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TERAPIA GÊNICA IN SITU EM CONDIÇÕES NEURODEGENERATIVAS: UMA REVISÃO DA PESQUISA CLÍNICA

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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharel(a) em Biomedicina.

Orientador: Prof. Dr. Guilherme Baldo
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RESUMO

As condições neurodegenerativas são de difícil tratamento, em parte pelo acesso limitado ao cérebro imposto pela barreira hematoencefálica. Uma vez que a terapia gênica surgiu como uma abordagem promissora não apenas para doenças monogênicas com envolvimento neurológico, mas também para doenças complexas e multifatoriais, como a doença de Alzheimer e a doença de Parkinson, a introdução de material genético terapêutico *in situ* (i.e. diretamente no sistema nervoso central) se apresentou como uma alternativa para superar este desafio. Tal estratégia levou ao desenvolvimento de diversos estudos de terapia gênica que agora estão atingindo a fase clínica. Essa revisão tem como objetivo apresentar os avanços clínicos na área da terapia gênica *in situ* para o sistema nervoso central, com foco em doenças neurodegenerativas e doenças lisossomais. Também são destacadas as principais características dos vetores virais e das rotas de administração utilizadas. Os estudos concluídos até o momento tiveram sucesso em demonstrar um bom perfil de segurança, no entanto, a maioria dos resultados são de ensaios abertos e os dados de eficácia são considerados preliminares.

Palavras-chave: Terapia gênica in situ. Doenças neurodegenerativas. Doenças lisossomais. Vetores virais.

ABSTRACT

Neurodegenerative conditions are difficult to treat in part due to the limited access to the brain imposed by the blood-brain barrier. As gene therapy emerged as a promising approach not only for monogenic disorders with neurological involvement but also for complex, multifactorial diseases such as Alzheimer's Disease and Parkinson's Disease, the delivery of genetic material *in situ* (i.e. directly into the central nervous system) presented an alternative to overcome this challenge. Such strategy led to the development of several gene therapy studies that are now reaching clinical phases. This review aims to present clinical progress in the field of *in situ* gene therapy for the central nervous system, focusing on neurodegenerative diseases and lysosomal storage disorders. Also, the main characteristics of viral vectors and administration routes employed are highlighted. Completed studies so far successfully demonstrated a good safety profile, yet most published data are from open-label trials and thus efficacy results are considered preliminary.

Keywords: In situ gene therapy. Neurodegenerative diseases. Lysosomal storage disorders. Viral vectors

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1 INTRODUÇÃO COMPREENSIVA

1.1 TERAPIA GÊNICA

A terapia gênica consiste na transferência de material genético para as células de um paciente com objetivo terapêutico, podendo ser classificada em duas principais abordagens: *ex vivo* e *in vivo*. Na terapia gênica *ex vivo*, células do paciente (usualmente células tronco hematopoiéticas) são extraídas, modificadas geneticamente *in vitro* e então reintroduzidas no indivíduo; enquanto na terapia gênica *in vivo* o material genético é administrado diretamente no paciente (NARDI; TEIXEIRA; SILVA, 2002).

A transferência do material terapêutico é realizada pelos chamados vetores, que são divididos em virais e não virais. Na construção dos vetores virais, o genoma original do vírus é substituído por um cassete de expressão contendo o gene de interesse, sendo mantidas apenas as sequências virais necessárias para o empacotamento do genoma. Essa estratégia reduz a patogenicidade do vírus, já que impossibilita sua replicação, enquanto mantém a sua capacidade de transdução celular (BENSKEY M.J. *et al.,* 2019). Atualmente, os principais vírus utilizados como vetores para terapia gênica são os retrovírus, lentivírus, adenovírus e vírus adeno-associados. Quanto aos vetores não virais, sistemas lipídicos ou poliméricos podem ser utilizados para carregar um plasmídeo contendo o gene terapêutico. Esses sistemas apresentam menor imunogenicidade, maior capacidade de empacotamento e facilidade de produção em comparação aos vetores virais, no entanto, sua baixa eficiência de transfecção e expressão não sustentada do transgene limitam seu uso na prática clínica (DONDE; WONG; CHEN, 2017).

Inicialmente, a terapia gênica tinha como foco o tratamento de doenças monogênicas através da introdução de uma cópia funcional do gene mutado. Diferentes estratégias foram sendo desenvolvidas para atender outras condições com etiologias complexas e multifatoriais, como câncer e doenças cardiovasculares, infecciosas e neurodegenerativas (OLIVEIRA et al. 2018). Entre essas estratégias estão a introdução de genes que codificam fatores protetivos ou proteínas envolvidas em vias de sinalização, o silenciamento de genes utilizando RNA de interferência e a edição genômica (WANG; GAO, 2014).

Independente da estratégia escolhida, na terapia gênica *in vivo* os vetores podem ser administrados sistemicamente por via intravenosa ou diretamente no tecido alvo, caracterizando a chamada terapia gênica *in situ*. Utilizando essa metodologia, é possível atingir efeito terapêutico injetando uma menor dose de vetor, reduzir a chance de transdução

em outros tecidos que não o tecido alvo, evitando toxicidade, além de contornar respostas imunes que possam ser ativadas contra o vetor (PEREZ *et al.*, 2020).

A seguir, serão abordadas as principais condições neurodegenerativas que estão sendo avaliadas em estudos clínicos empregando terapia gênica *in situ*.

