

Encapsulated Cell Therapy for The Treatment of Epilepsy

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Abstract

Contemporary antiepileptic drugs are ineffective in approximately 30% of the patients. These patients continue to experience seizures and, in many cases, seizures increase in frequency and are associated with significant cognitive decline and psychiatric disorders. The delivery of trophic factors such as glial cell-derived neurotrophic factor (GDNF) to the CNS has tremendous potential for treating a range of diseases including epilepsy. We have recently tested a clinically-validated, implantable cell encapsulation system (EC) that delivers high levels of GDNF in a selective, long-term and stable manner to the epileptogenic area of pilocarpine treated rats. As such, this therapeutic technology platform combines the potency of de novo in situ synthesis of cell-derived GDNF with the safety of an implantable, biocompatible, and retrievable medical device. The de novo synthesized source of very high levels of GDNF in the brain region of interest proved able to significantly reduce generalized seizures frequency, improved cognitive performance and normalized anatomical alterations associated with chronic epilepsy.

Keywords: cell encapsulation, GDNF, epilepsy

Introduction

Epilepsy is a devastating neurological condition that affects about 50 million people of all ages and ethnicities. The affected individual's daily activities, education, employment, and social life is considerably disrupted due to the sudden and unpredictable severity of seizures as well as the adverse effects of the majority of medications. Even with the best pharmacotherapy options, approximately 30% of patients do not benefit from drug treatment. Together with serious cognitive and psychiatric co-morbidities, the personal, familial, and societal impact of epilepsy is almost impossible to calculate.

Direct, local delivery of therapeutic factors to the disease locus may optimize the chances of obtaining efficacy by maximizing treatment of the impacted brain region and avoiding the unwanted side-effects that occur with systemic drug administration. Here we briefly discuss several potential therapeutic proteins of growth factors with an emphasis on recent studies using polymer encapsulated cells as the delivery system.

Neurotrophic Factors and Epilepsy

Glial cell line-derived neurotrophic factor (GDNF)

In 1993 Lin and colleagues reported the presence of dopaminergic neurotrophic activity in conditioned media derived from primary glial cells, several cell lines with properties of glia and the characterization of the GDNF secreted by a specific (rat B49) glial cell line [1]. Later, it was reported that GDNF promoted neurotransmitter release from dopaminergic neurons in vivo [2] and in vitro [3], as well as from motoneurons in vitro [4]. This was heralded as a breakthrough as available treatments were aimed at simply increasing dopaminergic transmission while GDNF also prevented neuronal degeneration and increased the functional activity of the remaining dopaminergic cells.

Together these findings made GDNF a potential therapeutic agent for various central nervous system diseases, including Parkinson's disease [5], stroke [6] and motoneuron injury [7].

GDNF belongs, together with Neurturin (NRTN), Artemin (ARTN) and Persephin (PSPN), to the GDNF-family ligand (GFL) which, in turn, is part of the transforming growth factor- β (TGF- β) superfamily [8]. Structurally, GDNF presents a typical motif, *cystine knot*, that is involved in protein stabilization and homodimer formation and consists of six cysteine residues that are linked together by three disulphide bonds. GDNF dimer is readily released from the neural cells and binds to GFR α 1 receptor. The GDNF-GFR α 1 complex then interacts with NCAM receptors or with two RET molecules, inducing their homodimerization and tyrosine autophosphorylation and initiating downstream signaling pathways [9,10].

GDNF has also emerged as a possible agent for epilepsy treatment, because GDNF family ligand and receptors are expressed in areas salient for seizure generation. Specifically, Kokaia and colleagues demonstrated a dynamic regulation of GDNF circuits after seizures important for the effectiveness of neuroprotective responses. In fact, following seizures, GDNF and c-Ret mRNA expression increased selectively in the dentate granule cells and hilar neurons, respectively, and GFR α -1 mRNA level in both neuron types, which would markedly enhance GDNF signaling in this circuit [11].

Brain-derived neurotrophic factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is one of the most widely distributed and extensively studied neurotrophins in the mammalian brain [12]. There is controversy regarding the role of BDNF in epileptogenesis. Croll and colleagues showed that overexpression of BDNF increased seizure susceptibility [13]. This observation was confirmed by a previous study where the status epilepticus (SE)

induced dramatic increase in BDNF expression that persisted for several days [14]. Furthermore, increased BDNF correlated with SE-induced activation of TrkB signaling [15]. A combination of genetic approaches showed that PLC γ 1 may be the dominant signaling effector of SE-induced TrkB activation promoting epileptogenic consequences [16]. In contrast, several studies revealed that SE-induced TrkB activation may promote neuroprotective effects by the activation of Shc/Akt signaling pathway, resulting in neuronal survival for mammalian neurons in vitro [17,18] and for peripheral sensory neurons in vivo [18]. Supporting a positive role for BDNF in seizure reductions, we recently reported that EC devices filled with genetically modified human cells engineered to release BDNF implanted into the hippocampus of pilocarpine-treated rats, decreased the frequency of spontaneous seizures by more than 80% [19].

