Epilepsy Research (2014) **108**, 1492—1500

journal homepage: www.elsevier.com/locate/epilepsyres

A potential effect of ganaxolone in an animal model of infantile spasms

Mi-Sun Yum[a], **Minyoung Lee**[a], **Tae-Sung Ko**[a,*], **Libor Velíšek**[b]

[a] *Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Republic of Korea*
[b] *Departments of Cell Biology & Anatomy, Pediatrics, and Neurology, New York Medical College, Valhalla, NY, USA*

Received 16 April 2014; received in revised form 4 August 2014; accepted 20 August 2014
Available online 2 September 2014

KEYWORDS
Ganaxolone;
Infantile spasms;
Animal model;
GABA;
Neurosteroid

Summary
Rationale: Infantile spasms (IS), a devastating epileptic encephalopathy of infancy, involve various etiologies associated with an unknown underlying common pathophysiology. The efficacy of adrenocorticotropic hormone (ACTH) as an IS therapy suggests a role for steroid hormones in treating IS. This study used an animal model of IS to test the efficacy of ganaxolone, a synthetic neurosteroid, promoting tonic $GABA_A$ inhibition.
Methods: The model of cryptogenic IS used in this study involved prenatal priming of rats with betamethasone (0.4 mg/kg i.p. at 08:30 and 18:30) on gestational day 15. To test the acute effects of ganaxolone, rats were pretreated with ganaxolone (10, 25, or 50 mg/kg i.p.) or vehicle (β-cyclodextrin, i.p.) 30 min prior to N-methyl-D-aspartate (NMDA)-induced spasms at postnatal day 15 (P15). To mimic human conditions, another group of rats was randomly divided and repeatedly treated with ganaxolone (20 mg/kg at 9:00 and 18:00 from P13-15) or vehicle after experiencing NMDA-triggered spasms at P12. Additional spasms were triggered on P13 and P15. We determined latency to the onset of spasms and the total number of spasms per 90-min observation period after the trigger at P15. On P19 and P21, behavioral tests were performed in rats with randomized repeated treatments.
Results: The 25 mg/kg and 50 mg/kg doses of ganaxolone significantly delayed the onset of spasms compared with the controls, and significantly decreased the number of spasms or suppressed their incidence. Ganaxolone had significant side effects in terms of sedation: all animals with the 50 mg/kg dose were sleeping during the test. Randomized ganaxolone treatment for 3 days also significantly delayed the onset and decreased the number of spasms triggered by NMDA on P15, and decreased exploratory behavior after multiple NMDA triggered spasms.

* Corresponding author at: Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, 86 Asanbyeongwon-gil, Songpa-gu, Seoul 138-736, Republic of Korea. Tel.: +82 23010 3390; fax: +82 2473 3725.
E-mail address: tsko@amc.seoul.kr (T.-S. Ko).

http://dx.doi.org/10.1016/j.eplepsyres.2014.08.015

Conclusion: Ganaxolone significantly suppresses the development of spasms in the rat model of cryptogenic IS. This synthetic neurosteroid active in an animal model of IS might contribute to the current armamentarium to treat human IS.

Introduction

Infantile spasms (IS; epileptic spasms representing developmental epileptic encephalopathy) are characterized by distinctive spasms in infancy, interictal hypsarhythmia, and progressive developmental delay. IS do not respond to classic antiseizure drugs. Adrenocorticotropic hormone (ACTH), vigabatrin, and glucocorticoids can alleviate symptoms in certain patients. However, the pathophysiological mechanisms of IS are unknown and the pharmacodynamic mechanisms of ACTH action remain obscure. Considering the grave developmental and cognitive outcomes of patients with IS and the serious side effects of current drugs of choice for IS treatment, a different therapeutic approach is needed to better reduce spasms and improve mental abilities in patients with IS. The efficacies of both ACTH and corticosteroids in IS suggest a possible role of neurosteroids in IS. Neurosteroids target tonic inhibitory conductance mediated by extrasynaptic $GABA_A$ receptors containing the delta subunit, as well as synaptic GABAergic inhibition (Monaghan et al., 1999; Nohria and Giller, 2007). The efficacy of vigabatrin, an inhibitor of GABA transaminase, in treating IS through elevation of the level of GABA in the brain also supports involvement of GABAergic inhibition in the IS (Riikonen, 2014). Based on this information, we speculated that ganaxolone (GNX) a synthetic neurosteroid, might be another useful candidate for IS treatment.

