Gene Therapy for Epilepsy

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Introduction

Epilepsy is the most common serious brain disorder: it is estimated to affect 50 million people worldwide, with a prevalence of 1–2% of the population (World Health Organization, 2009). The term "epilepsy" actually includes a large group of genetic and acquired chronic neurological disorders whose single common feature is a persistent increase of neuronal excitability occasionally and unpredictably expressed as a seizure. An epileptic seizure is "the transient occurrence of signs and/or symptoms due to abnormal, excessive or synchronous neuronal activity" (Fisher et al., 2005). Seizures can be of two types: generalized, when occurring in and rapidly engaging bilaterally distributed networks, or focal, when occurring within networks limited to one hemisphere (Berg et al., 2010). Etiologically, epileptic syndromes are classified as genetic (when resulting from a known genetic defect), structural/metabolic (when resulting from a structural or metabolic lesion), and of unknown cause (Berg et al., 2010). Genetic epilepsies are most often associated with generalized seizures, whereas most structural epilepsies are associated with focal seizures that originate within or around the lesion area.

There is a significant unmet medical need in epilepsy. First, no truly antiepileptogenic therapy is currently available. None of the antiepileptic drugs in clinical use can prevent the development of epilepsy in cases in which the cause of the epileptogenic lesion is identifiable (e.g., head trauma, episode of status epilepticus [SE], stroke, brain infection). Second, pharmacological therapy is unsatisfactory: one third of the patients treated with antiepileptic drugs continue to experience seizures. Furthermore, in patients in whom seizures are well controlled, drugs may exert debilitating side effects and, in time, refractoriness to their therapeutic effects may develop. For some patients with focal seizures that are refractory or become refractory to pharmacological therapy, one final option is the surgical resection of the epileptogenic region. Third, there is a need for disease-modifying therapies. Antiepileptic drugs do not prevent the progression of the disease, and we lack therapies that can ameliorate or prevent the associated cognitive, neurological, and psychiatric comorbidities or epilepsy-related mortality.

Possible Gene Therapy Interventions

At least 30% of the epilepsies are believed to be of genetic origin. At first glance, it may seem that these diseases are good candidates for gene therapy, but this is not the case. Only rare forms of epilepsy are caused by a single mutant gene, whereas more common types result from the inheritance of two or more susceptibility genes (Berkovic et al., 2006). Moreover, the pathology in these cases often affects a large part of the brain and, thus, would require widespread gene transfer; however, currently available gene therapy methods provide only localized effects.

Researchers are attempting to develop strategies for globally delivering genes to the brain by crossing the blood–brain barrier (BBB) after administering vectors in the peripheral blood. One such strategy is to employ a pathway used by many circulating endogenous molecules, such as transferrin or insulin, to reach neurons and glia (de Boer and Gaillard, 2007; Simionescu et al., 2009). After these ligands bind on the luminal side of the capillary endothelial cell membrane, a caveolar vesicle is formed, engulfing the receptor and the bound conjugate. The caveola and its cargo are then transported across the endothelial cell cytoplasm, from the luminal to the abluminal side, via an intracellular transport mechanism known as transcytosis. For gene therapy, a vector can be conjugated to a ligand (such as a single-chain antibody against the transcytosis receptor or a peptide) that mimics the natural ligand for the receptor, e.g., transferrin or insulin. The vector–ligand conjugate remains intact and unmodified while in transit and is therefore released intact into the interstitial space. Recently, adeno-associated virus (AAV) vectors (Di Pasquale and Chiorini, 2006; Foust et al., 2009) have been shown to undergo transcytosis of the rodent BBB. However, much work remains to be done to prove that this approach can be applied to the treatment of genetic epilepsies.

Meanwhile, epileptic syndromes that are characterized by a focal lesion appear to be much better candidates for gene therapy. As described above, most of these diseases have an identifiable cause, and it is thought that these damaging insults set in motion a cascade of neurobiological events that eventually lead to epilepsy: a phenomenon termed "epileptogenesis." Thus, these forms of epilepsy offer the opportunity for intervention at different levels: preventive (antiepileptogenic), symptomatic (antiseizure), and disease-modifying (Fig. 1).

