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Interaction of pregabalin with valproate in the mouse maximal electroshock-induced seizure model: an isobolographic analysis

Interakcja pregabaliny z walproinianem w modelu maksymalnego wstrząsu elektrycznego u myszy: analiza izobolograficzna

INTRODUCTION

Pregabalin (PGB) is a third-generation antiepileptic drug (AED) recently licensed as an adjunct therapy for partial (simple and complex) seizures with or without secondary generalization in adults [1,4,5,19]. In clinical settings, PGB is concomitantly administered with classical AEDs to suppress seizures in patients with refractory epilepsy [4,5,19].

Isobolographic analysis is a statistical and mathematical method applied to precisely characterize interactions between drugs in both preclinical and clinical studies. With type I isobolographic analysis, it has been documented that PGB additively interacted with various classical and second-generations AEDs, including carbamazepine, phenytoin, phenobarbital, lamotrigine, oxcarbazepine and topiramate in the mouse maximal electroshock (MES)-induced tonic seizure model [9-12]. Moreover, with type II isobolographic analysis, it has been documented that PGB interacted synergistically with gabapentin and tiagabine, and interacted additively with levetiracetam and vigabatrin in the mouse MES model [unpublished data].

The aim of this study was to determine the interaction of PGB with valproate (VPA – a classical AED used in patients with generalized tonic-clonic seizures and partial onset seizures) in the mouse MES model. Generally, the mouse MES model is considered as an animal model of tonic-clonic seizures and partial convulsions with or without secondary generalization in humans [7]. Thus, it was appropriate to determine the interaction profile of PGB with VPA in the mouse MES model.

Additionally, we investigated the combinations of PGB with VPA in relation to impairment of motor coordination, long-term memory and muscular strength by the use of the chimney test, step-through passive avoidance task and grip-strength test, respectively. Finally, to ascertain whether the observed interaction was pharmacodynamic in nature or that pharmacokinetic interaction also contributed, total

brain VPA concentrations were measured with fluorescence polarization immunoassay.

MATERIAL AND METHODS

A n i m a l s a n d e x p e r i m e n t a l c o n d i t i o n s. All experiments were performed on adult male albino Swiss mice (weighing 22-26 g) purchased from licensed breeder (Dr. T. Gorzkowska, Warszawa, Poland). The mice were kept in colony cages with free access to food and tap water under standardized housing conditions (natural light-dark cycle, temperature of 21 ± 1ºC, relative humidity of 55 ± 3%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 mice. Each mouse was used only once. All tests were performed between 9.00 a.m. and 3.00 p.m. Procedures involving animals and their care were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. 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 described in this manuscript were approved by the Local Ethics Committee at the Medical University of Lublin (License no.: 21/2007).

D r u g s. The following AEDs were used in this study: PGB (Lyrica®, Pfizer Ltd., Sandwich, Kent, UK) and VPA (magnesium salt; kindly donated by ICN-Polfa S.A., Rzeszow, Poland). PGB was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water, while VPA was directly dissolved in distilled water. The AEDs were administered by intraperitoneal (i.p.) injection in a volume of 0.005 ml/g body weight as follows: PGB – 60 min and VPA – 30 min before seizures, motor coordination, grip-strength and long-term memory tests, as well as before brain sampling for the measurement of AED concentrations. The route of systemic (i.p.) administration and the pretreatment times before testing of PGB and VPA in the mouse MES model and all behavioral tests were based upon information about their biological activity from the literature and our previous experiments [8-13]. Moreover, these pretreatment times were considered as the times to the peak of maximum anticonvulsant effects for the studied AEDs.

M a x i m a l e l e c t r o s h o c k s e i z u r e t e s t. The protective activities of PGB and VPA administered separately were evaluated and expressed as their median effective doses (ED_{50} in mg/kg), protecting 50% of mice against MES-induced seizures (fixed current intensity of 25 mA, maximum stimulation voltage of 500 V). Electroconvulsions were produced by a current (0.2 s stimulus duration) delivered via standard auricular electrodes by a Hugo Sachs generator (Rodent Shocker, Type 221, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension. The animals were administered with different drug doses so as to obtain a variable percentage of protection against MES-induced seizures, allowing the construction of a dose-response relationship curve (DRRCs) for PGB and VPA administered alone, according to Litchfield and Wilcoxon [6]. The anticonvulsant activity of the mixture of PGB with VPA at the fixed-ratio of 1:1 was evaluated and expressed as median effective doses

($ED_{50\ mix}$ values) against MES-induced seizures. This experimental procedure has been described in details earlier [8-13].

