International Journal of Epilepsy 3 (2016) 68–74

Contents lists available at ScienceDirect

International Journal of Epilepsy

journal homepage: http://www.journals.elsevier.com/international-journal-of-epilepsy

Research paper

# To evaluate the anti-kindling effect of allopregnanolone alone and its interaction with sodium valporate in pentylenetetrazole induced kindling model

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ARTICLE INFO

*Article history:*
Received 18 January 2016
Accepted 4 October 2016
Available online 24 October 2016

*Keywords:*
Allopregnanolone
PTZ kindling
Sodium valproate
Combination
Side effects

ABSTRACT

*Background and purpose:* Studies in the animal models of epilepsy have suggested the anti-seizure effects of neuroactive steroids and its derivatives in kainic acid and pilocarpine induced limbic seizures and status epilepticus in mice, but no such studies have been reported in the published literature on the role of allopregnanolone in chemical kindling model and its interaction with sodium valproate. The purpose of this study was to evaluate the interaction between sodium valproate and allopregnanolone in pentylenetetrazole induced kindling model in rats.

*Methods:* In a PTZ kindled Wistar rat model, sodium valproate and allopregnanolone were administered 30 min before the PTZ injection. The PTZ injection was given on alternate day till the animal became fully kindled or till 10 weeks. The parameters measured were latency to develop kindling and incidence of kindling, histopathological study of hippocampus, hippocampal anti-oxidant parameters and hippocampal DNA fragmentation studies.

*Results:* In this study, the combination of low dose of allopregnanolone with low dose of sodium valproate showed a similar beneficial effect to that of a higher dose of sodium valproate in significantly reducing the number of kindled animals (0/8) as compare to PTZ control group (5/8) as well as the seizure scores and the histopathological scores. The combination significantly reduces oxidative stress by significantly decreasing the MDA levels, and increasing the SOD levels and GSH levels in the hippocampus of rats as compared to PTZ control group. So all these data suggest the antiepileptic effect of the combination and confers the synergistic interaction between the allopregnanolone and sodium valproate.

*Conclusions:* It can be concluded that by choosing this combination the dose of sodium valproate can be reduced and thereby reduces the incidence of adverse effects caused by sodium valproate and hence proves to be a useful combination clinically. This study has lead the basis to further investigate the various combinations of neurosteroids and valproate in the process of epileptogenesis with better side effect profile.

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## 1. Introduction

Epilepsy is a chronic neurological disorder having a prevalence of about 1%.[1] It is characterized by the recurrent appearance of spontaneous seizures due to neuronal hyperactivity in the brain.[2] Epileptogenesis is the transformation of normal neuronal network into an hyperexcitable one.[3] Some of the underlying mechanism in the initiation and progression of epilepsy after an initial brain insult includes oxidative stress from increased reactive oxygen radicals, apoptosis induction, inflammation, immune modulation and blood brain barrier dysfunction.[4,5] It is to be noted that despite the increasing availability of newer antiepileptic agents, seizures in one-third patients remains refractory to medical therapy[6] and epilepsy remains as an ongoing health problem. Among various types of epilepsy, temporal lobe epilepsy (TLE) and drug-resistant type of adult focal epilepsy are the most common.[2]

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http://dx.doi.org/10.1016/j.ijep.2016.10.001
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Nuclear factor erythroid-2-related factor 2 (Nrf2) is the primary transcription factor that gets activated in response to oxidative stress and increases the expression of anti-oxidant enzyme such as SOD (superoxide dismutase), catalase, glutathione peroxidase (GSH-Px) and glutathione S-transferase.[4] Animal models of epilepsy are an important pre-requisite to study the process of epileptogenesis and are used to develop drugs that are anti-epileptogenic. Kindling is a chronic model of epilepsy where sub-convulsive stimulus (either chemical or electrical), if applied repetitively and intermittently, will ultimately lead to the generation of full-blown convulsions.[7] It has the advantage of both an epileptogenic and a spontaneous seizure model.

Valproic acid (VPA) is a broad spectrum antiepileptic drug (AED) and are effective against most of the seizure types including primary generalized tonic–clonic seizures, partial seizures, absence seizure, atonic seizures, etc.[8] But it is associated with adverse effects and the most commonly reported adverse effects of valproate include hepatotoxicity, gastrointestinal disturbances, tremor, body weight gain, platelet disorders and encephalopathy symptoms.[9] Studies have demonstrated the protective effect of neurosteroids against seizures induced by GABA-A receptor antagonists, including pentylenetetrazole and bicuculline, and are effective against pilocarpine-induced limbic seizures and seizures in kindled animals.[10] Allopregnanolone (3α-hydroxy-5α-pregnan-20-one) is a neurosteroids which is the secondary metabolite of progesterone, and has also demonstrated the potent anticonvulsant effects against the secondary generalized component of the amygdale-kindled seizures in rats.[11]

Apart from the anticonvulsant activity, the endogenous neurosteroids play a role in regulating epileptogenesis.[12] In the kindling model, it was demonstrated that the development and persistence of limbic epileptogenesis are impaired in mice lacking progesterone receptors.[13] Neurosteroids also play a key role in the pathophysiology of catamenial epilepsy, stress-sensitive seizure conditions, temporal lobe epilepsy, alcohol-withdrawal seizures and in status epilepticus.[14] So, it can be hypothesized that neurosteroid replacement with natural or synthetic neurosteroids may be useful in the treatment of epilepsy.