Choice of vector and route of delivery

The vector types employed thus far in epilepsy studies have been AAV (different serotypes) and herpes simplex virus (HSV) (Table 1). Typically, the route of delivery has been the stereotactic injection of the vector into the epileptogenic region (the hippocampus, in most instances). This approach

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ensures high levels of transgene expression and a limited immune response. (Note, however, that the surgical procedure may induce breakdown of the BBB and penetration of lymphocytes.)

Scientists have taken advantage of the biological properties of the different viruses to calibrate the spread of the viral particles in order to adequately cover the target area while limiting the number of injections and their volume. For example, Paradiso et al. (2009) have taken advantage of the retrograde transport of HSV to deliver therapeutic genes bilaterally after injection into one hippocampus, HSV being transported contralaterally by commissural fibers. Richichi et al. (2004) found degrees of spread around the injection site with different AAV serotypes.

Researchers have tested other routes of administration to obtain sufficiently specific accumulation of the transgene in the region of interest without facing the technical hurdles of direct intracerebral administration. In this respect, intranasal delivery is a feasible approach that has been tested using a replication-defective HSV-2 vector to deliver the anti-apoptotic gene *ACP10PK* (Laing et al., 2006).

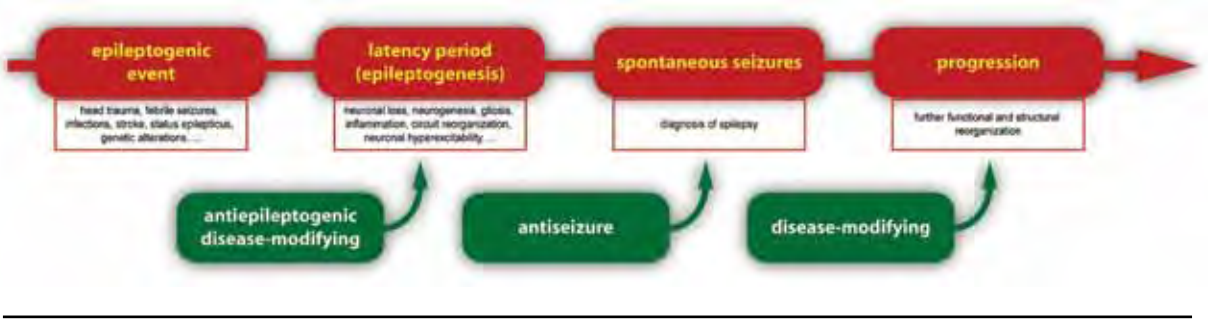

Figure 1. Natural history of acquired focal epilepsy (red) and possible therapeutic interventions (green).

