



EXPERIMENTAL EVALUATION OF MEDIAN EFFECTIVE DOSE OF RETIGABINE (TROBALT®) IN THE MAXIMAL ELECTROSHOCK TEST AND MAXIMAL PENTYLENETETRAZOLE TEST IN RATS

E. Apostolova*, V. Kokova, L. Peychev

Department of Pharmacology and Drug toxicology, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria

ABSTRACT

Retigabine is a novel antiepileptic drug that influences M-type potassium currents. It is active in broad range of animal seizure models. **PURPOSE.** The aim of the present study is to determine the median effective dose (ED₅₀) of Retigabine in two seizure models – maximal electroshock (MES) test and maximal pentylenetetrazole (MPTZ) test in rats. **METHODS.** Male Wistar rats (10 groups of 6 rats) were treated orally with Retigabine in doses 5, 7, 12, 20, 25 mg/kg (MES test) and 35, 60, 70, 90, 100 mg/kg (MPTZ test) respectively. ED₅₀ MES and ED₅₀ MPTZ were evaluated 30 minutes after p.o. administration of Retigabine (Trobalt®, tab. 200 mg) by the method of Litchfield and Wilcoxon. PTZ was injected i.p. in dose 100 mg/kg. **RESULTS.** Our results showed ED₅₀ Retigabine = 18 mg/kg in the MES test and ED₅₀ Retigabine = 64 mg/kg in the i.p. MPTZ test. The drug suppress electrically and chemically induced seizures – models of generalized tonic – clonic seizures. **CONCLUSIONS.** Retigabine inhibits generalized seizures in MES test (18 mg/kg, p.o.) and in i.p. MPTZ test (64 mg/kg, p.o.).

Key words: Retigabine; MES; PTZ; Litchfield and Wilcoxon; Animals

INTRODUCTION

Around 50 million people in the world are affected by epilepsy - a serious neurologic disorder. Classical antiepileptic drugs (Phenobarbital, Phenytoin, Carbamazepine, Ethosuximide, Primidone, Valproate) have effect on 65-70% of patients with epilepsy. Other 25-30% do not respond to available drugs. Some newer antiepileptic drugs (AEDs) proved to be useful in the treatment of patients with refractory epilepsy (1).

Retigabine (**Figure 1**) - N-[2-Amino-4-(4-fluorbenzylamino)-phenyl] carbamin acid ethyl ester was approved by EMEA in January 2011 under the trade name Trobalt® (GlaxoSmithKline) (2).

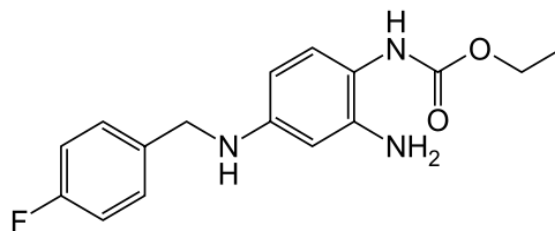


Figure 1. Retigabine – chemical structure.

Retigabine acts as activator of voltage – gated potassium channels (“M-channels”). It induces neuronal hyperpolarization and stabilization of the membrane resting potential (3). Another mechanism of action involves GABA. Kapetanovic MP et al. (1995) (4) showed increased synthesis of GABA in rat hippocampal slices. The enhanced chloride current, induced by GABA is not caused by activation of benzodiazepine receptors (5). Sills GJ et al. (2000) (6) reported that Retigabine diminish the concentrations of glutamate and glutamine in mouse brain.

*Correspondence to: Elisaveta Apostolova, bul. “V. Aprilov” 15 A, Plovdiv, Bulgaria, tel. 032602089, e-mail: elisaveta_apostolova30@yahoo.com

Retigabine is found to be effective in various animal models – MES, PTZ, picrotoxin, kainate, NMDA, genetic epilepsy model, which explains the broad spectrum of anticonvulsant activity (7).

Purpose

In the present study we determine the median effective dose (ED_{50}) of Retigabine in two seizure models – MES test and PTZ test in rats.

MATERIALS AND METHODS

Animals

Sixty male Wistar rats, body weight 180-200 g were used for the experiments. They were weighed, numbered and divided in 10 groups of 6. Rats were kept under standard laboratory conditions ($22 \pm 1^\circ\text{C}$, humidity 45% and 12-h light cycle). The rodents received food and water ad libitum. All experiments were approved by the Bulgarian Food Safety Agency.

