Encapsulated Neuroprotection
to the Rescue—or How to Safely Protect
a Brain From Seizing

Long-Term, Targeted Delivery of GDNF From Encapsulated Cells Is Neuroprotective and Reduces Seizures in the Pilocarpine Model of Epilepsy
Paolone G, Falcicchia C, Lovisari F, et al. J Neurosci. 2019;39(11):2144-2156. doi:10.1523/JNEUROSCI.0435-18.2018. Epub 2019 Jan 21.

Neurotrophic factors are candidates for treating epilepsy, but their development has been hampered by difficulties in achieving stable and targeted delivery of efficacious concentrations within the desired brain region. We have developed an encapsulated cell technology that overcomes these obstacles by providing a targeted, continuous, de novo synthesized source of high levels of neurotrophic molecules from human clonal ARPE-19 cells encapsulated into hollow fiber membranes. Here, we illustrate the potential of this approach for delivering glial cell line–derived neurotrophic factor (GDNF) directly to the hippocampus of epileptic rats. In vivo studies demonstrated that bilateral intrahippocampal implants continued to secrete GDNF that produced high hippocampal GDNF tissue levels in a long-term manner. Identical implants robustly reduced seizure frequency in the pilocarpine model. Seizures were reduced rapidly, and this effect increased in magnitude over 3 months, ultimately leading to a reduction in seizures by 93%. This effect persisted even after device removal, suggesting potential disease-modifying benefits. Importantly, seizure reduction was associated with normalized changes in anxiety and improved cognitive performance. Immunohistochemical analyses revealed that the neurological benefits of GDNF were associated with the normalization of anatomical alterations accompanying chronic epilepsy, including hippocampal atrophy, cell degeneration, loss of parvalbumin-positive interneurons, and abnormal neurogenesis. These effects were associated with the activation of GDNF receptors. All in all, these results support the concept that the implantation of encapsulated GDNF-secreting cells can deliver GDNF in a sustained, targeted, and efficacious manner, paving the way for continuing preclinical evaluation and eventual clinical translation of this approach for epilepsy. SIGNIFICANCE STATEMENT: Epilepsy is one of the most common neurological conditions affecting millions of individuals of all ages. These patients experience debilitating seizures that frequently increase over time and can associate with significant cognitive decline and psychiatric disorders that are generally poorly controlled by pharmacotherapy. We have developed a clinically validated, implantable cell encapsulation system that delivers high and consistent levels of GDNF directly to the brain. In epileptic animals, this system produced a progressive and permanent reduction (>90%) in seizure frequency. These benefits were accompanied by improvements in cognitive and anxiolytic behavior and the normalization of changes in CNS anatomy that underlie chronic epilepsy. Together, these data suggest a novel means of tackling the frequently intractable neurological consequences of this devastating disorder.

Unilateral Ex Vivo Gene Therapy by GDNF in Epileptic Rats
Nanobashvili A, Melin E, Emerich D, et al. Gene Ther. 2019;26(3-4):65-74. doi:10.1038/s41434-018-0050-7. Epub 2018 Nov 21.

Temporal lobe epilepsy is the most common type of epilepsy in adults. This neurological disorder is characterized by focal seizures originating in the temporal lobe, often with secondary generalization. A variety of pharmacological treatments exist for patients having focal seizures, but systemically administered drugs offer only symptomatic relief and frequently cause unwanted side effects. Moreover, available drugs are ineffective in one-third of the patients with epilepsy. Thus, developing more targeted and effective treatment strategies for focal seizures, originating from, for example, the temporal lobe, is highly warranted. In order to deliver potential antiepileptic agents directly into the seizure focus, we used encapsulated cell biodelivery (ECB), a specific type of ex vivo gene therapy. Specifically, we asked whether unilateral delivery of glial cell line–derived neurotrophic factor (GDNF), exclusively into the epileptic focus, would suppress already established spontaneous recurrent seizures (SRS) in rats. Our results show that GDNF delivered by ECB devices unilaterally into the seizure focus in the hippocampus effectively decreases the number of SRS in epileptic rats. Thus, our study demonstrates that focal unilateral delivery of neurotrophic factors, such as GDNF, using ex vivo gene therapy based on ECB devices could be an effective antiepileptic strategy providing a base for the development of a novel, alternative, treatment for focal epilepsies.
Commentary

The safe, localized, and sustained delivery of therapeutics into the brain is one of the most pressing problems in the hunt for more effective treatment strategies for epilepsy. Preclinical studies have provided evidence for the potential therapeutic efficacy of many novel approaches but, so far, it has been difficult to safely “translate” those into clinical use. Recently, 2 preclinical studies by Nanobashvili et al and Paolone et al have reported exciting progress toward solving the problem to deliver a therapeutic safely into epileptic brains.

Therapeutic strategies that harness endogenous neuroprotective mechanisms of the brain are particularly promising. Signaling through neurotrophic factors, for example, may provide neuroprotection by counteracting negative effects of epilepsy-associated cell death and mossy fiber sprouting in the brain. An especially interesting neurotrophic factor that could provide therapeutic neuroprotection is glial cell line–derived neurotrophic factor (GDNF).