1.2 DOENÇAS NEURODEGENERATIVAS

Doenças associadas ao avanço da idade, como a doença de Alzheimer (DA) e a doença de Parkinson (DP), têm se tornado mais prevalentes no mundo devido ao envelhecimento da população (PEDEN; IRONSIDE, 2012). Apesar das diferentes apresentações clínicas, ambas as condições são caracterizadas por agregados de proteínas mal dobradas que levam à perda gradual de neurônios e suas conexões sinápticas em áreas específicas do cérebro (SOTO; PRITZKOW, 2018).

A DA atinge aproximadamente 35 milhões de pessoas, sendo responsável por 60 a 70% dos casos de demência (World Health Organization, 2020). O déficit cognitivo, que envolve perda de memória, desorientação e mudanças comportamentais (ATRI, 2019), está associado a emaranhados neurofibrilares e placas extracelulares formados pelas proteínas tau e β-amilóide, respectivamente (TIWARI et al., 2019). Esses agregados se desenvolvem inicialmente no lobo temporal medial e em áreas subcorticais, sobretudo no núcleo basal de Meynert, córtex entorrinal, hipocampo e amígdala (COUGHLAN *et al.*, 2018) . A partir do núcleo basal de Meynert, neurônios colinérgicos se projetam para o todo o córtex cerebral; a morte desses neurônios e a consequente redução dos níveis de acetilcolina leva ao aparecimento dos sintomas (LIU *et al.,* 2015). O principal tratamento para a DA, os fármacos inibidores da acetilcolinesterase, é sintomático e seu benefício é observado apenas em uma parcela dos pacientes (FORLENZA, 2015).

Após a DA, a doença de Parkinson é a segunda doença neurodegenerativa mais comum, com aproximadamente 10 milhões de casos no mundo (BALL *et al.*, 2019). Sua patofisiologia é marcada pela presença de agregados de alfa-sinucleína na substância negra pars compacta, área do mesencéfalo com uma grande população de neurônios dopaminérgicos. A perda desses neurônios resulta na redução de dopamina nos núcleos da base, especialmente no corpo estriado, dando origem a manifestações motoras como bradicinesia, tremores, rigidez e distúrbios da marcha (AXELSEN; WOLDBYE, 2018). O corpo estriado, composto por estruturas denominadas núcleo caudado e putâmen, também é atingido na doença de Huntington, uma doença neurodegenerativa monogênica causada pelo

aumento do número de repetições do trinucleotídeo CAG no gene da huntingtina, que resulta em uma proteína mutante prejudicial (JIMENEZ-SANCHES *et al.*, 2017). Os principais tratamentos disponíveis atualmente para a DP incluem reposição de dopamina com levodopa e estimulação cerebral profunda; no entanto, essas terapias não impedem a progressão da doença e estão associadas a efeitos adversos como flutuações motoras e disartria (AXELSEN; WOLDBYE, 2018).

Em sua maioria, os casos de DA e DP são esporádicos e resultam de uma complexa associação entre fatores de risco genéticos e ambientais. Apenas 5–10% dos pacientes apresentam a forma familiar destas condições (DENG; WANG; JANKOVIC, 2018) (ECHEVERRY, Marcela *et al.,* 2018). Sendo assim, não há um alvo terapêutico específico para essas doenças neurodegenerativas – abordagens usualmente utilizadas incluem a administração de genes que codificam fatores promotores de sobrevivência neuronal ou enzimas envolvidas na síntese de neurotransmissores (OJALA; AMARA; SCHAFFER, 2015).

1.3 DOENÇAS LISOSSÔMICAS

As doenças lisossômicas (DL) são um grupo de aproximadamente 70 doenças metabólicas raras causadas por mutações em genes que codificam proteínas lisossomais. Como consequência da disfunção dessas proteínas, macromoléculas não degradadas se acumulam de forma gradual dentro dos lisossomos, resultando em manifestações multissistêmicas e progressivas como hepatoesplenomegalia, deformidades esqueléticas e comprometimento neurológico (PLATT et al., 2018). Atualmente, o tratamento disponível para algumas dessas condições é a terapia de reposição enzimática, na qual uma enzima recombinante análoga à enzima funcional é administrada via intravenosa no paciente periodicamente (MARCÓ; HAURIGOT; BOSCH, 2019). A principal limitação dessa abordagem é a inabilidade da enzima de atravessar a barreira hematoencefálica, não trazendo, portanto, melhora dos sintomas neurológicos (EDELMANN; MAEGAWA, 2020).

As lipofuscinoses ceróides neuronais (LCN), também conhecidas como doença de Batten, são exemplos de DL com envolvimento neurológico grave. Essas condições são caracterizadas por déficits motores, demência, crises convulsivas e perda de visão progressiva, resultando em morte prematura (JOHNSON *et al.*, 2019). As LCN diferem principalmente quanto à ordem de aparecimento dos sintomas e idade do paciente – ambos aspectos que dependem da mutação encontrada em um dos 13 genes associados. Em sua

maioria, os casos têm início durante a infância (MOLE et al. 2018). Declínio das funções motoras e crises convulsivas também são sintomas característicos da mucopolissacaridose tipo III (ou Síndrome de Sanfilippo), outra DL marcada pela neurodegeneração. As primeiras manifestações da doença ocorrem entre o primeiro e o quarto ano de vida principalmente como atraso no desenvolvimento (MARCÓ; HAURIGOT; BOSCH, 2019), seguido de hiperatividade, distúrbios do sono e déficit cognitivo (ZHOU *et al.*, 2020).

