Getting rid of the catastrophe: 
frontier research in infantile spasms

Tomonori Ono, M.D., Ph.D.,1,2, Aristea S. Galanopoulou M.D. Ph.D.2,3 and Solomon L. Moshé M.D.2,3,4

1Department of Neurosurgery, National Nagasaki Medical Center, Omura, Nagasaki, Japan
2Saul R. Korey Department of Neurology, Albert Einstein College of Medicine, Bronx, New York, USA
3Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York, USA
4Department of Pediatrics, Albert Einstein College of Medicine, Bronx, New York, USA

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Summary

Infantile spasms (IS) are an age-specific epileptic syndrome with overall poor outcome and are recognized as one of the ‘catastrophic epilepsies’. Current conventional therapies including adrenocorticotropic hormone and vigabatrin often fail to control the spasms and improve the long-term outcome, especially in cases with structural/metabolic etiologies. To improve this situation, new treatments with a disease modifying potential must be identified. Recent translational studies have led to the development of several animal models of IS that reflect their multiple etiologies. Among these, the multiple hit model has been used to screen new promising therapies that, in the future, may be explored in clinical trials.
Introduction

Infantile spasms (IS) or West syndrome is an age-specific epileptic syndrome characterized by recurrent spasms, often accompanied by arrest or regression of psychomotor development as well as specific electroencephalographic (EEG) patterns showing interictal hypsarrhythmia and ictal electrodecremental responses [1]. IS typically begin in the first year of life and often transform into other intractable epilepsies in later life. Depending on the etiology, IS have been classified into structural/metabolic, genetic, and etiology-unknown [2]. More than 200 possible causes have been described but the majority of IS are linked to pre-existing structural/metabolic brain pathologies such as cortical dysplasia, tuberous sclerosis complex, known metabolic disorders, and hypoxic/ischemic brain damage [1,3]. These structural/metabolic IS are frequently refractory to conventional therapies including adrenocorticotropic hormone (ACTH) and vigabatrin, although studies have suggested that successful initial treatment with these drugs results in better epilepsy and cognitive outcome in the etiology-unknown subgroup [4,5]. Moreover, these drugs are sometimes toxic. To overcome this pessimistic status of the disorder, it is important to understand the pathophysiological mechanism of the disorder and to develop innovative, effective, non-toxic treatments to promptly stop the seizures and the regression. This will require the identification of appropriate model systems to be used for finding new treatments and screening for efficacy in preclinical studies. However, few basic research studies focusing on IS have been reported mainly due to a lack of appropriate animal models. Recently, several research groups have developed novel animal models of IS, identified some of the mechanisms involved, and tested the efficacy of new drugs. In this paper, we review these experimental studies and the current state of research on IS.

Experimental models of IS

A valid animal model would not only enhance the understanding of disease pathophysiology but also encourage the development of new diagnostic approaches, permit testing of novel treatments, and help to devise strategies to ameliorate the cognitive and epileptic consequences [6]. Optimally, the model should mimic the human disorder, including features of: 1) unprovoked spasms or myoclonic seizures in early postnatal development; 2) EEG correlates of seizure events (ictal decremental response); 3) abnormal interictal EEG (“hypsarrhythmia”) reflecting generalized epileptic encephalopathy; 4) response to clinically relevant treatments (such as ACTH and/or vigabatrin); and 5) behavioral/cognitive sequelae [7]. However, there are issues regarding these proposed criteria. Many forms of IS are refractory to ACTH, and thus it is important to have models refractory to ACTH in order to identify novel treatments. In terms of response to treatment, refractoriness to ACTH is one of the biggest
clinical problems and response to ACTH may not be a suitable criterion to be included in studies to find new treatments. Because the definition of hypsarrhythmia includes the presence of multifocal and high amplitude discharges, identification of hypsarrhythmia on experimental EEG may be extremely difficult in rat or mouse pups, because placement of multiple electrodes in the brain is limited by the head size and fragility of skull bones. Therefore, modeling of hypsarrhythmia may be restricted to larger animals at present. Finally, progression of other epilepsies should be considered in testing long-term treatment outcome.

Some novel animal models of IS have been published (Table). One model involves intracerebroventricular administration of picomolar quantities of corticotrophin releasing hormone (CRH) into neonatal rats during the first or second week of life [8,9]. This model is based on the hypothesis that stress increases endogenous CRH release which plays a role in the development of infantile seizures, and the hypothesis that ACTH may suppress IS by reducing CRH expression. Although long-term cognitive deficits have been described in this model, the animals manifest limbic seizures rather than spasms.

