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Stiripentol in a dose-dependent manner elevates the threshold for maximal electroshock-induced seizures in mice

Jarogniew J. Łuszczki[1,2], Monika Dudra-Jastrzębska[2], Marta Andres-Mach[2], Remigiusz Czernecki[1,3], Beata Barcicka-Kłosowska[1], Marta Misiuta-Krzesińska[1], Jakub Zwoliński[1] , Damian Filip[1]

[1] *Department of Pathophysiology, Medical University, Lublin, Poland*
[2] *Department of Physiopathology, Institute of Agricultural Medicine, Lublin, Poland*
[3] *Department of Neurology, Neuropsychiatric Hospital, Kielce, Poland*

Abstract: The aim of the study was to determine the effect of stiripentol (STP) on the threshold for maximal electroshock (MEST)-induced seizures in mice. STP is a novel anti-epileptic drug recently approved for the treatment of patients with myoclonic seizures and children with Dravet syndrome. Electroconvulsions were induced in mice by means of an alternating current (50 Hz, 500 V, ear-clip electrodes, 0.2 s stimulus duration, tonic hind limb extension taken as the endpoint). Linear regression analysis of STP doses and their corresponding threshold increases allowed determination of threshold increasing doses by 20% and 50% (TID_{20} and TID_{50} values) which elevate the threshold in drug-treated animals over the threshold for control animals. Results indicate that STP administered systemically (i.p.), 60 min. before the MEST test, increased in a dose-dependent manner the threshold for MEST-induced seizures in mice. STP at 200 mg/kg significantly elevated the threshold for MEST-induced seizures ($P<0.001$). Similarly, STP at lower doses of 50, 100 and 150 mg/kg also increased the threshold for MEST-induced seizures, although these results did not attain statistical significance. The experimentally-derived TID_{20} and TID_{50} values for STP were 103.2 and 195.8 mg/kg, respectively. Based on this pre-clinical study, one can ascertain that STP dose-dependently increased the threshold for MEST-induced seizures, allowing determination of TID_{20} and TID_{50} values.

Key words: stiripentol, threshold for maximal electroshock-induced seizures, TID_{20}, TID_{50}, mice

INTRODUCTION

Evaluation of the anticonvulsant effects produced by drugs or agents is based on experimental (pre-clinical) studies showing the efficacy of these compounds in animal models of epilepsy. Of these models, the maximal electroshock-induced seizure threshold (MEST) test can determine whether or not the examined agents influence the threshold for maximal electroconvulsions in animals. To unequivocally assess the anti-seizure potential of agents or drugs in this test, one denotes doses increasing the threshold by 20% and 50% (TID_{20} and TID_{50} values, respectively) that elevate the threshold in drug-treated animals over the threshold for control animals [1, 2]. These values uniformly describe the anti-seizure effects of drugs or agents in pre-clinical studies. To determine TID_{20} and TID_{50} values, linear regression analysis of drug doses and their corresponding thresholds is used. The assessment of dose-response relationship with linear regression is a standard and common procedure in pharmacological studies, especially to evaluate the anti-electroshock potential of drugs or agents [3].

Stiripentol (STP {4,4-dimethyl-1-[3,4-(methylenedioxy)-phenyl]-1-penten-3-ol} – a third-generation anti-epileptic drug) inhibits GABA metabolism by blocking GABA-transaminase activity [4] and reducing synaptosomal uptake of GABA [5], leading to an increase in GABA brain content. Moreover, STP markedly increases the mean open duration of $GABA_A$ receptor-dependent chloride channels by a barbiturate-like mechanism [6]. In clinical practice, STP has been reported to decrease the frequency of both partial and generalized seizures, including typical and atypical absences [7, 8]. Recently, STP has reached the status of an orphan drug for children with severe myoclonic epilepsy in infancy and Dravet syndrome [8]. Experimental evidence indicates that STP suppresses seizures in various animal models, including maximal electroshock (MES)-, pentylenetetrazole (PTZ)-, bicuculline-, cocaine- and strychnine-induced seizures in rodents [4, 9, 10]. Therefore, it was of pivotal importance to determine the TID_{20} and TID_{50} values for STP in the MEST test in mice.

MATERIALS AND METHODS

Animals and experimental conditions. All experiments were performed on adult male Swiss mice weighing 22-26 g. The mice were kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light/dark cycle; temperature 21 ± 1°C). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 mice each. Each mouse was used only once. All tests were performed between 09.00-14.00. Procedures involving animals and their care were conducted in conformity with

Corresponding author: Dr. Jarogniew J. Łuszczki, Department of Pathophysiology, Medical University of Lublin, Jaczewskiego 8, 20-090 Lublin, Poland.
E-mail: jarogniew.luszczki@am.lublin.pl or jluszczki@yahoo.com

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current European Community and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures listed below were conformed to the *Guide for the Care and Use of Laboratory Animals* and approved by the First Local Ethics Committee in Lublin (License No. 420/2003/446/2003).

