Challenges of Gene Therapy for Neurodegenerative Disorders

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ARTICLE HISTORY

Received: July 13, 2020
Revised: September 12, 2020
Accepted: September 29, 2020
DOI: 10.2174/1566523220999201105150442

INTRODUCTION

Progressive dysfunction of neurons in certain parts of the central nervous system (CNS) is the primary characteristic of neurodegenerative diseases, which leads to neuronal malfunctioning and, eventually, death [1, 2]. With an increase in drug target research, the number of probable clinical interventions have improved. However, various drugs and their associated targets are only capable of temporary or symptomatic relief and hardly deal with the underlying pathology of neurodegenerative disease [3, 4]. In the case of neurodegeneration, inherited genetic mutations are often responsible [5]. Gene therapy focuses on the delivery of genetic materials, which encodes potential therapeutic molecules into patient's cells [6]. Hence, it is paramount to characterize the potential pathogenic targets for gene therapy of neurodegenerative diseases. For the treatment of neurodegenerative disorders by gene therapy, substantial numbers of clinical trials have been performed. Nonetheless, several clinical trials have failed to achieve the desired therapeutic results, probably owing to the lack of biodistribution of the applied intervention in the target tissue [7, 8]. Despite the failures, there are several reports of promising results in experimental models of many neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's chorea (HC), and amyotrophic lateral sclerosis (ALS) [9-13]. Several genetic alternations are slight and can disturb a single gene, but these single-gene mutations can exert a large impact because of the alteration of the instructions of the gene for protein synthesis. In fact, such single-gene mutations can cause various rare inherited neurological diseases [14].

Performing successful gene delivery is a challenging task in general and in sensory organs, especially the brain [8, 13]. Optimization of treatment efficiency and patient safety is primarily determined by the combination of delivery routes and selection of delivery vectors. Access to the therapeutic gene possesses serious obstacles, such as physiological barriers that compartmentalize CNS and sensory tissues like the blood-brain barrier (BBB) [15]. For the treatment of brain diseases, adeno-associated viral (AAV) vectors represent a promising gene therapy platform [16]. After intravenous injection, AAV9 effectively penetrate these barriers. This property helps to treat multifocal disorders as it facilitates widespread expression in CNS and acts as a stimulus for further development [17-19]. AAV variants have been discovered by capsid engineering research like AAV-AS, AAV-B1, and AAV.PHP.B, which has shown superiority over AAV9. The intravenous injection might result in delivery to different parts of the body, which has some limitations and also has a few benefits too [20, 21]. Even though intravenous delivery offers noninvasive and technically feasible, generation of antibodies against AAVs, the requirement of large dose, and associated safety challenges are major drawbacks of intravenous delivery of AAVs. The benefits of local vector delivery over systemic vector administration are evident. The intraparenchymal injection is specific to the region of interest and delivers therapeutic genes straight to the neurons and brain, and rarely distributing to peripheral organs [11, 22, 23]. Vectors like AAV1, AAV8, AAV9, and AAVrh.10 which do not bind heparin sulfate proteoglycans (HSPGs), diffuse over larger areas after intraparenchymal injection than vectors like AAV2, AAV-D88, and AAV6, which bind HSPGs [24]. Canavan disease has been treated with AAV2-ASPA and PD has been treated with AAV2; AAV2-AADC provides an appropriate vehicle to limit diffusion while securing/getting satisfactory delivery [25].
Adenovirus (Adv) has been used as gene therapy vectors for the treatment of several neurodegenerative diseases however, Adv is well-tolerated and has few adverse effects [26]. In contradistinction to AAV and Adv capsids, via reverse transcription, the retrovirus/lentivirus (LVs) can completely integrate deoxyribonucleic acid (DNA) into the host genome, thereby facilitating to improve stability and express the longer transgene in vivo. Although this integrative feature of LVs assures highly stable and long-term expression of the transgene, it involves insertional mutagenesis. However, the gene designer can make safer LVs by gene editing in specific integration sites [27]. The viral genome can be divide into multiple plasmids to increase the safety of LVs, thus constructing recombinant virus generation highly unlikely [28]. Moreover, pseudotyping by envelope glycoproteins can redirect viral particles to specific targets [29-33]. Because LVs can effectively introduce foreign DNA into neurons, they have been inspected in various neurodegenerative diseases, including AD and PD [34-38].