Studies in the animals have demonstrated the anti-seizure effects of neurosteroids and its derivatives in kainic acid and pilocarpine induced limbic seizures and status epilepticus in mice. To the best of our knowledge till now no study has been reported in the published literature on the role of allopregnanolone in chemical kindling model and its interaction with sodium valproate. Thus the present study has been contemplated with the aim of establishing the anti-kindling effect of allopregnanolone and its interaction with sodium valproate in chemical kindling model in rats.

## 2. Materials and methods

### 2.1. Experimental animals

All animal procedures and the experimental protocols were approved by the institutional animal ethics committee (IAEC) of the institute before the start of the study. Young male Wistar rats (150–200 g) were used for the present study. The animals were maintained at 23 ± 2 °C with a relative humidity of 65 ± 5% in 12 h light/dark cycle. Animals had free access to standard pellet chow diet and tap water ad libitum. Animals were acclimatized to the laboratory conditions for 7 days prior to experimentation.

### 2.2. Grouping

A total of 48 ($n$ = 8 in each group) adult male Wistar rats (150–200 g) were divided into the following groups: (1) saline control group (0.9% NaCl, w/v), (2) DMSO control group (0.1%, w/v), (3) saline + PTZ group (35 mg/kg), (4) sodium valproate (200 mg/kg) + PTZ group (35 mg/kg), (5) allopregnanolone (0.5 mg/kg) + PTZ group (35 mg/kg) and (6) allopregnanolone (0.5 mg/kg) + sodium valproate (100 mg/kg) + PTZ group (35 mg/kg); where NaCl – sodium chloride, PTZ – pentylenetetrazole, DMSO – dimethyl sulphoxide.

### 2.3. Drug preparation and dosing scheduled

PTZ was dissolved in 0.9% saline and injected intraperitoneally (i.p.) in a volume of not exceeding 10 ml/kg at a sub convulsive dose of 35 mg/kg. Allopregnanolone (0.5 mg/kg) was dissolved in a 0.1% solution of DMSO and was injected subcutaneously (s.c.) 30 min before PTZ administration. Sodium valproate (200 mg/kg) was dissolved in 0.9% saline and was administered intraperitoneally 30 min before PTZ injection. All the drugs were given every alternate day until the animal develops kindling or up to 10 weeks. All chemicals used in the present study were in analytical grade and were procured from Sigma Pharmaceutical Industrial Co.

### 2.4. Pentylenetetrazole (PTZ) induced kindling in rats

#### 2.4.1. Procedure

PTZ was injected i.p. and after each injection of PTZ, the rat was placed singly in isolated transparent plexiglass cages and was observed for 1 h and the intensity of convulsions was rated according to the Racine 5-point scale[15] as follow: 0 – no response; 1 – ear and facial twitching; 2 – myoclonic jerks without rearing; 3 – myoclonic jerks with rearing; 4 – turn over into side position, tonic–clonic seizures; 5 – turn over into back position, generalized tonic–clonic convulsions.

#### 2.4.2. Endpoints

An animal was considered kindled, when it exhibits, stage 4 of seizure score on three consecutive trials. Latency to develop kindling in each group, the percentage (%) of animals being kindled in each group and the percentage (%) of animals suffering from mortality in each group were recorded.

#### 2.4.3. Collection of samples

When the animal became fully kindled (exhibits, stage 4 of seizure score on three consecutive trials), on the next day, it was sacrificed by decapitation under the overdose of anesthetic agent. The hippocampus was carefully dissected out of the brain.

### 2.5. Studies with hippocampus

#### 2.5.1. Histopathology of hippocampus using hematoxylin and eosin (H&E) strain

Hippocampus was carefully dissected out of the brain and fixed in 10% formalin and was subjected to histopathological studies using hematoxylin and eosin (H&E) stain. A semi quantitative histopathological score was used to determine the relative percentage of damaged neurons as follows[16]: normal, no injury = 0; rare neuronal injury (<5 clusters) = 1; occasional neuronal injury (5–15 clusters) = 2; frequent neuronal injury (>15 clusters) = 3; diffuse neuronal injury = 4.

#### 2.5.2. Study of oxidative stress parameters in hippocampus

*2.5.2.1. Measurement of thiobarbituric acid – reactive substances.* The extent of lipid peroxidation was estimated according to the method of Okhawa et al. (1979).[17] Tissue homogenate was prepared in a ratio of 1 g of wet tissue to 9 ml of phosphate buffer (pH 7.2) using a homogenizer. To 0.1 ml of the hippocampal tissue

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