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Original research article

Pre- and post-exposure talampanel (GYKI 53773) against kainic acid seizures in neonatal rats

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ABSTRACT

Background: AMPA receptors play an important role in the neurobiology of neonatal epilepsy. The present study evaluated the effect of talampanel, a potent and selective non-competitive antagonist of AMPA receptors, against kainic acid-induced continuous seizures (status epilepticus) and other behavioral abnormalities in neonatal rats.

Methods: Kainic acid was administered at doses of 2 or 4 mg/kg, *ip* to induce seizures and status epilepticus in postnatal 7 days old rat neonates in pre- and post-exposure studies, respectively.

Results: Intraperitoneal administration of kainic acid (2 or 4 mg/kg) resulted in forelimb/hind-limb scratching defined as automatism, continuous generalized tonic–clonic seizures with loss of righting reflex suggesting status epilepticus and tonic extension. Pre-exposure of talampanel (2.5–10 mg/kg, *ip*) 30 min before kainic acid did not affect the onset of kainic acid convulsions. Talampanel at 20 mg/kg, *ip* delayed the commencement of tonic extension, but not status-induced by kainic acid. In contrast, talampanel (5 and 10 mg/kg, *ip*) when administered 5 min after kainic acid (4 mg/kg, *ip*) postponed the onset of status epilepticus and tonic extension compared to vehicle treated group. Furthermore, talampanel (10 mg/kg, *ip*) but not GYKI 52466 (20 or 50 mg/kg, *ip*; a non-competitive AMPA/kainate receptor antagonist) stopped the ongoing status epilepticus when administered 10 min after the administration of kainic acid. However, seizures re-occurred after 35.98 ± 2.36 min.

Conclusion: The present results suggested that talampanel is protective in kainic acid-induced neonatal status epilepticus model; however, the time of administration is a crucial factor in determining its effectiveness.

Introduction

Neonatal seizures are associated with higher risks of mortality and morbidity and considered as a medical emergency [1]. Neonatal brain is more sensitive to seizures due to augmented excitatory neurotransmission and reduction in inhibitory input [2,3]. Although phenobarbitone and phenytoin are the first-line drugs of choice for the management of neonatal seizures, including status epilepticus; however, these molecules have limited efficacy [4]. Therefore, there is an utmost need to explore different receptor systems in the management of seizures during the neonatal period.

AMPA receptors consist of a combination of four major subunits (GluR1-4) and maintain fast excitatory synaptic transmission [5]. These receptors are over-expressed in oligodendrocytes of 7 days old rat pups [6] and plays an important role in the pathophysiology of neonatal seizures [7]. The GluR2 subunit of AMPA receptors is responsible for the impermeability of receptor channels towards calcium ions. In immature neurons, there is a low expression of AMPARs GluR2 compared to other sub-units [8–11], leading to more permeability of calcium ion and reducing the time interval between individual excitatory post synaptic potential (EPSP) [12]. There are numerous findings depicting the role of AMPA receptors in the neurobiology of neonatal seizures [7,13–16]. However, there is only a single finding that has explored the effect of talampanel (GYKI 53773 {(R)-7-acetyl-5-(4-aminophenyl)-8,9-dihydro-8-methyl-7H-1,3-dioxolo[4,5-h][2,3] benzodiazepine}), a potent and selective non-competitive inhibitor of AMPA receptors, in animal models of neonatal seizures [17]. Pretreatment of talampanel 30 min before hypoxia was found to protect postnatal 10 days old rat pups against hypoxia-induced seizures [17]. Talampanel easily penetrates the blood brain barrier (BBB) [18] and found useful in refractory partial seizures [19]. Talampanel is an anticonvulsant agent in preclinical and

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clinical findings. However, the effect of talampanel in the management of neonatal status epilepticus has not been explored yet. AMPA receptors undergo plastic changes with the expression of GluR2-lacking AMPA receptors during status epilepticus thus leading to enhance expression of calcium permeable AMPA receptors [20].

With this background, we have evaluated the anticonvulsant profile of talampanel in an animal model of neonatal status epilepticus. We have used kainic acid to induce continuous seizure phase or status epilepticus in postnatal 7 days old rat pups and explored the effect of talampanel when administered both pre- and post-kainic acid. The effect of talampanel in animals undergoing status epilepticus was compared with its parent compound, GYKI 52466. GYKI 52466 is another non-competitive AMPA/kainate receptor antagonist that is found to be protective in animal models.