Uma vez que as DL são doenças monogênicas que afetam o sistema nervoso central como um todo, e não áreas específicas (MARCÓ; HAURIGOT; BOSCH, 2019), a principal estratégia de terapia gênica empregada é a administração da versão correta do gene mutado utilizando vias de injeção e vetores que apresentam ampla biodistribuição.

1.4 JUSTIFICATIVA

Considerando que condições neurodegenerativas têm um enorme impacto na qualidade de vida dos pacientes e suas famílias, e que os tratamentos disponíveis atualmente não são capazes de impedir a progressão da doença, a busca por novas estratégias terapêuticas se faz necessária. Estudos utilizando terapia gênica *in situ* para essas condições chegaram à fase clínica nos últimos anos e vêm aumentando consideravelmente. Dessa forma, uma revisão dos produtos de terapia gênica utilizados em ensaios clínicos até o momento reúne informações relevantes para a literatura da área e pode contribuir para o desenvolvimento de novas terapias.

1.5 OBJETIVOS

1.5.1 Objetivo geral

Apresentar os principais avanços clínicos da terapia gênica *in situ* para condições neurodegenerativas.

1.5.2 Objetivos específicos

a) Explorar os principais ensaios clínicos realizados até o momento em doenças neurodegenerativas e doenças lisossômicas

b) Abordar as especificidades dos vetores desenvolvidos a partir de vírus adeno-associados e lentivírus

c) Comparar vantagens e desvantagens das vias de administração intravenosa, intraparenquimal e via fluido cerebrospinal

2 ARTIGO CIENTÍFICO

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Título: *In situ* gene therapy for neurodegenerative disorders: a clinical overview

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In situ gene therapy for neurodegenerative disorders: a clinical overview

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Short Title: Brain in situ gene therapy

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Abstract

Background: Neurodegenerative conditions are difficult to treat in part due to the limited access to the brain imposed by the blood-brain barrier. As gene therapy emerged as a promising approach not only for monogenic disorders with neurological involvement but also for complex, multifactorial diseases such as Alzheimer's Disease and Parkinson's Disease, the delivery of genetic material *in situ* (i.e. directly into the central nervous system) presented an alternative to overcome this challenge. Such strategy led to the development of several gene therapy studies that are now reaching clinical phases. **Summary:** This review presents recent clinical progress in the field of *in situ* gene therapy for the central nervous system, focusing on neurodegenerative diseases and lysosomal storage disorders. Also, the main characteristics of viral vectors and administration routes employed are highlighted. Clinical studies were obtained from the ClinicalTrials.gov database in November 2020. Published data so far demonstrated a good safety profile, but efficacy results are yet to be established. **Key messages:** *In situ* gene therapy for the central nervous system is a field that reached clinical trials in recent years and most published results are from open-label trials. Advances in areas such as biomarkers investigation and vector engineering will certainly contribute to better outcomes.

Introduction

Neurodegenerative diseases are a group of debilitating disorders characterized by progressive loss of neurons and their synaptic connections, leading to neurological function decline [1]. Alzheimer's disease (AD) alone affects nearly 35 million people worldwide and represents an enormous burden in the quality of life of patients and their families, as well in health care costs [2]. Other common neurodegenerative disorders include Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). In most cases these conditions occur in a sporadic form and arise from multifactorial aspects, presenting a complex pathophysiology not yet completely understood [3]. Neurodegeneration is also a component of rare monogenic diseases and occurs in two-thirds of lysosomal storage disorders (LSDs). LSDs are caused by mutations in genes encoding lysosomal hydrolases, which results in the accumulation of substrates within lysosomes and ultimately, a multisystemic disease.

A major challenge in treating these neurological conditions is the limited access to the central nervous system (CNS) due to the blood-brain barrier (BBB). The available treatment for some LSDs, the intravenous enzyme replacement therapy, is unable to address the neurological symptoms as the enzymes cannot cross the BBB [4]. To overcome this, administration of therapeutic agents directly into the CNS is under evaluation as an alternative to intravenous, systemic delivery. Depending on the disease presentation, *in situ* delivery can be done into the brain parenchyma or the cerebrospinal fluid (CSF), using either intrathecal, intracerebroventricular, or intra-cisterna magna routes. Additionally, current therapies for neurodegenerative diseases and most LSDs cannot revert or stop disease progression, highlighting the need for novel therapeutic strategies.

Gene therapy emerged with the promise of treating diseases by delivering genetic material into the cells of a patient – mostly, the normal copy of a mutated gene [5]. While LSDs are ideal candidates to be treated using gene therapy since they are caused by single-gene mutations, neurodegenerative diseases face the additional challenge of not having a specific therapeutic target. Therefore, a commonly used strategy is to deliver genes that can support cell survival, such as neurotrophic factors [6, 7].

For gene therapy to be successful, the delivery vehicle (called vector) is also a key factor besides the therapeutic transgene and administration route. Viruses are widely used as vectors since they can efficiently introduce their genetic material into host cells. For therapeutic use, their wild-type genes that allow viral replication are replaced with the transgene of interest [8]. Regarding CNS application, the chosen vector must be capable of transducing non-dividing cells and provide stable, long-lasting transgene expression following a single treatment, as repeated administration is not feasible. For these reasons, adeno-associated viruses (AAVs) and lentiviruses are currently the preferred delivery vehicles for CNS gene therapy.

This review aims to present recent clinical progress in the field of *in situ* gene therapy for the CNS, focusing on neurodegenerative diseases and LSDs. Also, the main characteristics of viral vectors and delivery routes employed are highlighted.

