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Perspective

Preventing epileptogenesis: A realistic goal?

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ABSTRACT

The definition of the pathologic process of epileptogenesis has considerably changed over the past few years due to a better knowledge of the dynamics of the associated molecular modifications and to clinical and experimental evidence of progression of the epileptic condition beyond the occurrence of the first seizures. Interference with this chronic process may lead to the development of novel preventive therapies which are still lacking. Notably, epileptogenesis is often associated with comorbid behaviors which are now considered primary outcome measures for novel therapeutics. Anti-epileptogenic interventions may improve not only seizure onset and their frequency and severity but also comorbidities and cell loss, and when applied after the onset of the disease may provide disease-modifying effects by favorably modifying the disease course. In the preclinical arena, several novel targets for anti-epileptogenic and disease-modifying interventions are being characterized and validated in rodent models of epileptogenesis. To move proof-of-concept anti-epileptogenesis studies to validation in preclinical trials and eventually to clinical translation is a challenging task which would be greatly facilitated by the development of non invasive biomarkers of epileptogenesis. Biomarker discovery together with testing potential novel drugs would provide a major advance in the treatment of human epilepsy beyond the pure symptomatic control of seizures.

1. Introduction

1.1. New definition of epileptogenesis

Epileptogenesis is characterized by the development and extension of brain tissue capable of generating spontaneous seizures, thus resulting in the development of an epileptic condition and/or progression of the epilepsy after it is established [1]. Epileptogenesis can be triggered by congenital, genetic or acquired insults, such as neurotrauma, stroke, infections, status epilepticus. The current definition takes into consideration the new knowlegde related to the cellular, molecular and functional changes occurring in seizure-prone brain areas both before and during the development of epilepsy, as emerging from clinical [2] and basic science investigations in animal models [3–5]. Consequently, the concepts related to epileptogenesis have evolved significantly over the past few years. The complexity of this process has been acknowledged as well as the evidence that epileptogenesis is not a stepwise process rather a *continuum* of modifications [6]. Moreover, epileptogenesis is not merely limited to the prodromal phase preceding the onset of spontaneous seizures, but also underlies the development of the disease after its diagnosis [1]. This concept has been perceived by William Gowers in 19th century that empirically set that "seizures beget seizures".

This novel understanding of epileptogenesis is compatible with clinical evidence reporting that human epilepsy has a progressive course in a significant percent of cases [2]. This concept is not only mechanistically but also therapeutically relevant since it means that epileptogenesis can potentially be targeted also after spontaneous seizures arise. In accord, anti-epileptogenic interventions could be designed not only for preventing the onset of the disease after risk factors are evaluated but also for improving the related pathological outcomes after the disease is diagnosed (i.e., disease-modifying treatments). Another relevant aspect emerging from basic science investigations relates to comorbidities such as anxiety, cognitive deficits and depression, that are often associated with epilepsy [7,8]. There is intense research to understand the mechanisms leading to comorbidities giving insights that they may share common molecular events with the hyperexcitability phenomena underlying seizure generation. Notably, animal models clearly show that these comorbid behaviours often arise before the development of spontaneous seizures indicating that they are not

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a mere consequence of seizures or the anticonvulsive treatments [9–16].

1.2. Mechanistic and therapeutic implications

A consensus definition of epileptogenesis is crucial for designing therapeutic interventions based on specific targets and for searching mechanistic biomarkers of this process that are still lacking. In fact, the development of effective therapies to prevent or treat epileptogenesis remains an urgent unmet clinical need. Clinical trial designs for novel therapeutics able to prevent (anti-epileptogenic) or favorably change the disease course (disease-modifying) are likely to hinge on discovering non-invasive biomarkers that allow early identification of patients at high risk of developing the disorder, as well as patients with a progressive course of the disease, including the development of comorbidities and pharmacoresistance [17]. Both aspects are challenging and rely upon a better understanding of the mechanisms of epileptogenesis and their dynamics during disease development. In this frame, one should consider that comorbidity may not be eliminated by either the anti-epileptogenic or disease-modifying treatment unless the treatment is targeted against the common mechanistic pathway for the disease and comorbidities. Yet, once the mechanistic pathway of comorbidity (after being triggered) splits from that of the disease, no efficacy of either treatment should be expected.