Isobolographic analysis of interactions. The percent protection of animals against MES-induced seizures per dose of an AED administered alone and the DRRC for each investigated AED in the MES test were fitted using log-probit linear regression analysis according to Litchfield and Wilcoxon [6]. Subsequently, from the respective linear equations the median effective doses (ED_{50}s) of AEDs administered alone were calculated. To precisely and correctly analyze the experimental data with isobolography, the test for parallelism of DRRCs for PGB and VPA based on the log-probit analysis was used [8-13]. The test for parallelism was performed according to Litchfield and Wilcoxon [6], as previously described in details [13]. In this test PGB had its DRRC non-parallel to that of VPA. Therefore, the interactions between PGB and VPA against MES-induced seizures were analyzed according to the methodology described by Tallarida [16], and Łuszczki [10-13]. Based upon the ED_{50} values denoted previously for the AEDs administered alone, median additive doses of the mixture of PGB with VPA – i.e., doses of the mixture, which theoretically should protect 50% of the animals tested against MES-induced seizures ($ED_{50\ add}$) were calculated from two equations of additivity presented by Tallarida [16]. For the lower line of additivity the equation at a 50% effect for the combination of PGB with VPA is as follows: $y = ED_{50\_VPA} - [ED_{50\_VPA} / (ED_{50\_PGB} / x)^{q/p}]$; where y – is the dose of VPA; x – is the dose of PGB; p and q – are curve-fitting parameters (Hill coefficients) for VPA and PGB, respectively. Similarly, for the upper line of additivity the equation at a 50% effect for the combination of PGB with VPA is: $y = ED_{50\_VPA} [(ED_{50\_PGB} - x)/ ED_{50\_PGB}]^{q/p}$. To calculate the curve-fitting parameters (p and q), probits of response for VPA and PGB administered alone were transformed to % effect. Proportions of PGB and VPA in the mixture were calculated only for the fixed-ratio combination of 1:1, as recommended earlier [10-13,16], and the mixtures of PGB with VPA were administered to animals. The evaluation of the experimentally derived $ED_{50\ mix}$ at the fixed-ratio of 1:1 was based upon the dose of the mixture protecting 50% of animals tested against MES-induced seizures in mice. Finally, to determine the separate doses of PGB and VPA in the mixture, the $ED_{50\ mix}$ values were multiplied by the respective proportions of AEDs (denoted for purely additive mixture). Further details regarding these concepts and all required equations allowing the calculation of standard error (S.E.) for $ED_{50\ add}$ values have been published elsewhere [8-13].

Measurement of total brain antiepileptic drug concentrations. Total brain concentrations of VPA were determined in mice that were administered PGB + VPA at doses corresponding to the fixed-ratio combination of 1:1 from the MES test. Mice were killed by decapitation at times chosen to coincide with that scheduled for the MES test and whole brains were removed from skulls, weighed, harvested and homogenized using Abbott buffer (2:1 vol/weight; Abbott Laboratories, North Chicago, IL, USA) in an Ultra-Turrax T8 homogenizer (Staufen, Germany). The homogenates were centrifuged at 10,000 g for 10 min. and the supernatant samples (75 µl) were analyzed by fluorescence polarization immunoassay using a TDx analyzer and reagents (VPA) exactly as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA).

Total brain concentrations of VPA were expressed in μg/ml of brain supernatants as means ± S.E. of at least 8 separate brain preparations.

C h i m n e y t e s t. The chimney test of Boissier et al. [2] was used to quantify the adverse effect potential of the two AEDs on motor performance in mice. In this test, the animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length), and motor performance impairment was indicated by the inability of the mice to climb backward up the transparent tube within 60 s. The acute adverse effect potentials of two AEDs administered alone and in combination at the fixed-ratio of 1:1 were determined for AEDs administered at doses corresponding to their ED_{50} and $ED_{50\ mix}$ values from the MES test.

