Translating regenerative medicine techniques for the treatment of epilepsy

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Abstract:

Epilepsy is considered a chronic neurological disorder and is accompanied by persistent and diverse disturbances in electrical brain activity. While antiepileptic pharmaceuticals are still the predominant treatment for epilepsy, the advent of numerous surgical interventions has further improved outcomes for patients. Despite these advancements, a subpopulation continues to experience intractable seizures which are resistant to current conventional and nonconventional therapeutic options. In this review, we begin with an introduction to the clinical presentation of epilepsy before discussing the clinically relevant laboratory models of epilepsy. Finally, we explore the implications of regenerative medicine – including cell therapy, neuroprotective agents, and electrical stimulation – for epilepsy, supplemented with our laboratory's data. This paper is a review article. Referred literature in this paper has been listed in the references section. The datasets supporting the conclusions of this article are available online by searching various databases, including PubMed. Some original points in this article come from the laboratory practice in our research center and the authors' experiences.

Keywords:

Central nervous system disorders, electrical stimulation, epilepsy, neurogenesis, neuroprotective agents, regenerative medicine, stem-cell therapy

Introduction

Defined by the existence of various irregularities in brain electrical activity (seizures), epilepsy is a chronic neurological disorder which affects a diverse population of patients.[1] Antiepileptic drugs are the initial treatment option for patients with epilepsy, yet approximately 20%–40% of patients display "refractory" epilepsy and do not respond favorably to these pharmaceuticals.[2] Excision of causative tissue may be a viable treatment modality for patients with temporal lobe epilepsy (TLE) – the most common form of epilepsy – accompanied by mesial temporal sclerosis, or for patients with lesion-induced epilepsy.[3] Alternatively, patients who exhibit refractory epilepsy and are not candidates for surgical intervention may

benefit from alternative therapies such as electrical stimulation, magnetic stimulation, adrenocorticotropic or immunoglobulin medications, ketogenic diet, and psychobehavioral therapy.[3] Even with various, patient-specific combinations of the aforementioned treatment modalities, many patients remain encumbered by persistent epileptic seizures, emphasizing the need for innovative therapeutic strategies to treat epilepsy.[4] One such novel intervention strategy which has been proposed is stem-cell therapy.[5] Induced pluripotent stem cells (iPSC) present a promising candidate donor cell for transplantation, yet critical concerns related to their tumorigenicity and irregular electrical activity intrinsic to the epileptic patient-derived cell should first be addressed.[6,7] Interestingly, iPSCs harvested from epileptic patients may be valuable in identifying new pathological mechanisms and treatment targets for this disease.[8]

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Here, we review relevant rodent models of epilepsy and discuss current progress in the treatment of epilepsy, with a focus on regenerative therapies including cell therapy, neuroprotection, and electrical stimulation. Moreover, due to its prevalence, TLE will receive the majority of our attention.

Murine Modeling of Epilepsy and Epileptogenesis

Two traditional explanations exist for the development of epilepsy – the recurrent excitation hypothesis and the recurrent inhibition hypothesis.[9] Recurrent excitation hypothesis proposes that seizures originate from abnormal excitatory circuitry mainly induced by mossy fiber sprouting, resulting in hyperexcitability of dentate granule cells.[10] Alternatively, the recurrent inhibition hypothesis holds that epileptic foci in the dentate granule cells result from a lack of inhibitory input.[10] Importantly, both hypotheses have displayed merit and have received support from various studies. In either case, epileptogenesis is thought to involve three progressive stages: an initial insult which precipitates pathological alterations, a latent asymptomatic period, and a chronic symptomatic phase.[11] Rodent models of epilepsy have been widely utilized in the characterization of TLE pathogenesis, and other subcategories of epilepsy. Many of the pathological symptoms which occur in epileptic patients are reproduced in TLE models, such as loss of inhibitory gamma-aminobutyric acid (GABAergic) interneurons, formation of abnormal neuronal circuit, loss of excitatory neurons in discrete hippocampal regions, changes in expression of multiple receptors/ion channels, and a consequential hyperexcitability due to the loss of excitation/inhibition balance.[12]

