Neuroscience Letters 664 (2018) 91–97

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Effect of zonisamide on refractory epilepsy during pregnancy in lamotrigine resistant kindled rats

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ARTICLE INFO

Keywords:
Lamotrigine-resistant kindled rats
Pregnancy
Refractory epilepsy
Zonisamide

ABSTRACT

Drug-resistant epilepsy with uncontrolled severe seizures despite state-of-the-art medical treatment continues to be a major clinical problem. Pregnancy is a state where drug pharmacokinetic changes are more pronounced and more rapid than any other period of life. The current study investigated the effect of zonisamide (ZNS) on refractory epilepsy during pregnancy in lamotrigine-resistant kindled rats. Fifty-six lamotrigine (LTG)-resistant kindled Wistar rats were divided into five experimental (four pregnant and one non-pregnant) and 2 positive controls (pregnant and non-pregnant) groups and eight intact Wistar rats were put in the negative pregnant control group. Experimental groups received daily ZNS 50 mg/kg by oral gavage and 30 min later, pentylenetetrazol (PTZ) (30 mg/kg) was injected intraperitoneal (i.p) on Gestational Days 10–15 (in rats with or without ZNS or methanol and ethyl acetate as a ZNS solvent challenge in days −5 to 0) or Days 15–20 and for six days in the non-pregnant group. The positive control groups received the ZNS solvent for the same number of days, but the negative pregnant control group did not receive any treatment. Epilepsy was significantly controlled by ZNS in the experimental groups compared to the positive control groups. It was concluded that ZNS can control refractory epilepsy during pregnancy and increase pregnancy survival in refractory epileptic rats.

1. Introduction

More than 30% of patients with partial epilepsy are resistant to traditional antiepileptic drugs (AEDs) [14]. Therefore, there is a definite need for pharmacotherapies that will effectively manage seizures in a population of patients with pharmacoresistant epilepsy. Development of AEDs for the effective treatment of pharmacoresistant epilepsy has proven to be particularly difficult, partly because of a lack of suitable preclinical animal models. Despite the introduction of several new AEDs since 1993, pharmacoresistant epilepsy continues to represent a significant clinical problem. In recent years, several models have been identified that display one or more characteristics of pharmacoresistance. More recently, it has been demonstrated that treatment with LTG to prevent chemical kindling acquisition leads to subsequent resistance to LTG [24].

The pharmacological treatment of epilepsy is more challenging during pregnancy. The treatment involves drug exposure to two individuals: the mother with epilepsy and the unborn foetus/es. Foetal loss has been reported in conjunction with prolonged seizures, such as status epilepticus [26], and frequent tonic-clonic seizures during pregnancy are associated with poorer cognitive development of the child [18]. On the other hand, AEDs can be teratogenic, thereby increasing the risk of congenital malformations as well as of adverse cognitive outcomes [28]. Therefore, the usual recommended treatment strategy is to review and possibly revise treatment well before conception and select the most appropriate AED for the individual woman, taking efficacy as well as teratogenic risks into account.

Valproate (VPA) is now known to be associated with a significantly increased risk of anatomic teratogenesis compared with baseline population rates and other AEDs [7,12].

Carbamazepine (CBZ), LTG, phenytoin (PHT) and levetiracetam (LEV) seem to have a risk of major congenital malformations [7,12]. Recent data on topiramate (TPM) has raised concern about its teratogenic potential, particularly a specific association with oral clefting [11,12].

ZNS is an AED that was approved for human use for the first time in Japan in 1989. Evidence from animal models has shown that its effect against partial onset seizures is due to the drug's ability to block voltage-gated sodium channels. Its effect against absences of seizure is most likely explained by the ability to inhibit voltage-gated T-type

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https://doi.org/10.1016/j.neulet.2017.11.020
Received 5 July 2017; Received in revised form 6 October 2017; Accepted 8 November 2017
Available online 11 November 2017
0304-3940/