Methods

Animals

Offspring of Wistar breeder rats bred in Animal House facility of Institute of R&D, Gujarat Forensic Sciences University, Gandhinagar, India, was used for the present study. These pups were housed with the mother in polycarbonate cage until 7 days old. The vivarium was maintained under controlled laboratory conditions (temperature, 22–26 °C; humidity, 40–50%) with an artificial 12-h light/dark cycle. Pups/litter of both sexes, 7 days old, weighing 8–12 g were separated from their mother on the day of experiment and brought to the experimental room 30 min before the experiment. In a preliminary experiment, we have used postnatal 5 days old rat pups to evaluate different phases of kainic acid-induced seizures. In kainic acid (4 mg/kg, *ip*) treated group, the onset time of status epilepticus in male and female neonates was 1029.25 ± 84.11 s and 1022.75 ± 69.93 s, respectively and the difference was not statistically significant (unpaired *t*-test). Therefore, we have used both the sexes in the present protocol and each group has an equal sex distribution. The experiments were performed during the light phase (between 0900 and 1500 h) of the light/dark cycle. Each animal was used once. Each group consists of 6–11 animals. Experimental protocols were approved by the Institutional Animal Ethics Committee of Gujarat Forensic Sciences University, Gandhinagar, Gujarat, India and conducted according to the Indian National Science Academy Guidelines (INSA) for the use and care of experimental animals.

Kainic acid-induced seizures in neonatal rats

Kainic acid (2 or 4 mg/kg) was administered *via* an intraperitoneal route to induce seizures in rat neonates (postnatal 7 days old rats; PN7). Animals were observed for a total of 3 h after kainic acid. Various phases of convulsions *viz.* forelimb/hind-limb scratching defined as automatism, continuous generalized tonic–clonic seizures with loss of righting reflex suggesting status epilepticus and tonic extension were measured. Status epilepticus was defined as continuous clonic seizures involving both forelimbs and hind-limbs and continual loss of righting reflex. Absence of the above-mentioned criterion was considered as an interruption from status epilepticus. In a pre-exposure study, kainic acid was administered at a lower dose of 2 mg/kg, *ip* that induced seizures in neonatal rats, but no mortality. In the post-exposure study, kainic acid was administered at a higher dose of 4 mg/kg, *ip* that has shown to induce mortality in more than 60% of the animals. It may not be appropriate to use the term "post-treatment" when administered 5 or 10 min after kainic acid challenge. However, for better understanding of the results, we have differentiated the study in two parts, pre- and post-exposure in the present manuscript.

Mortality is an important parameter to be noted in neonatal seizures. In one retrospective study, Pisani and colleagues have reported mortality of 20 out of 106 newborns that had video-confirmed EEG seizures [21]. It has become important to determine if talampanel could prevent status-epilepticus induced mortality and therefore used higher dose of kainic acid in the second part of the manuscript. All treatments were blinded to the observer.

Treatment schedule

The following molecules were used for the present study: Kainic acid (Sigma Aldrich, MO, USA), talampanel (Sigma Aldrich, MO, USA), GYKI 52466 (Sigma Aldrich, MO, USA). Kainic acid or GYKI 52466 was dissolved in saline while talampanel was prepared using 1 drop of Tween80® and the solution was prepared to its final volume with saline. All the compounds were administered *via* an intraperitoneal route using a method described by Hornick in 1986 [22]. A dose volume of 10 ml/kg was used. The vehicle of talampanel was prepared using Tween 80 and saline in a similar fashion. The vehicle of GYKI 52466 was kept as normal saline.

The treatment schedule has been shown in Fig. 1.

The pre-exposure study will give us an idea if talampanel could be used as a prophylactic agent in patients suffering from status epilepticus. In an early phase of post-exposure study, a dose of

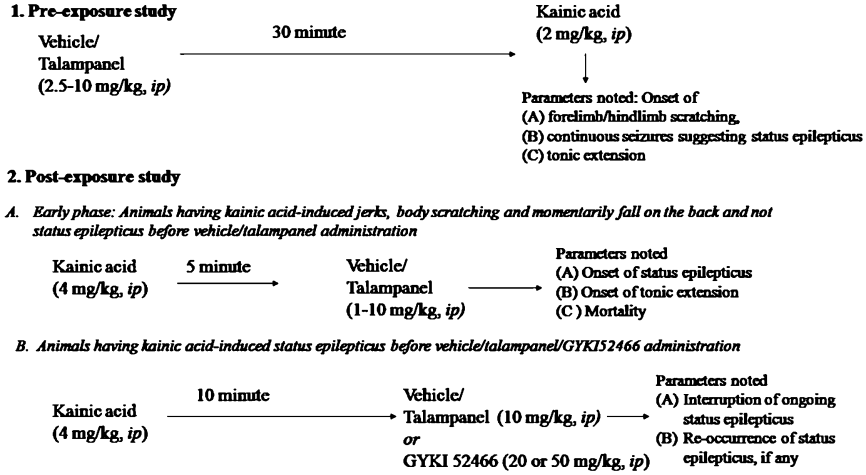

Fig. 1. Treatment schedule.