Single gene mutation/molecular mechanism is not enough to explain CNS disorders; their pathophysiology most often multifactorial origin. Consequently, the large genetic payload capacity of viral vectors was most frequently exceed through the therapeutic gene(s) delivery [39]. Regarding this payload capacity, herpes simplex virus type 1 (HSV-1) vectors would be the best alternative to solve this issue, which has a large genomic size and a capacity to host a large proportion of external DNA. It is important to bear in mind that the 1st generation of replication-defective HSV-1 vectors had some serious shortcomings, such as short-term expression of the transgenes and toxicity [40]. Several studies have been conducted objectively to overcome these limitations. For instance, the deletion of α genes from mutant HSV-1 vectors dismiss viral replication and significantly decrease cytotoxicity [41]. Deleting of ICP4 and ICP27 immediate-early (IE) genes gave rise to the 1st (ΔICP4) and 2nd (ΔICP4/27) generation of HSV-1 vectors [42, 43]. These early generations can establish a prolonged-expression without the capability to reactivate; however, the residual presence of ICP0 liable for the cytotoxicity of transduced cells. The result of deleting this ICP0 gave rise to the 3rd-generation vectors that were free from toxicity while exerted an extremely short duration of expression of the transgenes [44]. By installing the transgene expression coding sequence into the viral latency-associated transcript locus, a new-fashioned HSV-1 vector (Fig. 1) had constructed to solve this hurdle; a genome site remains protected through the insulator sequences from silencing during latency, which shields the locus against epigenetic modifications [45]. In in vitro infection in various cell types, this new generation vectors allow a large payload capacity and free from toxicity [46]. In case of in vivo injecting of new engineered HSV vectors into several brain areas of naive rats provided a stable and long-period of neuron-specific expression of transgenes inserted in the ICP4 locus, without any sign of toxicity, indicating that ICP4 locus may be suitable to obtain a sustainable and long-term transgene expression in neurons [47].

In clinical trials, the use of viral vectors, including AAVs, LVs, and Adv for the delivery of therapeutic genes is common. However, these viral vectors have some serious limitations, which include restricted loading capacity, obscure vector production, broad tropism, and indifferent inflammatory responses (Fig. 1) [16, 48-51]. Safety-related issues (Fig. 1) of viral vectors can be avoided by using non-viral vectors [52-54]. Lipid-based vectors are the most widely utilized non-viral vector [55]. Neutral lipids including 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), and cholesterol are commonly utilized helper lipids to enhance transfection efficiency and liposome stability [56]. The key attributes of cationic lipids used for gene therapy, which include 1,2-dioleoyl-3-dimethylammonium-propane (DODAP), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), and 3β-[N′(N,N′-dimethylaminoethane)carbamoyl] cholesterol (DC-cholesterol), have three major areas such as linking groups, hydrophobic tails, and cationic cap groups [52, 57]. The core limitations of cationic lipids are their substandard pharmacokinetic biodistribution because of their imprecise binding, fast clearance, and cytotoxicity [52, 58]. In order to circumvent these limitations, augmented cationic lipids with suitable pKa values have been invented [53, 55]. Lipidoids (i.e. materials similar to lipid), exosomes, and magnetic nanoparticles have also gained attention as gene delivery tools, especially for neurodegenerative disorders [59-61].
The physiological barriers that group CNS and sensory tissues, including BBB (Fig. 1), pose significant difficulties in therapeutic gene delivery. AAV9 can efficiently enter these physiological barriers following the intravenous injection. This characteristic of AAV9 facilitates pervasive expression in CNS to cure several multifocal disorders and functions as a stimulus for technological advancement and development [59-61]. Furthermore, capsid fabrication related investigation has identified AAV variants that appear to be better than AAV9, for instance, AAV-B1, AAV-AS, and AAV.PHP.B, which indicates a substantial development in Intravenous Delivery (IVD) [16] The IVD route may result in delivery of the gene therapy vehicles to several body tissues that has a potential disadvantage, however, in certain cases, may offer significant improvements. The IVD of AAVs is noninvasive and technically viable. Still, due to the requirement of a substantial quantity of doses, antibody generation against AAVs, and associated safety concerns are major obstacles in their clinical application.

Local administration of vectors has apparent benefits over systemic delivery. The intraparenchymal injection is well-tolerated and delivers the required genes directly to the nervous system, with slight chances of biodistribution to peripheral systems [11, 22, 62]. Vectors that are not capable of binding with heparin sulfate proteoglycans (HSPGs), such as AAV8, AAV1, AAV9, and AAVrh.10, may diffuse widely to nonspecific areas after intraparenchymal injection, as opposed to vectors that bind HSPGs, like AAV-DJ88, AAV2, and AAV6 [24]. For ailments like Canavan disease, that was treated with PD and AAV2-ASPA, or AAV2, and AAV2-AADC provides a unique and suitable vehicle to reduce the diffusion with efficient delivery [25].