Adeno-associated virus vectors

Adeno-associated virus (AAV) is a widely used vector in clinical trials targeting the central nervous system. AAV is a non-enveloped, single-stranded DNA virus with a 4.7 kb genome consisting of two terminal inverted repeats (ITRs) flanking the rep and cap genes [9].

For gene delivery, a recombinant AAV is produced by replacing these wild-type sequences with an expression cassette containing the therapeutic gene, while the ITRs are maintained to ensure packaging [10]. The cap and rep genes, which encode capsid subunits and proteins required for viral replication, respectively, are supplied in another plasmid (in trans), allowing vector formation [9]. AAV has the ability to remain in an episomal form in the transduced cells, leading to long-term transgene expression with a very low rate of genomic integration [10].

Based on their capsid proteins, AAVs are classified into serotypes. Each serotype has a different pattern of cell transduction (i.e. tropism), determined by interactions of the viral capsid with host cells receptors. For example, when delivered into the brain parenchyma, AAV1, 2, 9 and rh.10 are highly neurotropic, while AAV5 transduces mainly neurons, but also astrocytes to some extent, and AAV4 targets ependymal cells [11, 9]. In particular, serotypes 9 and rh.10 exhibit widespread transduction within the CNS, as a consequence of their ability to travel along axons with high efficiency (discussed below) [11]. These two serotypes are also capable of crossing the BBB following intravenous injection, leading to the possibility of reaching the brain without invasive procedures. Considering the physiological changes of the blood-brain barrier during development [3], the timing of AAV delivery represents another factor involved in vector distribution – while IV administration of AAV9 in neonatal mice resulted in extensive neuronal transduction, including motor neurons, in adult animals the expression was limited to astrocytes [12].

AAV2 was the first serotype used for *in vivo* gene delivery, following almost three decades of research in its basic biology [13]. As a result of this extensive work, serotype 2 was the vector of choice in early clinical trials, and until today the AAV2 genome is commonly used in a method called pseudotyping, where a hybrid AAV is generated by packing the ITRs from one serotype into the capsid of another. The AAV2 ITRs combined with the AAV5 capsid, resulting in a vector denominated AAV2/5, demonstrates greater distribution in the brain than the AAV2/2 vector [14], which is particularly relevant when targeting diseases that affect multiple CNS areas.

In addition, AAV capsids can be engineered to present novel features that are desirable for clinical application. These modifications are generated by two main strategies: rational design and directed evolution. In rational design, specific amino acid residues are modified based on a previous understanding of capsid structure and its interactions [15]. For example, the finding that phosphorylation of surface tyrosines prevents the virion from reaching the nucleus led to the development of an AAV2 vector with phenylalanine residues instead of

tyrosine, which resulted in improved transduction efficiency [16]. A version of this vector is currently under clinical evaluation for an ocular condition (NCT02416622). Alternatively, the directed evolution approach involves the construction of large libraries of capsid mutants, followed by rounds of selective pressure to isolate variants with the property of interest (for example, the ability to evade immune responses) [17].

In summary, AAV vectors are the leading choice for CNS gene therapy as they are nonpathogenic, mediate stable transgene expression in non-dividing cells, and can be designed to improve their performance for specific therapeutic needs. Beyond that, results from clinical trials have demonstrated that AAV-mediated gene delivery has a good safety profile. There are, however, a few setbacks in the use of such vectors. Besides their limited carrying capacity of about 5 kb of genetic material [8], part of the human population has pre-existing immunity against AAVs due to natural exposure. An estimated 72% of healthy adults present neutralizing antibodies against AAV2, while for AAV9 a prevalence of 47% was observed [19]. As immune responses against the viral capsid can highly impact *in vivo* transduction [20], seropositive patients are often excluded from many clinical trials.

Lentiviral vectors

Lentivirus belongs to the retroviruses family and, as such, has an enveloped, single-stranded RNA genome. Recombinant lentiviral vectors (LV) are usually constructed from the human immunodeficiency virus type 1 (HIV-1) or the non-primate equine infectious anemia virus (EIAV) [7] by placing the therapeutic expression cassette between the long terminal repeats (LTRs), while the genes necessary for virion production (gag, pol, env, and rev) are provided in helper plasmids and accessory genes are entirely removed [21]. LV can efficiently integrate into the host genome, allowing long-term transgene expression, and in contrast to other retroviruses, can infect non-dividing cells. As integration holds the possibility of insertional mutagenesis, self-inactivating vectors were designed to overcome this safety issue. These third-generation vectors lack viral promoter and enhancer sequences in their LTRs, relying on an internal promoter to initiate transcription – thereby, the risk of upregulating nearby genes is diminished, as well as potential interference between promoters [22].

Moreover, lentiviruses can be pseudotyped similarly to AAVs, though in this case the tropism is determined by the envelope proteins instead. The most commonly used envelope for LV pseudotyping is the vesicular stomatitis virus glycoprotein (VSV-G), as it broadens the

lentiviral tropism to almost all mammalian cells and presents high stability [23]. Besides the aforementioned features that set lentivirus as a suitable vector for CNS gene therapy, its packaging capacity of 8-9 kb [8] is a major advantage over AAVs.

Administration routes

The finding that AAV9 can cross the BBB opened the possibility of reaching the CNS in a non-invasive manner. Systemic delivery of AAV9 is already FDA approved for the treatment of Spinal Muscular Atrophy type 1, a monogenic disease that leads to death in early infancy due to loss of motor neurons and muscle weakness [24]. There are a few issues that hamper intravenous (IV) delivery though. Systemic injection requires a high vector dose to achieve a therapeutically relevant concentration in the CNS and thus can result in off-target tissue transduction and toxicity, besides exposing the vector to neutralizing antibodies [25].

