Original Article

EXPERIMENTAL STUDY ON THE DEVELOPMENT OF TOLERANCE AND WITHDRAWAL SYNDROME TO PENTYLENETETRAZOLE-INDUCED SEIZURES IN RATS TREATED WITH RETIGABINE

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Summary

The aim of the study was to evaluate seizure liability in rats against the convulsant pentylenetetrazole (PTZ) during a 14-day treatment with retigabine (RTG). The seizure liability was also studied on the1st, 2nd and 3rd day after the abrupt determination of its administration. Male Wistar rats were divided in groups of 10 and treated orally for 14 days (1st – 6th group with distilled water, and 7th – 12th group with retigabine (RTG) at a dose of 60 mg/kg bw The tolerance to the anticonvulsant effect of RTG was studied using subcutaneous injection of PTZ (120 mg/kg bw) on the 1st and 14th day. To determine the possible neuronal hyperexcitability on the 1st, 2nd and 3rd day after termination of drug administration, a lower dose of PTZ (65 mg/kg bw) was used. According to our results there was no change in the anticonvulsant activity of RTG during the whole period of treatment. The study on withdrawal syndrome showed slightly decrease of the effect on the first day after the last treatment, but with no significant difference to the controls. The anticonvulsant effect of RTG on the 2nd and 3rd day was close to that in the control group. Our results showed no development of tolerance on subchronic treatment with RTG. There was no significant change in the neuronal hyperexcitability on the 1st, 2nd and 3rd day after the termination of treatment. Based on these results we can suggest that RTG has no potential to develop withdrawal syndrome.

Key words: retigabine, tolerance, withdrawal, PTZ, rats

Introduction

Epilepsy is a serious neurological disorder and its pharmacotherapy often requires long term treatment with antiepileptic drugs. The prolonged exposure of the body to chemical substances leads to adaptive changes, which results in development of tolerance and withdrawal syndrome. Repeated drug administration can lead to tolerance (i.e. progressive reduction in drug effectiveness) to its effects [1].

Another phenomenon related to long term therapy is the development of withdrawal syndrome. It includes symptoms which are opposite of the drug effects. In the case of antiepileptics the withdrawal syndrome is manifested with reduced seizure threshold (observed in animal models) and higher

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Received: October 19, 2014
Revision received: February 05, 2015
Accepted: May 18, 2015

risk of relapse (observed in clinical practice) [2].

Retigabine is an anticonvulsant, approved by the European Medicines Agency in January 2011 under the trade name Trobalt® (Glaxo SmithKline) [3]. The mechanism of action of retigabine is complex. The drug activates low-threshold voltage-gated potassium channels, causing hyperpolarization of the membrane potential. It may influence the neuronal mediation of γ-aminobutyric acid (GABA), glutamate, glutamine and dopamine [4, 5]. There are little experimental data regarding development of tolerance and withdrawal syndrome after treatment with retigabine.

Pentylenetetrazole is often used in screening for antiepileptic activity of drugs in development. The chemical, given parenterally, leads to myoclonic jerks, but higher doses are associated with tonic-clonic seizures [6]. According to Rostock et al. [7], retigabine is active against chemically induced seizures (PTZ test) in rats and the test can be used to evaluate the development of drug tolerance and withdrawal syndrome.

The aim of this study was to evaluate seizure liability in rats against the convulsant pentylenetetrazole (PTZ) during a 14-day treatment with retigabine (RTG). The seizure liability was also studied on the1st, 2nd and 3rd day after the abrupt determination of its administration.

Materials and Methods

All experiments were approved by the Animal Health and Welfare Directorate at Bulgarian Food Safety Agency with Permit No 88/09.01.2014.

Animals

Male Wistar rats (weight of 180–200g) were divided into 12 groups (n=10). They were treated orally for 14 days as follows: groups 1, 2, 3, 4, 5 and 6 (controls) – treated with Aqua destillata in the presence of 0.1% Tween 20; groups 7, 8, 9, 10, 11 and 12 – treated with retigabine at a dose of 60 mg/kg bw

Rats were kept under standard laboratory conditions (temperature 22 ±1°C, humidity 45% and 12-h dark/light cycle). The rodents received food and water ad libitum.

