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GENE THERAPY IN REFRACTORY EPILEPSY

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Abstract

Epilepsy is one of the most common neurological disorders affecting a significant portion of the world population. Approximately 30% of epilepsy cases are not effectively treated with anti-seizure medications (ASMs), leading to pharmacoresistant epilepsies, which affect up to 0.3% of the global population. Current therapies available to patients with refractory epileptic seizures include electrical stimulation, or surgery, with approximately two-thirds of patients achieving a seizure-free state. Gene therapy emerges as a novel and innovative approach, presenting a paradigm shift in the treatment landscape. Various treatment strategies tested in in vivo models are discussed, including overexpression of potassium channels, kainate receptor downregulation using RNA interference and others. As the field progresses, ongoing research and upcoming clinical trials are expected to contribute to our understanding of gene therapy's role in revolutionising the management of pharmacoresistant epilepsy.

KEYWORDS: Adeno-associated virus (AAV), gene therapy, Lentivirus, neurological disorders, refractory epilepsy, temporal lobe epilepsy (TLE), viral vectors

INTRODUCTION

Epilepsy affects around 50 million people worldwide[1] making it one of the most common neurological disorders. Approximately 30% of epilepsy cases are not effectively treated with anti-seizure medications (ASMs),[2] leading to pharmacoresistant epilepsy, which affects up to 0.3% of the world population.[3] Patients with refractory epilepsy experience 4-7 times higher mortality rates and an increased risk of injury compared to those with medication-controlled epilepsy.[4] Additionally, this population exhibits higher unemployment rates and lower psychosocial abilities.[4] Current therapies available to patients with refractory epilepsy include electrical stimulation as a palliative method7, with mean reduction in seizure frequency ranging from 45-65%,[5] or epilepsy neurosurgery, with approximately two-thirds of patients achieving a seizure-free state.[6] However, not every case of refractory epilepsy can be addressed by neurostimulation or neurosurgery[7], and the outcomes may not always lead to controlled epilepsy. This suggests a need for a more efficient strategy to combat refractory epilepsy. In this paper, gene therapy will be discussed as a potential treatment for refractory epilepsy.

GENE THERAPY STRATEGIES

The European Medicines Agency (EMA) defines that a gene therapy medicinal product is a biological medicinal product which fulfils the following two characteristics: "(a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence; (b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence."[9] The first concepts of using clinical-grade viruses for therapeutic gene transfers emerged in the early 1970s.[8] Decades of research on genetic processes in cells, their relationship to disease, and the potential side effects of treatments have led to the approval of 10 gene therapies by the EMA as of January 1, 2022.[10] Additionally, numerous others are currently in clinical trials, including two gene therapies for refractory epilepsy.[11,12]

While nonviral delivery systems for gene therapy exist,[13] current research on gene therapy in epilepsy primarily relies on viral delivery systems. Two therapies awaiting clinical trials utilise the adeno-associated virus (AAV) vector and Lentivirus as vehicles to transfer genes into targeted cells.[11,12]

The strategy of gene therapy in treating epilepsy differs from the 'most intuitive' use of gene therapy, which involves using vectors to transfer 'healthy' copies of a gene to cells with pathological variants or missing genes. In focal epilepsy, a consistent group of neurons with increased intrinsic activity initiates seizures. Specifically, the most common form of refractory focal epilepsy is temporal lobe epilepsy (TLE).[2] The goal of using gene therapy in focal epilepsy is to achieve a reduced intrinsic excitability of epileptogenic zones. This can be accomplished in various ways: by encoding neuropeptide Y (NPY), utilising microRNA silencing technology, overexpressing potassium channels or by employing gene expression mechanisms to up-regulate potassium channel expression.[16]