Drugs. STP (Laboratoires Biocodex, Gentilly, France) was suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA) and administered intra-peritoneally (i.p.) in a volume of 0.005 ml/g body weight at 60 min. before MEST-induced seizures. The times for administering the drugs to the mice was based on their activity, from literature, and confirmed by our previous experiments [11].

Maximal electroshock seizure threshold (MEST) test. Electroconvulsions were produced by means of an alternating current (0.2 s stimulus duration, 50 Hz, maximum stimulation voltage of 500 V) delivered via ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The electrical system of the stimulator was self-adjustable so that changes in impedance did not result in alterations of current intensity (i.e., the system provides constant current stimulation). The criterion for the occurrence of seizure activity was the tonic hind limb extension (i.e., the hind limbs of animals outstretched 180° to the plane of the body axis). To evaluate the threshold for maximal electroconvulsions, at least 4 groups of mice, consisting of 8 animals per group, were challenged with electroshocks of various intensities to yield 10-30%, 30-50%, 50-70%, and 70-90% of animals with seizures. A current intensity-response relationship curve was constructed according to the log-probit method of Litchfield and Wilcoxon [12], from which a median current strength (CS_{50} in mA) was calculated. Each CS_{50} value represents the current intensity required to induce tonic hind limb extension in 50% of the mice challenged.

After administration of a single dose of STP to 4 groups of animals, the mice were subjected to electroconvulsions (each group with a constant current intensity). The threshold for maximal electroconvulsions was recorded for 4 different doses of STP: 50, 100, 150 and 200 mg/kg. Subsequently, the percentage of increase in CS_{50} values for animals injected with increasing doses of STP over the control (vehicle-treated animals) was calculated. The doses of STP and their resultant percentage of threshold increase over the control (vehicle-treated animals) were graphically plotted in rectangular coordinates of the Cartesian plot system, and examined with least-squares linear regression analysis [13]. From linear regression equation the TID_{20} and TID_{50} values were determined as recommended by Swinyard et al., [1] and Löscher et al. [2]. The experimental procedure has been described in more detail in our earlier studies [11, 15].

Statistical analysis. The CS_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [12]. Subsequently, the 95% confidence limits were transformed to their standard errors (SE) according to Luszczki et al. [14]. Statistical analysis of data was performed with one-way ANOVA followed by the Tukey/Kramer test for multiple comparisons, as documented earlier [13, 15].

RESULTS

Effect of STP on the threshold for MEST-induced tonic seizures. STP administered i.p., 60 min. before electroconvulsions, increased in a dose-dependent manner the threshold for MEST-induced seizures in mice. The experimentally-derived CS_{50} value for STP (200 mg/kg) was 9.68 mA and significantly different from that of control animals – 6.19 mA ($P<0.001$) (Table 1). STP at lower doses of 50, 100 and 150 mg/kg also elevated the threshold for MEST-induced tonic seizures by 6.6%; 16.3% and 28.8%, respectively; however, the results did not attain statistical significance with the Tukey/Kramer *post-hoc* test (Table 1). The equation for dose-threshold increase relationship for STP was as follows: $y = 0.324\ x - 13.45$ ($r^2 = 0.937$); where y – threshold increase in %, x – the drug dose, and r^2 – coefficient of determination. The experimentally-derived TID_{20} and TID_{50} values for STP were 103.2 mg/kg and 195.8 mg/kg, respectively, in the MEST-induced seizures.

Table 1 Effect of stiripentol (STP) on the electroconvulsive threshold in mice

Treatment (mg/kg)	CS_{50} (mA)	N	TI (%)
Control	6.19 ± 0.447	16	-
STP (50)	6.60 ± 0.484	24	6.6
STP (100)	7.20 ± 0.449	16	16.3
STP (150)	7.97 ± 0.444	24	28.8
STP (200)	9.68 ± 0.376 ***	16	56.4
F(4;91) = 8.007; P<0.0001			

Values presented as median current strengths (CS_{50} ± SEM) necessary to evoke seizure activity (tonic hind limb extension) in 50% of animals tested.
N – number of animals tested at current strengths with effect ranging between 4-6 probits. Statistical evaluation of data was performed by one-way ANOVA followed by *post-hoc* Tukey/Kramer test. Threshold for control animals was considered as a baseline (reference) value, allowing subsequent calculation of percentage of threshold increase (TI) in animals after STP administration.

