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Review

Gene therapy for epilepsy

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ABSTRACT

Gene therapy may represent an effective alternative to standard pharmacological approaches for certain forms of epilepsy. Currently, the best candidates for this therapeutic approach appear to be epilepsies characterized by a focal lesion. Gene therapy has been attempted to produce antiepileptogenic (prevention of development of epilepsy in subject at risk after having received an epileptogenic insult), antiseizure (reduction of frequency and/or severity of seizures), and disease-modifying (alteration of the natural history of the disease) effects. An example of gene therapy aimed at producing antiepileptogenic effects is a combination therapy based on the supplementation of the neurotrophic factors brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 2 (FGF-2). Antiseizure effects have been obtained by increasing the strength of inhibitory signals (by supplementing specific $GABA_A$ receptor subunits or inhibitory neuropeptides like galanin or neuropeptide Y) or by reducing the strength of excitatory signals (by knocking down NMDA receptor subunits). This review summarizes the results obtained to date using gene therapy in epilepsy models and discusses the challenges and the opportunities that this approach can offer for the treatment of human epilepsies.

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1. Introduction

There is a significant unmet medical need in epilepsy [1–3]. First, no truly antiepileptogenic therapy is currently available. None of the antiepileptic drugs in clinical use can prevent development of epilepsy in cases in which the cause of the epileptogenic lesion is identifiable [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 which seizures are well controlled, drugs may exert debilitating side effects and, in time, refractoriness to their therapeutic effects may develop. For some of the patients with focal seizures that are or have 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 the epilepsy-related mortality. Gene therapy may help to address these needs: genetic interventions supplying therapeutic gene products in the epileptic brain may potentially represent an effective alternative to standard pharmacological approaches [4].

2. 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, while more commonly, they are due to inheritance of two or more susceptibility genes [5]. Moreover, the pathology in these cases often affects a large part of the brain and, thus, would require widespread gene transfer, but currently available gene therapy methods provide only localized effects. Attempts are being made to develop strategies for globally delivering genes to the brain by crossing the blood–brain barrier (BBB) after administration of 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 [6,7]. Following the binding of these ligands 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 (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

Abbreviations: AAV, adeno-associated virus; ADK, adenosine kinase; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; FIB, fibronectin; FGF-2, fibroblast growth factor 2; GAL, galanin; GDNF, glial cell line-derived neurotrophic factor; HSV, herpes simplex virus; LV, lentivirus; NMDA, N-methyl-D-aspartate; NPY, neuropeptide Y; NTFs, neurotrophic factors; SE, status epilepticus; SRSs, spontaneous recurrent seizures.

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natural ligand for the receptor, e.g., transferrin or insulin. The vector–ligand conjugate remains unmodified while in transit and is, therefore, released intact into the interstitial space. Recently, adeno-associated virus (AAV) vectors [8,9] have been shown to undergo transcytosis in the rodent BBB. However, much work remains to be done to prove that this approach is applicable to the treatment of generalized epilepsies.

Meanwhile, epileptic syndromes with focal onset appear to be much better candidates for gene therapy. These may be genetic or lesional. Many of those characterized by a focal lesion 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. Thus, these forms of epilepsy offer the opportunity for intervention at different levels: preventive (antiepileptogenic), symptomatic (antiseizure), and disease-modifying (Fig. 1).

3. Choice of the vector and route of delivery

The success of gene therapy depends on the effectiveness of the gene delivery tools, and viral vectors remain the reference approach. No other delivery method can rival the highly evolved mechanisms viruses possess to bring foreign genetic material into cells and alter cell functions. The vector types employed thus far in epilepsy studies have been based on AAV, lentivirus (LV), and herpes simplex virus (HSV). Each of these has advantages and disadvantages. Adeno-associated virus-based vectors are safe, are nonpathogenic, and afford long-term gene expression, but they have limited capacity, are difficult to target, require high doses for effective gene delivery, and are readily eliminated by preexisting immunity [10]. Lentivirus-based vectors have the ability to insert novel genetic material into the cell chromosome which is essential for dividing cells to avoid therapeutic gene loss; however, insertional mutagenesis poses a potential problem with their use [11]. Herpes simplex virus-based vectors have a large payload capacity and have potential for effective gene targeting and sustained transgene expression. However, HSV-based vectors suffer from toxicity and inflammatory problems based on leaky viral gene expression, which requires complete vector genome silencing that can impact transgene expression [4,12]. All viral vectors can be affected by innate immune responses to vector introduction that can, together with preexisting antiviral immunity, engender immune-mediated inflammatory processes and limit vector delivery, gene expression, and redosing [13].