G r i p – s t r e n g t h t e s t. The effects of PGB, VPA administered alone and in combination at the fixed-ratio of 1:1 at their ED_{50} and ED_{50mix} values from the MES test, on skeletal muscular strength in mice were quantified by the grip-strength test of Meyer et al. [15]. The time before the commencement of the grip-strength test (after drug administration) was identical to that for the MES test. The grip-strength apparatus (BioSeb, Chaville, France) comprised a wire grid (8 x 8 cm) connected to an isometric force transducer (dynamometer). The mice were lifted by the tails so that their forepaws could grasp the grid. The mice were then gently pulled backward by the tail until the grid was released. The maximal force exerted by the mouse before losing grip was recorded. The mean of 3 measurements for each animal was calculated and subsequently, the mean maximal force of 8 animals per group was determined. The skeletal muscular strength in mice was expressed in Newtons (N) as means ± S.E. of at least 8 determinations.

L i g h t – d a r k, s t e p – t h r o u g h p a s s i v e a v o i d a n c e t a s k. Each animal was administered an AED either singly or in combination at the fixed-ratio of 1:1 on the first day before training. The acute adverse effect potentials of PGB, VPA administered alone and in combination at the fixed-ratio of 1:1 were determined for AEDs administered at doses corresponding to their ED_{50} and ED_{50mix} values from the MES test. The time before the commencement of the training session (after drug administration) was identical to that for the MES test. Subsequently, animals were placed in an illuminated box (10 x 13 x 15 cm) connected to a larger dark box (25 x 20 x 15) equipped with an electric grid floor. Entrance of animals to the dark box was punished by an adequate electric footshock (0.6 mA for 2 s). The animals that did not enter the dark compartment were excluded from subsequent experimentation. On the following day (24 h later), the pre-trained animals were placed again into the illuminated box and observed up to 180 s. Mice that avoided the dark compartment for 180 s were considered to remember the task. The time that the mice took to enter the dark box, was noted and the median latencies (retention times) with 25th and 75th percentiles were calculated. The step-through passive avoidance task gives information about ability to acquire the task (learning) and to recall the task (retrieval). Therefore, it may be regarded as a measure of long-term memory [18].

S t a t i s t i c s. Both ED_{50} and $ED_{50\ mix}$ values (with their respective 95% confidence limits) for PGB and VPA administered alone and in combination at the fixed-ratio of 1:1 in the MES-induced seizure test were calculated by computer-assisted log-probit analysis according to Litchfield and

Wilcoxon [6]. In the isobolographic analysis for non-parallel DRRCs, the experimentally derived $ED_{50\ mix}$ value for the mixture of PGB with VPA at the fixed-ratio of 1:1 was statistically compared with the respective theoretically additive $ED_{50\ add}$ values by using the unpaired Student's t-test. Total brain AED concentrations were statistically analyzed using the unpaired Student's t-test. Qualitative variables from the chimney test were compared by use of the Fisher's exact probability test, whereas, the results obtained in the passive avoidance task were statistically evaluated using Kruskal-Wallis nonparametric ANOVA. The results from the grip-strength test were verified with one-way ANOVA. All statistical tests were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). Differences among values were considered statistically significant if $P<0.05$.

RESULTS

ANTICONVULSANT EFFECTS OF PREGABALIN AND VALPROATE ADMINISTERED SEPARATELY AND IN COMBINATION IN THE MOUSE MAXIMAL ELECTROSHOCK-INDUCED SEIZURE MODEL

PGB administered alone (i.p., 60 min. before the test) at doses ranging between 50 and 250 mg/kg produced a clear-cut anticonvulsant effect and the ED_{50} value for PGB was 142.14 ± 32.54 mg/kg (Table 1). Similarly, VPA administered singly (i.p. 30 min. before the test) produced a definite anticonvulsant activity in the mouse MES model and the ED_{50} value for VPA amounted to 269.71 ± 12.85 mg/kg (Table 1). The test for parallelism of DRRCs between PGB and VPA revealed that the AEDs had their DRRCs non-parallel to one another (Table 1; Fig. 1). The combination of PGB with VPA at the fixed-ratio of 1:1 exerted the anticonvulsant activity in the MES test and the experimentally derived $ED_{50\ mix}$ value from the DRRC for the mixture of both AEDs was 129.69 ± 17.46 mg/kg (Table 2).