To circumvent these issues, alternative routes of delivery based on injection directly into the brain parenchyma or the cerebrospinal fluid (CSF) are being investigated (shown in fig. 1). Intraparenchymal administration is widely employed in clinical trials, being particularly useful in disorders that affect limited brain areas, such as Parkinson's Disease (PD) [26]. Currently, MRI-guided convection-enhanced delivery (CED) is the gold standard technique to efficiently infuse vectors into the brain. CED promotes infusate flow within the interstitial fluid by establishing a pressure gradient through a cannula placed in the targeted area [27]. This procedure, combined with MRI (magnetic resonance imaging), allows precise targeting and real-time monitoring of distribution, ensuring that the infusate did not reflux to the cannula track or leaked into surrounding areas [27, 28]. Although the invasiveness of the procedure, direct administration into the parenchyma provides high local transgene expression with low viral dose [25]. Distribution can be further amplified as a result of axonal transport, in which vector particles can travel along axons and transduce beyond the injection site [26]. Anterograde transport involves uptake of vectors by cell bodies, intact transport along axons, and transduction in distal cells; while viral particles that go under retrograde transport are captured by axonal terminals and transported back to the cell body, where the transgene expression occurs [29, 27]. The axonal transport is highly dependent on the AAV serotype [30].

Upon CSF delivery, it comprises intrathecal (IT), intracerebroventricular (ICV), and intra-cisterna magna (ICM) routes. Lumbar IT injection is a less invasive, commonly used method in the clinic, and its application in gene transfer leads to widespread spinal cord

transduction, including motor neurons and dorsal root ganglia [3]. However, distribution to the brain is limited possibly due to dilution of the infusate and fluid dynamics within the CSF. When administered into the lateral ventricles, the vector moves in favor of the flow, resulting in broader CNS transduction [3]. Suboccipital injection into the cisterna magna also presents a good distribution pattern, yet the risk of medullary injury drawbacks its implementation in clinical practice [26,3].

Clinical trials for neurodegenerative diseases

Clinical trials for neurodegenerative diseases are summarized in Table 1. Alzheimer's Disease is characterized by the accumulation of misfolded proteins in the form of amyloid plaques and neurofibrillary tangles, leading to loss of cholinergic neurons and impairment in memory, cognition and behavior, ultimately resulting in dementia. The mainstay treatment, cholinesterase inhibitors, can only provide symptomatic relief for some patients [31].

The first gene therapy strategy to reach clinical trials was the delivery of nerve growth factor (*NGF*), an endogenous peptide with neuroprotective effects. Patients with mild to moderate AD received the *NGF* gene packaged into an AAV2 vector through bilateral stereotactic injections targeting the nucleus basalis of Meynert – a basal forebrain area that is the primary source of cholinergic input to the cerebral cortex (NCT00087789) [32]. Even though no benefit on cognition was observed after 24 months in the phase II study (NCT00876863), the procedure was safe and the research team confirmed the viability of sham-surgery control. The efficacy results may have been influenced by vector mistargeting, as no technique such as MRI-CED was employed to verify adequate delivery [33].

A more recent trial proposes intracisternal administration of AAVrh.10 coding *APOE2* (human apolipoprotein 2) in AD patients that are *APOE4* homozygotes (NCT03634007). It was hypothesized that the expression of the *APOE2* variant would play a protective role in these individuals, as carriers of two *APOE4* alleles have an increased risk to develop AD [34]. Another study that is currently recruiting participants proposes the expression of telomerase to treat age-related diseases, including AD. This novel trial aims to lengthen telomeres using AAV-hTERT administered both intravenously and intrathecally (NCT04133454). Intrathecal gene transfer was initially performed in an AAV9 trial for giant axonal neuropathy (GAN), a rare monogenic neurodegenerative disorder presented in early childhood (NCT02362438).

Parkinson's Disease (PD) is a synucleinopathy characterized by the progressive loss of dopaminergic neurons in the substantia nigra, leading to reduced levels of dopamine in the

striatum and then impairment in motor and cognitive function. Levodopa is the first therapy choice for symptom relief in PD, however, as the disease progresses, medication efficacy diminishes and patients experience dyskinesias and motor fluctuations. Gene therapy for PD has focused mainly on two strategies: provide neurotrophic support to the neurons or increase neurotransmitter synthesis [35].

One of the first efforts towards PD was to deliver the glutamic acid decarboxylase (*GAD*) gene into the subthalamic nucleus using AAV2 (NCT00195143). *GAD* is an enzyme involved in the synthesis of GABA, an inhibitory neurotransmitter that can diminish subthalamic hyperactivity and thus alleviate PD symptoms. Results of the phase II trial indicated improvement in motor scores in the AAV2-GAD group compared with the sham-surgery group for up to 12 months [36].

