Epilepsia, 54(Suppl. 6):43–45, 2013
doi: 10.1111/epi.12275

Gene therapy in status epilepticus

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Summary

Gene therapy in human disease has expanded rapidly in recent years with the development of safer and more effective viral vectors, and presents a novel approach to the treatment of epilepsy. Studies in animals models have demonstrated that overexpression of inhibitory peptides can modify seizure threshold, prevent the development of epilepsy, and modify established epilepsy. More recently there has been a flurry of studies using optogenetics in which light-activated channels expressed in neurons can transiently change neuronal excitability on exposure to light, thereby enabling the development of closed loop systems to detect and stop seizure activity. The treatment of status epilepticus presents its own challenges. Because of both the delay in gene expression following transfection and also the necessity of using focal transfection, there are a limited number of situations in which gene therapy can be used in status epilepticus. One such condition is epilepsia partialis continua (EPC). We have used gene therapy in a model of EPC and have shown that we can "cure" the condition. Recent evidence suggesting that gene therapy targeting subcortical regions can modify generalized or more diffuse epilepsies, indicates that the range of situations in status epilepticus in which gene therapy could be used will expand.

KEY WORDS: Epilepsia partialis continua, Animal models, Optogenetics, Subcortical regions, Viral vector.

The initial concept of gene therapy involved the replacement of a defective gene, using active or inactive viruses or virus-like particles carrying the "healthy" DNA (Friedmann & Roblin, 1972). There are few genetic conditions for which such an approach was considered suitable, and deaths from the use of pathogenic viral vectors (in particular adenovirus) stifled early research (Romano, 2006). In the last decade, gene therapy has undergone a resurgence, largely as a result of the development of effective and safe means of transfecting cells. To date, there have been >1,800 clinical trials approved for gene therapy (Ginn et al., 2013), and such therapy has expanded from replacing defective genes to overexpressing or "knocking down" healthy genes in order to treat disease.

Gene Therapy in Epilepsy

Gene therapy in epilepsy is still in its infancy. Initial studies overexpressing inhibitory peptides such as galanin or neuropeptide Y (NPY) were shown to increase seizure threshold or to modify the development of epilepsy (Haberman et al., 2003; Noè et al., 2008). A decrease in galanin was proposed to be a mechanism by which seizure activity progressed to status epilepticus; administering galanin into the hippocampus prevented such a progression from occurring (Mazarati et al., 1998). Loss of adenosinergic mechanisms has also been shown to be important for the progression to status epilepticus (Young & Dragunow, 1994; Hamil et al., 2012). Although gene therapy resulting in increased adenosine release has not yet been used, a similar approach in which stem cells are genetically engineered to release adenosine has met with some success in treating seizures (Boison, 2009).

There is growing evidence that such approaches may prevent the development of epilepsy and can also be used to treat established epilepsy, but gene therapy to treat status epilepticus brings with it particular hurdles. Foremost among these is the time over which gene expression occurs. Gene expression using viral vectors takes days to weeks. This is completely at odds with the rapid time scale over which emergency treatment of status epilepticus takes place. Furthermore, gene therapy is usually accomplished either ex vivo in which case cells are modified and then placed in vivo, or by focal application of a vector in vivo. Both of these approaches use focal

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treatment and would therefore be ideally aimed at a focal status epilepticus. Gene therapy would ideally treat a chronic focal status epilepticus such as epilepsia partialis continua (EPC; Wykes et al., 2012). EPC is chronic, drug-resistant, and usually affects eloquent cortex (Cockerell et al., 1996), making the risks of surgery high with a high chance of a focal deficit.

Gene Therapy in Epilepsia Partialis Continua

We developed a rodent model of EPC by injecting tetanus toxin into the motor cortex (Nilsen et al., 2005). Tetanus toxin decreases neurotransmitter release and predominantly affects inhibitory, γ-aminobutyric acid (GABA)ergic synapses. The loss of inhibition results in focal seizure activity, which progresses over about 3–7 days. After time, the tetanus toxin is cleared but the seizure activity continues. Neurophysiologic studies demonstrate that neuronal excitability increases after injection of tetanus toxin, and this increased excitability endures (Wykes et al., 2012). The clinical phenotype consists of tonic posturing of the limb (similar to that observed in human EPC), but clonic movements are not so obvious. There is a dose effect, such that at high doses of tetanus toxin, there is marked limb posturing, weight loss, and occasional sudden death associated with a more severe seizure. Lower tetanus toxin doses result in almost continuous electroencephalography (EEG) seizure activity but with much subtler clinical manifestations, and far fewer adverse effects (Wykes et al., 2012). We continuously monitored seizure activity for weeks using a wireless transmitter sampling at 512 Hz, permitting monitoring of activity up to 160 Hz (Chang et al., 2011).

