

NOVEL THERAPEUTIC STRATEGIES FOR EPILEPSY—RELEASING THE GENE GENIE

Neuropeptide Y Gene Therapy Decreases Chronic Spontaneous Seizures in a Rat Model of Temporal Lobe Epilepsy. Noè F, Pool AH, Nissinen J, Gobbi M, Bland R, Rizzi M, Balducci C, Ferraguti F, Sperk G, During MJ, Pitkänen A, Vezzani A. *Brain* 2008;131(Pt 6):1506–1515. Temporal lobe epilepsy remains amongst the most common and drug refractory of neurological disorders. Gene therapy may provide a realistic therapeutic approach alternative to surgery for intractable focal epilepsies. To test this hypothesis, we applied here a gene therapy approach, using a recombinant adeno-associated viral (rAAV) vector expressing the human neuropeptide Y (NPY) gene, to a progressive and spontaneous seizure model of temporal lobe epilepsy induced by electrical stimulation of the temporal pole of the hippocampus, which replicates many features of the human condition. rAAV-NPY or a control vector lacking the expression cassette (rAAV-Empty) was delivered into the epileptic rat hippocampi at an early progressive stage of the disease. Chronic epileptic rats were video-EEG monitored to establish pre-injection baseline recordings of spontaneous seizures and the effect of rAAV-NPY versus rAAV-Empty vector injection. Both non-injected stimulated controls and rAAV-empty injected rats showed a similar progressive increase of spontaneous seizure frequency consistent with epileptogenesis. The delivery of rAAV-NPY in epileptic rat brain leads to a remarkable decrease in the progression of seizures as compared to both control groups and this effect was correlated with the NPY over-expression in the hippocampus. Moreover, spontaneous seizure frequency was significantly reduced in 40% of treated animals as compared to their pre-injection baseline. Our data show that this gene therapy strategy decreases spontaneous seizures and suppresses their progression in chronic epileptic rats, thus representing a promising new therapeutic strategy.

COMMENTARY

Currently available antiepileptic drugs (AEDs) fail to prevent seizures in approximately 30% of people with epilepsy. These agents also are associated with significant dose-related adverse effects, occasional idiosyncratic reactions, and do not appear to influence the development or progression of epilepsy. There is no evidence that the plethora of new AEDs introduced over the past 2 decades has improved the prognosis of seizure disorders and little to suggest that compounds currently in the developmental pipeline will alter this outlook (1). Resective surgery is an effective alternative to pharmacological treatment, but only a small number of people with refractory epilepsy are suitable. Novel therapeutic strategies to address the significant clinical burden of drug-resistant epilepsy are required. Among those in current usage or under clinical consideration are vagal nerve stimulation, deep brain stimulation, transcranial magnetic stimulation, focal intracerebral drug delivery, cell transplantation, and gene therapy (2,3). All bypass the blood–brain barrier, reported to be a major contributor to the failure of conventional drug treatment in refractory epilepsy, and most can be applied with neuroanatomical selectivity, potentially avoiding adverse effects arising from normal, nonepileptic brain regions.

Gene therapy was originally developed as a means of replacing defective copies of individual genes in mature, proliferating

cells—thereby restoring normal function. It is currently undergoing clinical evaluation in numerous cancers, monogenic diseases (e.g., cystic fibrosis and hemophilia), and HIV-borne infection (4). The development of gene therapy for neurological conditions has been comparatively slow, mostly because of difficulties in identifying effective strategies for delivering genes across the blood–brain barrier and vectors capable of transducing postmitotic neurons (5). Another obstacle has been the relative lack of understanding about underlying molecular defects in the majority of CNS disorders; however, it is now apparent that gene therapy can be employed to boost endogenous neuroprotective pathways, without necessarily correcting the causative pathology. In terms of epilepsy, gene therapy remains the subject of early preclinical investigation, but pilot data suggest that it holds promise for both alleviation of seizures and disease modification (6).