Table 1. Several ongoing clinical trials of gene therapy for neurodegenerative disorders.

<table>
<thead>
<tr>
<th>ClinicalTrials.gov Identifier</th>
<th>Gene Therapy</th>
<th>Actual Enrollment</th>
<th>Disorders</th>
<th>Phase</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00876863</td>
<td>CERE-110 (adeno-associated virus serotype 2, AAV2) – nerve growth factor</td>
<td>49</td>
<td>Alzheimer's disease</td>
<td>II</td>
<td>[73]</td>
</tr>
<tr>
<td>NCT03065192</td>
<td>Adeno-associated virus serotype 2 (AAV2) – aromatic-L-amino-acid decarboxylase</td>
<td>16</td>
<td>Parkinson's disease</td>
<td>I</td>
<td>[74]</td>
</tr>
<tr>
<td>NCT01621581</td>
<td>Adeno-associated virus serotype 2 (AAV2) – glial-derived neurotrophic factor</td>
<td>25</td>
<td>Parkinson's disease</td>
<td>I</td>
<td>[75]</td>
</tr>
<tr>
<td>NCT02418598</td>
<td>Adeno-associated virus serotype 2 (AAV2) – aromatic-L-amino-acid decarboxylase</td>
<td>2</td>
<td>Parkinson's disease</td>
<td>II</td>
<td>[76]</td>
</tr>
<tr>
<td>NCT00400634</td>
<td>Adeno-associated virus serotype 2 (AAV2) – neurturin</td>
<td>58</td>
<td>Parkinson's disease</td>
<td>II</td>
<td>[77]</td>
</tr>
<tr>
<td>NCT00627588</td>
<td>Lentivirus – aromatic-L-amino-acid decarboxylase</td>
<td>15</td>
<td>Parkinson's disease</td>
<td>I</td>
<td>[78]</td>
</tr>
<tr>
<td>NCT00643890</td>
<td>Adeno-associated virus serotype 2 (AAV2) – glutamic acid decarboxylase</td>
<td>44</td>
<td>Parkinson's disease</td>
<td>II</td>
<td>[79]</td>
</tr>
<tr>
<td>NCT02519036</td>
<td>Antisense oligonucleotides (ASOs) – mutant huntingtin messenger RNA</td>
<td>46</td>
<td>Huntington's disease</td>
<td>II</td>
<td>[80]</td>
</tr>
<tr>
<td>NCT03225833</td>
<td>Antisense oligonucleotides (ASOs) – mutant huntingtin mutant pre-messenger RNA</td>
<td>60</td>
<td>Huntington's disease</td>
<td>II</td>
<td>[81]</td>
</tr>
<tr>
<td>NCT01041222</td>
<td>Antisense oligonucleotides (ASOs) to superoxide dismutase 1</td>
<td>33</td>
<td>Amyotrophic lateral sclerosis</td>
<td>I</td>
<td>[82]</td>
</tr>
<tr>
<td>NCT01801709</td>
<td>Adeno-associated virus serotype rh10 (AAVrh10) – arylsulfatase A</td>
<td>5</td>
<td>Metachromatic leukodystrophy</td>
<td>II</td>
<td>[83]</td>
</tr>
<tr>
<td>NCT02240407</td>
<td>Adeno-associated virus serotype 9 (AAV9) – lysosomal acid α-glucosidase</td>
<td>7</td>
<td>Pompe's disease</td>
<td>I</td>
<td>[84]</td>
</tr>
<tr>
<td>NCT00976352</td>
<td>Adeno-associated virus serotype 1 (AAV1) – lysosomal acid α-glucosidase</td>
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<td>Pompe's disease</td>
<td>II</td>
<td>[85]</td>
</tr>
<tr>
<td>NCT02122952</td>
<td>Adeno-associated virus serotype 9 (AAV9) – survival motor neuron</td>
<td>15</td>
<td>Spinal muscular atrophy</td>
<td>I</td>
<td>[86]</td>
</tr>
<tr>
<td>NCT02292537</td>
<td>Antisense oligonucleotides (ASOs) targeting survival motor neuron 2 splicing</td>
<td>126</td>
<td>Spinal muscular atrophy</td>
<td>III</td>
<td>[87]</td>
</tr>
</tbody>
</table>
Additional delivery routes involve the administration of vehicles into the different cerebrospinal fluid (CSF) compartments. Intrathecal infusion of AAVs is particularly appropriate for delivering vectors to sensory neurons in a motor neuron or dorsal root ganglia and has been tolerated well in several preclinical studies [63-65]. Intriguingly, AAVs, including AAVrh.10 and AAV9, chiefly target motor neurons of the spinal cord after intrathecal injection in nonhuman primates like cynomolgus macaques (Macaca fascicularis) and rhesus macaques (Macaca mulatta) [66]. To date, the subpial administration has been examined in the laboratory conditions only; therefore, several dosing optimization related studies are warranted before its application in neurodegenerative disorders [71]. Some preclinical studies have indicated that intracisternal and intracerebroventricular injection has also induced substantial expression of transgenes in cerebral tissues and spinal cord, which has eased clinical symptoms in various models of neurodegenerative disorders, such as spinal muscular atrophy ALS, and AD [67-69]. Besides, Ahmed et al. [70] have demonstrated efficient and permanent transduction of motor neurons following the intrauterine injection of Integration-Deficient Lentiviral Vectors (IDLVs), showing the ability of IDLVs to be used as an effective tool to cure neurodegenerative diseases.