Table 1. Anticonvulsant effects of pregabalin (PGB) and valproate (VPA) administered singly against maximal electroshock (MES)-induced seizures in mice

Drug	ED_{50}	n	CFP	q/p
PGB	142.14 ± 32.54	32	1.354 (p)	-
VPA	269.71 ± 12.85	24	6.933 (q)	5.120
#Test for parallelism: PGB vs. VPA S.R. = 2.120 f ratio S.R. = 1.333 S.R. > f ratio S.R., the examined two DRRCs are non-parallel.				

Results are presented as median effective doses (ED_{50} values in mg/kg ± S.E.) of PGB and VPA administered singly against MES-induced seizures in mice. The drugs were administered systemically (i.p.), as follows: PGB – 60 min and VPA – 30 min before the MES-induced seizures. n – total number of animals used at doses whose expected anticonvulsant effects ranged between 4 and 6 probits (16% and 84%); CFP – (q and p) curve-fitting parameters; q/p – ratio of q and p values; S.R. – slope function ratio for the respective two-drug combination (i.e., S_{PGB}/S_{VPA}), where: S_{VPA} and S_{PGB} – are slopes for the AEDs administered alone; f ratio S.R. – factor for slope function ratio for the respective two-drug combinations. Test for parallelism of two dose-response relationship curves (DRRCs) was performed according to Litchfield and Wilcoxon [6]. #All detailed calculations required to perform the test for parallelism of two DRRCs were presented elsewhere [8-13].

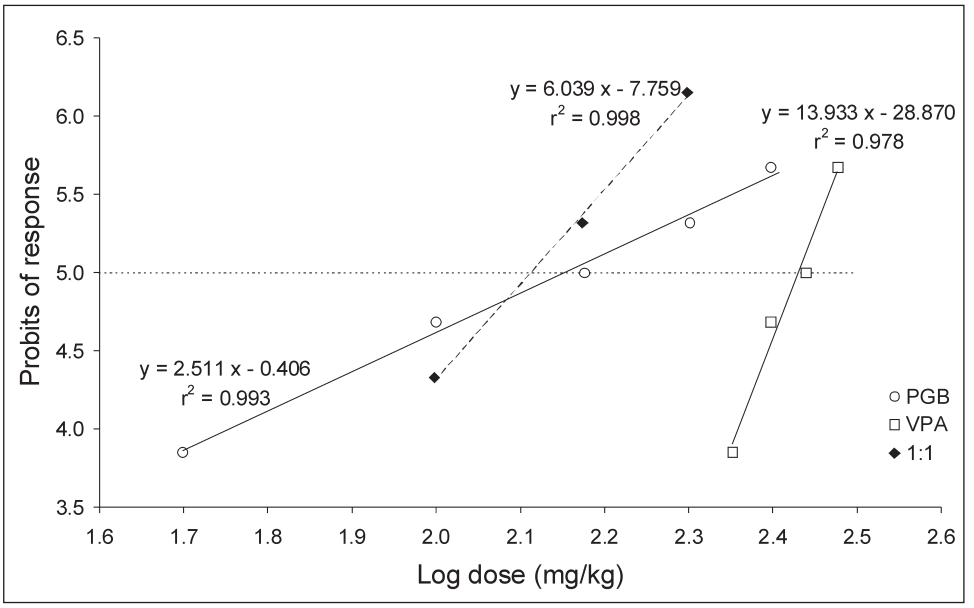

Fig. 1. Log-probit dose-response relationship curve (DRRC) analysis of pregabalin (PGB) and valproate (VPA) administered alone and in combination against maximal electroshock (MES)-induced seizures in mice

Doses of PGB and VPA administered alone and in combination at the fixed-ratio of 1:1 were transformed to logarithms, whereas the protective effects offered by the AEDs against MES-induced seizures were transformed to probits according to Litchfield and Wilcoxon [6]. Linear regression equations of DRRCs for PGB and VPA administered alone and in combinations are presented on the graph; where y – is the probit of response; x – is the logarithm (to the base 10) of an AED dose or a dose of the mixture of PGB with VPA; and r^2 – coefficient of determination. Test for parallelism revealed that the experimentally determined DRRC for PGB was non-parallel to that for VPA when administered alone. For more details see Table 1.