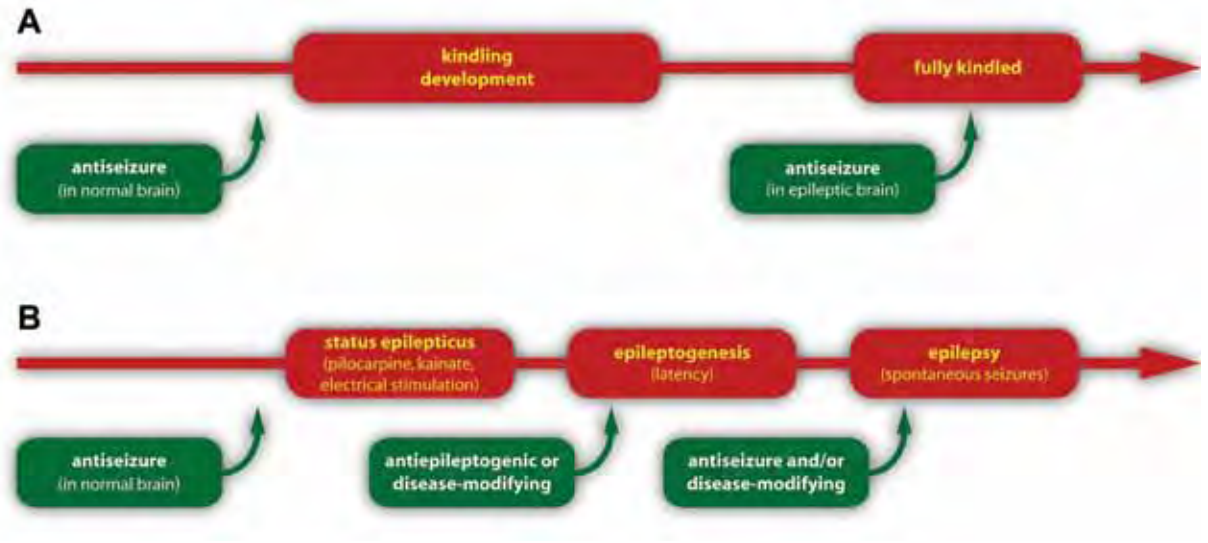

Figure 2. Murine models of acquired epilepsy employed in gene therapy studies. The time of gene transfer and its therapeutic significance are shown in green.

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Unfortunately, the transgene expression that resulted was not specific to the area of interest and, further, its level of expression was low. More recently, Gray et al. (2010) reported having identified an AAV clone capable of crossing the seizure-compromised, though not the intact, BBB. This finding opens up the possibility of creating vectors that may selectively target the brain areas involved in seizure activity after peripheral administration.

Models and endpoints employed in gene therapy studies

Research into gene therapy for epilepsy has been conducted essentially in two types of models. In the kindling model (Fig. 2*A*), the repeated administration to a discrete limbic brain area of an initially subconvulsive electrical stimulation induces seizures that progressively intensify in duration and severity, from focal to secondarily generalized. Kindling can be evoked by stimulating different areas of the brain, including the amygdala, hippocampus, and piriform cortex. Second, chemically (pilocarpine or kainate) or electrically (self-sustained SE) evoked SE (Fig. 2*B*) are models in which induction of an epileptogenic insult (SE) is followed by a latency period during which the animals are apparently well, and then by spontaneous recurrent seizures (SRSs), i.e. epilepsy. This situation closely mimics the one occurring in humans who acquire structural epilepsies.

These two models allow the exploration of the three main intervention levels identified above and shown in Figure 1: antiepileptogenic (preventing the development of epilepsy in subjects at risk after having received an epileptogenic insult), antiseizure (reducing the frequency and/or severity of seizures), and disease-modifying (altering the natural history of the disease). However, special care should be taken in choosing the model and the endpoint for evaluating treatment effectiveness in order to correctly allocate the results in terms of translation to clinical relevance. We will adopt here the conservative approach proposed by Dudek (2009). When gene transfer is performed before SE or kindling stimulation, therapeutic effects should be considered as antiseizure even when parameters relative to latency, SRSs, or kindling development are altered, because it is essentially impossible to guarantee that the treatment did not alter the initial SE or suppress each individual stimulus-evoked seizure during kindling. Accordingly, we will consider as potentially antiepileptogenic only treatments in which gene therapy was applied after the epileptogenic insult. Even in this scenario, undisputable evidence of an antiepileptogenic effect comes from prolonged observation (lasting several months) of treated animals and verifying that the effect is maintained well after termination of transgene expression or overexpression. Otherwise, it seems more appropriate to define the effect as disease-modifying. A disease-modifying effect may also be documented as either neuroprotection or arrest of disease progression during the chronic phase (when SRS frequency progressively increases).