In the quest to characterize underlying mechanisms of TLE, various techniques have been employed including hyperthermia, trauma, hypoxia for newborn animals, chemo convulsants, electrical stimulation, tetanus toxins, and genetic manipulations.[13] Among the two most established models of TLE are the poststatus epilepticus model and kindling model. Status epilepticus, in humans, describes an acute, prolonged (5+ min) epileptic crisis; this if often accompanied by additional seizures after a latency period.[14] Poststatus epilepticus models use a severe insult, such as those induced by the chemoconvulsants kainic acid (KA) or pilocarpine, to mimic status epilepticus and provoke periodic seizures within the following weeks.[15,16] Thereafter, the periodic seizures can be tracked, and therapeutic interventions probed for beneficial effects. Both intrahippocampal and intraperitoneal administration of KA have been used to induce TLE models, and pose distinct advantages/disadvantages; systemic administration is convenient but highly toxic, resulting in increased specimen mortality while intrahippocampal

delivery requires invasive surgery, yet has a lower mortality rate.[17]

These chemoconvulsant models of TLE are valuable, but kindling models offer a distinct advantage due to their high success rate, lower mortality rate, and recapitulation of clinical symptomology. Kindling models use mild, recurrent electrical stimulation of the perforant pathway to target brain regions commonly responsible for clinical epileptogenesis, including the hippocampus, amygdala, and other causative loci.[13] Important for the ongoing progression of epileptic research is a thorough understanding of the benefits and drawbacks of each model. Indeed, many of the epileptic mechanisms and therapeutic strategies discussed here were uncovered by the appropriate utilization of these models.

Cell Therapy for Treating Epilepsy

In an attempt to substitute the damaged/irregular hippocampal neurons, a number of transplantable cell types have been examined in epilepsy models including fetal hippocampal cells, neuronal precursor cells from medial ganglionic eminences, and various neural stem cells (NSCs).[18-22] Embryonic stem cell-derived GABAergic neurons displayed notable efficacy in replacing GABAergic neurons of epileptic animals.[23,24] Similarly, GABAergic inhibitory interneurons transplanted into the hippocampus moderated epileptic hyperactivity, seizures, and additional abnormal behavioral metrics.[25] This was in contrast to transplantation of the same cells into the basolateral amygdala, which rescued the hyperexcitability deficit but afforded no favorable effects on seizure activity.[25] These findings shed light on possible mechanisms of epileptogenesis and also indicate inhibitory interneurons as potential therapeutic targets. In addition to direct cell replacement, stem cell transplantation may mediate functional benefits in epilepsy by targeting various pathogenic mechanisms through antithetical therapeutic pathways – promoting angiogenesis, synaptogenesis, neurogenesis, and anti-inflammatory effects to rescue/preserve damaged hippocampal tissue.[26,27]

Our laboratory investigated the effects of intrahippocampal transplant of adult-derived NSCs in a KA-induced model of epilepsy.[27] Two weeks following intraventricular KA infusion, transplanted rats displayed a reduction in abnormal electrical activity, as measured by electrophysical recording.[27] Furthermore, graft survival was detected in the CA3 region 5 weeks posttransplantation, with signs of migration into the subgranular zone.[27] The majority of transplant cells expressed GFAP (a marker of astrocytic phenotype), yet a subpopulation of transplant cells expressed Neuronal Nuclei (NeuN) (a phenotypic marker of mature neurons);[27] these

findings were accompanied by immunohistochemical evidence showing normalization of abnormal mossy fiber sprouting and a preservation of GABAergic inhibitory neurons.[27] In other studies, transplanted NSCs have been found to secrete important trophic factors such as stem cell factor which may have further contributed to the therapeutic effects reported.[28] Stem/progenitor cells positive for cellular kit (c-kit), a receptor for stem cell factor, confer neuroprotection by increasing astrocytic glutamate transporter GLT1 and consequently reducing extracellular glutamate.[29] Moreover, NSCs may promote neurogenesis of endogenous stem cells which contribute to the reparative effort.[30] These various therapeutic effects of NSC transplantation may work cooperatively to promote the survival of damaged cells in the epileptic brain and moderate the hyperactive, abnormal neural circuitry.

