinations. Many of the motor side effects are related to the progressive loss of

dopaminergic neurons with disease progression and chronic nonphysiological dopaminergic therapy. In addition, neurodegeneration of PD involves more than nigrostriatal dopamine neurons and the involvement of other systems results, consequently, in non-motor symptoms. There are three basic approaches of PD therapy including gene therapy: replacement of dopamine, compensation for abnormal circuits, and disease modification by intervening pathophysiology.

Current treatment strategies for advanced PD focus on these fluctuations, trying to deliver continuous and localized dopaminergic stimulation. Continuous dopaminergic stimulation is being addressed by various pharmacological preparations that release slowly. The localized delivery has been tried by transplantation of dopamine-producing cells. The first clinical trials of this approach for PD utilized embryonic human nigral neurons taken at a stage of development when they have started to express their dopaminergic phenotype and transplanted into the striatum, a strategy used to replace the nigral dopaminergic neurons that are lost in the disease (10). Two multicenter, NIH-funded, double-blind, controlled trials were initiated a decade ago and showed disappointing results. The functional improvements were not significantly better than medications and moreover off-phase dyskinesias were observed in a significant proportion of the transplanted patients (10–12). Recent reports have further provided insights in the biology of grafted neurons in the striatum. They have shown that subjects with PD who had 11–16 years of long-term survival of transplanted fetal dopaminergic neurons developed the pathologic hallmark of PD, alpha-synuclein-positive Lewy bodies in grafted neurons, indicating that the disease is an ongoing process that can propagate from the host to graft cells in the striatum in a similar way to dopamine neurons in the substantia nigra (13, 14). This raises a possibility that therapy that does not address fundamental pathophysiology may be very limited.

Gene therapy using *ex vivo* approach attempts to produce alternative sources of implantable cells that provide dopamine by genetic modification. Such genetic modification can be combined with stem cells to augment their phenotype to a more desirable one. More recently *in vivo* gene therapy approaches have been promising in terms of long-term sustained gene expression and safety. Viral vectors are the predominant vehicles used for gene delivery in mammalian systems. Vectors that are useful for gene delivery to the central nervous system (CNS) include adenovirus, adeno-associated virus (AAV), herpes simplex viruses (HSV) and lentiviruses (LV) (15). Adeno-associated viruses are benign viruses that are capable of transfecting CNS cells with great efficiency (16). They have been shown to have a highly efficient transduction of rodent neurons in focal regions following direct interstitial injection of AAV with different serotypes (17, 18), although the rate of transduction may not be the same in primates (19). The immune response to brain directed AAV appears to be minimal (20).

Preclinical trials involving viral mediated gene transfer have been promising using various target enzymes (6). Dopaminergic nerve terminals in the striatum produce dopamine from two sequential enzymatic steps involving TH and AADC. GTPCH is essential to produce tetrahydrobiopterin, which is an absolute requirement for TH activity (21). This prompted the evaluation of replacement gene therapy of these enzyme genes, either as single genes or in combination with others (2, 22). TH and GTPCH co-expression is essential for L-DOPA

delivery (21, 23). The utility of adding AADC to TH and GTPCH is controversial, some reporting a detrimental effect by feedback inhibition (24)). Use of AADC gene therapy alone to enhance the efficacy of L-DOPA conversion to dopamine is likewise controversial, some reporting no additional benefit in rodent models using genetic modified fibroblasts (24) and others reporting increase in dopamine production and phenotypic improvement both in 6-OHDA-lesioned rats (25, 26) and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-(MPTP)-lesioned monkeys (27) using AAV vector. In addition, the vesicular monoamine transporter (VMAT-2), which transports dopamine into the synaptic vesicles and is important for dopamine storage and release, has been used as an adjunctive gene therapy target (28). Attempts have been made to use co-expression of three or four genes (TH, AADC, GCH with or without VMAT-2) in order to achieve higher levels of dopamine production and regulation in striatal neurons using an LV vector. In the 6-OHDA-lesioned rat, sustained expression of each enzyme and effective production of dopamine were detected, as well as phenotypic improvement (29). The critical issues of dopamine replacement therapy are the ability to regulate the precise therapeutic levels of dopamine, which is not easily achievable with current gene therapy technologies and the limitation of dopamine targeted therapy for overall symptoms of PD that include many symptoms not associated with dopamine deficit.

The first Phase I clinical trial has been completed utilizing bilateral putamenal infusions of low dose AAV - AADC vector in five patients with PD. PET results showed an average 30% increase in fluorodopa uptake in the putamen as evidence of sustained gene expression after gene transfer, and preliminary analysis of clinical data indicates a modest improvement in this open label, unblinded study. This gene therapy approach has been well tolerated and initial findings demonstrate the safety of the therapy in low doses (30). AADC gene therapy has the advantage of being able to regulate the dopamine levels by adjusting L-DOPA administration and may increase dopamine conversion from exogenously administered L-DOPA, but is not likely to blunt the fluctuating levels of dopamine.