***P<0.001 vs. control (vehicle-treated) animals.

DISCUSSION

The objective of the study was to determine the TID_{20} and TID_{50} values for STP in the MEST test. Linear regression analysis of STP doses and their corresponding threshold increasing values over the threshold for control animals in the MEST test revealed that there was a close relationship between the doses of STP and their biological effects in terms of seizure suppression in mice (Figure 1). Both TID_{20} and TID_{50} values for STP were considerably lower than the experimentally-derived ED_{50} value of STP in the MES test – 277.7 (254.9-302.5) mg/kg [16]. Undoubtedly, the MEST test allows preselection of the agents or drugs possessing anti-seizure properties in both pre-clinical studies on animals and clinical settings in patients with epilepsy. Moreover, it has been documented that STP produced anti-convulsant action in PTZ-induced seizures in mice; the ED_{50} value for STP in the PTZ test was 221.3 (155.1-315.9) mg/kg [11]. The TID_{20} value for STP, as determined in PTZ-induced seizure test, was 205 mg/kg (data not shown), which suggests that STP also has a strong anti-convulsant potential against myoclonic seizures in clinical settings.

It is important to note that the linear regression analysis was used in this study to determine the doses of STP increasing the threshold for electroconvulsions in animals by a fixed,

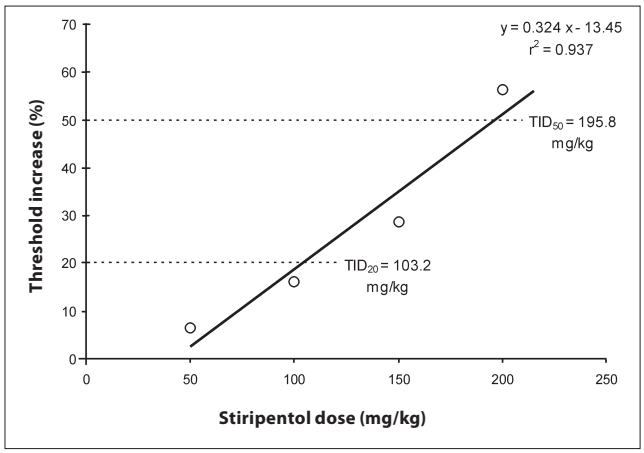

Figure 1 Dose-threshold increase relationship for stiripentol (STP) in maximal electroshock seizure threshold (MEST) test in mice.

Points on the graph represent threshold increasing doses of STP, experimentally denoted in the MEST test in mice. Linear regression analysis allowed determination of the equation for dose-threshold increase relationship for STP, as follows: y = 0.324 x – 13.45 (r2 = 0.937); where y – threshold increase in %, x – drug dose, and r2 – coefficient of determination [13]. From this equation one denotes the TID20 and TID50 (threshold increasing doses by 20% and 50%) for the MEST test. In this study, these values were 103.2 and 195.8 mg/kg, respectively. STP was administered i.p. 60 min. before threshold evaluation.

previously established percentage. With the log-probit method followed by one-way ANOVA alone (without linear regression analysis), one can only determine the effects exerted by the drugs tested at various doses. Therefore, to determine other effects, more experimental groups were required. In contrast, linear regression analysis allows prediction of the doses increasing the threshold for electroconvulsions by the respective percentage, without testing additional groups of experimental animals. Moreover, the application of linear regression analysis allows calculation of the drug doses that increase the threshold by 1-100%, i.e., TID_1 - TID_{100} values. In our opinion, the additional application of linear regression analysis during the evaluation of threshold for electroconvulsions in preclinical studies, can extend the results by predicting doses of drugs increasing the threshold by any effects ranging between 1-100% over the control animals (usually by 20 and 50%). Moreover, with the linear regression analysis, one can assess the same effect for various agents and drugs, contributing to the evaluation of their anti-seizure potency by comparing their TID_{20} or TID_{50} values. In the case of the log-probit method and one-way ANOVA, the effects obtained experimentally for the respective drug doses are unpredictable because the threshold differs considerably for various drugs, and even for control animals. It is therefore difficult to denote the same effects in order to compare the anti-convulsant potential of the examined drugs. It is noteworthy that linear regression analysis substantially extended the results presented in this study, providing more reliable data. Thus, it seems that the log-probit method, one-way ANOVA, and linear regression analysis with calculation of TID_{20} and TID_{50} values should definitely be applied in pre-clinical studies for the precise determination of the potency of the examined agents or drugs influencing the threshold for electroconvulsions.

CONCLUSIONS

Based on this pre-clinical study, one can ascertain that STP suppressed MEST-induced tonic seizures in mice, and thus the effectiveness of STP against MES- and PTZ-induced seizures in mice confirms the clinical use of STP in patients with myoclonic seizures and with Dravet syndrome. The linear regression analysis used in conjunction with calculation of TID_{20} or TID_{50} values should be added to other methods for evaluating threshold for electroconvulsions in mice, because it facilitates comparison of the anti-convulsant potency of various drugs tested in pre-clinical studies.

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