Drugs

Retigabine (Trobalt® 100 mg, distributed by GlaxoSmithKline) was dissolved in distilled water containing 0.1% Tween 20. The solution was prepared to allow oral administration of 0.01 ml/g bw. PTZ was dissolved in distilled water.

Experimental procedures

Evaluation of development of tolerance using maximal PTZ test

On the first day groups 1st and 7th were treated and PTZ at a dose of 120 mg/kg bw was injected subcutaneously 30 minutes after treatment. The 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: 1st degree – sudden muscle twitches or behavior changes; 2nd – myoclonic twitches; 3rd – repeated clonic seizures of forelimbs, without loss of righting reflexes; 4th – generalized clonic seizure of fore- and hindlimbs with loss of righting; 5th – tonic forelimb seizure; 6th – tonic fore- and hindlimb seizure. Animals, not experiencing stage 5th or higher seizure degree were considered as protected.

The same procedure was conducted on the 14th day of treatment on groups 2nd and 8th. The percentage of protected animals in each group was statistically calculated using χ^2-test.

Evaluation of withdrawal syndrome after 14th day treatment using PTZ seizure test

Groups 3, 4, 5, 6, 9, 10, 11 and 12 were treated once a day at 9:00 a.m. for 14 days. Groups 3 and 9 were tested on the last day of treatment (day 0) with PTZ (dose 65 mg/kg bw). The test was conducted 30 minutes after oral administration of the substances. 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 same scale. The drug administration was discontinued. On the 1st day after withdrawal groups 4 and 10 were tested using the same procedure. On 2nd day and 3rd day were tested respectively groups 5 and 11(day 2) and 6 and 12 (day 3) and the sizure degree was determined. Animals, not experiencing stage 5 or higher seizure scores were considered as protected. The percentage of protected animals in each group was statistically calculated using χ^2-test.

Statistical analysis

Alternative statistical analysis was conducted with the program INSTAT 2.02 (PraphPad

Software, Inc, CA, USA), χ^2-test. PHARCAP was used for SEM evaluation. The results were considered significant at $p<0.05$.

Results

Evaluation of development of tolerance using maximal PTZ test

A single dose of retigabine led to a significant increase of the percentage of protected animals (60% ± 15.49%) when compared to controls (0%±3.15%), $\chi^2=5.95$. The antiepileptic effect of retigabine was significantly higher (80%±12.65%) as compared to controls (0%±3.15%), $\chi^2=10.21$ on the 14th day of treatment (Fig. 1).

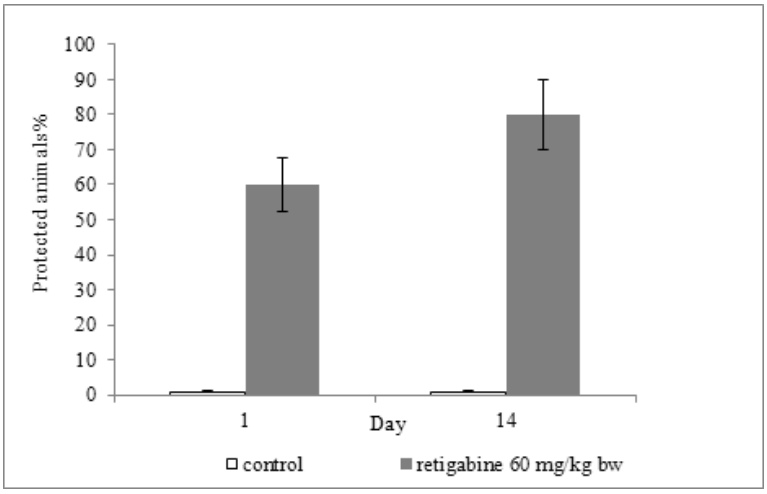

Figure 1. Changes in the percentage of protected animals against PTZ after a single dose retigabine and 14 day treatment. (*p=0.0108 compared with controls on 1-st day; †p=0.007 compared with controls on 14-th day)

Evaluation of withdrawal syndrome after 14 days treatment using electroshock seizure test

As shown on Fig. 2, there was no significant difference between the percentages of protected animals in the control group during the tests on all four days. On the first day after the drug withdrawal this percentage was lower in the group, treated with retigabine, as compared to controls. When comparing the percentage of protected animals in the group, treated with retigabine, a tendency to decrease was observed on the first day after the withdrawal, as compared with the last day of treatment.

