Available online at www.sciencedirect.com
ScienceDirect
Experimental Neurology 209 (2008) 72–81
Experimental Neurology
www.elsevier.com/locate/yexnr

Preclinical development of gene therapy for Parkinson's disease

Grégory Porras, Erwan Bezard *

CNRS UMR 5227, Universite Victor Segalen-Bordeaux 2, 33076 Bordeaux, France

Received 21 May 2007; revised 12 July 2007; accepted 7 August 2007
Available online 22 August 2007

Abstract

Multiple targets and pathways may be amenable to the development of gene therapy approaches for Parkinson's disease. This article discusses some of the cellular and brain circuit pathways relevant to Parkinson's disease that would be clinically amenable to gene therapy. Approaches could be classified according to two main categories, i.e. symptomatic vs. neuroprotective/neurorestorative strategies. Examples of the different possibilities currently in development are given and feature both dopaminergic and non-dopaminergic symptomatic treatments of parkinsonian symptoms and/or L-DOPA-induced side effects, anti-apoptotic neuroprotective strategies and growth-factor delivery for neuroprotection/neurorestoration. While gene therapy has been mostly used so far for enhancing the expression of the target gene, the use of dominant negative or siRNA opens new possibilities. This, combined with the key feature of gene delivery that offers access to intracellular signalling pathways, is likely to further expand the number of proposed targets to be studied.

Keywords: Dopamine transmission; Basal ganglia; Striatum; Subthalamic nucleus; Globus pallidus; Apoptosis; Growth factor

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by the death of dopaminergic neurons in the substantia nigra (SN) that provide dopamine input to the striatum (Ehringer and Hornykiewicz, 1960). Dopamine replacement with L-3,4-dihydroxyphenylalanine (L-DOPA) serves to increase available dopamine in the neurotransmitter-depleted denervated striatum (Fahn, 2006). Although effective in the early stages of PD, this symptomatic therapy loses efficacy over time due to the developments of severe side effects (Brotchie et al., 2005). Since available pharmacological therapies are unable to arrest, or reverse the progression of loss of dopaminergic neurons, the possible application of gene therapy techniques provides interesting perspective in the treatments for PD. Of the variety of available delivery systems, viral vectors have recently been widely used for transferring genes to specific brain regions. Vectors that have useful features for gene delivery to the central nervous system (CNS) and more specifically for an application to PD include different types: adenovirus (AdV), adeno-associated virus (AAV), herpes simplex viruses (HSV) and lentiviruses (LV) (Davidson and Breakefield, 2003; see Mandel et al., 2008). At present, several approaches for PD gene therapy are in progress.

The most conservative one consists of managing the symptoms by acting on the altered dopamine neurotransmission in basal ganglia. A number of studies have indeed focused on the restoration of dopamine in the denervated striatum (Muramatsu et al., 2002; Shen et al., 2000). This strategy would modify dopamine levels in the striatum by introducing gene-expressing essential enzymes for dopamine production (Azzouz et al., 2002) or more recently, gene-altering expression of dopaminergic signalling

Abbreviations: 6-OHDA, 6-hydroxydopamine; AADC, aromatic L-amino acid decarboxylase; AAV, adeno-associated virus; AdV, adenovirus; BH_4, tetrahydrobiopterin; CNS, central nervous system; D_2, dopamine D_2 receptor; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GAD_{65}, glutamate decarboxylase 65 kDa isoform; GAD_{67}, glutamate decarboxylase 67 kDa isoform; GCH, guanosine triphosphate cyclohydrolase I; GDNF, glial cell line-derived neurotrophic factor; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; HSV, herpes simplex virus; IAP, inhibitor apoptosis protein; L-DOPA, L-3,4-dihydroxyphenylalanine; LID, L-DOPA-induced dyskinesia; LV, lentivirus; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; SN, substantia nigra; STN, subthalamic nucleus; TH, tyrosine hydroxylase; VMAT-2, vesicular monoamine transporter-2; XIAP, X-chromosome-linked inhibitor of apoptosis protein.

* Corresponding author. CNRS UMR 5227, Université Bordeaux 2, 146 rue Leo Saignat, Bordeaux 33076, France. Fax: +33556901421.
E-mail address: erwan.bezard@u-bordeaux2.fr (E. Bezard).

0014-4886/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2007.08.003

pathway (Bezard et al., 2005). At odds with such dopamine-related approaches, non-dopaminergic strategies have been designed for normalizing the pathological activity of the output structures of the basal ganglia such as the subthalamic nucleus (STN; Luo et al., 2002), the internal and the external segment of the globus pallidus (GPi and GPe, respectively).

Another therapeutic approach consists of over-expressing trophic factors that provide support for the dying dopaminergic nigral cells from the SN. Specific neurotrophic factors, including glial cell line-derived neurotrophic factor (GDNF; Bjorklund et al., 2000; Kordower et al., 2000; Lin et al., 1993) and brain-derived neurotrophic factor (BDNF; Hyman et al., 1991; Klein et al., 1999), have been found to enhance survival and function of dopaminergic neurons in animal models of PD and can also interfere with both apoptotic and necrotic forms of cell death.