The secretion of various neurotrophic/neuroprotective factors by stem cells has been recognized as a leading mechanism of transplantation therapy.[31] With this in mind, our laboratory has developed an interest in investigating the effects which encapsulated stem cell transplantation have in the epileptic brain. Beyond allowing researchers to focus on the secretory mechanisms of stem cell therapy, stem cell encapsulation is associated with a number of distinct, clinically-relevant advantages; physical barriers prevent tumorigenesis while still allowing the cells to receive nutrients from the surrounding environment through the semipermeable membrane. The membrane also allows the distribution of secreted trophic factors which can exert therapeutic effects on the surrounding tissue. Cells which are encapsulated can be tailored or modified to secrete specific therapeutic factors or lack replicative senescence. Encapsulation also prevents immunologic rejection by host defenses, as the capsule hinders immunocompetent cells from accessing transplant epitopes. Due to this immune privilege, a more diverse array of transplantable stem cell types are safe and viable, including xenografts and conventionally immunogenic cell types. The secretion of trophic factors directly from encapsulated stem cells may be superior to direct trophic factor infusion through a mini-pump system, as the degradation of these proteinaceous molecules is reduced and dynamic host-graft interactions are still permitted with encapsulation. Chief among the stem cell donor sources which have been demonstrated to exhibit potent secretory effects are mesenchymal stem cells (MSCs) and umbilical cord blood cells.[32,33]

Nonencapsulated stem cell transplantation has also been tested in epilepsy models; genetically-modified MSCs which were prompted toward an inhibitory GABAergic phenotype amended functional deficits in a pilocarpine model of epilepsy.[34] Another study demonstrated the efficacy of intravenous mononuclear MSCs in ameliorating pilocarpine-induced epileptic activity.[26] In both studies, MSCs transplantation was associated with neuroprotection and neurorestoration mediated by anti-inflammatory effects.[26,34] In rats receiving MSCs 3 weeks following pilocarpine injections, the number of doublecortin + neuronal precursor cells decreased.[34] As an increase in abnormal neurogenesis is associated with acute phase epileptogenesis, this reduction in neurogenesis can be interpreted as a positive outcome. Rats receiving MSCs 10 months after pilocarpine injection, however, displayed an increase in doublecortin + neuronal precursor cells; the neuronal death associated with chronic epilepsy pathology suggests that the increase in neurogenesis at this later time point could be a positive indication for the restorative capacity of MSC therapy. Importantly, the necessity of this dynamic downregulation then upregulation of neurogenesis emphasizes the importance for cellular crosstalk between the epileptic brain and transplanted cells. The details of this relationship between neurogenesis and epilepsy development/progression are still shrouded in controversy regarding its therapeutic and pathological implications.[35] Moreover, the role of environmental queues, the timing of the neurogenesis-to-neurodegeneration transition, and its corresponding processes are yet to be established.

The advent of iPSCs and their potential within nervous system diseases has incited a burgeoning sub-field of research within the regenerative medicine community.[36] Notably, methods have been described for prompting human iPSCs into a primitive neural stem cell phenotype within 7 days.[37] Thereafter, it was shown that these induced NCSs could differentiate into specialized subtypes of neurons such as motor neurons, dopaminergic neurons, and GABAergic neurons.[37] Thus, these induced stem cells could present a promising and versatile donor source for the treatment of epilepsy. As mentioned previously, isolating stem cells from diseased patients could be a valuable tool in characterizing the causative pathological mechanisms of epilepsy, and in developing novel drug targets. The potential to develop *in vitro* epilepsy models amenable to pharmaceutical screening assays has been demonstrated. After developing iPSCs from skin fibroblasts of patients with Dravet syndrome – an infantile-onset epileptic condition – iPSCs were differentiated into neurons which assumed predominantly GABAergic phenotypes, with electrophysiological characteristic consistent with neurons derived from murine epilepsy models.[38] Complimenting this, an *in vitro* model established by similar protocol was responsive to the common anti-epileptic drug phenytoin (Dilantin), replicating the therapeutic response *in vitro* which is observed in humans.[39] Together, these studies demonstrate a powerful research opportunity: establishing an *in vitro*