2. Mechanisms of epileptogenesis and anti-epiletogenesis strategies

A large number of animal models of pediatric and adult epilepsies has become available for studying the molecular mechanisms contributing to epileptogenesis [18]. The complexity of epileptogenesis, and its potential heterogeneity in the human condition, make these investigations and their clinical translatability very challenging. Several recent reviews report in detail which mechanisms have been demonstrated to contribute to epileptogenesis in animal models [3–5,19]. These studies describe changes in specific molecules and cell signaling pathways after the inciting epileptogenic event with differing temporal profile of induction, persistence and recovery. Many of these alterations continue to occur after the onset of spontaneous seizures and have been validated in human epileptic foci surgically resected from patients affected by various forms of drug-resistant epilepsy. Pharmacological or genetic interventions targeting such molecules and pathways can affect either the onset and/or the development and severity of the ensuing epilepsy, thus demonstrating their role in the process of epileptogenesis. The most studied and better validated molecules implicated in epileptogenesis include those related to: immunity and inflammation (e.g. cytokines and danger signals such as IL-1beta, HMGB1 and TNF-alpha, Cox-2 and prostaglandins, complement system), TGF-beta signaling activated by albumin in astrocytes following alterations in the blood brain barrier (BBB) permeability, extracellular matrix proteins, adenosine kinase, mTOR, neurotrophic kinase receptors such as TrkB receptors activated by BDNF, JAK/STAT signaling pathways, and a variety of acquired and congenital channalopathies and epigenetic factors (Fig. 1). Some level of functional interaction exists among these molecular pathways suggesting that discovery of nodal points of intersection may facilitate the development of drugs controlling the broad cascade of pathologic events taking place during epileptogenesis. We refer to previous reviews for more details [3–5,19].

This incremental list of potential mechanisms underscores the importance of non neuronal cells such as astrocytes, microglia, and endothelial cells of the BBB, as key contributors to neuronal network dysfunctions leading to seizures and comorbidities [20,21]. Additionally, these molecules represent potential targets for anti-epileptogenesis interventions and potential sources of mechanistic biomarkers of epileptogenesis.

2.1. The focus on inflammatory mechanisms as potential targets

Because of our expertise in the field of immunity and inflammation, and considering that an exhaustive literature review of the preclinical anti-epiletogenesis interventions is not the focus of this article, we will report here about specific anti-inflammatory treatments in animal models of epileptogenesis, as an example of anti-epileptogenesis approaches [19]. Whether different aetiological factors induce distinct or overlapping epileptogenic mechanisms remains unknown, however, the search for such pathogenic mechanisms in animal models has shown that neuroinflammation in seizure-prone brain regions is a common feature of various forms of drug-resistant symptomatic epilepsies in humans and animal models [22,23]. Neuroinflammation is determined by the synthesis and release of pro-inflammatory molecules with neuromodulatory properties by glia, neurons and the BBB endothelium, in the context of innate immunity activation. *De novo* status epilepticus and other potential epileptogenic injuries (e.g. neurotrauma, stroke, CNS infections, gene mutations, i.e., GAERS rats with spike-and-waive discharges [24] or cystatin B mutation in a model of progressive myoclonus epilepsy [25]) trigger neuroinflammation, which therefore represents a consistent feature of epileptogenesis irrespective of the initial insult.

Which are the triggering factors of neuroinflammation during epileptogenesis, and whether common factors ignite inflammation following differing epileptogenic injuries are still open questions. Basic knowledge of the mechanisms of inflammation by innate immunity activation, and experimental findings in models of seizures and epileptogenesis, suggest that one pivotal generator of neuroinflammation in epileptogenesis is likely to be the activation of toll-like receptors (TLR) by endogenous ligands, namely the alarmins/danger signals [26,27]. These are endogenous molecules constitutively available that are released by brain cells and leukocytes upon tissue injury. One of such molecules is High Mobility group Box 1 (HMGB1) which is released by various epileptogenic injuries and is induced in human brain tissue from various forms of pharmacoresistant epilepsy [22,23,26,27]. The activation and assembly of the inflammasome, a multiprotein complex which includes the cysteine protease ICE/caspase-1, is required for the biosynthesis and release of both the biologically active form of IL-1beta and HMGB1. Extracellular ATP-mediated stimulation of P2X7 receptors and reactive oxygen species are powerful inducers of ICE/Caspase-1, and they are both commonly produced during epileptogenesis [26,27].

Notably, it is well established that HMGB1 as well as other neuroinflammatory molecules such as IL-1beta, TNF-alpha, PGE2 and the complement system, contribute to ictogenesis by promoting neuronal network hyperexcitability *via* activation of specific transcriptional and post-translational molecular mechanisms in neurons and glia, thereby reducing seizure threshold and promoting seizure generation and recurrence [26,27]. In accord, anti-inflammatory treatments are anticonvulsive in some human pediatric and adult epilepsies [23,28], and specific anti-inflammatory drugs significantly reduce acute symptomatic seizures and chronic spontaneous seizures in animal models of existing epilepsy [23,28]. Additionally, target-specific anti-inflammatory treatments in SE models are providing increasing evidence of anti-epileptogenic and neuroprotective effects, thus supporting that neuroinflammation is implicated in epileptogenesis [19,29–32]. Table 1 reports a list of successful anti-inflammatory interventions tested as preventive treatments in animal models of status epilepticus-induced epileptogenesis. In general,