ISOBOLOGRAPHIC ANALYSIS OF INTERACTION BETWEEN PREGABALIN AND VALPROATE IN THE MOUSE MAXIMAL ELECTROSHOCK-INDUCED SEIZURE MODEL

The isobolographic analysis of interaction for non-parallel DRRCs revealed that the mixture of PGB with VPA at the fixed-ratio of 1:1 exerted additive interaction in the MES test in mice (Figure 2). The experimentally derived $ED_{50\ mix}$ value for this fixed-ratio combination was 129.69 ± 17.46 mg/kg, whereas the additively calculated ED_{50} add values were 99.67 ± 42.56 mg/kg (for the lower $ED_{50\ add}$) and 312.19 ± 56.31 mg/kg (for the upper ED_{50} add; Table 2). The $ED_{50\ mix}$ value did not significantly differ from the $ED_{50\ add}$ values (Table 2, Fig. 2).

Table 2. Isobolographic analysis of interactions (for non-parallel DRRCs) between pregabalin (PGB) and valproate (VPA) at the fixed-ratio of 1:1 against maximal electroshock (MES)-induced seizures

AED combination		ED_{50}	n	PGB	VPA
PGB + VPA	$ED_{50\ mix}$	129.69 ± 17.46	16	44.76	84.93
	#$ED_{50\ add}$	99.67 ± 42.56	52	34.40	65.27
	&$ED_{50\ add}$	312.19 ± 56.31	52	107.74	204.45

Data are presented as median effective doses (ED_{50} values in mg/kg ± S.E.) for two-drug mixtures, determined either experimentally ($ED_{50\ mix}$) or theoretically calculated ($ED_{50\ add}$) from the equations of additivity, protecting 50% of the animals against MES-induced seizures. The actual doses of PGB and VPA that comprised the mixtures at the fixed-ratio of 1:1 for the $ED_{50\ mix}$ and $ED_{50\ add}$ values are presented in separate columns. PGB – dose of PGB in the mixture; VPA – dose of VPA in the mixture; n – total number of animals used at those doses whose expected anticonvulsant effects ranged between 16% and 84% (i.e., 4 and 6 probits). Total number of animals were determined either experimentally (nmix) or theoretically from the equation of additivity (nadd = n_PGB + n_VPA – 4); # – $ED_{50\ add}$ value calculated from the equation for the lower line of additivity; & – $ED_{50\ add}$ value calculated from the equation for the upper line of additivity. Statistical evaluation of data was performed with unpaired Student's t-test.

