Hacking the Brain: Gene Therapy as a Tool to Combat Epilepsy
INTRODUCTION

Epilepsy is a brain disorder predominantly characterized by recurrent and unpredictable seizures.\(^1\) Generalized epilepsy with febrile seizures plus (GEFS+) is a subtype of autosomal dominant epileptic syndromes characterized by over 90 mutations of the sodium channel voltage gated type 1 alpha subunit (SCN1A) gene.\(^2,3\) GEFS+ pathogenesis is mostly genetic. One form of GEFS+ is Dravet syndrome, a rare and lethal form of epilepsy also known as Severe Myoclonic Epilepsy of Infancy (SMEI). 80% of patients with Dravet Syndrome have mutations in the SCN1A gene.\(^4\) These mutations confer either hypoexcitability, a repressed reaction to stimuli, or hyperexcitability, an excessive reaction to stimuli, in the sodium ion channel Na\(_{1.1}\). As a result, afflicted individuals have a greater susceptibility to seizures due to a widespread dysfunction of their neural network inhibition.\(^2,3\)

Multiple causal factors are associated with epileptogenesis. Since the early 1990s, pharmacological approaches have had limited progress in treating epileptic symptoms, with no decrease in drug-resistant epilepsy cases.\(^5,6\) This provides the impetus for the development of new, more effective treatments, namely in the form of gene therapy.\(^7\)

Gene therapy is the process of delivering nucleic acids including adenine, thymine, guanine, and cytosine in a packaged vector to patient cells.\(^4\) Replication-defective recombinant herpes simplex virus type 1 (HSV-1) is an effective and unique vector for neuronal gene therapy. It is a virus that preferentially infects the brain by spreading across synapses, establishing latent infection, and evading immune responses.\(^3,9\) HSV-1 is able to disguise itself upon infection to allow the DNA vector to be conserved in neurons for extremely prolonged periods of time, thereby maintaining long-term neuronal infection. Lastly, HSV-1 has a large genome containing few spliced genes, allowing for the insertion of large foreign genes for gene therapy applications.\(^10,11\)

Zinc finger nucleases (ZFNs) are a recent development in genetic engineering for the cleavage of DNA strands. ZFNs combine the nonspecific cleavage domain of FokI endonucleases, restriction enzymes that break phosphodiester bonds between nucleotide bases, with zinc finger proteins to achieve site specific gene targeting and editing. Subsequent non-homologous end joining occurs after cleavage activity.\(^12\) When paired with a viral vector, ZFNs allow for a targeted combinatorial method of gene editing that has powerful potential for therapeutic applications. ZFNs have also been cleared through Phase II of clinical trials, lending towards their credibility as a safe and scalable system for genetic editing. If our proposed treatment method involving ZFNs is successful in a rat model, it can be further applied to humans and potentially serve as a therapy for this form of epilepsy.

ABSTRACT

Since its inception in 1972, gene therapy has demonstrated remarkable progression and advancement. Initially through the use of engineered viral vectors and later through the use of complex RNA-based editing tools, the fundamental processes and techniques used in gene therapy have grounded themselves on a watershed of discovery. A potential application of gene therapy is in epilepsy, which is a prevalent set of neurological disorders characterized by epileptic seizures of numerous divergent pathologies. Though many epileptic disorders are idiopathic in nature, the genetic predisposition of generalized epilepsy with febrile seizures plus (GEFS+) has been well documented in scientific literature. GEFS+ is believed to be caused by a mutation in the SCN1A gene, coding for a subunit of a specific ion-gated sodium channel on bipolar and pyramidal neurons. In a rat model of GEFS+, we propose the use of herpes simplex virus type 1 as a vector to deliver zinc finger nucleases and a corrected SCN1A gene sequence to simultaneously knock out and replace the mutated SCN1A gene. This experimental design would demonstrate the prospects of epileptic gene therapy in the rodent brain and, by extension, validate the plausibility of genetic editing procedures in a clinical setting.
**RESEARCH DESIGN AND RESULTS**

The following series of experiments outline a ‘proof-of-concept’ genetic treatment on a rat model for the specific form of epilepsy being studied. These animal subjects are characterized by GEFS+ type 2 symptoms, which are inducible via hyperthermia, due to a mutation in their Scn1a gene and are therefore known as hyperthermia-induced seizure-susceptible (Hiss) rats. The treatment is a solution of standard artificial cerebrospinal fluid (CSF) harboring HSV-1 vectors containing Scn1a-specific-ZFN DNA and the correct Scn1a gene. The expressed ZFNs will cleave the mutated Scn1a gene in the host DNA. The HSV-1 vector has a large DNA capacity of 150 kilobase pairs (kbp) that can allot for the rat Scn1a gene and the smaller ZFN.