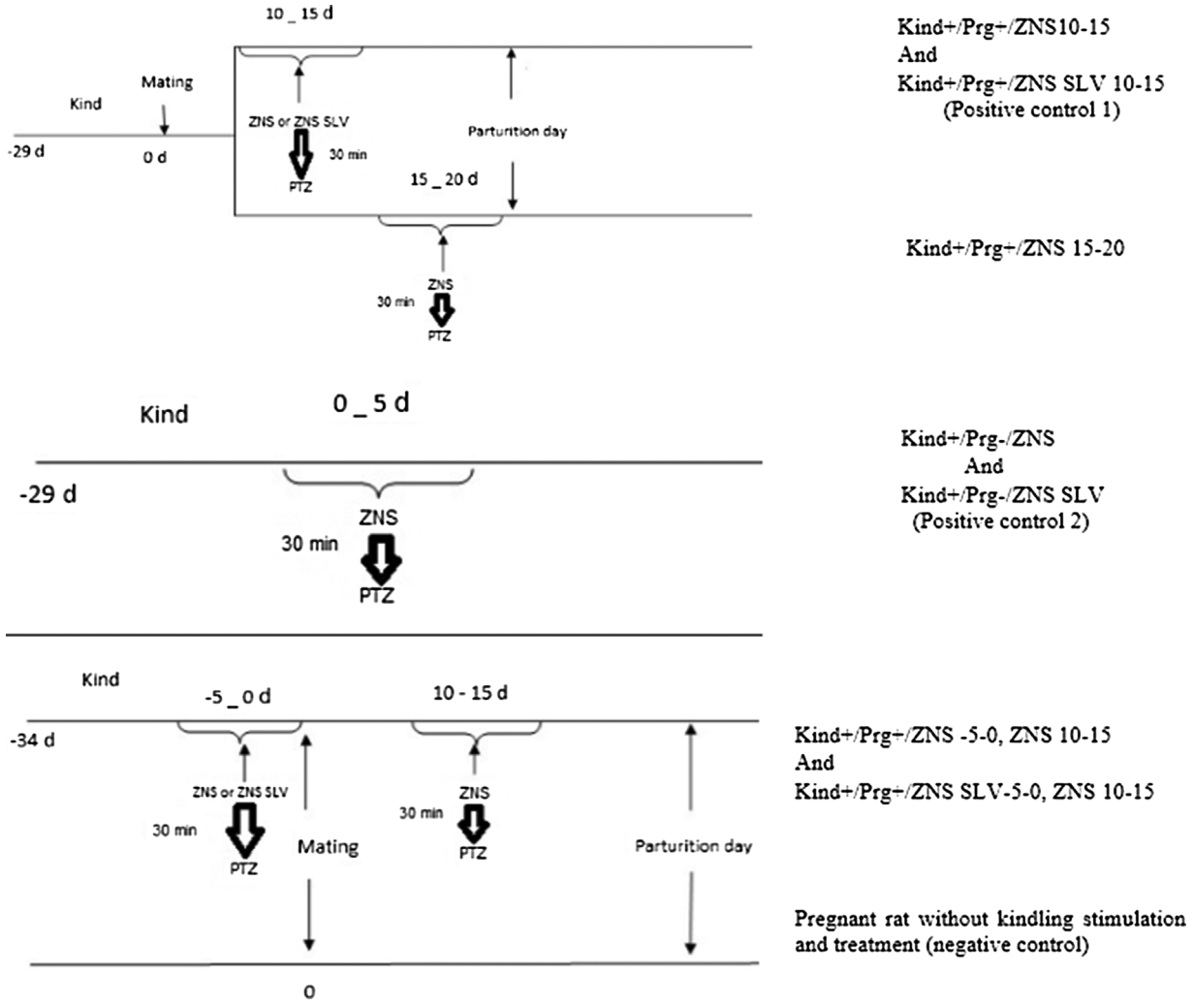

Fig. 1. Diagrammatic representation of eight treatments and control groups. LTG-resistant kindling in rats was induced by giving a LTG (5 mg/kg i.p.) 1 h before every PTZ (30 mg/kg i.p.) injection every alternate day for mean 29 days. Rats were considered to be fully kindled when they were observed to have 3 consecutive stage 5 seizure. Drug treatment was discontinued once the rats were fully kindled. Two days following the last kindling session, fully kindled animals received higher dose of LTG (15 mg/kg i.p) before PTZ challenge and should showed stage 5 seizure. The day on which observation of vaginal plaque or spermatozoa were found in the vaginal smear was designated as embryonic day 0 (0 d). ZNS or Methanol and ethyl acetate as ZNS solvent gavaged daily (50 mg/kg) 1 h before i.p. injection of PTZ (30 mg/kg). Eight rats were included in each group.
LTG: Lamotrigine, PTZ: Pentylenetetrazol, ZNS: Zonisamide.

calcium channels. Additionally, the drug enhances extracellular GABA and decreases extracellular glutamate. It is also a weak carbonic anhydrase inhibitor, but this is hardly a part of its mechanism of action [5].

ZNS has some advantages over some other AEDs as it is shown to be effective both against focal and generalized seizures [3]. Based on our knowledge, in conjunction with ZNS effects on refractory epilepsy during pregnancy, there is no information available.

The complex interaction between epilepsy and pregnancy has been traditionally discussed under three heads viz. Effect of pregnancy on epilepsy, effect of epilepsy on pregnancy and effect of epilepsy and AEDs on foetus [27].

Therefore, the aim of this study was A) Evaluation of ZNS effect on refractory epilepsy during pregnancy in rats B) Effect of ZNS on pregnancy survival in refractory epileptic rats C) Effect of ZNS on refractory epilepsy during pregnancy with history of ZNS consumption.

2. Material and methods

2.1. Animals

Sixty four Wistar rats (200–240 g) were purchased from Shahmirzad Laboratory Animal Research Center in Shahmirzad, Semnan, Iran. The animals were maintained in the animal house under controlled conditions (12 h light-and-dark cycles, at 21 °C with 50% relative humidity) with laboratory chow and water provided *ad libitum*. Before mating, the experimental and positive control groups were kindled by LTG (Daroupakhsh Co., Iran) resistant model (n = 56) and the negative control group was intact.

2.2. LTG-resistant kindling procedure

According to previous study [24] Chemical kindling in rats was induced by giving an i.p. injection of subconvulsant dose of 30 mg/kg PTZ (Sigma, USA), one hour after saline injection, every alternate day for a maximum of 74 days. For induction of LTG-resistant kindled rat, animals received LTG (5 mg/kg i.p.) 1 h before every PTZ challenge every alternate day for a maximum of 74 days and were observed for 30 min; seizure score was recorded according to the modified Racine scale [9] as follows: stage 0, no response; stage 1, grooming and hyperactivity; stage 2, head nodding and tremor; stage 3, bilateral forelimb clonus; stage 4, clonus with rearing; and stage 5, generalized clonic seizures with loss of loss of postural control. Rats were considered to be fully kindled when they were observed to have 3