The second approach for PD therapy addresses abnormal circuitry that result from the primary dopamine deficit. Denervation and chronic therapy produce abnormalities in the pathways of the basal ganglia, downstream from the nigrostriatal dopaminergic system. Changes in the circuitry are likely to be contributing to the motor response fluctuations. Surgical ablative lesions or deep brain stimulation of various components of the basal ganglia can compensate for these changes (31). Alternatively, gene therapy of glutamic acid decarboxylase was employed to produce inhibitory neurotransmitter GABA in animal models (32) and in Phase 1 clinical trials (33).

The third approach is to address the neurodegenerative aspect of PD by transfer of various neurotrophic factors, including glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), found to enhance survival and function of dopaminergic neurons in animal models and now being assessed in phase I clinical trials (34, 35). Eventually one could also envision gene therapy that would directly intervene in the pathogenetic pathway of PD. Such approaches would potentially be most beneficial for PD by addressing dopamine and non-dopamine degeneration, but may have less direct relevance to PNDs.

3. Gene therapy for PND

PNDs are inborn errors of metabolism resulting from single gene mutations and thus are, in principle, good candidates for gene therapy using viral vector-mediated gene transfer. In contrast to the fairly localized nigrostriatal degeneration noted in PD, PNDs have a widespread network of monoamine deficiency. It may be necessary to deliver the gene to multiple central and peripheral sites in the case of TH deficiency, AADC deficiency and disorders of tetrahydrobiopterin metabolism. As a result, local viral injection may not be sufficiently effective and the issues of appropriate targets and levels of gene delivery are critical for the success of the therapy. Other issues result from the specific characteristics of the different PNDs. In TH deficiency, the treatment delivering L-DOPA directly to the dopaminergic neurons could bypass the defective enzymatic activity. Since L-DOPA is easily diffusible, TH gene therapy in relatively small areas of brain may be sufficient to restore catecholamine levels in wider areas of CNS. AADC deficiency poses a complex problem. The treatment target includes dopamine, norepinephrine, epinephrine, and serotonin delivery. These are not easily diffusible and the precursor administration will not be sufficient as the enzymatic deficiency precludes the utilization of precursors such as L-DOPA or 5-hydroxytryptophan. Therefore, this disease would require more direct delivery of neurotransmitters in multiple specific targets in the CNS. AADC gene would be delivered near target cells that have neurotransmitter receptors, for example, striatal delivery of AADC to produce dopamine locally, as done in PD. Given the high circulating levels of precursors and their properties to diffuse relatively easily to cells, AADC gene therapy may produce enough dopamine for therapeutic efficacy.

The BH₄ pathway deficiencies comprise of a group of diseases with variable phenotypes. Treatment for autosomal dominant GTPCH-I deficiency is very effective by administration of L-DOPA and would not require gene therapy. For disorders with more severe phenotypes such as autosomal recessive GTPCH-I and SR deficiency, gene therapy will need to be targeted into the affected neurons since BH₄ does not diffuse easily in and out of cells. PTPS deficiency would require both peripheral and central gene therapy. The peripheral gene therapy may be delivered into the liver.

As in PD gene therapy research, valid animal models suitable to address these delivery issues as well as to simulate the developmental aspects of pediatric neurotransmitter diseases are essential for pre-clinical gene therapy trials prior to initiation of human studies. Such models have been limited and developed only for some deficiencies with variable results. The complete transgenic TH knockout mouse dies at midgestation period, apparently of cardiovascular failure (36). TH knockout with tissue-specific rescue of TH expression in adrenergic but not in dopaminergic cells resulted in a dopamine-deficient mouse (37). If left untreated, it becomes hypoactive and aphagic a few weeks after birth, and dies from starvation. The clinical phenotype may imitate some aspect of human TH deficiency including parkinsonian motor symptoms and a good response to L-DOPA (37). The *hph-1* mouse is an animal model for GTPCH-I deficiency (38). Chemical mutagenesis affects a locus closely linked to the GCH gene and affects the stability of GTPCH-I mRNA (39). Biochemical validity of this model has been demonstrated with low brain levels of BH₄, monoamine metabolites and TH protein within the striatum (38). However, they have only

transient low GTPCH-I activity and hyperphenylalaninemia in the first 3 weeks after birth, and no significant phenotypic abnormality has been recorded. The $Pts^{-/-}$ mouse is a knockout model for PTPS deficiency (40). Unlike humans with this disease, the mice die perinatally with hyperphenylalaninemia and BH_4 deficiency and can survive if treated with BH_4 , L-DOPA and 5-hydroxytryptophan. Phenotypically, treated mice show severe dwarfism, hypotonia, hypersalivation, and temperature instability. Many models will have to be created for other PNDs. For example, a model for AADC deficiency has yet to be developed. A knockout mouse would be expected to be a recessive lethal phenotype, as the TH knockout, but a knockdown mouse may be a more valid model for the human condition.