The experimental method involves an *ex vivo* study using rat brain slices and an *in vivo* study using rats. For *in vivo*, the treatment solution will be injected directly into the hippocampus of rats using stereotaxic surgery, a method of injection using stereotaxic coordinates that map to specific brain structures. Direct injection circumvents processes in the bloodstream and the blood-brain barrier that could act as confounding variables in the experiment. An overview of the treatment design is shown in Fig. 2.

**Vector**

The transgenes for the correct donor Scn1a (pCMV-Scn1a-mKO2) (see Figure 2.a) and the ZFN pair (pCMV-ZFN) (see Figure 2.b) will be enclosed in a replication defective HSV-1 vector. To minimize possible adverse effects, the less pathogenic KOS HSV-1 strain is chosen, while cytotoxic and replication-essential genes are removed. The expressed ZFN will knock out mutant Scn1a in targeted hippocampal cells, while the new fluorescent correct sodium channel subunit will serve as a replacement. The mKO2 fluorescent protein is used as a reporter to verify the gene’s successful transfection and expression. This novel use of HSV-1 and ZFNs offers the ability to correct the Scn1a gene and alleviate GEFS+ type 2 symptoms in Hiss rats, a specific rat model for this epilepsy.

**Ex Vivo**

Our *ex vivo* experiments are divided into two broad categories: electrophysiology and expression testing. The electrophysiology testing will first use hippocampal slices in artificial CSF prepared from 12 day-old Hiss rats and wild-type F344/NSlc rats. Slices are chosen and allocated to obtain one F344/NSlc set (1) and five Hiss sets. The five Hiss brain slice sample sets are: (2) Mutant Control - Untreated cell culture; (3) Vehicle Control - ‘Empty’ HSV-1 vector; (4) ZFN Test - HSV-1 carrying Scn1a specific ZFNs; (5) Reporter Control - HSV-1 with a gene for the mKO2 fluorescent protein; and (6) Treatment - HSV-1 carrying the ZFN, mKO2 reporter, and the correct Scn1a cDNA. Whole-cell patch clamp electrophysiology measurements for the F344/NSlc slices and various Hiss slices will act as controls to determine baseline electrical recordings for comparison with the test groups.

Next, our expression measurements will measure RNA transcription. Quantitative reverse transcriptase PCR (qRT-PCR) will be utilized to determine the difference in transcription of Scn1a RNA between the control F344/NSlc and untreated Hiss slices and the four test groups, the treated Hiss slices. Western blots for the alpha subunit of Na$_{+}$1.1 channels will then measure the level of the protein expression in each group. Fluorescent microscopy should detect the mKO2 reporter in cultures (5) and (6) to determine the quantity and location of successfully transfected cell populations in the brain slices. Expected results, per group, are shown in Table 1.

**In Vivo**

Our *in vivo* testing will use experimentally naïve Hiss rats and experimentally naïve wild-type F344/NSlc rats (line from which the mutated Hiss line was derived), all starting at an age of four weeks. Stereotaxic surgery will inject the viral vector precisely into the hippocampal region. Weekly tests will involve inducing epileptic seizures by situating the rats in a hot water bath at 45°C and scoring the intensity of seizures on the 0–8 stage Moshe Racine scale. Unlike some methods, hot water baths can induce seizures in rats of up to 10 weeks of age. Rat behaviour will be videotaped at all times to monitor the presence and intensity of spontaneous seizure activity over the length of the experiment. At 10 weeks of age, rats will be euthanized with CO$_{2}$ and their brains will be removed and flash frozen prior to sectioning. Expected results are shown in Table 1. Animals are to be housed and tested in compliance with guidelines provided by the Guide to the Care and Use of Experimental Animals.

**DISCUSSION**

The Hiss rat model used in this experiment has several limitations. Firstly, it focuses on a single missense mutation – a change in one nucleotide base resulting in a single substituted amino acid in the
Further testing of the therapy on different species and other cell populations will be required for any human applications in the future.

**CONCLUSION**

Gene therapy is one of the most rapidly developing fields in science, and this proposal presents the foray of this technique into the field of epilepsy, a combination that has not been heavily explored in previous literature. Given the novelty of delivering ZFNs in conjunction with the therapeutic gene, our study would also represent a major advancement for gene therapy technology. If successful, this therapy would have major implications in the treatment of debilitating seizures caused by mutations in the SCN1A gene, including Dravet Syndrome. It would set a precedent for future investigation into the use of gene therapy as a treatment for genetic brain diseases, and would be a hallmark in genetic editing science.

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