Glial cell line–derived neurotrophic factor increases transiently after seizure. Exogenous GDNF infused into the brain shortly before seizure induction reduces seizure susceptibility in rats, suggesting that the initial GDNF surge after a seizure is neuroprotective. Several preclinical studies showed that sustained supplementation of GDNF through virus-mediated gene therapies or stem cell implantation reduces seizure frequency in animal models of epilepsy, but, so far, none of these approaches have been deemed safe for humans. Nanobashvili et al and Paolone et al have now taken a step further toward a GDNF-based therapy in epilepsy: they used an elegant method for localized and sustained delivery of GDNF to the brain, called encapsulated cell delivery (ECB), which strongly reduced seizure frequency in epileptic rats and, after further research, could be suitable for use in humans in the future.

The benefits of their system are evident: An inert capsule containing living cells engineered to secrete human GDNF and capable of exchanging nutrients, oxygen, and growth factors with its surroundings is directly inserted into the affected brain area. This strategy prevents the body’s natural immune response to exogenous cells and proteins, provides a sustained source of GDNF over weeks and months, and restricts the delivery of the seizure-suppressing factor to brain regions most affected by epilepsy.

The 2 studies demonstrate that this strategy reduces the frequency of SRSs in 2 different rat models of acquired epilepsy. Nanobashvili and colleagues used a focal lesion model in which the proconvulsant kainic acid was injected unilaterally into the hippocampus. After the rats developed recurrent spontaneous seizures, seizure frequency was monitored by continuous video-electroencephalogram (EEG) recording for 2 weeks. Then, the capsule containing GDNF-producing cells was implanted into the side of the lesion, and animals were video-EEG monitored for another 2 weeks. Mice implanted with devices that produced GDNF showed a reduction in seizure frequency compared to the pretreatment phase as well as compared to those mice implanted with a control device. The study by Paolone and colleagues used systemic injection of the proconvulsant pilocarpine to induce epilepsy and implanted 2 devices bilaterally into the hippocampus. Here, the GDNF-releasing capsules reduced the frequency of spontaneous seizures for months.

Both studies convincingly showed the seizure-suppressing effect of the strategy, but Paolone and colleagues went a step further: They demonstrated that the treatment also reversed several cellular and cognitive deficits in the epileptic rats including impaired memory, neurodegeneration, and abnormal neurogenesis. Although it is unclear whether these changes are secondary to the reduced seizure burden or an independent effect of GDNF, they provide strong support that this strategy has the potential to comprehensively improve epilepsy-associated symptoms.

One interesting aspect of the Paolone et al study is that seizure burden remained reduced even after the device was removed. It is unclear, though, how lasting this effect is: the monitoring period postremoval was only very brief (20 days), making it difficult to truly assess the long-term outcome. Moreover, the seizure frequency postremoval was significantly higher than preremoval and, perhaps most importantly, there seemed to be a slight increase in seizure frequency between days 1 to 10 and 10 to 20 postremoval. Epilepsy can be self-perpetuating in that every seizure causes molecular and cellular changes that increase the likelihood of having another seizure. The fact that seizures increased after device removal argues in favor of a further exacerbation of epilepsy over longer postremoval periods.

Several issues need to be addressed before a similar strategy could be applied in humans. The reoccurrence of seizures after device removal seems to require permanent implantation; however, it is unclear how long the cells can survive in the device. The capsule enables exchange of oxygen and nutrients, but there is no mechanism to remove dying or dead cells. One concern is that with the expected increasing amounts of necrotic cells within the capsule the benefits of GDNF secretion might be outweighed by the negative effects of molecules released by the dying cells. This could be easily overcome by replacing the device periodically, but comprehensive studies are needed to establish how long the encapsulated cells are beneficial to reduce seizures to avoid unnecessary repeated surgeries.

Glial cell line–derived neurotrophic factor expression increases after an induced seizure indicating that altered GDNF is an effect of, rather than a cause for, epilepsy. Glial cell line–derived neurotrophic factor supplementation, therefore, does not seem to target a disease-causing effect but instead augments an endogenous neuroprotective mechanism. This raises the question whether abnormally increased GDNF would lead to compensatory mechanisms over time, by, for example, a reduction in the receptors for GDNF, which could lead to reduced efficacy and seizure recurrence.

These concerns do not negate the exciting promise of this novel strategy in epilepsy. The possibilities using the ECB approach seem to be endless: what if cells are being engineered...
to secrete multiple neurotrophic factors shown to be diminished in epilepsy? Such a strategy may reduce potential compensatory effects of a monotherapy. Other secreted “therapeutics” could be delivered to the brain, such as microRNAs, a class of small molecules that regulate the expression of mRNAs and are promising novel therapeutic targets in epilepsy. MicroRNAs can be secreted in microvesicles as a means of transfer between glia cells and neurons, opening the possibility to engineer cells that secrete microvesicles containing antiepileptic microRNAs.

The two discussed studies are important for at least 2 reasons: first, they support earlier findings that sustained supply of the neurotrophic factor GDNF reduces seizure frequency and associated symptoms in epilepsy. Second, and maybe of even greater interest, they show that ECB is a promising strategy for sustained delivery of antiepileptic molecules to the brain. Such a strategy may be advantageous compared to in vivo gene therapy because, unlike a virus-mediated gene transfer, this method is reversible, localized, and renewable and may thus be safer for use in humans.

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