Delivery of the gene encoding L-amino acid decarboxylase (*AADC*), the enzyme that converts levodopa into dopamine, was performed using MRI-CED to monitor the distribution of AAV2 particles within the putamen (NCT01973543). It resulted in an enhanced response to levodopa at 6 and 12 months [37], and a randomized, sham-surgery controlled phase II study is currently ongoing to further evaluate VY-AADC efficacy (NCT03562494). Taking advantage of the ability of lentiviral vectors to carry larger payloads, Prosavin encodes not only *AADC* but also two other key enzymes in dopamine synthesis, tyrosine hydroxylase (*TH*) and GTP-cyclohydrolase 1 (*CH1*) (NCT00627588) [18]. Data from an 8-year follow-up indicates long-term safety and sustained motor improvements, however, the effects are within the placebo range described in other trials (NCT01856439) [38]. Also, there was an indication that the dopamine level achieved was suboptimal, even in the higher dose cohort. For that reason, a vector with an optimized expression cassette was developed and is currently under evaluation in a phase I/II trial (NCT03720418) [39]. A lentiviral vector is also being assessed for X-adrenoleukodystrophy, a genetic peroxisomal disorder that leads to demyelination and adrenal complications (NCT03727555).

Regarding the neurotrophic support strategy, putaminal delivery of AAV2 encoding the glial cell-derived neurotrophic factor (*GDNF*) is under evaluation in two open-label phase I trials for PD (NCT01621581, NCT04167540). A structural and functional analogue of GDNF, neurturin, was assessed in an earlier AAV2-mediated therapy (NCT00252850). Preliminary results suggested motor function improvement at 1 year following stereotactic injections into the putamen [40], however, evidence of efficacy was not maintained when the trial advanced to a randomized, double-blinded, sham surgery-controlled phase [41]. Also, a recent clinical trial is addressing the most important known genetic factor for PD, mutations

in the GBA1 gene [42]. The study aims to evaluate intracisternal administration of an AAV9 vector encoding for the lysosomal enzyme beta-glucocerebrosidase (NCT04127578).

And finally, an RNA interference-based therapy is being tested for the first time in humans for Huntington's Disease. Delivered into the striatum, the AMT-130 gene therapy encodes a micro-RNA designed to silence the huntingtin gene, which carries the CAG trinucleotide repeat expansion that causes the disease (NCT04120493) [43].

Clinical trials for lysosomal storage disorders

Clinical trials for LSDs are summarized in Table 2. LSDs are a group of rare monogenic diseases characterized by intracellular accumulation of substrates, as a result of a deficiency in lysosomal enzymes. Therapies for these conditions can rely on the "cross-correction mechanism", which allows lysosomal enzymes secreted from transduced cells to be internalized by non-modified cells in the neighborhood, thus achieving therapeutic benefit even with a small percentage of correction [44].

The mucopolysaccharidosis (MPS) are a subset of LSDs in which the metabolism of glycosaminoglycans is impaired. While most MPS exhibit severe multisystemic clinical manifestations, MPS III, also known as Sanfilippo syndrome, presents mainly neurological dysfunctions already at an early age [45]. Gene therapy for MPS III type A aims to deliver the N-sulfoglycosamine sulfohydrolase (SGSH) gene. An open-label trial conducted by Lysogene also delivered the gene encoding a sulfatase-modifying factor (SUMF1), that plays a role in activating the catalytic site of SGSH (NCT01474343). Both genes were carried into an AAVrh10 vector injected into the brain parenchyma and published results indicate safety of the procedure and moderate cognitive benefit [46]. Currently, a multi-center phase II/III trial is evaluating the delivery of the SGSH gene alone (NCT03612869). For Sanfilippo type B syndrome, intraparenchymal administration of an AAV5 vector that encodes alpha-acetylglucosaminidase proved to be safe and led to improvement in cognitive scores only in the younger patient, a 20-month old child (NCT03300453) [47]. As MPS I and MPS II also present CNS manifestations [4], two dose-escalating studies are assessing safety of intracisternal injection of an AAV9 vector (NCT03580083, NCT03566043). The goal is to deliver the alpha-L-iduronidase gene or the iduronate-2-sulfatase gene for patients with MPS I or MPS II, respectively.

Other LSDs, such as the late infantile neuronal ceroid lipofuscinosis (LINCL), also reached clinical trials. This form of Batten Disease is characterized by deficiency in the

tripeptidyl peptidase-I (TPP-I) enzyme, which leads to neurodegeneration and death by the age of 8 to 12 years [48]. Reported data of an open-label study indicated slowing of disease progression after six months of intraparenchymal injection of an AAV2-CLN2 vector (NCT00151216) [49]. At the moment, AAVrh10-mediated delivery of CLN2 is under evaluation (NCT01161576). Moreover, Amicus Therapeutics launched two trials proposing intrathecal administration of an AAV9 vector encoding the CLN3 gene or the CLN6 gene in patients with other forms of Batten Disease (NCT03770572, NCT02725580). No results are available yet.

At last, a single trial is evaluating lentiviral vectors for *in situ* treatment of a LSD. The study aims to deliver the gene encoding arylsulfatase A (ARSA) for patients with metachromatic leukodystrophy, a disease characterized by severe demyelination due to substrate accumulation in Schwann cells, oligodendrocytes, and neurons (NCT03725670).

Conclusions

In situ gene therapy for the central nervous system is a field that reached clinical trials in recent years. A good safety profile was established for administration routes and viral vectors, yet most published data so far are from open-label studies and therefore efficacy results should be considered carefully. Late diagnosis of neurodegenerative conditions is an important setback, as intervention at advanced stages of neurodegeneration may have minimal or no therapeutic effect. In this sense, the identification of biomarkers may lead to improved outcomes and contribute to unbiased assessment of open-label studies. Also, especially for AD and PD, a better understanding of disease pathophysiology will certainly uncover new therapeutic targets. Finally, AAV vector engineering holds a great promise in the field, as highly desirable features for clinical application can be added, such as antibody evasion.