Typically, the route of delivery in epilepsy studies has been the stereotactic injection of the vector in the epileptogenic region (the hippocampus in most instances). This approach ensures a high level of transgene expression and a limited immune response (although 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, the retrograde transport of HSV can be used to deliver therapeutic genes bilaterally after injection in one hippocampus (HSV being transported contralaterally by commissural fibers) [14]; different AAV serotypes display different attitude to spread around the site of injection [15].

Other routes of administration have been tested in an attempt 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 antiapoptotic gene ACP10PK [16]. Unfortunately, the transgene expression was not specific in the area of interest and, further, its level of expression was low. More recently, an AAV clone has been identified that is capable of crossing the seizure-compromised, but not the intact, BBB [17]. This finding discloses the possibility of creating vectors that may selectively target the brain areas involved in seizure activity after peripheral administration.

4. Models and endpoints employed in gene therapy studies

Research on gene therapy for epilepsy has been conducted essentially in two types of models (Fig. 2). First, kindling: a model in which 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, including the amygdala, hippocampus, and piriform cortex. Second, chemically (pilocarpine or kainate) or electrically (self-sustained status epilepticus) evoked SE: these 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 followed by spontaneous recurrent seizures (SRSs), i.e., epilepsy. This situation closely mimics the one occurring in humans with acquired structural epilepsies.

These models allow exploring the three main intervention levels identified above, namely: antiepileptogenic (prevention of development of epilepsy in subjects at risk after having received an epileptogenic insult), antiseizure (reduction of frequency and/or severity of seizures), and disease-modifying (alteration of the natural history of the disease). However, special care should be taken in the choice of the model and of the endpoint for evaluation of effectiveness in order to correctly allocate the results in terms of translation to clinical relevance. In this respect (Fig. 2), a proposed conservative approach will be adopted here [18]. 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, only treatments in which gene therapy was applied after the epileptogenic insult will be considered as potentially antiepileptogenic. Even in this case, indisputable evidence of an antiepileptogenic effect comes from prolonged (at least a few months) observation of treated animals and verification that the effect is maintained well after termination of transgene (over)expression. If this level of evidence is not available, the effect should not be considered antiepileptogenic, and it should be more

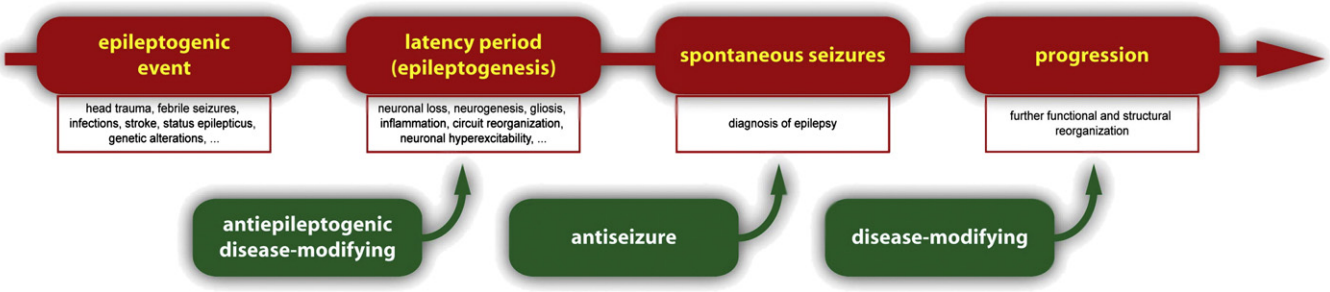

Fig. 1. Natural history of acquired focal epilepsy (in red) and possible therapeutic intervention (in green).