We used three different strategies to treat this model of status epilepticus (Wykes et al., 2012). In the first we used an optogenetic strategy to overexpress the light-activated chloride-pump, halorhodopsin, in pyramidal cells. Such a strategy had been previously used in ex vivo tissue and cell cultures (Tønnesen et al., 2009). In that study, activating halorhodopsin reduced pyramidal cell excitability and terminated stimulation-induced epileptiform bursts and seizure-like activity induced with the $GABA_A$ receptor antagonist picrotoxin. We extended this method to our in vivo model of EPC. The second strategy was to determine if permanently reducing the excitability of a subset of pyramidal cells in the focus during the epileptogenic process prevented the occurrence or severity of seizures. This approach is not so relevant to the clinical problem (i.e., treatment of established EPC). Therefore, we pursued a third strategy of using the same method of reducing the excitability of a subset of pyramidal cells in the focus in previously established epilepsy.

There are a number of different viral vectors for efficient transfections of neurons (Warnock et al., 2011). Adenoassociated viral vectors (AAVs) are modified adeno-associated viruses. Adeno-associated viruses are small, nonpathogenic, DNA viruses that insert into the genome at a specific location. AAVs are modified so that they do not insert but form episomes, avoiding the putative problems of insertional mutagenesis. AAVs have a predilection for neurons. Two main handicaps face AAV therapy; the first is that AAVs can be used only to insert a limited amount of genetic material (i.e., small genes), and the second is that AAVs are immunogenic and their usefulness can be restricted by the presence of neutralizing antibodies (this is less of a problem with injection into the immune privileged central nervous system [CNS]). There have also been some concerns that gene expression may be time-limited. An alternative vector is a lentivirus vector. Lentiviruses are retroviruses that can transfect nondividing cells. They contain RNA, which is reversed transcribed and inserted into the genome. Although insertional mutagenesis is a concern, increasing numbers of studies have revealed that the risks are vanishingly low. The lentivirus is modified so that it is nonreplicating, and more recent modifications render the virus self-inactivating, so that active infection cannot take place. The advantages of lentivirus vectors are persistent transfection and the ability to transfect a larger amount of DNA. Lentivirus vectors also diffuse a shorter distance than AAVs. By selecting the appropriate gene promoter, it is possible to restrict gene expression to particular subsets of neurons (e.g., pyramidal cells). We used lentiviral vectors to transfect predominantly pyramidal neurons with either halorhodopsin or the potassium channel Kv1.1 (Wykes et al., 2012). We had previously shown that Kv1.1 overexpression in neurons in culture is an effective strategy for reducing neuronal excitability. Lentiviral transfection in vivo resulted in persistent gene expression for at least 6 months.

More than a week after tetanus toxin injection into the motor cortex, when seizure activity was well established, we were able to suppress this activity using light activation of halorhodopsin transfected into pyramidal cells in the focus. This provides a method of regulating seizure activity but is probably more relevant to a means of terminating or preventing individual seizures using a closed loop system. Such an approach has been used successfully in a model of temporal lobe epilepsy by either transiently reducing the excitability of halorhodopsin-transfected pyramidal cells with light pulses or increasing the activity of interneurons selectively expressing the light-activated channel, channelrhodopsin (Krook-Magnuson et al., 2013). Such an approach, however, is unlikely to be used in EPC in which a continuous reduction of neuronal excitability is required.

Using focal overexpression of Kv1.1, we were able to both prevent the development of EPC and, more impor-

tantly, stop seizure activity after it had become established. It is important to note that our strategy reduced the excitability of pyramidal neurons but did not interfere with normal motor function.

Could such an approach be expanded to other forms of refractory status epilepticus? The observations that optogenetic reduction of excitability of thalamic neurons using halorhodopsin can interrupt more generalized seizure activity (Paz et al., 2013) and that genetic manipulation of thalamic delta $GABA_A$ receptor-subunit expression using oligodendronucleotides can inhibit generalized spike-wave activity (Cope et al., 2009) indicate that gene therapy manipulation of subcortical structures may be an approach that could be used to target generalized refractory status epilepticus.

Conclusion

In conclusion, we have shown how different gene therapy approaches could be used to treat a chronic, refractory, focal status epilepticus. At present, our methods of gene transfection mean that such approaches are suitable only for refractory or chronic status epilepticus. However, targeting subcortical structures may expand the use of this treatment from very focal status epilepticus to more diffuse or generalized forms of refractory status epilepticus.

Disclosure

The authors have no conflicts of interest to declare in relation to this article. The authors confirm that they have read the Journal's position on issues involved in ethical publication and affirms that this report is consistent with those guidelines.

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