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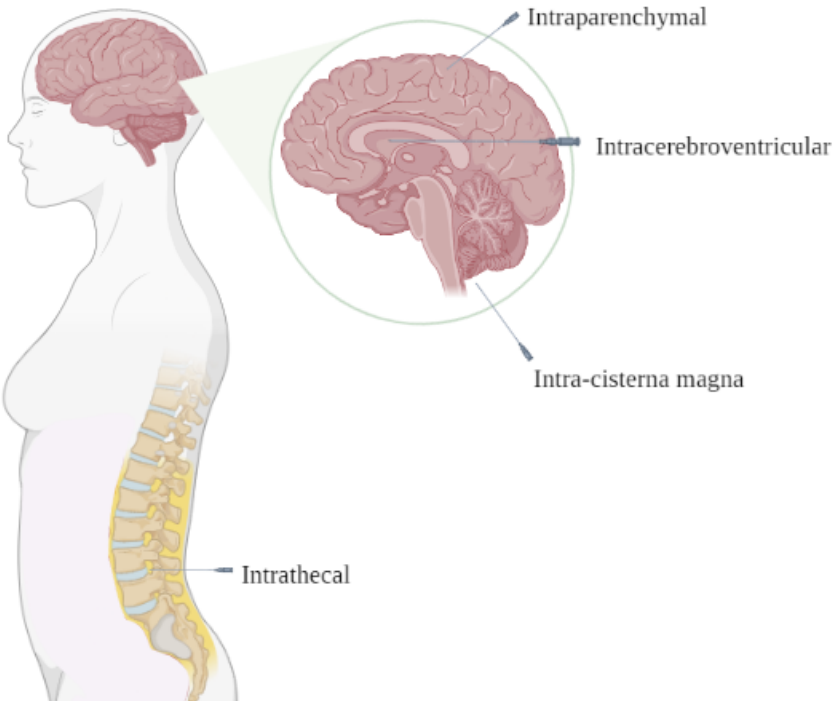

Figure 1. Overview of *in situ* delivery into the central nervous system. Vectors can be introduced directly into the brain parenchyma or the cerebrospinal fluid (CSF), via intrathecal, intracerebroventricular, or intra-cisterna magna routes.

Table 1. Clinical trials for neurodegenerative diseases

ID number	Status	Disease	Gene therapy	Genes	Vector	Administration route
NCT00087789	Completed	AD	CERE-110	*NGF*	AAV2	Intraparenchymal (nucleus basalis of Meynert)
NCT03634007	Recruiting	AD	LX1001	*APOE2*	AAVrh.10	Intracisternal
NCT04133454	Recruiting	AD	Libella Gene Therapy	*hTERT*	AAV	Intrathecal and intravenous
NCT00195143	Completed	PD		*GAD*	AAV2	Intraparenchymal (subthalamic nucleus)
NCT00252850	Completed	PD	CERE-120	*NTN*	AAV2	Intraparenchymal (putamen)
NCT00627588	Completed	PD	ProSavin	*AADC, TH, CH1*	Lentiviral	Intraparenchymal (putamen)
NCT01973543	Completed	PD	VY-AADC01	*AADC*	AAV2	Intraparenchymal (putamen)
NCT01621581	Active, not recruiting	PD		*GDNF*	AAV2	Intraparenchymal (putamen)
NCT04167540	Recruiting	PD		*GDNF*	AAV2	Intraparenchymal (putamen)
NCT04127578	Recruiting	PD	PR001A	*GBA1*	AAV9	Intracisternal
NCT04120493	Recruiting	HD	AMT-130	*miHTT*	AAV5	Intraparenchymal (striatum)
NCT02362438	Recruiting	GAN		*GAN*	AAV9	Intrathecal
NCT03727555	Recruiting	X-ALD		*ABCD1*	Lentiviral	Intraparenchymal

Abbreviatures: AADC, L-amino acid decarboxylase; AAV, adeno-associated virus; AD, Alzheimer's Disease; APOE2, apolipoprotein E2; CH1, cyclohydrolase 1; GAD, glutamic acid decarboxylase; GBA1, beta-glucocerebrosidase; GDNF, Glial Cell Line-Derived Neurotrophic Factor; HD, Huntington's Disease; hTERT, human telomerase reverse transcriptase; miHTT, microRNA targeting huntingtin mRNA; NGF, nerve growth factor; NTN, neurturin; PD, Parkinson's Disease; TH, tyrosine hydroxylase

Table 2. Clinical trials for lysosomal storage disorders

ID number	Status	Disease	Gene therapy	Genes	Vector	Administration route
NCT00151216	Completed	Batten Disease		*CLN2*	AAV2	Intraparenchymal
NCT01161576	Completed	Batten Disease		*CLN2*	AAVrh.10	Intraparenchymal
NCT02725580	Active, not recruiting	Batten Disease	AT-GTX-501	*CLN6*	AAV9	Intrathecal lumbar
NCT03770572	Active, not recruiting	Batten Disease	AT-GTX-502	*CLN3*	AAV9	Intrathecal lumbar
NCT03580083	Recruiting	MPS I	RGX-111	*IDUA*	AAV9	Intracisternal
NCT03566043	Recruiting	MPS II	RGX-121	*IDS*	AAV9	Intracisternal
NCT01474343	Completed	MPS IIIA	SAF-301	*SGSH and SUMF1*	AAVrh.10	Intraparenchymal
NCT03612869	Active, not recruiting	MPS IIIA	LYS-SAF302	*SGSH*	AAVrh.10	Intraparenchymal
NCT03300453	Completed	MPS IIIB		*NAGLU*	AAV5	Intraparenchymal
NCT03725670	Recruiting	MLD		*ARSA*	Lentiviral	Intraparenchymal