The administration of drugs directly into different CSF compartments is another promising delivery route. Numerous preclinical studies have shown that intrathecal injection of AAVs is appropriate for delivering vectors to sensory neurons in motor neurons or dorsal root ganglia has potential for clinical application [63-65]. Intriguingly, AAVs, including AAVrh.10 and AAV9, chiefly targets motor neurons of the spinal cord after intrathecal injection in nonhuman primates like cynomolgus macaques (Macaca fascicularis) and rhesus macaques (Macaca mulatta) [66]. To date, the subpial administration has been examined in the laboratory conditions only; therefore, several dosing optimization related studies are warranted before its application in neurodegenerative disorders [71]. Some preclinical studies have indicated that intracisternal and intracerebroventricular injection has also induced substantial expression of transgenes in cerebral tissues and spinal cord, which has eased clinical symptoms in various models of neurodegenerative disorders, such as spinal muscular atrophy ALS, and AD [67-69]. Besides, Ahmed et al. [70] have demonstrated efficient and permanent transduction of motor neurons following the intrauterine injection of Integration-Deficient Lentiviral Vectors (IDLVs), showing the ability of IDLVs to be used as an effective tool to cure neurodegenerative diseases.

Thus, choosing the appropriate delivery routes is the primary factor among several factors while considering the risk/benefit or the intervention. Owing to maximum delivery and minimum safety issues, local delivery routes are preferred for neurodegenerative disorders. In order to achieve broader efficacy of treatment than local administration routes, new tools and techniques are warranted which can accomplish result-oriented gene transfer globally. The existing delivery routes require higher doses, increasing the cost of treatment, enhancing the burden on the drug manufacturing companies, and increasing the toxicity risk. Furthermore, utilizing multiple routes of injection could be useful while treating a multi-organ associated disease [72].

CONCLUSION

In recent decades, copious amounts of preclinical and clinical studies (Table 1) associated with the development of novel gene therapy tools against the treatment and prevention of a variety of neurodegenerative diseases [1, 88]. Nonetheless, safety concerns related to gene therapy remains one of the biggest challenges in effective clinical application. Overexpression of the transgene may cause severe toxicity in the tissue of interest or nearby tissue. Moreover, the off-target expression in cells might have also serious toxic consequences. The reported toxic effect of failed gene therapy includes ataxia, impaired ambulation, damaged dorsal root ganglia, proprioceptive deficits, and elevated transaminases [88, 89]. Inherent host response may alter the dose, duration, and safety of the applied gene therapy approach. Existing adaptive immunity might also produce antibodies, which will neutralize the effect of the delivered transgene [90, 91]. Point or frameshift mutations in the transgene and genotoxicity are potential issues of gene therapy, which will reduce the potency of treatment, especially in the case of high dose vectors [18, 92, 93]. Therefore, the gene therapy tools to treat neurodegenerative disorders should be meticulously scrutinized before clinical use, security profiles and pharmacological effects must be evaluated along with the identification of potential patients who can get the benefit of the developed strategy.

AUTHORS’ CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This project was funded by the Pharmakon Neuroscience Research Network, Dhaka, Bangladesh.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors concede the support by the Pharmakon Neuroscience Research Network, Dhaka, Bangladesh.

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Perspective

Current Gene Therapy, 2021, Vol. 21, No. 1 9

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