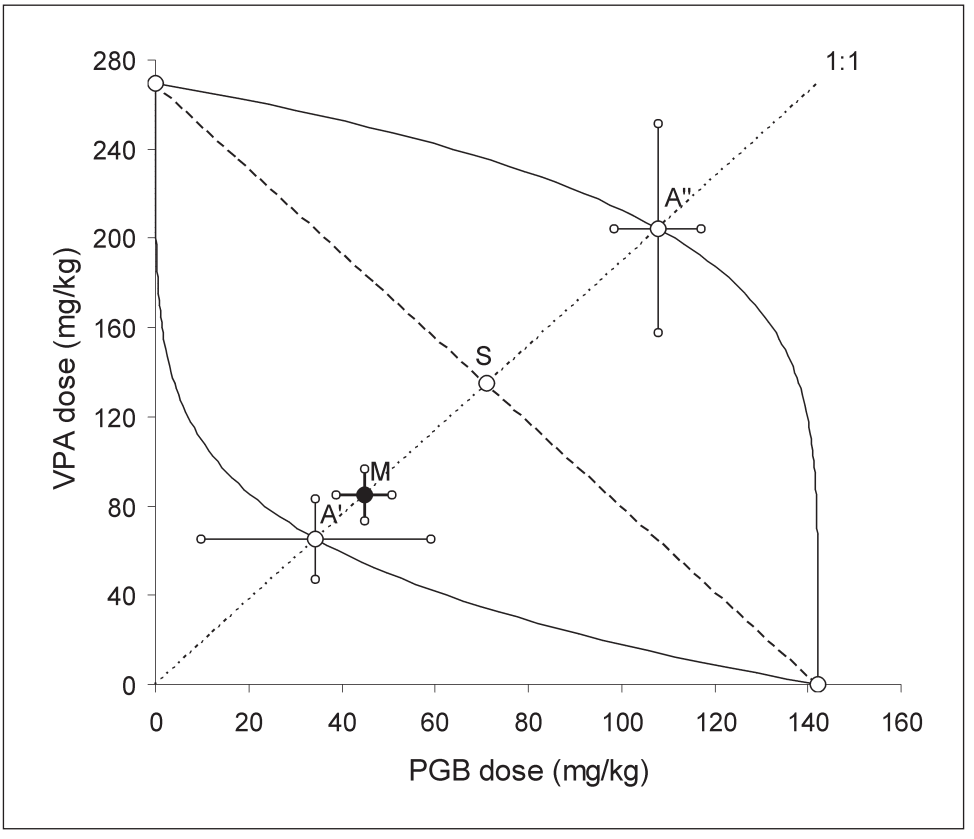

Fig. 2. Isobologram showing additive interaction between pregabalin (PGB) and valproate (VPA) against maximal electroshock (MES)-induced seizures in mice

The median effective dose (ED_{50}) for PGB is plotted graphically on X-axis, whereas the ED_{50} of VPA is placed on Y-axis. The lower and upper isoboles of additivity represent the curves connecting the ED_{50} values for PGB and VPA administered alone. The dotted line starting from the point (0; 0) corresponds to the fixed-ratio of 1:1 for the combination of PGB with VPA. The diagonal dashed line connects the ED_{50} for PGB and VPA on the X- and Y-axes. The closed circle (●) depicts the experimentally derived $ED_{50\ mix}$ (± S.E.), whereas the open circles (○) depict the theoretically calculated $ED_{50\ adds}$ (± S.E.) for total doses expressed as the proportions of PGB and VPA that produced 50% protection of animals against MES-induced seizures. The S.E. values are presented as horizontal and vertical error bars for the $ED_{50\ mixs}$ and $ED_{50\ adds}$. The points A' and A" depict the theoretically calculated $ED_{50\ add}$ values for both, lower and upper isoboles of additivity. The point M represents the experimentally-derived $ED_{50\ mix}$ value for total dose of the mixture expressed as proportions of PGB and VPA that produced a 50% anticonvulsant effect (50% isobole) in the mouse MES model. The sum of X and Y coordinates, for each point placed on the isobologram, corresponds to the respective ED_{50} values. The point S reflects the $ED_{50\ add}$ value denoted theoretically from the Loewe's equation for the fixed-ratio combination of 1:1. The experimentally derived $ED_{50\ mix}$ value is placed between the point A' and S, within the area of additivity bounded by two isoboles of additivity, indicating additive interaction between PGB and VPA in the mouse MES model. The X- and Y-coordinates for all points presented on the isobologram are as follows: A' (34.40; 65.27), A" (107.74; 204.45), S (71.07; 134.86), and M (44.76; 84.93).

Total brain antiepileptic drug concentrations

Pharmacokinetic estimation of total brain VPA concentration with fluorescence polarization immunoassay method revealed that PGB co-administered with VPA (at doses corresponding to the $ED_{50\ mix}$ values at the fixed-ratio of 1:1 from the MES test) did not significantly affect total brain concentration of VPA (Table 3).

Table 3. Total brain concentration of valproate (VPA) administered singly or in combination with pregabalin (PGB)

Treatment (mg/kg)	Total brain concentration (μg/ml)
VPA (84.93) + vehicle	75.3 ± 11.3
VPA (84.93) + PGB (44.76)	91.3 ± 13.5

Data are presented as means (± S.E.) and expressed as μg/ml of brain supernatants of eight determinations (n = 8). Estimation of total brain concentrations of VPA was performed with fluorescence polarization immunoassay. Statistical evaluation of data was performed with unpaired Student's t-test. Brain tissue samples were taken at times scheduled for the MES test.