Drugs

Retigabine (Trobalt[®] 200 mg, distributed by GlaxoSmithKline) was dissolved in distilled water containing 0.1% Tween 20. Retigabine was administered in doses 35, 60, 70, 90, 100 mg/kg b.w. in the MPTZ test and 5, 7, 12, 20, 25 mg/kg b.w. in the MES test. The solutions were prepared to allow oral administration of 0.01 ml/g b.w.

PTZ in dose 100 mg/kg b.w. was dissolved in saline.

MPTZ seizure test

The dose-response curve for MPTZ test was determined using 5 groups of 6 rats. They were treated orally as follows: 1st group – RTG 35 mg/kg b.w., 2nd group – RTG 60 mg/kg b.w., 3rd group – RTG 70 mg/kg b.w., 4th group – RTG 90 mg/kg bw and 5th group – RTG 100 mg/kg bw.

PTZ was injected intraperitoneally 30 minutes after the treatment with Retigabine. Animals were placed in clear plexiglass boxes after injection of PTZ. The seizure activity was observed for 30 minutes and the seizure stage was determined, using the scale: 1- sudden muscle twitches or behavior changes; 2- myoclonic twitches; 3- repeated clonic seizures of forelimbs, without loss of righting reflexes; 4- generalized clonic seizure of fore-and hindlimbs with loss of righting; 5- tonic forelimb seizure; 6- tonic fore- and hindlimb seizure. Animals, not experiencing stage 5 or higher seizure scores were considered as protected. ED_{50} was calculated by the method of Litchfield and Wilcoxon (8).

MES seizure test

For the MES test the animals were treated orally as follows: 1st group – RTG 5 mg/kg b.w., 2nd group – RTG 7 mg/kg b.w., 3rd group – RTG 12 mg/kg b.w., 4th group – RTG 20 mg/kg bw and 5th group – RTG 25 mg/kg bw. The animals were stimulated with corneal electrodes 30 minutes after the treatment with Retigabine. The apparatus was set on 50 Hz, 150 mA, 0.2 s, a stimulus caused stage 6 seizure in controls (Group 1). In order to minimize the pain caused by corneal stimulation Alcaine[®] was administrated in the eyes before the test. Animals were placed in clear plexiglass boxes after the stimulation. The seizure activity was observed for 30 minutes and the seizure stage was determined, using the same scale as in MPTZ test. ED_{50} was calculated by the method of Litchfield and Wilcoxon (8).

RESULTS

PTZ test

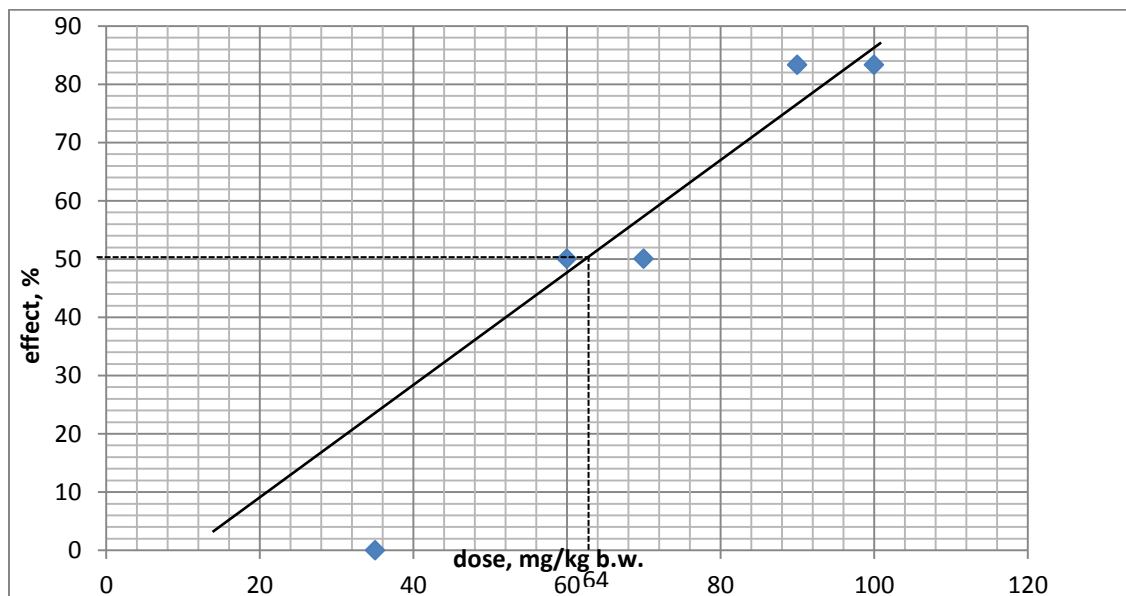


Figure 2. Dose – response curve of Retigabine (Trobalt[®]) in MPTZ test.

