2014 Epilepsy Benchmarks Area II: Prevent Epilepsy and Its Progression

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The prevention of epilepsy and its progression after initial diagnosis represents one of the most challenging goals of epilepsy research (1). Although epilepsy diagnoses lack a formal component of disease stage, anecdotal clinical observation suggests that individual patients usually fall into one of at least three types of presentation: an easily treated type that is responsive to the first or second antiseizure medications tried, a relapsing/remitting type in which seizure control is intermittent, and a drug-resistant type in which the patient never achieves seizure control with medication (2). As drug-resistant epilepsy is extremely difficult to treat, the successful prevention of epilepsy in at-risk patients may provide the best strategy to alleviate the negative impact of epilepsy. Benchmark II aims to establish therapeutic approaches for preventing the development and progression of epilepsy by 1) understanding epileptogenic processes related to genetic or neurodevelopmental causes, 2) understanding epileptogenic processes related to acquired brain injuries, 3) identifying biomarkers for monitoring epileptogenesis, 4) developing models closely aligned with etiologies of human epilepsies, and 5) identifying molecular targets that drive epileptogenesis or are opposed to it.

Key Advances in Area II
Understanding Epileptogenesis
Epileptogenesis is not a single process. It can be triggered by numerous genetic or nongenetic factors, and its course,

manifestations, and treatment response can be further modified by exogenous and endogenous factors that have age, sex, cell type, or stage-specific effects. This level of complexity complicates the identification of key target mechanisms that can infallibly be used to stop or prevent epilepsy or to improve outcomes. There is increasing interest in identifying personalized antiepileptogenesis strategies (3) and biomarkers (4) to guide treatment implementation. Since 2013, when the last Benchmark report was compiled, new epileptogenesis targets have earned the spotlight (e.g., microRNAs [miRNAs], new genes) and the role of a few well-studied targets or processes has been strengthened (mTOR, neuroinflammation/innate and adaptive immunity/blood–brain barrier [BBB]) supporting the idea that epileptogenesis is not necessarily exclusively neurocentric or brain-centric. New animal models, promising data on potential antiepileptogenic treatments (TrkB pathway inhibitors, ethosuximide), and candidate epileptogenesis biomarkers (electrophysiological, imaging, inflammatory, genetic) have also emerged.

The extensive interactions of complex epileptogenic pathways have, however, generated areas of convergence that can be utilized to develop treatment strategies with wider applicability. Pathways implicated in genetic epilepsies have proven to be valuable therapeutic targets for acquired epilepsies (e.g., mTOR inhibitors). Interneuronopathy has been implicated in various types of epilepsies (e.g., infantile spasms, Dravet syndrome). Interventions that are not specific for the affected gene have shown interesting antiepileptogenic effects in genetic syndromes. In the following sections, we will highlight a few of these advances that help set the pace for future directions in antiepileptogenesis research.

MiRNAs, small noncoding RNAs that regulate RNA translation and degradation, have differential expression patterns in human temporal lobe epilepsy (TLE) compared with unaffected individuals (5). Studies of acquired epilepsies in animal models examined miRNA changes in the hippocampus and cerebral cortex during the first 2 months after the insult. The greatest changes in miRNA populations occurred the first days after induced seizures. The majority of changes represented decreases in miRNAs and only a handful of increased miRNAs. The patterns of modified miRNAs were largely unique to each experimental seizure paradigm, as different profiles were observed for status epilepticus (SE) induced by pilocarpine, kindling, or kainic acid, reflecting high variability in the miRNA profile or hinting that multiple molecular pathways may underlie the spectrum of clinically defined epilepsies (6, 7). The miRNAs themselves may serve as drug targets. Antagomirs of miR-132 and miR-219 agomir that sequester or mimic, respectively, their endogenous miRNAs reduced long-term seizure frequency and severity when given during epileptogenesis (8, 9). The promising therapeutic use of antagomirs and agomirs leads to questions of identifying the targets of each miRNA, which in some cases can be >200 genes, and of efficient drug-delivery methods to the targeted brain region(s). The miRNAs measurable in blood may serve as biomarkers for monitoring the potential of developing epilepsy after injury.

Neuroinflammation and dysfunction of the BBB are often induced by epileptogenic insults and are detected in seizure foci in human drug-resistant epilepsy. Both events lead to neuronal hyperexcitability and contribute to epileptogenesis in various experimental models of acquired epilepsy (10, 11). Vascular injury leads to albumin extravasation into the brain parenchyma, which provokes astrocytic dysfunction and induces excitatory synaptogenesis (10), contributing to hyperexcitability and a chronic decrease in seizure threshold. In turn, activated astrocytes and reactive microglia generate neuroinflammation, which causes BBB damage and release of ictogenic cytokines (11, 12, 13). Notably, seizure activity per se can trigger and perpetuate both phenomena (11). In a recent study using two models of BBB breakdown, cortical superfusion of albumin or sodium deoxycholate, activation of albumin-mediated transforming growth factor beta (TGF-β) signaling was shown, which induced local inflammation and spontaneous seizures (10). The angiotensin II type 1 receptor antagonist, losartan, effectively blocked albumin-induced TGF-β signaling and prevented the development of recurrent seizures in a cohort of animals with impaired BBB (14). In a genetic mouse model of chronic diffuse astrogliosis provoked by conditional deletion of β1-integrin in radial glia, spontaneous seizures were observed (15). Finally, both neuroinflammation and BBB damage were found to be involved in some mechanisms of pharmacoresistance (16).