Abbreviatures: ARSA, arylsulfatase A; CLN, neuronal ceroid lipofuscinoses; GLB1, beta-galactosidase; IDS, iduronate-2-sulfatase; IDUA, α-L-iduronidase; MLD, Metachromatic leukodystrophy; MPS, mucopolysaccharidosis; NAGLU, alpha-N-acetylglucosaminidase; SGSG, N-sulfoglycosamine sulfohydrolase; SUMF1, sulfatase-modifying factor

3 CONCLUSÕES E PERSPECTIVAS

Os estudos de terapia gênica *in situ* para o sistema nervoso central têm chegado à fase clínica nos últimos anos. Os ensaios concluídos até o momento tiveram sucesso em demonstrar a viabilidade dos procedimentos e sua segurança, um dos principais objetivos dessa fase clínica inicial principalmente devido à realização de procedimentos invasivos. Em sua maioria, os resultados publicados são de ensaios abertos e, portanto, os dados de eficácia são considerados preliminares. Embora alguns deles já se mostrem promissores, é provável que mais alguns anos sejam necessários para a efetiva aprovação desses produtos de terapia gênica, uma vez que períodos prolongados são necessários para avaliar se o efeito terapêutico é sustentado.

A eficácia de tratamentos para condições neurodegenerativas muitas vezes é prejudicada pelo diagnóstico tardio dessas doenças, uma vez que em estágios avançados de neurodegeneração as intervenções podem ter seu efeito terapêutico bastante reduzido. Nesse sentido, a identificação de biomarcadores pode levar a melhores desfechos e ainda contribuir para avaliações menos subjetivas nos ensaios clínicos. Por fim, o desenvolvimento de novos vetores adeno-associados com características aprimoradas para o uso clínico (como evasão do sistema imune) é uma grande promessa para melhores resultados terapêuticos.

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ANEXO A – NORMAS DE PUBLICAÇÃO DA REVISTA NEURODEGENERATIVE DISEASES

Review Article

Review Articles are considered reviews of research or summary articles. They are state-of-the-art papers covering a current topic by experts in the field. They should give evidence on and provide answers to a well-defined aspect or question in a particular area. Review Articles must include a critical discussion of the reported data and give a clear conclusion with potential impacts on the standard of care.

Article Preparation

The preferred word processing program for manuscripts is Microsoft Word. Page and line numbering should be activated, and the level of subheadings should be indicated clearly.

Footnotes should be avoided. When essential, they should be numbered consecutively and appear at the foot of the appropriate page.

Abbreviations (with the exception of those clearly well established in the field) should be explained when they are first used both in the abstract and in the main text.

The manuscript, tables, figures, and Submission Statement must be submitted in separate files.

Title Page

The first page should contain a short and concise title plus a running head of no more than 80 characters. Abbreviations should be avoided.

Below the title, list all the authors' names as outlined in the article sample, which can be downloaded under Article Types. Each listed author must have an affiliation, which comprises the department, university, or organization and its location, city, state/province (if applicable), and country.

Place the full postal address of the corresponding author at the bottom of the first page, including at least one telephone number and e-mail address

Keywords relevant to the article should be listed below the corresponding author information.

Abstract

Abstract should summarize the main points and reflect the content of the article. It should be written in a clear and concise way and be structured using the following subheadings: Background, Summary, and Key Messages. Abbreviations used in the main text may be introduced and used. Use neither bibliographic references nor references to figures or tables in the Abstract.

Please refer to the Author Guidelines for more information about the maximum accepted word count of the Abstract in your chosen journal. Where no specific word count is provided, an abstract of between 200‑400 words is permitted.

Figures should be mentioned in the manuscript text as follows:

Without round brackets: "…shown in Figure 1…" or "…shown in Figures 1 and 4…" or "…shown in Figures 2–6…" always with capital letters and written out.

With round brackets: "(shown in Fig. 1)" or "(shown in Fig. 1, 4)" or "(shown in Fig. 2–6)", always abbreviated as "Fig." followed by the number or numbers after a full stop and a space.

In the Legend: "Fig. 1." or "Fig. 1. a", always abbreviated as "Fig." followed by the number after a full stop and a space

Please note that the actual figures and all tables should be uploaded as separate items in their original file format.

References

References in the text should be identified using Arabic numerals [in square brackets]. References should be listed using the Vancouver style. The reference list should include only those publications which are cited in the text, arranged numerically in the order in which they are cited. The authors' surnames should be followed by their initials with no punctuation other than a comma to separate individual authors. A maximum of 6 authors should be listed (followed by "et al." if there are more than 6 authors). Material submitted for publication but not yet accepted should be referred to as "unpublished data" and should not be included in the reference list.

Statements

All papers must contain the following statements after the main body of the text and before the reference list:

Conflict of Interest Statement: Authors are required to disclose any possible conflicts of interest. All forms of support and financial involvement (e.g. employment, consultancies, honoraria, stock ownership and options, expert testimony, grants or patents received or pending, royalties) which took place in the previous three years should be listed, regardless of their potential relevance to the paper. Also the nonfinancial relationships (personal, political, or professional) that may potentially influence the writing of the manuscript should be declared. If there is no conflict of interest, please state: “The authors have no conflicts of interest to declare.”

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