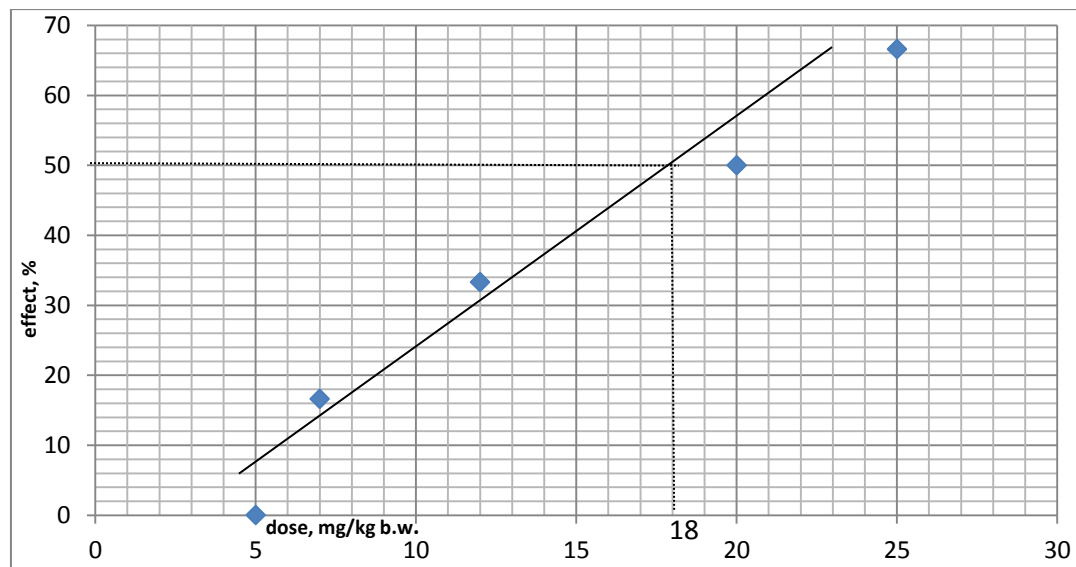
MES test

Figure 3. Dose – response curve of Retigabine (Trobalt®) in MES test.

Retigabine showed efficacy in MES test with $ED_{50} = 18$ (10,6-30,6) mg/kg b.w., when administrated orally in rats (Figure 3).

DISCUSSION**MPTZ test**

PTZ administrated intraperitoneally is a model of acute seizure, used for antiepileptic drugs screening. There was no data in the literature regarding the dose in i.p. PTZ model of epilepsy. According Large CH et al. (2012) Retigabine is effective against seizures, induced by s.c. PTZ ($ED_{50} = 68$ mg/kg b.w., p.o.) (9). Our experiments showed similar result when PTZ was injected intraperitoneally ($ED_{50} = 64$ mg/kg b.w., p.o.). Both models (s.c. and i.p. PTZ) are using the same dose (100 mg/kg b.w.), but the onset time of the seizure is shorter when PTZ is given i.p. due to rapid resorption (10).

MES test

Rostock A. et al. (1996) performed similar test, but the result for ED_{50} was 2.87 (1,9-4,1) mg/kg b.w. (7). The difference may be caused by the used substances. Our experiments were conducted using Trobalt® 200 mg and Rostock A. et al. were using substance, synthesized by ASTA Medica, Frankfurt. The presence of inactive ingredients in tablets may lead to a higher ED_{50} of Trobalt in comparison to a substance. Moreover we dissolved the tablets in distilled water in the presence of 0.1% Tween 20 (Polysorbate 20) v/s 0,5% Hydroxyethylcellulose

in the other case. The different co-solvent may influence the observed effect.

CONCLUSIONS

Retigabine inhibits generalized seizures in MES test (18 mg/kg b.w., p.o.) and in MPTZ test (64 mg/kg b.w., p.o.). Results may be different if using Retigabine as substance instead of Trobalt®.

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