Effects of pregabalin, valproate and their combination on motor performance, long-term memory, and muscular strength of animals in the chimney, step-through passive avoidance and grip-strength tests

When PGB and VPA were co-administered at doses corresponding to their $ED_{50\ mix}$ values at the fixed-ratio of 1:1 from the MES-induced seizure test, motor performance of animals as assessed by the chimney test was unaffected (Table 4). Furthermore, the combination of PGB with VPA did not impair long-term memory as determined in the passive avoidance test (Table 4). Similarly, PGB concomitantly administered with VPA at the fixed-ratio of 1:1 had no significant impact on skeletal muscular strength of the animals as assessed by the grip-strength test (Table 4). Moreover, it was found that the control (vehicle-treated) mice and animals receiving PGB or VPA alone (at doses corresponding to their ED_{50} values from the mouse MES model) did not show any significant sings of impaired motor coordination, long-term memory or muscular skeletal strength, as assessed in the chimney, passive avoidance and grip-strength tests, respectively (Table 4).

Table 4. Effects of pregabalin (PGB), valproate (VPA), and their combinations at the fixed-ratio of 1:1 on long-term memory in the passive avoidance task, muscular strength in the grip-strength test, and motor performance in the chimney test in mice

Treatment (mg/kg)	Motor performance (%)	Retention time (s)	Grip-strength (N)
Vehicle	100	180 (180; 180)	96.5 ± 6.23
PGB (142.14) + vehicle	100	180 (180; 180)	96.3 ± 6.01
VPA (269.71) + vehicle	100	180 (175.5; 180)	97.2 ± 6.09
PGB (44.76) + VPA (84.93)	100	165.5 (135.5; 180)	95.2 ± 6.01

Results are presented as: 1) percentage of animals without impairment of motor coordination in the chimney test in mice; 2) median retention times (in s; with 25th and 75th percentiles in parentheses) from the passive avoidance task, assessing long-term memory in mice; 3) mean strengths (in newtons ± S.E.) from the grip-strength test, assessing skeletal muscular strength in mice. Each experimental group consisted of 8 mice. Statistical analysis of data from the passive avoidance task was performed with nonparametric Kruskal-Wallis ANOVA, whereas the results from the grip-strength test were analyzed with one-way ANOVA. The Fisher's exact probability test was used to analyze the results from the chimney test. All drugs were administered intraperitoneally at times scheduled from the MES test, and at doses corresponding to their ED_{50} values (when administered alone) and $ED_{50\ mix}$ values at the fixed-ratio of 1:1 (when administered in combination) against MES-induced seizures in mice (for more details see the legend to Table 1 and 2).

DISCUSSION

Results indicate that PGB combined with VPA at the fixed-ratio of 1:1 exerted the additive interaction in the mouse MES model. Pharmacokinetic verification of total brain AED concentrations revealed that PGB did not alter total brain concentrations of VPA in experimental animals. From a theoretical point of view, PGB has an ideal pharmacokinetic profile because the drug neither binds to plasma proteins nor replaces the AEDs from plasma proteins [1, 17]. PGB undergoes a negligible (2%) metabolic transformation in the liver and the drug is excreted virtually unchanged by the kidneys. PGB neither inhibits nor activates liver enzymes such as cytochrome P450 system [1,17]. Considering the favorable pharmacokinetic profile of PGB, it is unlikely that VPA would be able to affect total brain PGB concentrations in experimental animals.

To explain the exact characteristics of interaction between PGB and VPA in the mouse MES model, one should consider their anticonvulsant mechanisms of action. PGB binds with high affinity and specificity to the α2δ subunit of P/Q-type voltage-gated calcium channels and, by decreasing Ca^{2+} influx at nerve terminals, the drug reduces the release of excitatory neurotransmitters in the brain [17]. As regards the anticonvulsant activity of VPA, the drug blocks use- and voltage-dependent neuronal sodium channels, and therefore, it limits repetitive firing of action potentials in neurons [14]. VPA also facilitates the effects of GABA [14]. Hence, one can hypothesize that the blockade of calcium channels in neurons exerted by PGB additively cooperated with the blockade of sodium channels evoked by VPA.