Fibroblast Growth Factor (FGF2)

FGF-2 is a polypeptide composed of 146 amino acids, originally purified from the bovine pituitary and named due to its fibroblast growth property [20]. FGF-2 reduces brain cellular damage and improves functional recovery in experimental models of stroke, epilepsy, and traumatic brain and spinal cord injury proving to have neuroprotective effects [21].

Some studies have shown that FGF-2 overexpression reduces seizure induced-cell death and a prolonged administration of FGF-2 also reduced long-term behavioral deficits [22,23].

Paradiso and colleagues tested the combined action of FGF-2 and BDNF using viral vectors to locally supplement the two NTFs post epileptogenic damage. In vitro, these vectors increased the proliferation of neural progenitors and favored their differentiation into neurons. In vivo, the localized delivery of FGF-2/BDNF expressing vectors in a lesioned hippocampus, reduced epileptogenesis and spontaneous recurring seizures while also increasing local neurogenesis [24].

Tracolimus (FK506)

FK506, a calcineurin inhibitor and potent immunosuppressant used to prevent allograft rejection, exhibits neuroprotective effects in central nervous system diseases including epilepsy [25-27]. FK506 prevents kainic acid-induced seizures by inhibiting the sprouting of mossy fibers in the hippocampus [28] and attenuates pilocarpine-induced seizure and neuronal loss in the hippocampus of rats via free radical reduction and inhibition of inflammatory factors [29]. Moreover, FK506 treatment significantly increased the latency period to seizures and decreased the maximal intensity of seizures. FK506 also prevents and restored cognitive dysfunction by inhibiting reactive astrogliosis in pilocarpine-induced status epilepticus rats [30].

Vascular Endothelial Growth Factor (VEGF)

The vascular endothelial growth factor (VEGF) is a crucial regulator of angiogenesis, vascular permeability and it exerts a direct trophic effect on neuronal and glial cells in the central nervous system [31]. Moreover, it has been implicated in several neurological disorders, including epilepsy [32] although it appears to possess both pro- and anti-epileptic effects. Cabral and colleagues reported overexpression of VEGF-A, VEGF-B, and VEGF-C, their specific and accessory receptors in temporal neocortex of pharmacoresistant Temporal Lobe Epilepsy (PR-TLE) patients [33]. In contrast, additional studies [34,35], particularly those by Nicoletti and colleagues, showed that local infusion of Flt-Fc, a VEGF trap, increased neuronal loss after status epilepticus, while infusion of exogenous VEGF into the hippocampus

protected against neuronal loss. These findings suggest the possibility that the up-regulation of endogenous VEGF after seizures may serve a neuroprotective role, and that the VEGF receptor system has potential as a novel therapeutic pathway for the development of exogenous ligands to prevent cell loss after severe seizures [36].

Therapeutic Potential of GDNF and New Delivery Systems

GDNF and other neurotrophic factors are candidates for treating epilepsy, but their development has been interfered by difficulties in achieving stable and targeted delivery of efficacious concentrations within the desired brain region [37]. Numerous approaches are under investigation for delivering GDNF and other molecules directly to the brain including viral vectors, cell transplants and cell encapsulation systems (EC).

Virus-mediated gene therapies

The direct introduction of different genes inside the brain of epileptic patients, each designated to manage electrochemical activities that lead to epileptic insults, has potential for the treatment of the disease [38]. Yoo and colleagues demonstrated that Adenoviral vector-mediated overexpression of GDNF (Ad-GDNF) controls kainic acid (KA)-induced hippocampal cell loss and suppresses generalized tonic-clonic seizures. Pre-inoculation with Ad-GDNF into the KA-treated rat hippocampus 7 days before KA injection, suppressed tonic-clonic convulsions, significantly reduced the numbers of apoptotic cells and increased GAD-67 (GABA synthesizing enzyme) and Bcl-2 expression [39].

Similar results have been reported by Kanter-Schlifke and colleagues. They reported that recombinant adeno-associated viral (rAAV) vector-based GDNF overexpression in the hippocampus suppressed generalized seizure activity in kindled and SE-treated animal models of Temporal Lobe Epilepsy (TLE). Transduction with rAAV-GDNF attenuated seizure severity and convulsive activity in both kindled and SE-treated animals. These effects did, however, occur despite the fact that epileptogenesis was not affected [40]. Taken together, these data suggest that that long-term local increase of GDNF levels in the hippocampus could be a possible way of suppressing epileptic activity.