Preliminary *in vitro* studies in gene therapy for various PND associated enzymatic deficiencies have been reported. Successful correction of BH_4 deficiency due to PTPS deficiency was achieved by using retrovirus mediated transfer of the PTPS cDNA into human fibroblast cultures established from PTPS patients (41). Comparable results were achieved with double transduction of human cDNAs for GTPCH-I and PTPS (42), and single transduction of GTPCH-I and PTPS cDNAs together with a selective marker coupled in a single transcript by the use of triple-cistronic, retroviral vectors (43), resulting in successful production and release of BH_4 by PTPS deficient human fibroblasts. Adenoviral GTPCH-I gene transfer increased BH_4 levels and NO production in rat (44) and human (45) endothelial cells. Only one *in vivo* gene therapy experiments was reported which injected a recombinant triple-cistronic AAV2 vector expressing PAH, GTPCH and PTPS into the muscles of *Pah(enu2)* transgenic mouse model for phenylketonuria (PKU). This therapy led to stable and long-term reduction of blood phenylalanine and reversal of PKU-associated coat hypopigmentation (46).

An important limitation of the above approaches is the developmental aspect of some of these diseases. In addition to their activity as neurotransmitters, these molecules also have an important role in the development of the normal brain. The serotonergic system is widely distributed in the mammalian brain, and is one of the earliest networks to develop in the embryo (47). The serotonergic system is critical for the migration and outgrowth of neurons during early embryonal stages. High levels of serotonin were detected within embryonal cells before and during early neurite outgrowth, raising the possibility that serotonin might serve a trophic role during development before assuming its adult functions as a neurotransmitter (48, 49). Dopamine may have a lesser role in embryogenesis, and dopamine depleted embryos are able to produce structurally normal nigrostriatal projection (37) yet dopamine may still play a role in normal maturation of striatal neurons (50). This would imply that a substantial portion of the changes noted with the severely affected children with dopamine or serotonin deficiency result from abnormal developmental processes *in utero*, that may be irreversible by gene therapy that replaces neurotransmitters in a later stage of life. The embryonal lethality of many of the knockout animal models for PNDs further underscores the developmental aspect of these diseases. Therefore we need to understand the pathophysiology of the disease and determine the proper timing of gene therapy intervention early enough to be able to forestall the developmental abnormalities. Alternatively, understanding abnormal circuitry that results from the developmental changes and therapeutic approaches to compensate for these changes may restore many clinical features.

Summary

PNDs are inborn errors of metabolism resulting from single gene mutations without signs of neurodegeneration, and thus, they may be good candidates for *in vivo* gene therapy to replace the missing neurotransmitters. The genes involved in many of the currently known monoamine PNDs have been utilized in pre-clinical and now in phase I clinical trials, and have shown promise in their ability to restore dopaminergic activity in specific areas of the CNS. Future research should be conducted into the specific issues regarding PND, such as targeting and distribution of vectors to various parts of the CNS. Furthermore, the challenge is to understand the pathophysiology of PNDs including potential developmental abnormalities so that neurotransmitter replacement therapy is initiated at a time that can forestall the developmental changes and other novel therapeutic approaches are designed to deal with the developmental abnormalities.

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reductase; BH₄ - tetrahydrobiopterin; qBH₂, quinonoid dihydrobiopterin; P4C - pterin-4a-carbinolamine; PCD - pterin-4a-carbinolamine dehydratase; DHPR- dihydropteridine reductase; PAH - phenylalanine hydroxylase; TH - tyrosine hydroxylase; 5-HTP - 5-hydroxytryptophan; TrH- tryptophan hydroxylase; AADC - aromatic L-amino acid decarboxylase; B6 - pyridoxal 5'-phosphate; 3-OMD - 3-O-methyldopa; VLA - vanillylactic acid; DβH - dopamine beta hydroxylase; DOPAC - dihydroxyphenylacetic acid; 3-MT - 3-methoxytyramine; COMT- catechol-O-methyl transferase; SAM - S-adenosylmethionine; MAO - monoamine oxidase; AldDH - aldehyde dehydrogenase; PNMT - phenyletanolamine N-methyltransferase; 5-HIAld – 5- hydroxyindole acetaldehyde; MHPG - 3-methoxy-4-hydroxyphenylglycol; DHMAL – 3,4- dihydroxymandelaldehyde; DHMA – 3,4- dihydroxymandelic acid; 5-HIAA - 5- hydroxyindole acetic acid; HVA - homovanillic acid; VMA - vanillylmandelic acid.