"Are We There Yet?" Quest for Treatment of Refractory Epilepsy

Epilepsy Currents
2019, Vol. 19(1) 57-58

DOI: 10.1177/1535759718822843
journals.sagepub.com/home/epi
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Biochemical Autoregulatory Gene Therapy for Focal Epilepsy

Lieb A, Qiu Y, Dixon CL, Heller JP, Walker MC, Schorge S, Kullmann DM. *Nat Med*. 2018;24:1324-1329. doi:10.1038/s41591-018-0103-x

Despite the introduction of more than one dozen new antiepileptic drugs in the past 20 years, approximately one-third of people who develop epilepsy continue to have seizures on mono- or polytherapy. Viral-vector-mediated gene transfer offers the opportunity to design a rational treatment that builds on mechanistic understanding of seizure generation and that can be targeted to specific neuronal populations in epileptogenic foci. Several such strategies have shown encouraging results in different animal models, although clinical translation is limited by possible effects on circuits underlying cognitive, mnemonic, sensory, or motor function. Here, we describe an autoregulatory antiepileptic gene therapy, which relies on neuronal inhibition in response to elevations in extracellular glutamate. It is effective in a rodent model of focal epilepsy and is well tolerated, thus lowering the barrier to clinical translation.

Commentary

In short, we are not there yet. The longer version is much more complicated. In the beginning of this study, the authors review some alarming facts about epilepsy painting a dire picture. In fact, the picture is significantly darker. According to the most recent United Nations estimates, the current world population is 7.6 billion as of September 2018 (https://population.un.org/wpp/DataQuery/). If the 2015 US prevalence of epilepsy per the recent *Centers for Disease Control and Prevention* report of 1.2%[1] is applied to the world population, the number of people having epilepsy worldwide exceeds 91 million. Then, let's use the most recent study following therapy resistance that shows that approximately 36% of patients with epilepsy remain with seizures despite trying additional medications (up to 11 consecutive drugs).[2] So the picture is really depressing. One more little thing: The authors use the term "antiepileptic drugs," which semantically gives a glimmer of hope that in those 64% of seizure-free patients we are in fact capable of treating epilepsy. Again, we are not there yet. As many of new drugs we have, we are still merely in the seizure suppression mode, not in epilepsy treatment. Thus, we still need to search for new epilepsy treatment approaches and drugs really bearing antiepileptogenic effects.

The authors provide a very brief, yet nice overview of the most current clinical and new experimental treatment approaches with all the pros and especially the cons. The major negative aspects of new treatment approaches (optogenetics, chemogenetics[3]) are long latency to onset of action, interference with normal brain function, frequent false positives, and invasiveness.[4,5] In open-loop treatments (standard pharmacology, chemogenetics), the problem is low specificity of the treatment and therefore, likely interference with normal brain function. In current closed-loop systems such as optogenetics or focal drug delivery, an invasive approach is required with negatives of possible infection as well as accuracy of seizure recognition algorithms. So what may be a feasible approach? Epilepsy frequently results from an imbalance between excitatory drives and inhibition. No matter, what is primary, eventually there is net increase in excitatory glutamate. Thus, it would be advantageous to use the excess of glutamate released during a seizure to activate some inhibitory mechanisms.

Indeed, there is a glutamate-gated chloride channel (GluCl) in the *Caenorhabditis elegans* (*C elegans*[6]) similar to Drosophila.[7] And if excess glutamate can increase Cl^- influx into the postsynaptic neuron, it may lead to hyperpolarization and cessation of firing. The first obstacle is that the sensitivity of the natural channel to glutamate is very low. The authors were, however, able to engineer the GluCl to enhance its glutamate sensitivity (eGluCl). Electrophysiology studies confirmed sensitivity in the range of glutamate concentrations occurring during seizures. The authors then performed lentivector transfection of eGluCl in M1 sensorimotor cortex of Sprague-Dawley rats, and using mYFP tag they found that these channels are localized primarily extrasynaptically (showing up within 3 days and persisting for at least 8 months).

Extrasynaptic localization of the channel offers an advantage since there will be no mingling of eGluCl in the synaptic transmission necessary for normal brain functioning. Only excess activity and excess glutamate release (such as during a seizure) will lead to a transmitter spillover affecting extrasynaptic receptor sites including the eGluCl channels.[8] After successful transfection with either lentivector-containing eGluCl or inactive lentivector-containing green fluorescent protein (GFP), the authors decided to test efficacy in a model of acute seizures. Therefore, they infused pilocarpine twice intracortically to test the eGluCl efficacy. Seizures were followed with cortical electroencephalogram (ECoG) in the same area of M1. Those rats with eGluCl had shorter ECoG coastline, which takes into consideration changes in both amplitude and frequency of the ECoG. The coastline represents the length of the ECoG curve which equals sum of the distances of the consecutive ECoG data points. The GFP animals displayed increase in cortical ECoG coastline. In the second part of the experiment, efficacy of eGluCl was tested in a chronic model of epilepsy after intracortical tetanus toxin injection.[9] eGluCl significantly decreased number of seizures compared to GFP and also compared to prelentivector period. There was no interictal effect of eGluCl at least measured by interictal ECoG coastline. In nonepileptic animals, there was no effect of eGluCl on the ECoG at least in comparison with the ineffective GFP and no effects on behavior assessed in accelerating rotarod, and number of steps walked on an elevated grid. Finally, GluCl (as well as eGluCl) provides an opportunity to use ivermectin for chemical activation.[10] Thus, positive controls were introduced to the experiment. In nonepileptic animals, ivermectin was able to decrease the length of the ECoG coastline. Ivermectin also increased the latency to fall from the accelerating rotarod and decreased number of steps on the elevated grid. The overall data indicate that the eGluCl does not affect brain function during normal condition and for the effect needs an activation either by excess glutamate or by an exogenous drug.

In conclusion, the authors demonstrate in a straightforward study that there is a way for implementing closed loop (or if you wish on demand) control of epileptic seizures by gene therapy. The treatment was quite well tolerated without any obvious interference with normal brain functions. This publication opens door to extensive and serious consideration of gene therapy for suppression of pharmaco-resistant seizures and (eventually) epilepsy. There are also minor problems. Statistics not always matches experimental design. The behavioral section especially the grid-walking test had insufficient method description, explanation, and interpretation likely due to space constraints. This test, designed to reveal motor coordination problems, usually counts number of slips on the grid,[11,12] rather than the number of steps (number of steps may be related to anxiety). Further, the authors in their assessment of latency to fall of the rotarod compare improvements seen here after ivermectin to improvements seen in rodents after M1 ischemic lesions (possible analogy to the M1 transfection site lesion?). Yet the effect in animals with M1 ischemic lesions is attributed to the decreased tonic inhibition[13] while the treatment paradigm used in this study enhances tonic inhibitory currents. In this respect, a look at the chloride transporter activity might prove useful and might provide an explanation for seemingly incongruent outcome of behavioral tests after administration of ivermectin.

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