Cell-based therapies

Stem cell grafting-based therapies may be advantageous for treating chronic epilepsy thanks to the potential ability of stem cells to replace lost neuronal populations and to repair disrupted brain circuitries. Waldau and colleagues reported that neural stem cells (NSCs) derived from the embryonic medial ganglionic eminence (MGE; MGE-NSC) grafted into the hippocampus is an effective approach for suppressing spontaneous recurrent motor seizures (SRMS) in chronic TLE. That is, epileptic animals exhibited considerable reductions in seizure frequency, duration and severity. Furthermore, MGE-NSC grafting increased local GABAergic cell populations, increased GDNF tissue levels in epileptic animals and restored GDNF expression in hippocampal astrocytes [41]. In another studies, transplantation of bone marrow mononuclear cells (BMMCs) into epileptic animals has been found to be neuroprotective. In particular, Zanirati and colleagues, showed that BMMCs transplantation increased levels of several neurotrophic factors including GDNF. In fact, in the hippocampus of epileptic rats, GDNF mRNA levels were increased at 3 and 7 days post transplant while the expression of GDNF protein resulted augmented at 7 and 14 days post transplant [42].

Cell encapsulation systems (EC)

Cell encapsulation systems the EC combine the potency of de novo in situ synthesis of cell-derived GDNF with the safety of an implantable, biocompatible, and retrievable medical device. This system is based on implanting trophic factor-secreting cells encapsulated into a biocompatible matrix and separated from the host brain tissue by a polymer membrane. The membrane pores allow for oxygen and nutrients to enter and sustain the encapsulated cells while also allowing GDNF to leave the capsule and diffuse into the surrounding brain tissue. Furthermore, the pores are immunologically restrictive and prevent immunological interference with cell viability [43].

As shown below in **Figure 1**, this system is capable of providing long-term sustained delivery to the brain.

Several studies have addressed the potential of this system as a possible tool for the treatment for epilepsy. One of the first studies was conducted by Kanter-Schlifke and colleagues reporting that encapsulated genetically modified cells could release GDNF over at least 5 weeks in the hippocampus while modestly suppressing seizures in kindling animals. More recent studies have repeated and extended these studies using human derived ARPE-19 cells modified to produce significantly higher levels of GDNF [45,46]. Nanobashvili and colleagues used a focal lesion model generated through unilateral injections of proconvulsant kainic acid into the hippocampus. Following to the latent interval (2 weeks) during which the neural re-arrangements leading to spontaneous recurring seizures occur, EC devices loaded with GDNF-producing cells were implanted ipsilateral to the lesion. Rats implanted with devices showed significant reductions in seizure frequency compared to control rats [46].

Our group recently demonstrated not only the efficiency of sustained supplementation of GDNF in reducing seizures numbers in rat animal models of epilepsy but also that the EC-GDNF treatment reversed several cellular and cognitive deficits including impaired memory, neurodegeneration, and abnormal neurogenesis. Rats received bilateral implants EC-GDNF devices 20 days following the induction of status epilepticus (SE). In control rats (i.e. rats implanted with non-engineered parental cells or empty devices) the number of seizures continued unlimited, while in implanted EC-GDNF rats, seizures numbers were reduced by approximately 80% within 2 weeks and by more than 90% within 3 months. Importantly, these changes in behavior occurred with improvements in cognitive and anxiety-like behavior but without any associated alterations in general physiology (activity,

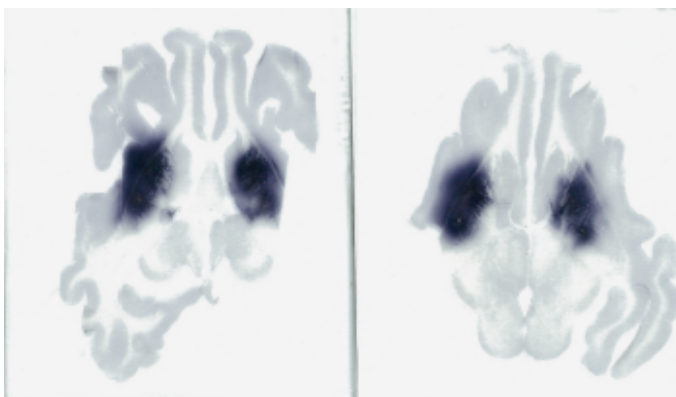


Figure 1: Serial sections illustrating immunohistochemistry of human GDNF distribution when delivered from encapsulated GDNF-secreting cells for 6 months. Widespread distribution of GDNF was observed at least 5-10 mm in each direction from the implant site.

weight gain, and circadian rhythmicity) rendering interpretations of the benefits specific to the treatment itself and not to any non-specific underlying mechanism. Anatomically, GDNF reversed the losses in hippocampal volume, reversed the loss of GABAergic parvalbumin-positive hippocampal cells, prevented the decreased density of NCAM receptors and the expression of pRET [37].

Conclusions

In conclusion, for the treatment of chronic diseases like epilepsy, it is necessary to use not only a direct and local delivery of therapeutic factor but also as long-term treatment such as an EC system to maximize treatment of the lesioned brain region avoiding the unwanted side-effects due to more widespread drug delivery achieved with systemic administration. Moreover, the EC treatment can be terminated by removal of EC devices from the brain and this latter aspect is important safety aspect for possible future clinical trials.

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