With each therapy – symptomatic *versus* neuroprotective strategies – the important objective is to develop the necessary technology to efficiently and specifically deliver the therapeutic agent to the target cells. We here review most of the strategies in preclinical development.

Symptomatic treatments

DA replacement therapy

The dopaminergic biosynthetic pathway includes the enzymes tyrosine hydroxylase (TH), which catalyzes the synthesis of L-DOPA, and aromatic L-amino acid decarboxylase (AADC), which converts L-DOPA to dopamine. Additionally, TH has a requirement for a cofactor, tetrahydrobiopterin (BH_4), the biosynthesis of which is rate-limited by GTPcyclohydrolase 1 (GCH; Elsworth and Roth, 1997; Nagatsu and Ichinose, 1999). Finally, a vesicular monoamine transporter (VMAT-2) transports DA into synaptic vesicles (Liu et al., 1992). Dopamine and TH, AADC and GCH enzymes are anterogradely transported from the substantia nigra to the striatum (Nagatsu et al., 1997). The loss of dopaminergic nerve terminals in the striatum of PD is associated with a depletion of striatal TH, AADC and GCH activity as well as a decrease in the level of BH_4 (Nagatsu and Ichinose, 1999).

As TH is the rate-limiting enzyme in dopamine synthesis, preliminary studies have first investigated TH replacement gene therapy. In 6-OHDA-lesioned rats, the intrastriatal transduction of an AdV- or HSV-encoding TH has been shown to decrease rotational asymmetry induced by apomorphine (Horellou et al., 1994; Leone et al., 2000). Efficient behavioural and biochemical recovery persisted up to 1 year after gene transfer with HSV vector (During et al., 1994). Issues that were raised about this last study included the side effects caused by this vector system, possible neurotoxicity, reduced long-term expression, and expression of TH only (Isacson, 1995; Neve and Geller, 1996; Mandel et al., 1998). For example, in the case of intrastriatal viral injections of HSV, toxic reactions to the viral infection have been reported (Pakzaban et al., 1994; Monville et al., 2004) and this striatal damage may account for the reductions in HSV-induced rotational reductions observed (Isacson, 1995). How critical During's study was, the authors put an end to the debate by developing new HSV vector with low side effects, stable expression that resulted in biochemical and behavioural improvement in the 6-OHDA rat model of PD (Sun et al., 2003, 2004).

Transduction with AAV–AADC in animal models of PD leads to an increase in the capacity of the striatum to decarboxylate exogenous L-DOPA. Efficient and long-term expression of the transgene in the striatum, restores local dopamine production (>50%) and achieves behavioural recovery in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-(MPTP)-lesioned monkeys (Bankiewicz et al., 2000, 2006) or 6-OHDA-lesioned rats (Leff et al., 1999; Sanchez-Pernaute et al., 2001). Interestingly, AAV–AADC transgene in MPTP-lesioned monkey brain after convection-enhanced delivery was shown to be distributed not only in the striatum but also in all the basal ganglia structures (Hadaczek et al., 2006). Co-expression of TH and AADC by using one (During et al., 1998) or two separate (Fan et al., 1998; Hadaczek et al., 2006) AAV vectors have been shown to significantly increase the intracellular dopamine level in the striatum. Better behavioural recovery was also observed with this double transduction rather than with AAV–TH or AAV–AADC alone (During et al., 1998; Fan et al., 1998). As discussed above, similar results were observed with the HSV vector that co-expresses TH and AADC (Sun et al., 2003). In the parkinsonian striatum, GCH activity and endogenous levels of BH4 are also reduced (Nagatsu and Ichinose, 1999). Co-transduction of AAV–TH and AAV–GCH in 6-OHDA-lesioned rat striatum produces a significant increase in dopamine which may have functional recovery in apomorphine-induced rotational behaviour if the levels of striatal L-DOPA are to reach relevant levels (Mandel et al., 1998; Kirik et al., 2002).

A number of studies together suggest that co-expression of at least four genes (*TH*, *AADC*, *GCH* and *VMAT-2*) will be required for efficient production and regulated, vesicular release of dopamine from striatal neurons. Remarkable efficacy has been observed in animal models when all three genes are co-expressed in the striatum. This was demonstrated with AAV vectors (Shen et al., 2000; Muramatsu et al., 2002), but three different vectors carrying each of the genes were used because of the size limitation of AAV. Due to this restriction of the number of genes that could be delivered by AAV vectors, the advantage of the higher capacity of LV vector has been exploited to build a single vector expressing all three enzymes (Azzouz et al., 2002). The delivery of this LV vector into the DA-denervated striatum of the 6-OHDA-lesioned rat, sustained expression of each enzyme and effective production of dopamine were detected, resulting in significant reduction of apomorphine-induced rotational behavioural (Azzouz et al., 2002). VMAT-2 plays a critical role in dopamine storage by packaging dopamine into synaptic vesicles and regulating its sustained release. The co-expression of a VMAT-2 with specific DA biosynthetic enzymes (TH–AADC–GCH) in a HSV vector supported higher levels of DA and reduced apomorphine-induced rotation than did the vector that co-expresses the three enzymes (Sun et al., 2004).

Non-dopaminergic strategies

Striatum level

Besides normalizing dopamine levels, researchers are attempting to control the pathological changes that affect the striatum