Another model of IS involves intraperitoneal injection of N-methyl-D-aspartate (NMDA) into rats on postnatal day 15 [10,11]. NMDA causes behavioral spasm-like seizure consisting of whole body tonic flexion with forward body-arching. The seizure is often accompanied by a diffuse attenuation of the EEG amplitude similar to human electrodecremental pattern. Learning and memory impairments appear in adult rats subsequent to NMDA-induced seizures during infancy [11]. In this model, pretreatment with a single high dose of porcine ACTH₁₃₉ causes an obvious reduction in susceptibility to NMDA-induced seizures [12] and pretreatment with vigabatrin significantly decreases the incidence of flexion seizures in a dose-dependent manner [13].

On the other hand, if the pups are prenatally exposed to betamethasone or restraint stress on gestational day 15, injection of NMDA on postnatal day 10-15 triggers spasm-like seizures significantly earlier and in greater numbers [14-16]. “Pre-treatment” with repetitive ACTH injections decreases the number of seizures and improves the mortality [15,16]. The lack of preexisting structural pathology has led to the proposal that this model may be a model of cryptogenic (unknown etiology) IS.

Intracerebral chronic infusion of tetrodotoxin (TTX) using an osmotic pump for several weeks starting from postnatal day 10 leads to the development of spontaneous recurrent brief spasm-like seizures in adulthood [17]. The seizures are characterized by very brief (1–2 s duration) extensor or flexor spasm of the body musculature, sometimes accompanied by forelimb pawing movements, associated with ictal EEG pattern consisting of initially generalized, high-amplitude, slow waves followed by an electrodecremental with superimposed fast activity.
The majority of the animals that develop spasms also exhibit a high voltage, chaotic background pattern resembling the hypsarrhythmic pattern observed in human children with IS. In this model, however, the seizures occur on postnatal day 21 at the earliest, which is equivalent to the juvenile and adult periods. To date, there is no report on the therapeutic effects of any agent on spasms in this model. Regarding the pathophysiological mechanisms, ictal EEG recording with spectral analysis and band pass filtering showed that the earliest and most intense high frequency discharges typically occur contralateral to the side of TTX infusion [18]. Interictally, high frequency oscillations (HFOs) occur most frequently during hypsarrhythmia and non-rapid eye movement sleep, from multiple areas but most prominently contralateral to the TTX infusion site, similar to the ictal findings. In addition, microwire recordings show that neuronal unit firing increases abruptly with the generation of HFOs during both ictal and interictal discharges [19]. These results may imply that neocortical networks are more abnormally excitable, particularly in the hemisphere contralateral to TTX infusion (neocortical hyperexcitability hypothesis). However, subcortical contributions also require elucidation because IS may be caused by abnormal interaction between cortical and subcortical structures [20].

Two genetic mouse models of IS with loss of function of the Aristaless-related homeobox (ARX) gene have been generated. Loss of ARX gene function has been associated with a variety of neurologic syndromes in humans involving mental retardation and epilepsy, including IS [21]. The conditional ARX knockout mouse model, in which loss of ARX function is observed in interneurons, manifests limbic seizures in early life and spasm-like seizures in adulthood [22]. The ARX knockin mouse model with a triple repeat expansion previously described in human patients manifests spasms in early life, and subsequent expression of other seizures and cognitive and behavioral deficits [23]. Immunohistochemistry revealed reduction of calbindin- and calretinin-labeled cells in the neocortex of ARX knockout mice [22] and reduction of calbindin and neuropeptide Y (NPY) interneurons as well as cholinergic neurons in the cortex and hippocampus of ARX knockin mice [23]. These findings may suggest that the impairment of GABAergic interneurons (interneuronopathy) [24] is an important mechanism underlying the pathogenesis of these developmental epilepsies.

Another model of chemically-induced spasms is produced in a mouse genetic model of Down syndrome (Ts65Dn) [25]. IS occur in approximately 10% of patients with Down syndrome [26]. In the model mice, administration of baclofen or g-butyrolactone, which is the prodrug of the GABA_B receptor agonist g-hydroxybutyrate, between 1 week and 2 months of age causes clusters of extensor spasms accompanied by polyspike-wave bursts and electrodecremental responses on EEG. Higher expression of GABA_B receptors in specific brain sites including the thalamus...
and the medulla oblongata together with GABA<sub>B</sub>-mediated effects may be the fundamental mechanism of spasmsogenesis in this model. Moreover, pretreatment with rodent ACTH<sub>1-24</sub> fragment, vigabatrin, valproic acid, and ethosuximide suppresses chemically-induced flexion spasms [25]. In contrast, spasms in this model are not affected by porcine ACTH<sub>1-39</sub>.