epilepsy model that faithfully reflects *in vivo* pathology allows for high throughput screening of compounds which can effectively moderate neuron hyperexcitability. Another exciting ramification of this *in vitro* screening method is the prospect of determining drugs with anti-epilepto*genic* effects (i.e., drugs which prevent the development of epileptic characteristics, as opposed to antiepileptic drugs which minimize already existing hyperexcitability). Pharmaceuticals of this nature could offer the potential to preemptively impede the development of epilepsy in certain high-risk patients.[40-42]

Neuroprotective Agents for Treating Epilepsy

A variety of neurotrophic factors have been vetted as potential therapeutic options for the treatment of TLE. Overexpression of brain-derived neurotrophic factor (BDNF) and fibroblast growth factor within the hippocampus lessened cell death, increased neurogenesis, and provided anti-inflammatory effects in a pilocarpine-induced status epilepticus model.[43] When insulin-like growth factor-1 (IGF-1) was coadministered with KA in a chemoconvulsant model of TLE, IGF-1 mice displayed a reduction in hippocampal neurogenesis (a favorable outcome, given the acute phase measurement), a decrease in seizure activity, downregulation of cellular-level neurodegenerative markers, and improvement in cognitive metrics.[44] Innovative growth factor-based therapies also include modulating the mammalian target of rapamycin (mTOR) signaling pathway, which has been implicated in pharmacological hindering of epileptogenesis.[45,46]

Discrepancies exist in the literature regarding the appropriateness of BDNF in treating epileptic conditions; when BDNF interacts with the tropomyosin receptor kinase B (TrkB) receptor, the downstream signaling pathway may promote epileptogenesis.[47] Furthermore, analysis of mossy fiber pathways in the hippocampus reveals that seizures are associated with a drastic upregulation of BDNF and an increase in BDNF-TrkB signaling.[48] Supporting this harmful role, intraventricular administration of BDNF at either 1 or 3 μg/h for 7 days provoked spontaneous seizures while overexpression of BDNF worsened already-present seizure activity.[49,50] Finally, matrix metalloproteinase-9, which promotes the conversion of pro-BDNF to BNDF, has been revealed to facilitate epileptogenesis.[51] Conversely, certain studies have found anti-epileptic effects of BDNF treatment.[43] Our investigations have found that continuous low-dose (200–300 pg/h) BDNF administration through encapsulated BDNF-secreting cells exerted anti-epileptic effects.[52] Outcome measures verified behavioral and electrophysiological ameliorations in rats receiving BDNF treatment.[52] Immunohistochemical analysis showed an increase of neuronal precursor cells (doublecortin+) within the dentate gyrus and a preservation of mature neurons (NeuN+) in the CA1 and CA3.[52] Other studies support the notion that continuous low-dose BDNF may attenuate epileptic activity by increasing neuropeptide Y (NPY) expression.[53] Apparent from these studies is the importance of dosing and timing in the therapeutic usage of BDNF, particularly considering the BDNF upregulation seen in epileptic hippocampi.

Erythropoietin (EPO) is a well-characterized and widely-studied hormone which has the capacity for neuroprotection in diverse diseases of the central nervous system, such as ischemic stroke and Parkinson's disease.[54,55] A number of studies have evaluated EPO for therapeutic effects in the epileptic brain. EPO conferred anti-epileptic effects in a model of febrile seizures by dampening postseizure inflammation and through molecular regulation, rescuing numerous seizure-induced molecular alterations.[56] Using a KA-induced epilepsy model, our laboratory reported that intraventricular infusion of EPO reduced mortality and improved behavioral metrics.[57] Furthermore, histological data showed a preservation of NeuN + mature neurons in the CA1 region and a suppression of abnormal neurogenesis.[57] Importantly, administration of an NPY Y2 receptor antagonist negated the therapeutic efficacy of EPO, indicating NPY's role in the therapeutic effects exerted by EPO.[57] Further, recent evidence was provided demonstrating the neurogenic and neuroprotective effect exerted by intraventricular NPY infusion in an epilepsy model.[58] Our laboratory found that adjunctive treatment of EPO with NSCs in a KA-induced model of epilepsy significantly increased the survival rate of NSCs and drastically decreased mossy fiber sprouting compared to all other groups.[27] Another study by our group found that infusion of carbamylated EPO Fc fusion protein conferred robust neuroprotective effects, yet without hematopoietic effects, in a model of Parkinson's disease.[59] Our group proposes that EPO may be a novel therapeutic agent for the treatment of epilepsy.