NPY GENE THERAPY

NPY is a 36-amino acid peptide expressed throughout the central and peripheral nervous system and has a modulatory effect on the excitability of neurons, among other functions.[17] When expressed in the hippocampus, NPY displays an inhibitory action on glutamate-releasing neurons.[18] Further research showed that to achieve an inhibitory effect in the hippocampus, NPY is required to bind to Y2 receptors located on the presynaptic membrane of granule cells, inhibiting calcium-mediated release of glutamate, thus providing an anti-epileptic effect.[19] Research by Woldbye et al. from 2010 has shown that the most promising strategy for using NPY in epilepsy gene therapy is to develop a combinatorial gene delivery of both NPY and Y2 receptor genes.[19] Nikitidou Ledri et al. in 2016 attempted to deliver NPY and Y2 genes using two separate AAV vectors,[20] and researchers Melin et al. in 2019 tested gene delivery of both genes in a single vector,[21] both resulting in a remarkable decrease in electroencephalogram (EEG) seizure frequency and duration in the kainic acid model of TLE in rats. Gene therapy regarding NPY requires further research on regulating the secretion of NPY and the expression of Y2 receptors in pathological and physiological processes of neurons.[17] However, there is currently only one licensed AAV gene therapy product that utilises NPY, named CG01 and owned by Spark Therapeutics. Nevertheless, the company has not announced any clinical trials for this product.[22]

MODIFYING KAINATE RECEPTOR EXPRESSION

Researchers backed by the world gene therapy leader UniQure are currently developing a potential treatment for TLE in which seizures are generated in the limbic system, such as the hippocampus.[15] Pathological network organisation has been discovered in the hippocampus, containing excitatory circuits which include dentate granule cells (DGC) with postsynaptic kainate receptors (KARs) which are in part responsible for recurrent and chronic seizures in TLE.[23] Previous research has demonstrated that reducing the expression of GluK2/GluK5-containing kainate receptors (KARs) in dentate gyrus cells (DGC) reduces chronic epileptic discharges.[23] The approach of this therapy involves using RNA interference to downregulate GluK2 expression in the human hippocampus via the translational repression of grik2mRNA, thereby reducing epileptic activity. The latest published research by Boileau in 2023 showed that this AAV9-mediated gene therapy reduced interictal-like epileptiform discharges (IEDs) in hippocampal slices derived from patients with TLE.[15]

SELF-REGULATED OVEREXPRESSION OF POTASSIUM CHANNELS

In 2019, a research group led by Snowball at University College London (UCL) decided to address the issue of increased excitability in focal epilepsy by inserting a gene encoding the voltage-gated potassium channel Kv1.1 in target neurons within the epileptogenic zone. The study was conducted on rats with chemically induced focal epilepsy originating in the hippocampus. This channel was engineered to have a short inactivation period, thereby decreasing the intrinsic excitability of target cells. The engineered potassium channel is under the control of a cell type-specific CAMK2A promoter and is packaged in a non-integrating lentiviral vector. This approach helps mitigate the risk of insertional mutagenesis caused by using viral gene therapy.[3]

In 2022, Qiu et al. affiliated with UCL presented another gene therapy strategy designed to down-regulate the excitability of overactive neurons in a closed loop. This innovative approach utilises an early gene promoter that responds to neuronal activity to drive the expression of potassium channels. In essence, neurons exhibiting increased activity, such as in seizure activity, are genetically engineered to express more potassium channels, thereby reducing their activity. Once seizures resolve, the genetic therapy switches itself off until excessive activity occurs again.[16] Tested in rodent models, the research demonstrated a greater decrease in seizure frequency than previously reported for gene therapy with constitutive promoters or with the widely used ASM,[16] highlighting significant potential for this strategy.

BENEFITS AND LIMITATIONS OF DIFFERENT GENE THERAPY STRATEGIES

Since clinical trials regarding viral gene therapy in refractory epilepsy are yet to begin,[11,12] this novel approach requires discussion about potential risks and how to mitigate them. The main feature of this type of therapy is that gene insertion into the cell genome cannot be reversed. While this feature is favourable for target cells with initiating seizure activity, viral delivery to undesired neurons could result in decreased intrinsic excitability of key parts of the brain, such as the hippocampus.