Gene therapy: antiepileptogenic effects

Based on the above criteria, there is no undisputable evidence so far of gene therapy strategies that can actually exert antiepileptogenic effects. A series of studies, however, although not yet providing a final proof, strongly supports this notion (Bovolenta et al., 2010; Paradiso et al., 2009, 2011). In both humans and animals, epileptogenesis is associated with focal pathological abnormalities, including cell death (most prominently, a loss of neurons in the hippocampus termed "hippocampal sclerosis"); axonal and dendritic plasticity; neurogenesis; neuroinflammation; and functional alterations in ion channel and synaptic properties. The molecular mechanisms underlying these cellular alterations are still poorly understood, but impairment in neurotrophic factor (NTF) support may be a key causal element (Simonato et al., 2006).

Among the NTFs, fibroblast growth factor 2 (FGF-2) and brain-derived neurotrophic factor (BDNF) may be particularly implicated in epileptogenesis. Both protect neurons from ongoing damage and, further, FGF-2 is a potent proliferation factor for neural stem cells, while BDNF favors their differentiation into neurons (Simonato and Zucchini, 2010). Thus, Paradiso et al. (2009) reasoned that supplementing FGF-2 and BDNF in the epileptogenic hippocampus could attenuate seizure-induced damage, enhance repair, and ultimately, alleviate epileptogenesis. To test this hypothesis, they developed a replication-defective HSV-1 vector expressing these two NTFs and injected it into one hippocampus four days after pilocarpine-induced SE, i.e., during latency and after the establishment of hippocampal damage. These conditions are similar to those of a person who, following the occurrence of an epileptogenic insult, is in the latency period preceding the beginning of spontaneous seizures. The HSV vector was retrogradely transported to the contralateral hippocampus, allowing bilateral expression of the transgenes. Transgene expression was transient, lasting approximately two weeks. However, short-term expression is an advantage in these specific

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settings because NTFs can trigger plastic changes that remain detectable when they are no longer expressed, whereas their long-term expression may be detrimental for brain function (Thoenen and Sendtner, 2002). The goal of this approach was to increase the extracellular levels of FGF-2 and BDNF by generating cells capable of constitutively but transiently secreting these factors; achievement of this goal was verified by performing *in vitro* and *in vivo* analysis of both NTFs processing and release.

Administering the vector expressing FGF-2 and BDNF slightly attenuated the ongoing cell loss, indicating that, *in vivo*, its neuroprotective effect is limited or may require more prolonged or higher-level transgene expression. In contrast, the effect on neurogenesis was remarkable: the proliferation of early progenitors was favored and led to the production of cells that entered the neuronal lineage of differentiation, while aberrant aspects of SE-induced neurogenesis were reduced. One month after SE, all untreated animals displayed hippocampal sclerosis and SRSs. Treated animals, in contrast, had a highly significant reduction of cell loss in the hippocampus, and in particular, a nearly complete preservation of somatostatin interneurons.

To verify that these beneficial effects were sufficient to ameliorate the outcome of the disease, animals were video-EEG monitored for 20 days, and the occurrence, severity, and duration of SRSs were recorded. As expected, all non–vector-injected pilocarpine rats exhibited SRSs. In contrast, rats treated with the vector displayed a highly significant improvement: a subset of animals never developed SRSs in the time frame of observation, and the average number of seizures per day and their severity were significantly reduced. Finally, the authors controlled the possible effect of FGF-2 and BDNF therapy on ictogenesis (generation of spontaneous seizures) in a separate group of animals. They found that the effect was negligible in this respect, arguing that the treatment interferes selectively with epileptogenesis (Paradiso et al., 2009).

Gene therapy: antiseizure effects

A primary logical target for the gene therapy of seizures in drug-resistant individuals consists of modulating excitability by either increasing the strength of inhibitory signals or reducing the strength of excitatory signals. One study focused on $GABA_A$ receptors. In the granule cells of the hippocampus of epileptic (pilocarpine) rats, the expression of $GABA_A$ alpha-1 subunits is decreased, while expression of alpha-4 subunits is increased compared with controls (Brooks-Kayal et al., 1998). This altered expression pattern may be critical for the generation of seizures. Thus, Raol et al. (2006) designed an AAV2 vector containing the alpha-4 subunit gene promoter to drive alpha-1 expression. They injected this vector into the hippocampus two weeks before pilocarpine SE, obtaining increased alpha-1 expression in the granule cells, increased latency time, and decreased number of rats developing SRSs in the first four weeks after SE. Although these effects may be interpreted as antiepileptogenic, it cannot be ruled out that the vector attenuated SE and only secondarily protected from SRSs.

