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Review

Gene therapy for Parkinson's disease: Disease modification by GDNF family of ligands

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ABSTRACT

Gene transfer is a promising drug delivery method of advanced therapeutic entities for Parkinson's disease. One advantage over conventional therapies, such as peripheral delivery of the dopamine pre-cursor L-DOPA, is site-specific expression of proteins with regenerative, disease-modifying and potentially neuroprotective capacity. Several clinical trials have been performed to test the capacity of glial-cell line derived neurotrophic factor and neurturin to rescue degenerating dopaminergic neurons in the substantia nigra and their axon terminals in the striatum by delivery of these neurotrophic factors either as purified protein or by means of viral vector mediated gene delivery to the brain. Although gene therapy approaches tested so far have been shown to be safe, none met their primary endpoints in phase II clinical trials designed and powered to test the efficacy of the intervention. Within the scope of this review we aim to describe the state-of-the-art in the field, how different technical parameters were translated from pre-clinical studies in non-human primates to clinical trials, and what these trials taught us regarding important factors that may pave the way to the success of gene therapy for the treatment of Parkinson's disease.

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1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the decrease in dopamine (DA) input to the striatum resulting in a debilitating loss of motor control and the symptoms of tremor, rigidity, akinesia and postural imbalance. Another hallmark of the disease is the accumulation of protein inclusions, Lewy bodies, in several nuclei in the brain including, but not limited to, the DA neurons in substantia nigra pars compacta (SN). For the majority of PD patients the etiology is unknown although possible risk factors include old age, toxin exposures and genetic factors (Alves et al., 2013; Wirdefeldt et al., 2011). Today, a causal genetic mutation can be determined in about 10% of cases.

Symptomatic treatment of PD is focused on supporting the DA tone and function in the striatum - the main target nuclei for DA projection neurons in SN. The precursor of DA, L-3,4-dihydroxyphenylalanine (L-DOPA), is used for symptomatic relief since DA has a very short half-life, causes hemodynamic effects when given peripherally and is unable to cross the blood–brain barrier (BBB) due to its polarity. Endogenously synthesized L-DOPA normally derives from the dietary amino acid tyrosine, a reaction catalyzed by the tyrosine hydroxylase (TH) enzyme. The enzymatic activity of TH is dependent on the presence of the co-factor 5,6,7,8-tetrahydro-L-biopterin (BH4). L-DOPA is then further converted

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into DA by L-aromatic amino acid decarboxylase (AADC). In the normal intact brain AADC activity is far greater than TH and is found in multiple cell compartments other than DA neurons, e.g., serotonergic cells, thus the rate limiting step in synthesis of DA is the activity of the TH enzyme.

L-DOPA medication is given in combination with benserazide or carbidopa to inhibit the peripheral AADC activity and minimize the conversion of L-DOPA precursor to DA outside the brain. Although the therapeutic benefit of L-DOPA is striking in the beginning of treatment, troubling side effects appear in the majority of patients after some years. These side effects involve involuntary movements, so called dyskinesias, that are believed to emerge when the therapeutic window becomes narrower and the dose of L-DOPA needed for an anti-parkinsonian effect (typically within the motor domain) is increased due to increasing loss of DA neurons. Intermittent availability of L-DOPA after oral administration and fluctuations in drug levels in the brain cause the patients to experience rapid shifts from parkinsonian to dyskinetic state and vice versa. Sustained release formulations with L-DOPA have been explored to stabilize plasma levels of L-DOPA but the therapeutic benefit is less potent. Other attempts to increase the therapeutic benefit of L-DOPA aim at preventing the degradation of DA in the synapse by inhibition of the monoamine oxidase (MAO) and the catechol-o-methyl transferase inhibitor (COMT). Alternatives to L-DOPA include DA agonists that directly target the post-synaptic DA receptors. These agonists are often used to allow delayed initiation of L-DOPA therapy and thereby increase the total time of efficacious anti-parkinsonian treatment.

The concept of drug delivery by gene transfer, i.e. gene therapy, has many attractive attributes in the treatment of PD and other neurodegenerative diseases. First, many viral vectors are able to efficiently transduce brain cells resulting in long-term expression of the transgene. Secondly, intracerebral injection of vectors allows the expression of transgenes in specific brain areas, which reduces the off-target effects that are often associated with systemic drug administration. This specificity can be further improved by using specific vector serotypes and promoters to drive the expression of the therapeutic gene in restricted cell groups in the target area.