Electrical stimulation for treating epilepsy
Electrical stimulation has a long history within the clinic for treatment of epilepsy and other neurological conditions, yet its use has not been optimized. While the safety of electric stimulation is largely undisputed, with vagus nerve stimulation being employed regularly for refractory epilepsy, treatment is often expensive and the efficacy is variable.[60,61] Vagus nerve stimulation may exert therapeutic effects to multiple regional structures which surround the stimulated tissue including the locus ceruleus and raphe nuclei.[62,63] In the kindling model of epilepsy, vagus nerve stimulation slowed the rate of hyperpolarization in cerebral cortex neurons, and elevated the seizure threshold.[64] In addition,

electrical stimulation for two consecutive days caused nearly 50% increase in the number of hippocampal BrdU + cells, whereas stimulation for 1 month incited morphological evidence of newly formed neurons and BDNF upregulation in the CA3.[65,66] These investigations support the notion that electrical stimulation may counteract epileptic aberrations through cellular reorganization and neurotrophic mechanisms within the causal brain loci. Other stimulation modalities, including epidural stimulation, deep brain stimulation, and transcranial magnetic stimulation, have been explored for the treatment of epilepsy, but with inconsistent or controversial findings. In one of our laboratory studies using an amygdala-kindling model of epilepsy, chemical suppression of the anterior thalamic nucleus was effective in reducing behavioral dysfunction and neurogenic abnormalities of the hippocampus.[67] This implies that the anterior thalamic nucleus may be a beneficial target for electrical stimulation, being that its chemical suppression reduced off-target seizure activity. Brain regions such as the cerebellum, cerebral cortex, substantia nigra pars reticula, and subthalamic nucleus may also be targets responsive to electrical stimulation.[68-70]

Using a stroke model, we recently provided data for the neuroprotective and neuroregenerative effects of epidural and deep brain stimulation, which prompted angiogenesis, neurogenesis, anti-inflammatory effects, anti-apoptotic effects, and an upregulation of trophic factors.[71,72] We demonstrated that electrical stimulation, particularly at low frequencies, conferred therapeutic benefits.[71] In line with this, electrical stimulation of the spinal cord in Parkinson's disease increased neuroplasticity, presenting itself as a possible alternative treatment option.[73,74] While debate persists as to the efficacy and mechanisms of electrical stimulation, a substantial body of positive preclinical data merits ongoing investigations into its applications in epilepsy. Improving our understanding of the mechanisms which underlie epileptic pathology will compliment and facilitate the search for novel therapeutic regimens.

Conclusions

Current treatment options for epilepsy include anti-epileptic medications and traditional treatment modalities such as surgical intervention and electrical stimulation. However, the need for new treatment options for patients with refractory epilepsy still exists. Critical to the progress of epilepsy research has been the establishment of standardized models, highlighting the importance which basic science research plays in improving patient outcomes. Moreover, translational research efforts are critical in determining safety profiles and efficacy readouts for promising therapeutic options. Looking forward,

regenerative medicine is an exciting frontier for epilepsy, providing opportunities for innovative treatment options and new tools for the exploration of disease mechanisms and pathology. Our laboratory endorses the concept that basic and translation research efforts into cell therapies, neuroprotective agents, electrical stimulation, and other regenerative tools are worthy endeavors in the quest to find effect means of managing epilepsy.

Conflicts of interest
There are no conflicts of interest.

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