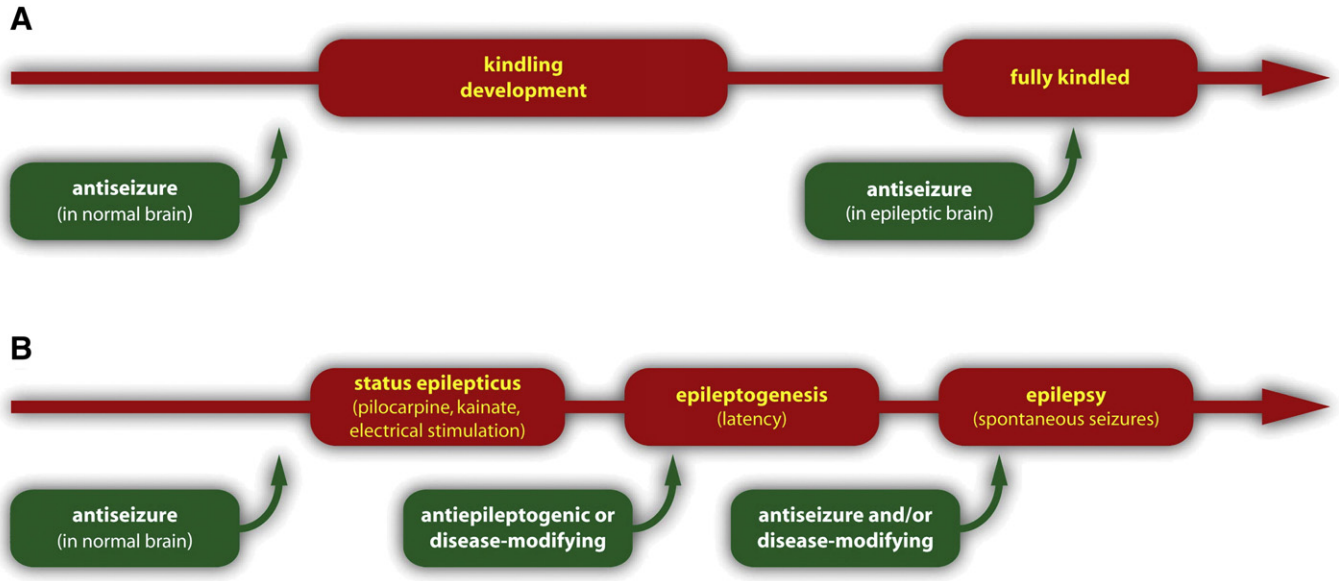

Fig. 2. Murine models of acquired epilepsy employed in gene therapy studies. The time of gene transfer and its therapeutic significance are in green.

appropriately defined as disease-modifying. A disease-modifying effect may also be documented as neuroprotection, arrest of disease progression in the chronic phase (progressive increase in SRS frequency), or reduction of comorbidities.

Below, the results of gene therapy studies in epilepsy models will be described and discussed. A schematic summary of the data is provided in Table 1.

5. Gene therapy: antiepileptogenic effects

Based on the above criteria, there is no demonstrated strategy that can actually exert antiepileptogenic effects. A series of studies, however, although not yet providing a final proof, strongly support this notion [14,19,20]. Both in 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 neurotrophic factors (NTFs) may be implicated because of their involvement in many of the cellular alterations associated with epileptogenesis [21,22]. In fact, NTFs can not only exert trophic effects suggesting involvement in cell death, neurogenesis, and axonal sprouting but they can also exert functional effects at the synaptic level, with distinct modulatory actions at excitatory and inhibitory synapses.

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 [22]. Thus, Paradiso et al. [14] reasoned that supplementation of 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 in one hippocampus 4 days after pilocarpine-induced SE, i.e., during latency and after the establishment of hippocampal damage. These conditions are similar to those of a patient that, following occurrence of an epileptogenic insult, is in the 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 2 weeks, but this is an advantage in these specific 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 [23]. The goal 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 in vitro and in vivo analyses of both NTF processing and release.