Haberman et al. (2002) tested out the idea of protecting from seizures by reducing the strength of excitatory signals. They did so by cloning in antisense an essential subunit for the functioning of NMDA receptors (NR1) in an AAV vector, under control of two different promoters. They found that, depending on the promoter, the cells expressing the transgene (those in which NMDA currents were downregulated) were either inhibitory interneurons or primary seizure output neurons; thus, the two different vectors had opposite effects on focal seizures (Haberman et al., 2002). This study underscores the importance of transducing a specific cell population anytime the transgene codes for a receptor (or a channel) that is expressed on both inhibitory and excitatory neurons.

As described for NTFs, one means of circumventing the problem of selectively targeting certain cell populations could be to express an inhibitory factor in a way that it is constitutively secreted from the transduced cells. If the receptors for that factor are present in the injected area, seizure control can be achieved without the need to target specific cells. Indeed, significant antiseizure effects have been obtained by overexpressing the NTF glial cell line–derived neurotrophic factor (GDNF) in the hippocampus (Kanter-Schlifke et al., 2007) and increasing hippocampal levels of the endogenous anticonvulsant adenosine with an AAV8 vector expressing the enzyme that catabolizes adenosine (adenosine kinase [ADK]) in antisense (Theofilas et al., 2011). However, the most promising results have been obtained with the inhibitory neuropeptides galanin (GAL) and neuropeptide Y (NPY).

Galanin

GAL is a 29-amino-acid neuropeptide released during seizures that inhibits glutamate release in the hippocampus (Lerner et al., 2008). Administration of GAL receptor agonists attenuates seizures, whereas pharmacological blocking exerts proconvulsant effects.

Table 1. Summary of the gene therapy studies in epilepsy.

Antiepileptogenic						
Gene	**Vector**	**Model**	**Site of injection**	**Timing**	**Results**	**Reference**
FGF-2 and BDNF	HSV-1	Pilocarpine	Hippocampus	Latency (4 days after SE)	DM: reduced cell loss, increased neurogenesis AE: reduced seizure frequency and severity	Paradiso et al., 2009
					DM: reduced neuroinflammation	Bovolenta et al., 2010
					DM: reduced mossy fiber sprouting	Paradiso et al., 2011
Antiseizure						
Gene	**Vector**	**Model**	**Site of injection**	**Timing**	**Results**	**Reference**
$GABA_A$ subunit alpha-1	AAV2	Pilocarpine	Dentate gyrus of the hippocampus	Before pilocarpine	AS: decreased % of animals with SRS at 4 weeks	Raol et al., 2006
NMDA subunit NR1 (antisense)		Inferior collicus stimulation	Inferior collicus	Before stimulation	AS or PC (depending on the promoter and the transduced cells)	Haberman et al., 2002
GAL	AAV2	Intrahippocampal kainate	Hilus of dentate gyrus in the hippocampus	Before kainate	AS: attenuation of seizures DM: reduced hilar cell loss	Haberman et al., 2003
		Inferior colliculus stimulation	Inferior colliculus	Before inferior collicus stimulation	AS: increased seizure threshold	
		Intrahippocampal kainate	Hippocampus	Before kainate	AS: reduction of seizure frequency and severity	Lin et al., 2003
		Intraperitoneal kainate	Piriform cortex	Before kainate	AS: reduction of seizing animals	McCown 2006
		Piriform cortex kindling	Piriform cortex	Fully kindled	AS: increased seizure threshold	
NPY	AAV2 AAV-1/2	Intrahippocampal kainate	Hippocampus	Before kainate	AS: delayed latency and reduction of seizure frequency	Richichi et al., 2004
		Rapid hippocampal kindling	Hippocampus	Before kindling	AS: retardation of kindling development	
	AAV2	Intraperitoneal kainate	Piriform cortex	Before kainate	AS: delayed latency	Foti et al., 2007
	AAV-1/2	Self-sustained SE	Hippocampus (bilateral)	In the chronic period (with spontaneous seizures)	AC: reduction of seizure frequency in a subset of rats DM: arrest in disease progression	Noè et al., 2008
	AAV-1/2	Rapid kindling	Hippocampus	Before kindling	AS: retardation of kindling development AR: no alteration in LTP	Sørensen et al., 2009
	AAV1	Intrahippocampal kainate	Hippocampus	Before kainate	AC: reduction of seizure frequency and dwuration AR: no alteration in learning and memory, anxiety, locomotor activity	Noè et al., 2010
Y2 receptor	AAV-1/2	Rapid hippocampal kindling; subcutaneous kainate	Hippocampus	Before kindling or kainate	AS: retardation of kindling development and reduction of kainate seizure frequency	Woldbye et al., 2010
NPY + Y2 receptor		Rapid hippocampal kindling	Hippocampus	Before kindling	AS: potentiation	
GDNF	AAV2	Hippocampal kindling Hilus of dentate gyrus		Before kindled	AS: no seizure generalization	Kanter-Schlifke et al., 2007
		hippocampal kindling		Fully kindled	AS: increased currents to evoke seizures	
		self-sustained SE		Before SE	AC: reduction of seizure severity and mortality	
ADK (antisense)	AAV8	ADK transgenic mice	Intra-CA3	Spontaneously seizing mice	AC: reduction of spontaneous seizures	Theofilas et al., 2011
ICP10PK (anti-apoptotic gene)	HSV-2	Intraperitoneal kainate	Intranasal	Before kainate	AC: prevention of seizures DM: prevention of neuronal loss and inflammation	Laing et al., 2006