**Multiple hit model: a model of structural (symptomatic) IS**

The experimental models described above are considered to be models for etiology-unknown (cryptogenic) or genetic (idiopathic) IS. On the other hand, structural/metabolic (symptomatic) IS caused by preexisting brain disorders comprise the largest proportion of cases and are more frequently refractory to conventional therapies [1,25]. Thus, it is crucial to develop successful animal models of symptomatic IS to study mechanisms and design safer and more effective treatments. Based on evidence that structural or functional abnormalities in cortical or subcortical structures or their connections may be implicated in the pathogenesis of IS [20], Scantlebury et al. [28], developed the multiple hit model of IS, in which rats receive intracerebral infusion of doxorubicin and lipopolysaccharide on postnatal day 3 and systemic injection of p-chlorophenylalanine on day 5. All rats manifest spontaneous flexion, extension or mixed flexion/extension spasms during the neonatal-infantile period, associated with ictal electrographic correlates and bilateral spikes and sharp waves. However, because of the small size and fragility of the skull at this young age, it is not possible to place more than 3 active electrodes. Therefore, it is not possible to evaluate hypsarrhythmia. Some animals subsequently develop other epileptic seizures after the period of spasms. These seizures include behavioral arrest events, wild running, tonic seizures, myoclonus with sudden drops, and stage 5 limbic seizures associated with forelimb clonus, rearing, and falling. Neurodevelopmental deficits in learning and memory, and autistic-like behaviors (indifference to other rats, increased grooming) are also observed after postnatal day 12.

In this model, daily ACTH<sub>1-24</sub> given after the onset of spasms does not affect the frequency of spasms, while vigabatrin transiently suppresses spasms on postnatal day 5. The underlying brain pathology consists of cortical and subcortical lesions including the white matter tracts, and is most prominent in the ipsilateral (drug-infused) hemisphere and periventricular regions. Histopathological studies reveal loss of GABAergic interneurons in the cortical layer 2/3 and 6, while the remaining GABAergic interneurons are morphologically abnormal (Galanopoulou, personal communication). Therefore, this new model is an exclusive structural lesion-associated (symptomatic) model of IS and will be useful to study the neurobiology and treatment of IS, including those that are refractory to conventional ACTH and vigaba-
Pulse rapamycin treatment suppresses spasms dose-dependently and improves cognitive performance, although it does not reduce the frequency of other seizures [29]. Histopathological analysis reveals that therapeutically effective rapamycin doses normalize the phosphorylation of S6 ribosomal protein (pS6), which indicates biologically overactivation of TORC1 complex of the mTOR pathway at perilesional cortical regions of rats with spasms [29]. These findings support the notion that pathological overactivation of TORC1 may be implicated in the pathogenesis of IS with acquired etiologies, and is not related to tuberous sclerosis.

Carisbamate is a novel neuro-modulating drug. Its efficacy has been assessed in the multiple hit model. The drug acutely reduces both behavioral spasms and electroclinical spasms during the first 2–3 post-injection hours without detectable toxicity [30]. Sodium channel blockade has been proposed to contribute to the antiepileptic action of carisbamate [31,32], but spasms are not suppressed by the sodium channel blocker phenytoin [30]. Another possibility may be an increase of chloride channel conduction [32]. Thus the mechanisms of action of carisbamate on suppression of spasms need to be investigated. Also, further evaluation of the effects of repetitive administration of carisbamate is required.

There is hope: challenge to get rid of catastrophe

Up to this day, IS has been difficult to treat with poor overall outcome. Many investigators have participated and are now researching how to get rid of the “catastrophe” of the disorder. The experimental approaches and encouraging results reviewed here promise that new potential treatments for this catastrophic disorder do exist. Further clinical and basic science studies are needed to identify etiology-specific differences in the pathogenesis and therapy of IS, which could reverse their intractability and improve outcome.

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Conflict of interests

Dr. Ono has no conflict of interest to declare. Dr. Galanopoulou has received a speaker’s honorarium from Novartis. Dr. Moshé has received consultancy fees from Eisai and an honorarium/travel reimbursement from GlaxoSmithKline. The Albert Einstein Col-
lege of Medicine holds a patent on the multiple-hit model of IS (#7863499). Research funding by Johnson & Johnson has been received for the investigation of carisbamate in the treatment of spasms in the multiple-hit model. There is no other conflict of interest.

### Table. Animal models of infantile spasms.

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<td>Evolution to other seizures Cognitive and behavioral deficits</td>
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IS, infantile spasms; CRH, corticotrophin releasing hormone; NMDA, N-methyl-D-aspartate; TTX, tetrodotoxin; ARX, Aristaless-related homeobox; P, postnatal day; EEG, electroencephalography; ACTH, adrenocorticotropic hormone. The clinically-equivalent categories of IS are based on the Report of the ILAE Commission on Classification and Terminology 2010 [2].

Spasm-inducing chemicals are usually injected intraperitoneally in all but the TTX, multiple-hit and ARX models.

* Lack of efficacy of rapamycin in the betamethasone/NMDA model may be dose- and age-related, as older animals and lower doses were used in this model [33].
References