We have previously developed a rat model of IS (Chachua et al., 2011; Velisek et al., 2007), which consists of prenatal priming with betamethasone and postnatal triggering of developmentally specific spasms with N-methyl-D-aspartic acid (NMDA) in infant rats. By using this animal model, we searched for additions in the armamentarium to treat IS. Another goal was to find a treatment that can also reverse the poor cognitive outcomes of IS. This study was designed to use an animal model of cryptogenic IS to assess the effects of GNX on the development of spasms and on the behavioral changes after multiple spasms.

Methods

Animals

Experiments were approved by the Institutional Animal Care and Use Committee of the Ulsan University College of Medicine and conformed to the Revised Guide for the Care and Use of Laboratory Animals [NIH GUIDE, 25(28), 1996]. Timed-pregnant Sprague-Dawley rats were purchased from an approved source (Orient Bio, Seoul, Korea). The rats were housed individually in the animal facility during the remainder of their pregnancy with free access to standard rat chow and water on a regular 12 h light-dark cycle with the lights on at 08:00. On gestational day (G) 15, pregnant rats received two injections of betamethasone 0.4 mg/kg (Sigma—Aldrich, St. Louis, MO) or the same volume of saline, each at 08:30 and 18:30. Delivery occurred consistently on G22, which was considered postnatal day (P) 0 for the offspring.

On P1, the offspring were identified and marked, and the size of each litter was reduced to 12 (six males and six females, if possible). Flexion spasms were triggered on P15 with intraperitoneal (i.p.) injection of 15 mg/kg NMDA (Sigma). In the multiple seizure groups for the randomized treatment, spasms were triggered on P12 (6 mg/kg NMDA), P13 (12 mg/kg) and P15 (15 mg/kg). Immediately after NMDA administration, the rats were observed for 90 min because our pilot experiments to establish appropriate NMDA doses demonstrated that the spasms would nearly disappear within this time frame. Latency to the onset of tailing (twisting tail movements), and to the onset of spasms was recorded, and the spasms per subject were counted as an expression of seizure severity.

GNX treatment paradigm

Ganaxolone (3α-Hydroxy-3β-methyl-5α-pregnan-20-one; Sigma) was used in two different regimens.

To test the acute pretreatment effect, different doses of GNX (10, 25, or 50 mg/kg i.p.) were injected 30 min before the trigger of spasms by NMDA on P15. To confirm the efficacy of GNX in non-primed animals, offspring from saline-injected rats were treated with 25 mg/kg of GNX i.p. 30 min before the NMDA triggered spasms. To mimic the condition of IS in children, where treatments start after the development of spasms, we used the following protocol: only offspring prenatally primed with betamethasone were used and the spasms were triggered in all rats by NMDA on P12. Once all rats experienced spasms, they were randomly divided into a treatment group and a vehicle group (controls). The treatment group received multiple doses of 20 mg/kg GNX from P13 through P15 morning (daily at 09:00 and 18:00) and the vehicle group received vehicle for 3 days from P13 to P15 at appropriate times. All rats received additional triggers of spasms on P13 and P15. On P15, the last dose of GNX or saline was given at 09:00 and the rats were monitored to determine the effect of the GNX on the spasms triggered by NMDA injection at 14:00.

EEG recording

Surgical implantation of the electrodes was done on P13 rats under ketamine/xylazine (50/7 mg/kg, i.p.) anesthesia. One screw serving as a reference in the nasal bone, the other screw as an electrical ground on the cerebellum, and two silver ball electrodes on the left frontal and right occipital cortex were inserted and covered with dental acrylic.