Results are classified as antiepileptogenic (AE), antiseizure (AS), proconvulsant (PC) and disease-modifying (DM). Evaluation of possible adverse reactions (AR) of the treatment is also reported.

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Transgenic mice with functional deletion of *GAL* and *galanin type 1* receptor genes have spontaneous seizures or enhanced susceptibility to seizures, whereas transgenic mice overexpressing *GAL* in seizure pathways are resistant to epilepsy. Several synthetic agonists of galanin type 1 and type 2 receptors have been shown to inhibit experimental seizures.

In order to obtain constitutive secretion of GAL from transduced cells in the seizure-generating area, Haberman et al. (2003) constructed an AAV vector in which the GAL coding sequence was preceded by the secretory signal sequence of fibronectin (FIB), a protein that is constitutively secreted. This vector was tested in two seizure models. After injection into the hippocampus, this vector attenuated kainate seizures and prevented kainate-induced hilar cell death; after injection in the inferior colliculus, it increased the seizure threshold in this area (Haberman et al., 2003).

Congruent with these findings, other studies have reported that AAV-mediated expression of GAL in the hippocampus reduces the frequency and severity of seizures caused by intrahippocampal injection of kainate (Lin et al., 2003) and that AAV-mediated expression of GAL in the piriform cortex reduces the number of seizing animals after peripheral administration of kainate (McCown, 2006). Notably, these effects were independent of the promoter driving GAL expression. Together, these studies support the notion of an antiseizure effect in normal animals (Fig. 2). To determine whether this effect may hold true in an epileptic brain, McCown (2006) injected the AAV-FIB-GAL vector into fully kindled rats, obtaining a significant elevation of seizure threshold. Thus, vector-derived GAL expression and constitutive secretion appear to be able to suppress epileptic seizure activity.

Neuropeptide Y

NPY is a 36-amino-acid neuropeptide that is overexpressed during seizures (Noè et al., 2009). Activation of the NPY Y2 and Y5 receptors inhibits glutamate release in the hippocampus and attenuates seizures. Transgenic rats overexpressing NPY show reduced seizure susceptibility, whereas knock-out mice lacking NPY or the Y2 or Y5 receptor gene are more vulnerable to chemically or electrically induced convulsions. In hippocampal slices from epileptic patients, NPY potently inhibits perforant path-evoked excitatory responses in granule cells.