Imaging of neuroinflammation and BBB damage is providing tools for developing biomarkers (13, 17, 18) to facilitate the clinical translation of new therapies for antiepileptogenesis and disease modification. Radiotracers binding to the translocator protein 18kDa (TSPO), a marker of microglial activation, have been used in both animal and clinical studies to detect inflammation. These include [^{18}F]PBR111-PET (18) and (R)-[^{11}C]PK11195-PET (19, 20) in animal studies as well as [^{11}C]PBR28-PET and [^{11}C]DPA-713-PET imaging in humans (21). The (R)-[^{11}C]PK11195-PET imaging showed increased TSPO binding in phenobarbital nonresponder rats with epilepsy after self-sustained status epilepticus, suggesting that this could be a marker of drug resistance (19). In TLE, [^{11}C]PBR28 PET and [^{11}C]DPA-713 PET imaging showed increased TSPO binding both ipsilateral and contralateral to seizure foci and found that ligand uptake asymmetry was greater in patients with mesial TLE (MTLE) (21).

Antiepileptogenic Strategies

Brain-derived neurotrophic factor (BDNF) belongs in the neurotrophin family of neurotrophic factors and binds to its high-affinity receptor tropomyosin-related kinase B (trkB) and the low-affinity neurotrophin receptor p75. Limbic seizures in rodents increased the expression of BDNF mRNA, and protein. BDNF has proconvulsant and proepileptogenic effects, affecting synaptic plasticity, enhancing excitatory synapses, and compromising inhibitory GABA$_A$ receptor signaling (reviewed in [22, 23]). Intraventricular administration of trkB receptor bodies to scavenge BDNF or conditional deletion of trkB, but not of BDNF, in synapsin-positive neurons in mice inhibits limbic epileptogenesis, suggesting that trkB signaling may be *necessary* for limbic epileptogenesis. Using a novel chemical-genetic approach, transient inhibition of trkB kinase activity was initiated after termination of SE induced by right amygdalar infusion of kainic acid (24). Transient trkB inhibition for 2 weeks after SE prevented spontaneous seizures, reduced hippocampal neuronal loss, and ameliorated anxiety-like behavior. Using a peptide (pY816) that uncouples trkB from phospholipase Cγ>1 (PLCγ1), it was shown that PLCγ1 is the downstream effector of epileptogenesis via trkB signaling (25). Data on BDNF or trkB in the hippocampus of patients after SE are lacking. In individuals with MTLE, increased BDNF expression and reduced neuronal trkB expression in the hippocampus have been described (22, 26). The higher trkB hippocampal expression in patients with MTLE and depression or psychosis (26) may render this subpopulation an interesting target population.

The previously demonstrated antiepileptogenic effects of early ethosuximide treatment in Wistar albino Glaxo rats of Rijswijk (WAG/Rij) rats (27) have been replicated in genetic absence epilepsy rats from Strasbourg (GAERS) (28). Ethosuximide treatment before the typical onset of spike-wave discharges led to a decreased number and/or duration of spike-wave discharges and improved anxiety-like behavior, even after cessation of treatment. These data indicate that time-limited exposure to ethosuximide has long-term effects in two models of generalized epilepsy. There are no clinical reports of antiepileptogenic effects of presymptomatic treatment with ethosuximide in humans as identification of pre-symptomatic persons at risk is currently challenging. Interestingly, in the Childhood Absence Epilepsy (CAE) study, a double-blind randomized controlled trial of initial therapy for children with childhood absence epilepsy, early treatment failure with ethosuximide or valproic acid was a risk factor for late occurrence of tonic clonic seizures (29).

Finally, as an endogenous anticonvulsant and a potent mediator of epigenetic regulation, adenosine is emerging as a key player in ictal and epileptogenic processes. Parallel

evidence suggests that ketogenic dietary therapy, in addition to its antiseizure effects, could be a useful antiepileptogenic strategy, possibly because of associated increases in adenosine signaling and epigenetic modification. In two different models of chronic epilepsy, a ketogenic diet successfully suppressed spontaneous seizures and attenuated the rate of epilepsy development and long-term expression of epilepsy (references in [30, 31]). Newer work reaffirmed these findings but also showed an association with increased hippocampal adenosine levels and found that disease modification extended beyond the active treatment period independent of seizure-suppressing effects during kindling (32, 33).