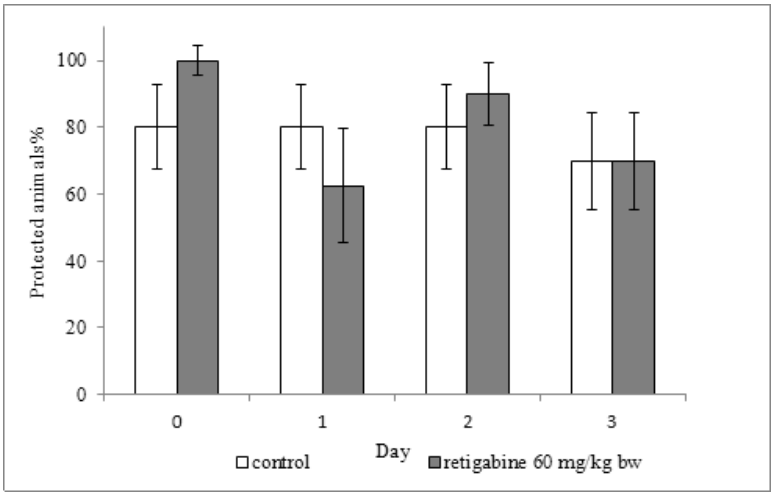

Figure 2. Changes in the percentage of protected animals against PTZ induced seizures on the 14-th day of treatment (day 0) and on the 1-st, 2-nd and 3-rd day after drug withdrawal

Discussion

Our results showed that retigabine, administrated orally in rats for 14 days in a dose of 60 mg/kg bw did not demonstrate the potential to develop tolerance. Retigabine maintained its effectiveness against chemically induced seizures after a 14-day treatment. There is no data in the literature regarding development of tolerance against chemically induced seizures in rats. Rostock et al. found no reduction in the seizure threshold, evaluated with maximal electroshock seizure threshold test. They carried out experiments with mice, treating them orally with retigabine at a dose of 15 mg/kg for 14 days [7]. Data from clinical trials have shown that no tolerance developed during chronic treatment with retigabine in view of its anticonvulsant effect [8].

When studying the development of dependence our results indicated a decrease in the percentage of protected animals treated with retigabine on the 1st day after drug withdrawal, but with no significant difference was fond as compared to controls on the same day. When comparing the percentage of protected animals within the group treated with retigabine, a tendency to decrease was observed on the 1st day after discontinuation of treatment as compared to the last day of drug administration.

The data indicated no presence of neuronal hyperexcitability after the termination of treatment. Rostock et al. (1996) studied the development of withdrawal syndrome as well. These authors found no rebound hyperexcitability on the 1st, 2nd and 6th day after drug withdrawal, using a maximal electroshock model [7]. There are some differences in the experimental procedure in both cases. Rostock et al. (1996) studied the effects on mice versus rats in our experiment, respectively the doses used were different (15 mg/kg bw vs 60 mg/kg bw). Our experiments were conducted using Trobalt® 200 mg, while Rostock A. et al. (1996) used a substance, synthesized by ASTA Medica. In spite of these differences, our results were similar.

Clinical trials have indicated that retigabine is not associated with tolerance, dependence, or withdrawal liability [9-11].

Conclusions

Retigabine shows no potential for development of tolerance against PTZ-induced seizures in rats after the 14th day of treatment. Spontaneous termination of treatment does not lead to withdrawal syndrome and neuronal hyperexcitability after subchronic treatment with the drug.

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