The effect of chronic overexpression of NPY in the hippocampus has been extensively studied. The NPY-coding gene has been transferred into the hippocampus using two types of vectors, based on AAV2 or AAV-1/2 (a vector consisting of a 1:1 mixture of AAV1 and AAV2 capsid proteins), eight weeks before intrahippocampal injection of kainate or rapid kindling. Researchers observed a decreased occurrence of seizures or a retardation in kindling development (Richichi et al., 2004). Similarly, bilateral piriform cortex infusions of AAV vectors that constitutively secrete NPY (AAV-FIB-NPY) increased latency to seizures after kainate administration (Foti et al., 2007). Moreover, AAV-induced overexpression in the hippocampus of the Y2 receptor has been found to exert seizure-suppressant effects per se and potentiate the effects of NPY overexpression (Woldbye et al., 2010). Together, these findings strongly support an antiseizure effect in the normal brain.

To evaluate whether the antiseizure effect was also present in the epileptic brain, Noè et al. (2008) injected the NPY-expressing AAV-1/2 vector bilaterally into the hippocampus of rats that were experiencing SRSs after electrically induced SE. They found a significant reduction in seizure frequency in 40% of the animals. Even more interestingly, they observed a remarkable decrease in the progression of seizures (in this model, the frequency of spontaneous seizures increases over time), i.e., a disease-modifying effect. More recent studies have explored the possible side effects that may be expected because of the many functions of NPY in the CNS. However, the NPY-expressing AAV-1/2 vector did not affect epilepsy-induced impairment of long-term potentiation (LTP), an indication that it will not further impair epilepsy-associated memory loss (Sørensen et al., 2009). Furthermore, an NPY-expressing AAV1 vector, while demonstrating potent anticonvulsant activity, did not cause alterations in learning and memory, anxiety, and locomotor activity behavioral tests (Noè et al., 2010). Taken together, the overall evidence supports the application of AAV-NPY gene therapy for human epilepsy.

Future Developments

Gene therapy offers a wealth of opportunities for epileptologists. Vectors can be tailored to the desired experimental needs in several respects:

- Spread from the zone of inoculation. For example, different degrees of spread for different AAV serotypes or retrograde transport for HSV. Also, new vectors may be available soon for peripheral administration with either selective localization in lesion areas (for the treatment of focal epilepsies) or widespread distribution in the brain (for the treatment of generalized epilepsies);

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- Duration of transgene expression. Relatively short-acting expression is achieved with HSV and long-lasting expression with AAV vectors; and
- Targeting specific cell populations (e.g., employing population-specific promoters).

In turn, patients with partial epilepsies selected for surgical resection of the epileptogenic area are ideal candidates for gene therapy. Their pathology is focal, optimal medical treatment has failed, and the success of surgery in leading centers (~70% seizure-free at one year) supports the hypothesis that local, sustained release of an inhibitory molecule might suffice to "silence" hyperactivity. In a way, tissue resection represents the most extreme form of cellular "silencing," so gene therapy may provide a realistic alternative. Gene transfer of inhibitory factors such as GAL or NPY into the epileptogenic area in patients selected for surgery does not require ad hoc stereotaxical intervention, because these patients undergo implantation of depth electrodes for diagnostic purposes before surgery. Also, gene transfer therapy has a built-in rescue procedure because, should it fail to produce any advantage, patients would simply undergo surgery as originally planned.

No doubt, accurately verifying gene therapy's safety and efficacy in nonhuman primates is needed before beginning studies in humans. However, clinical experience of gene therapy in humans with other diseases is encouraging. Once these last hurdles are overcome, the GAL and NPY gene therapy strategies for treating epilepsy will likely progress to Phase 1 clinical trials.

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