The adeno-associated viral (AAV) vector is the most frequently used vector for neurological disorders and there are currently 7 ongoing clinical trials for PD. AAVs belong to the *Parvoviridae* family and are single-stranded DNA viruses, where the recombinant vector is non-integrating and requires a helper virus to generate new virions. When the virulent genes are removed, leaving only the inverted terminal repeats (ITR), the AAV vectors are not considered to be pathogenic in humans - especially since modern production methods do not require co-infection with wild type helper virus (2016). AAV vectors are very suitable for CNS gene therapy since they transduce neurons with high efficiency. This can in particular be illustrated in cases where the therapeutic transgene is a cytoplasmic protein allowing the identification of transduced cells in the brain (Fig. 1). Neuronal transduction results in long-term transgenic expression and MPTP lesioned monkeys have shown persistent transgene expression for up to 8 years post-injection, which has

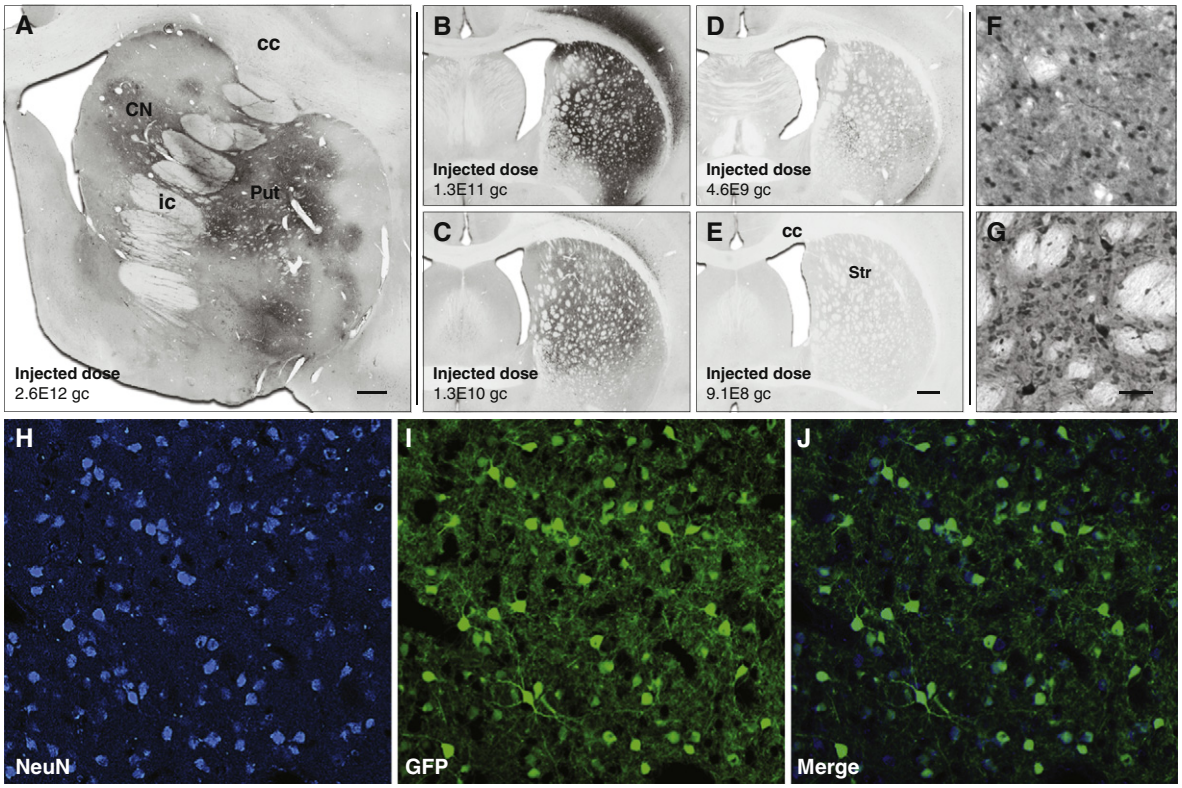

Fig. 1. Immunohistochemical staining for AAV vector-derived transgenic GTP cyclohydrolase 1 (GCH1) in rhesus monkey (A,F,H–J) or rat (B–E,G). GCH1 is the rate-limiting enzyme in the synthesis of the co-factor tetrahydrobiopterin - essential for efficient function of tyrosine hydroxylase (TH) and dopamine (DA) production. It is shown here as an example of a therapeutic transgene with cytoplasmic localization and thus different from neurotrophic factors reports the identify of transduced cells in the target region. All animals were injected with the same vector construct, encoding human GCH1 and TH, in the caudate and putamen. Only human GCH1, and not endogenous forms of the protein, was recognized by the antibody. In the rhesus monkey (A), injections were made at two sites in the caudate nucleus (10 + 5 μl) and at three sites in the putamen (10 + 10 + 5 μl) making a total of 40 μl injected per hemisphere. Injections in rodents were made at two sites (in total 5 μl) within the dorsal striatum. Injections of 1.3E11 gc resulted in wide-spread transduction including extrastriatal areas e.g. globus pallidus and overlying cortex (B). Decreasing the dose 10-fold resulted in similar transduction pattern in the striatum but much more limited spread to adjacent regions (C). Decreasing the dose to 4.6E9 and 9.1E8 gc resulted in sparse transduction with only few immunoreactive cell bodies. High-power images are taken from the transduced putamen in monkey (F) and from the dorsal striatum in the rat (G). Panels H–J are confocal images stained with NeuN (H) and GFP (I) taken from another monkey. Merged channel is shown in J and illustrates that the transduced cells are essentially all neuronal phenotype. Scalebar in A represent 1 mm, E represent 0.5 mm, G represent 50 μm. cc: corpus callusum, CN: caudate nucleus, gc: genomic copies, ic: internal capsule, Put: putamen, Str: striatum.
Panels modified from Cederfjäll et al. Scientific Reports, 2013.