Looking Forward: Challenges and Opportunities

A major factor influencing progress in developing preventive therapies for epilepsy is the availability of research tools and models that are directly relevant to human epilepsy. The revolution in genetics has been particularly fruitful in identifying an increasing number of mutations involved in the pathogenesis of seizures or epilepsy. Recent technological advances have produced genetic and induced models, which closely mimic the human condition, increasing the translational value of preclinical therapeutic studies. New animal models of early life epileptic encephalopathies and epilepsies, like West syndrome or Dravet syndrome (34), changed the practice of epilepsy research and therapy development, allowing the use of syndrome-specific, age-appropriate models and less reliance upon adult models of epilepsies. For example, knockout mouse models of sodium channel *SCN1A* have revealed unexpected pathophysiological mechanisms of epileptogenesis in Dravet syndrome, potentially involving loss of function of sodium channels preferentially in cortical GABAergic interneurons (35, 36). Transfections of the gene encoding SCN1A channels carrying Dravet syndrome mutations found in humans suggested that haploinsufficiency may be the cause (37). In addition to rodents, other model systems offer complementary approaches to investigating effects of genetic mutations and therapeutic interventions. Zebrafish harboring *Scn1a* mutations allow rapid drug screening of potential antiepileptic drugs for Dravet syndrome (38). In contrast to the mouse models, neurons derived from induced pluripotent stem cells from patients with Dravet syndrome reveal a gain of function with increased sodium currents and burst firing, presumed to be caused by a posttranscriptional mechanism (39). The variety of novel model systems provides the opportunity to explore the pathogenesis and develop potential preventive therapies for specific epilepsy syndromes.

The International League Against Epilepsy, American Epilepsy Society, and National Institute of Neurological Disorders and Stroke are partners in the Translational Taskforce of the International League Against Epilepsy in an effort to reassess strategies for preclinical antiepileptogenic therapy discovery (1, 3) by harmonizing practices, using preclinical common data elements and standardized interpretations of rodent video-EEG studies, performing systematic reviews of epilepsy models for translational research, and improving the infrastructure plan for multicenter, blinded, randomized, controlled studies. In Europe, the National Consortium for the Replacement, Refinement, and Reduction of animal models in research (https://www.nc3rs.org.uk/) attempts to improve the use of animal models with the goal of balancing the needs for increased experimental rigor and humane use of animals in research (40).

Considerable research has focused on the basic mechanisms of epileptogenesis and has identified a number of potential targets for the development of biomarkers. However, currently there are no validated biomarkers of epileptogenesis that would help identify patients at risk for developing epilepsy or for identifying which newly diagnosed patients may experience a relapsing/remitting, or an entirely drug-resistant, course of their disease. Validated biomarkers would also be useful in providing new information for the development of antiepileptogenic interventions (41). A higher CSF to serum IL-1β ratio, but neither concentration alone, and a heterozygous genotype in a single nucleotide polymorphism of the promoter region of the gene encoding interleukin-1β (IL-1β) were associated with greater risk of posttraumatic epilepsy in adults with moderate to severe traumatic brain injury (42). In addition, preliminary findings from a cohort of 162 Caucasian adults with traumatic brain injury suggest that genetic variation in the adenosine kinase gene *ADK* and the ecto-5'-nucleotidase gene *NT5E* may help explain variability in time to first seizure and risk of posttraumatic epilepsy, independent of previously identified risk factors (43). The availability of in vivo imaging PET modalities to monitor neuroinflammation is likely to provide insights into the role of these signals as biomarkers of epileptogenesis and possibly treatment response (18, 21). Use of EEG also shows promise as a biomarker of future infantile spasms and epilepsy in presymptomatic infants with tuberous sclerosis complex (44). It is interesting to note that a combination of biological measures (electrophysiological, molecular, genetic, or neuroimaging biomarkers) rather than a single measure increased the sensitivity and specificity to predict epilepsy in rats (45). Furthermore, the relatively easily accessible sampling of circulating molecules is a tremendous advantage in the search for biomarkers of epilepsy (46).

The search for epilepsy prevention therapies has been unsuccessful so far despite several decades of effort. We have learned over this time that the biology underlying epileptogenesis or epilepsy progression appears different from the biology driving seizures in the epileptic brain. An antiepileptogenic therapy would prevent the frank appearance of epilepsy in an at-risk individual, whereas disease modification therapy is designed to slow progression, alleviate cognitive or behavioral comorbidities of epilepsy, or convert medically intractable epilepsy to drug-responsive epilepsy. Both therapy classes attempt to target the mechanisms underlying the control of seizure threshold (47). A special challenge in this area is the development of etiologic animal models that are predictive of the human condition for antiepileptogenic treatments, currently a catch-22 in the absence of a therapeutically validated animal model for any form of epilepsy.

Nonetheless, there remains great value in studying the underlying biology of epilepsy and in testing novel therapeutic strategies in animal models of these disorders. A second challenge for epilepsy prevention therapy is the design of efficient clinical trials because epilepsy acquired after a triggering event can take years or decades to surface (48). Thus,

the identification of biomarkers predictive of ensuing epilepsy or progression of seizures, or predicting drug response, that would allow patient stratification is a high priority, and careful assessment of the feasibility of completing a proposed clinical trial is also essential to avoid wasting opportunities.

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