Administration of 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, favoring proliferation of early progenitors, leading to the production of cells that entered the neuronal lineage of differentiation and reducing some aberrant aspects of SE-induced neurogenesis. 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, with a nearly complete preservation of somatostatin interneurons. To verify that these beneficial effects were sufficient to ameliorate the outcome of the disease, animals were monitored through video-EEG for 20 days, and the occurrence, severity, and duration of SRSs were recorded. As expected, all nonvector-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; 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) by injecting the vector expressing FGF-2 and BDNF in animals that were already experiencing SRSs and testing if the treatment was effective in controlling seizures. In fact, the effect was negligible in this respect, arguing that the treatment interferes selectively with epileptogenesis [14].

6. Gene therapy: antiseizure effects

One first logical target for the gene therapy of individuals with drug-resistant seizures is the modulation of excitability by increasing the strength of inhibitory signals or reducing the strength of excitatory signals. One study focused on $GABA_A$ receptors. Expression of $GABA_A$ alpha-1 subunits is decreased while expression of alpha-4 subunits is increased in the granule cells of the hippocampus of epileptic (pilocarpine) rats compared with controls [24]. This altered expression pattern may

Table 1
Summary of the gene therapy studies in epilepsy.

Gene	Vector	Model	Site of injection	Timing	Results	Reference
Antiepileptogenic						
FGF-2 and BDNF	HSV-1	Pilocarpine	Hippocampus	Latency (4 days after SE)	DM: reduced cell loss, increased neurogenesis AE: reduced sz frequency and severity	Paradiso et al. (2009)
					DM: reduced neuroinflammation	Bovolenta et al. (2010)
					DM: reduced mossy fiber sprouting	Paradiso et al. (2011)
Antiseizure						
$GABA_A$ subunit alpha1	AAV-2	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 colliculus stimulation	Inferior colliculus	Before stimulation	AS or PC (depending on the promoter and the transduced cells)	Haberman et al. (2002)
Galanin	AAV-2	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 IC stimulation	AS: increased seizure threshold	
		Intrahippocampal kainate	Hippocampus	Before kainate	AS: reduction of seizure frequency and severity	Lin et al. (2003)
		Ip kainate	Piriform cortex	Before kainate	AS: reduction of seizing animals	McCown (2006)
		Piriform cortex kindling	Piriform cortex	Fully kindled	AS: increased seizure threshold	
NPY	AAV-2 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	
	AAV-2	Ip 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 SE: no alteration in LTP	Sorensen et al. (2009)
	AAV-1	Intrahippocampal kainate	Hippocampus	Before kainate	AC: reduction of seizure frequency and duration SE: no alteration in learning and memory, anxiety, and locomotor activity	Noè et al. (2010)
Y2 receptor	AAV-1/2	Rapid hippocampal kindling; sc 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	AAV-2	Hippocampal kindling	Hilus of dentate gyrus	Before kindling	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)	AAV-8	ADK transgenic mice	Intra-CA3	Spontaneously seizing mice	AC: reduction of spontaneous seizures	Theofilas et al. (2011)
ICP10PK (antiapoptotic gene)	HSV-2	Ip kainate	Intranasal	Before kainate	AC: prevention of seizures DM: prevention of neuronal loss and inflammation	Laing et al. (2006)
Kv1.1	LV	Tetanus toxin in the motor cortex	Cortex (seizure focus)	During or after the epileptogenic insult (together with tetanus toxin or 1 week after tetanus toxin)	AE: prevention of epileptiform events following administration during the epileptogenic insult DM: reduction of epileptiform events following administration in established epilepsy	Wykes et al. (2012)

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

be critical for the generation of seizures. Thus, Raol et al. [25] designed an AAV-2 vector containing the alpha-4 subunit gene promoter to drive alpha-1 expression. They injected this vector in the hippocampus 2 weeks before pilocarpine SE, obtaining increased alpha-1 expression in the granule cells, increased latency, and decreased number of rats developing SRSs in the first 4 weeks after SE. Although these effects may be interpreted as antiepileptogenic, the possibility that the vector attenuated SE and only secondarily protected from SRSs cannot be excluded.