Furthermore, gene insertion by a viral vector poses a risk of insertional mutagenesis. This challenge could be solved by following the example of researchers Morris and Schorge at UCL using a non-integrating lentiviral vector where genes are still expressed but not integrated in the cell genome or by using low-risk AAV vectors.[2] To specify gene expression only in target cells, researchers have also developed cell-specific promoters that prevent the gene encoding potassium channels from being expressed in non-target cells.[3]

Along with cell-specific promoters, the expression of inserted genes should involve a mechanism to regulate when these genes are expressed. Early gene promoters that activate during increased neuron activity are employed to address this issue in in vivo models.[16] Additionally, a theory utilising exogenous ligands has been proposed, necessitating the use of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs).[2] In this theory, an engineered ligand-gated channel would be expressed in target cells only when an exogenous ligand (taken orally, for example) is present. However, this approach would require non-ideal adherence to daily medication. Therefore, an approach using activity-dependent promoters seems more favourable and future efforts should focus on refining targeting strategies and conducting thorough safety evaluations before widespread clinical use.

The high costs of developing and delivering gene or stem cell therapy currently restrict its accessibility, especially in low- and middle-income countries. Future research should also focus on finding ways to reduce costs and ensure equitable access to all novel gene therapies for patients globally. The permanence of gene therapy poses a significant hurdle when testing in humans. If the therapy proves ineffective or causes unwanted effects, it cannot be reversed. The ethical implications of modifying an individual's genes, especially for neurological disorders, require careful consideration. Moving forward, it is crucial to have open and transparent discussions with patients about the potential risks and benefits. To make testing in humans more acceptable, starting with phase 1/2a trials is proposed.[2] These trials would enrol patients meeting the criteria for refractory epilepsy, for whom the last line of treatment currently would be neurosurgery to remove the region with initial seizure activity. In the event of unsuccessful gene therapy, patients would still have the option to undergo the surgical procedure.[11,12]

Gene therapy in refractory epilepsy has not been tested in humans to date; however, according to the International Clinical Trial Registry Platform (ICTRP), a phase 1/2a first-in-human study by uniQure France has commenced enrolment in November 2023.[12] Another phase 1/2a study by UCL is anticipated to begin in September 2024.[11]

CONCLUSION

Research has shown the promising role of gene therapy as a potential treatment for refractory epilepsy. Current gene therapy approaches mainly involve manipulating gene expression (voltage-gated potassium channels, KARs) or insertion of genes encoding certain molecules (such as NPY) to suppress abnormal neuronal activity in affected regions in TLE. The limitations of current therapeutic options, such as medication and surgical interventions, underscore the urgent need for more effective strategies. As the field progresses, ongoing research and upcoming clinical trials will undoubtedly contribute to our understanding of gene therapy's role in revolutionising the management of this complex neurological disorder and, hopefully, other brain-circuit disorders.

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GENSKA TERAPIJA REFRAKTORNE EPILEPSIJE

Sažetak

Epilepsija je jedan od najčešćih neuroloških poremećaja od kojeg obolijeva značajan dio svjetske populacije. Otprilike 30% slučajeva epilepsije nije učinkovito liječeno antiepileptičkim lijekovima, te farmakorezistentni oblici zahvaćaju do 0.3% globalne populacije. Trenutačne terapije dostupne pacijentima s refraktornim epileptičkim napadajima uključuju električnu stimulaciju ili kirurški zahvat, od kojih dvije trećine pacijenata nakon zahvata postiže stanje bez epileptičkih napadaja. Genska terapija novi je i inovativni pristup, predstavljajući novu mogućnost u liječenju. Razmatraju se različite strategije liječenja ispitane na in vivo modelima, uključujući prekomjernu ekspresiju kalijevih kanala, smanjenje izraženosti kainatnih receptora uporabom RNA interferencije i druge. Kako se područje razvija, očekuje se da će trenutna istraživanja i nadolazeća klinička ispitivanja doprinijeti našem razumijevanju uloge genske terapije u liječenju farmakorezistentne epilepsije.

KLJUČNE RIJEČI: genska terapija, Lentivirus, neurološki poremećaji, refraktorna epilepsija, epilepsija temporalnog režnja (TLE), virus povezan s adenovirusima (engl. AAV), virusni vektori