The most common method of gene delivery to the brain involves packaging of the molecular apparatus of gene expression into a deactivated viral vector, followed by direct focal micro-injection into the relevant brain region (3,4). This technique exploits the fundamental ability of viruses to invade host cells, insert their genetic code, and for that code to be expressed *de novo*. A number of viruses can be adapted for this purpose, although recombinant adeno-associated viruses (rAAVs) are currently preferred by virtue of their tropism for postmitotic neurons, low immunogenicity, and prolonged expression *in vivo* (4). To date, the range of candidate genes selected for preclinical studies of gene therapy for epilepsy has been somewhat limited (6). Initial investigations focused on inhibitory

neurotransmission mediated by GABA, with transduction of glutamic acid decarboxylase and subunits of the GABA_A receptor. Concerns regarding downregulation of GABA receptors and/or loss of GABAergic neurons under chronic epileptic conditions have, however, encouraged the search for more robust alternatives. In this regard, endogenous neuromodulators, such as galanin and neuropeptide Y (NPY), appear to be gaining favor (7,8). Both are known to possess anticonvulsant activity mediated by an inhibitory effect on presynaptic glutamate release, and when their genetic code is incorporated into host neurons by gene transduction, the resulting overexpression supplements the releasable pool of these peptides, thereby enhancing their neuroprotective potential.

A recent study by Noè et al. provided clear proof-of-principle for NPY-based gene therapy in epilepsy. It employed an experimental model of temporal lobe epilepsy in which a short period of acute status epilepticus was induced by electrical stimulation of the hippocampus, followed some weeks later by the onset of recurrent, unprovoked seizures that were recorded by 24-hour video-EEG monitoring. Once the chronic epileptic state had been established, animals were randomized to receive human NPY gene therapy, using the rAAV vector, or a control vector devoid of the NPY-expressing apparatus—administered by direct bilateral microinjection into the hippocampus. After a period of 4 weeks to allow for neuronal transduction and overexpression of the NPY gene, rats underwent further evaluation of seizure activity by 24-hour video-EEG. While control animals showed progressive increases in spontaneous seizure frequency consistent with the ongoing development of epilepsy, those receiving NPY gene therapy showed no overall change from baseline frequency. In addition to the attenuation of epileptogenesis, 40% of rats treated with the active human NPY gene experienced a reduction in daily seizure frequency and associated cumulative seizure duration, suggesting suppression of acute spontaneous seizure activity or regression of the epileptic state. Immunohistochemical processing of ex vivo tissue harvested shortly after video-EEG recording revealed that overexpression of NPY was restricted to the hippocampus, specifically observed in the inner molecular layer of the dentate gyrus and in multiple CA1/CA3 subfields, and that the extent of overexpression was directly correlated with the antiepileptic/antiepileptogenic effect.

This collaborative study, performed by a group of international investigators, provides convincing preliminary evidence of the potential utility of NPY-based gene therapy for epilepsy. The progressive nature of seizures in this particular animal model allowed the concomitant investigation of both antiseizure and antiepileptogenic effects. Furthermore, the administration of gene therapy several weeks after development of spontaneous seizures lent credence to the clinical applicability of the findings, particularly as comparative pharmacological studies typically require drug exposure prior to the initial in-

vestigation in order to demonstrate a neuroprotective action (9). The duration of efficacy after a single application remains to be investigated, as does the potential emergence of associated adverse effects in the longer term. It would also be interesting to know if a gene dose effect could be demonstrated, whether a selective NPY receptor agonist might not elicit similar findings, and if the reported efficacy can be reproduced in a model of nonprogressive chronic epilepsy.

From a clinical perspective, gene therapy for epilepsy is likely to remain technically and ethically demanding for some time. Safety is the overriding concern: the potential for deactivation of the viral vector, by insertional mutagenesis or reverse recombination, is the uppermost consideration (3–6). There are also issues around long-term, irreversible upregulation of gene expression leading to unexpected adverse effects and the requirement for focal delivery via stereotaxic neurosurgery, which is likely to restrict availability of future gene therapy to those patients with the most severely refractory epilepsy and possibly only to those who are unsuitable for surgery. Paradoxically, the technique may be initially investigated in patients with surgically remediable lesions, affording an opportunity to resect the injected tissue and limit long-term consequences should gene therapy prove ineffective (6). Despite these challenges, there would appear to be grounds for cautious optimism about the promise of gene therapy for epilepsy. Whether single gene transduction will ever be sufficient to treat a complex disorder arising from an organ with a remarkable and inherent capacity to compensate for manipulation remains to be seen.

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