The idea of protecting from seizures by reducing the strength of excitatory signals was tested in the inferior colliculus stimulation model by cloning in antisense an essential subunit for the functioning of N-methyl-D-aspartate (NMDA) receptors (NR1) in two AAV vectors, in which different promoters with different cell specificity controlled the transgene expression [26]. Depending on the promoter, the cells expressing the transgene (those where NMDA currents were downregulated) were either inhibitory interneurons or primary seizure output neurons, which led respectively to the inhibition of inhibitory or excitatory neurotransmission. As a consequence, the two vectors had opposite effects on focal seizures [26]. These observations underlie the importance of transducing a specific cell population any time the transgene codes for a receptor (or a channel) expressed both on inhibitory and excitatory neurons. Consistent with this idea, the LV vector-mediated overexpression of the potassium channel Kv1.1 preferentially in excitatory neurons had a therapeutic effect on neocortical epilepsy [27].

As described for NTFs, one means to circumvent this problem could be the expression of 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 could be achieved without a 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 [28] and increasing the hippocampal levels of the endogenous anticonvulsant adenosine with an AAV-8 vector expressing the enzyme that catabolizes adenosine (adenosine kinase, ADK) in antisense [29]. However, the most promising results have been obtained with the inhibitory neuropeptides galanin (GAL) and neuropeptide Y (NPY).

Galanin is a 29-amino acid neuropeptide released during seizures and inhibits glutamate release in the hippocampus [30]. Administration of GAL receptor agonists attenuates seizures, while pharmacological blocking exerts proconvulsant effects. Transgenic mice with functional deletion of GAL and galanin type-1 receptor genes have spontaneous seizures or enhanced susceptibility to seizures, while transgenic mice overexpressing GAL in seizure pathways are resistant to epilepsy. Several synthetic agonists of galanin type-1 and type-2 receptors inhibit experimental seizures. In order to obtain constitutive secretion of GAL from transduced cells in the seizure-generating area, Haberman et al. [31] 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 in the hippocampus, it attenuated kainate seizures and prevented kainate-induced hilar cell death and, after injection in the inferior colliculus, it increased seizure threshold in this area [31]. Coherently with these findings, other studies reported that AAV-mediated expression of GAL in the hippocampus reduces the frequency and severity of seizures caused by the intrahippocampal injection of kainate [32] and that AAV-mediated expression of GAL in the piriform cortex reduces the number of animals afflicted with seizures after peripheral administration of kainate [33]. 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 if this may also hold true in an epileptic brain, McCown [33] injected the AAV–FIB–GAL vector in 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 is a 36-amino acid neuropeptide that is overexpressed during seizures [34]. Activation of 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 patients with epilepsy, 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 in rats. The NPY-coding gene was transferred in the hippocampus using two types of vectors based on AAV-2 or AAV-1/2 (a vector consisting of a 1:1 mixture of AAV-1 and AAV-2 capsid proteins) 8 weeks before intrahippocampal injection of kainate or rapid kindling, respectively, resulting in a decreased occurrence of seizures or a retardation in kindling development [15]. Similarly, bilateral piriform cortex infusions of AAV vectors that constitutively secrete NPY (AAV–FIB–NPY) increased latency to kainate seizures [35]. Moreover, AAV-induced overexpression in the hippocampus of the Y2 receptor exerted seizure-suppressant effects per se and potentiated the effects of NPY overexpression [36]. Together, these findings strongly support an antiseizure effect in the normal brain.

To evaluate if this effect was also present in the epileptic brain, the NPY-expressing AAV-1/2 vector was injected bilaterally in the hippocampus of rats that were experiencing SRSs after electrically induced SE, and a significant reduction in seizure frequency was found in 40% of the cases [37]. It is even more interesting that a remarkable attenuation of the progressive increase in seizure frequency (i.e., a disease-modifying effect) was also observed. 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 LTP, an indication that it will not further impair epilepsy-associated memory loss [38]; furthermore, an NPY-expressing AAV-1 vector, while demonstrating a potent anticonvulsant activity, did not cause alterations in learning and memory, anxiety, and locomotor activity in behavioral tests [39]. Taken together, the overall evidence supports the application of AAV–NPY gene therapy for human epilepsy.

7. Future developments

Gene therapy offers a wealth of opportunities for epileptologists. Vectors can be tailored to the desired needs (1) in terms of spread from the zone of inoculation (different degrees of spread for different AAV serotypes, retrograde transport for HSV — and, maybe soon, new vectors will be available for peripheral administration with selective localization in lesion areas for the treatment of focal epilepsies, or widespread distribution in the brain for the treatment of generalized epilepsies); (2) in terms of duration of transgene expression (relatively short-lasting with HSV and long-lasting with AAV and LV vectors); and (3) in terms of targeting specific cell populations (for example, employing population-specific promoters).

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

There is no doubt that accurate verification of safety and scale-up studies is needed before beginning studies in humans, but gene therapy experience in humans with other diseases is encouraging [4]. Once these last hurdles are overcome, the GAL and the NPY gene therapy strategies will likely progress to phase I clinical trials.

Conflict of interest

The authors have nothing to disclose.

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References

[1] Galanopoulou AS, Buckmaster PS, Staley KJ, Moshe SL, Perucca E, Engel Jr J, et al. Identification of new epilepsy treatments: issues in preclinical methodology. Epilepsia 2012;53:571–82.
[2] Simonato M, Loscher W, Cole AJ, Dudek FE, Engel Jr J, Kaminski RM, et al. Finding a better drug for epilepsy: preclinical screening strategies and experimental trial design. Epilepsia 2012;53:1860–7.
[3] Simonato M, French JA, Galanopoulou AS, O'Brien TJ. Issues for new antiepilepsy drug development. Curr Opin Neurol 2013;26:195–200.

[4] Simonato M, Bennett J, Boulis NM, Castro MG, Fink DJ, Goins WF, et al. Progress in gene therapy for neurological disorders. Nat Rev Neurol 2013;9:277–91.
[5] Berkovic SF, Mulley JC, Scheffer IE, Petrou S. Human epilepsies: interaction of genetic and acquired factors. Trends Neurosci 2006;29:391–7.
[6] de Boer AG, Gaillard PJ. Drug targeting to the brain. Annu Rev Pharmacol Toxicol 2007;47:323–55.
[7] Simionescu M, Popov D, Sima A. Endothelial transcytosis in health and disease. Cell Tissue Res 2009;335:27–40.
[8] Di Pasquale G, Chiorini JA. AAV transcytosis through barrier epithelia and endothelium. Mol Ther 2006;13:506–16.
[9] Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol 2009;27:59–65.
[10] Goncalves MA. Adeno-associated virus: from defective virus to effective vector. Virol J 2005;2:43.
[11] Matrai J, Chuah MK, VandenDriessche T. Recent advances in lentiviral vector development and applications. Mol Ther 2010;18:477–90.
[12] Simonato M, Manservigi R, Marconi P, Glorioso J. Gene transfer into neurones for the molecular analysis of behaviour: focus on herpes simplex vectors. Trends Neurosci 2000;23:183–90.
[13] Nayak S, Herzog RW. Progress and prospects: immune responses to viral vectors. Gene Ther 2010;17:295–304.
[14] Paradiso B, Marconi P, Zucchini S, Berto E, Binaschi A, Bozac A, et al. Localized delivery of fibroblast growth factor-2 and brain-derived neurotrophic factor reduces spontaneous seizures in an epilepsy model. Proc Natl Acad Sci U S A 2009;106:7191–6.
[15] Richichi C, Lin EJ, Stefanin D, Colella D, Ravizza T, Grignaschi G, et al. Anticonvulsant and antiepileptogenic effects mediated by adeno-associated virus vector neuropeptide Y expression in the rat hippocampus. J Neurosci 2004;24:3051–9.
[16] Laing JM, Gober MD, Golembewski EK, Thompson SM, Gyure KA, Yarowsky PJ, et al. Intranasal administration of the growth-compromised HSV-2 vector deltaRR prevents kainate-induced seizures and neuronal loss in rats and mice. Mol Ther 2006;13:870–81.
[17] Gray SJ, Blake BL, Criswell HE, Nicolson SC, Samulski RJ, McCown TJ, et al. Directed evolution of a novel adeno-associated virus (AAV) vector that crosses the seizure-compromised blood–brain barrier (BBB). Mol Ther 2010;18:570–8.
[18] Dudek FE. Commentary: a skeptical view of experimental gene therapy to block epileptogenesis. Neurotherapeutics 2009;6:319–22.
[19] Paradiso B, Zucchini S, Su T, Bovolenta R, Berto E, Marconi P, et al. Localized overexpression of FGF-2 and BDNF in hippocampus reduces mossy fiber sprouting and spontaneous seizures up to 4 weeks after pilocarpine-induced status epilepticus. Epilepsia 2011;52:572–8.
[20] Bovolenta R, Zucchini S, Paradiso B, Rodi D, Merigo F, Navarro Mora G, et al. Hippocampal FGF-2 and BDNF overexpression attenuates epileptogenesis-associated neuroinflammation and reduces spontaneous recurrent seizures. J Neuroinflammation 2010;7:81.
[21] Simonato M, Tongiorgi E, Kokaia M. Angels and demons: neurotrophic factors and epilepsy. Trends Pharmacol Sci 2006;27:631–8.
[22] Simonato M, Zucchini S. Are the neurotrophic factors a suitable therapeutic target for the prevention of epileptogenesis? Epilepsia 2010;51(Suppl. 3):48–51.
[23] Thoenen H, Sendtner M. Neurotrophins: from enthusiastic expectations through sobering experiences to rational therapeutic approaches. Nat Neurosci 2002;5:1046–50 [Suppl.].
[24] Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. Nat Med 1998;4:1166–72.
[25] Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, et al. Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. J Neurosci 2006;26:11342–6.
[26] Haberman R, Criswell H, Snowdy S, Ming Z, Breese G, Samulski R, et al. Therapeutic liabilities of in vivo viral vector tropism: adeno-associated virus vectors, NMDAR1 antisense, and focal seizure sensitivity. Mol Ther 2002;6:495–500.
[27] Wykes RC, Heeroma JH, Mantoan L, Zheng K, MacDonald DC, Deisseroth K, et al. Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. Sci Transl Med 2012;4 [161ra152].
[28] Kanter-Schlifke I, Georgievska B, Kirik D, Kokaia M. Seizure suppression by GDNF gene therapy in animal models of epilepsy. Mol Ther 2007;15:1106–13.
[29] Theofilas P, Brar S, Stewart KA, Shen HY, Sandau US, Poulsen D, et al. Adenosine kinase as a target for therapeutic antisense strategies in epilepsy. Epilepsia 2011;52:589–601.
[30] Lerner JT, Sankar R, Mazarati AM. Galanin and epilepsy. Cell Mol Life Sci 2008;65:1864–71.
[31] Haberman RP, Samulski RJ, McCown TJ. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. Nat Med 2003;9:1076–80.
[32] Lin EJ, Richichi C, Young D, Baer K, Vezzani A, During MJ. Recombinant AAV-mediated expression of galanin in rat hippocampus suppresses seizure development. Eur J Neurosci 2003;18:2087–92.
[33] McCown TJ. Adeno-associated virus-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity in vivo. Mol Ther 2006;14:63–8.
[34] Noe F, Frasca A, Balducci C, Carli M, Sperk G, Ferraguti F, et al. Neuropeptide Y overexpression using recombinant adeno-associated viral vectors. Neurotherapeutics 2009;6:300–6.
[35] Foti S, Haberman RP, Samulski RJ, McCown TJ. Adeno-associated virus-mediated expression and constitutive secretion of NPY or NPY13-36 suppresses seizure activity in vivo. Gene Ther 2007;14:1534–6.
[36] Woldbye DP, Angehagen M, Gotzsche CR, Elbrond-Bek H, Sorensen AT, Christiansen SH, et al. Adeno-associated viral vector-induced overexpression of neuropeptide Y Y2 receptors in the hippocampus suppresses seizures. Brain 2010;133:2778–88.
[37] Noe F, Pool AH, Nissinen J, Gobbi M, Bland R, Rizzi M, et al. Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. Brain 2008;131:1506–15.
[38] Sorensen AT, Nikitidou L, Ledri M, Lin EJ, During MJ, Kanter-Schlifke I, et al. Hippocampal NPY gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP. Exp Neurol 2009;215:328–33.
[39] Noe F, Vaghi V, Balducci C, Fitzsimons H, Bland R, Zardoni D, et al. Anticonvulsant effects and behavioural outcomes of rAAV serotype 1 vector-mediated neuropeptide Y overexpression in rat hippocampus. Gene Ther 2010;17:643–52.