It is important to note that PGB is a structural analogue of the inhibitory neurotransmitter GABA with a pharmacological profile similar to that of gabapentin (GBP – a second-generation AED). Therefore, one can suggest that the interaction between PGB and VPA should be identical to that denoted for the combination of GBP with VPA in the mouse MES test. Experimental studies

have revealed that the interaction of GBP with VPA was both, supra-additive (synergistic) at the fixed-ratios of 1:3, 1:5, 1:7, and 1:10, and additive at the fixed-ratio of 1:20 in the mouse MES model [3]. Pharmacokinetic verification of free (non-protein bound) plasma concentrations of VPA in experimental animals revealed that the observed interaction in the mouse MES model was pharmacodynamic in nature [3]. Comparing the interactions of GBP and PGB with VPA, one can ascertain that the combinations of GBP with VPA were superior to that for PGB with VPA in the mouse MES model. The apparent discrepancy between the interaction profiles of PGB and GBP with VPA resulted from different isobolographic methods used for the analysis of interactions.

In experimental studies, the type II isobolographic analysis is applied if one of the investigated drugs in the mixture is virtually ineffective. Since GBP was considered as a virtually ineffective drug, type II isobolographic analysis of interaction was used to analyze the interaction between GBP and VPA in the mouse MES model [3]. Because types I and II isobolographic analysis considerably differ to each other, the fixed-ratios for the combinations of PGB with VPA and GBP with VPA also differ. This is why, the combination of GBP with VPA was investigated at several fixed-ratios of 1:3, 1:5, 1:7, 1:10 and 1:20, whereas the combination of PGB with VPA was examined only at the fixed-ratio of 1:1. Details concerning the isobolographic background were presented elsewhere [8-13].

As mentioned earlier, PGB interacted additively with several classical and second-generation AEDs, including carbamazepine, phenytoin, phenobarbital, lamotrigine, oxcarbazepine and topiramate in the mouse maximal electroshock (MES)-induced tonic seizure model [9-12]. Thus, it is not surprising that PGB interacted additively with VPA in this study.

Evaluation of acute adverse-effect profile for the combination of PGB with VPA at the fixed-ratio of 1:1 revealed that the AEDs in combination did not affect skeletal muscular strength in animals subjected to the grip-strength test. Moreover, the combination of PGB with VPA did not disturb long-term memory in mice challenged with the step-through passive avoidance task. Additionally, it was found that the combination of PGB with VPA at the fixed-ratio of 1:1 had no impact on motor performance in the chimney test in mice.

CONCLUSION

The combination of PGB with VPA can offer an additive interaction in preclinical studies. If the results from this study could be extrapolated into clinical trials, the combination of PGB with VPA would be beneficial for patients remaining refractory to currently available AEDs.

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SUMMARY

The aim of this study was to characterize the interaction between pregabalin (PGB – a third-generation antiepileptic drug) and valproate (VPA – a classical antiepileptic drug) in the maximal electroshock (MES)-induced seizure model in mice by using the type I isobolographic analysis for non-parallel dose-response relationship curves (DRRCs). Tonic hind limb extension (seizure activity) was evoked in adult male albino Swiss mice by a current (25mA, 500V, 50Hz, 0.2s stimulus duration) delivered via auricular electrodes. In the mouse MES model, PGB administered singly had its DRRC non-parallel to that for VPA. According to type I isobolographic analysis for non-parallel DRRCs, the combination of PGB with VPA at the fixed-ratio of 1:1 exerted additive interaction. Pharmacokinetic studies revealed that PGB had no impact on total brain concentrations of VPA in experimental animals. Moreover, PGB, VPA and their combination at the fixed-ratio of 1:1 did not alter motor performance, long-term memory or skeletal muscular strength in experimental animals. In conclusion, the additive interaction between PGB and VPA is worthy of consideration while extrapolating the results from this preclinical study to clinical settings.

Keywords: Pregabalin; valproate; isobolographic analysis; maximal electroshock; pharmacodynamic/pharmacokinetic interaction.

STRESZCZENIE

Celem pracy było scharakteryzować interakcję pomiędzy pregabaliną (PGB – lekiem przeciwpadaczkowym trzeciej generacji) a walproinianem (VPA – klasycznym lekiem przeciwpadaczkowym) w modelu maksymalnego wstrząsu elektrycznego (MES) u myszy przy użyciu typu I analizy izobolograficznej dla nierównoległych krzywych zależności dawka-efekt (DRRCs). Toniczny wyprost kończyn tylnych (aktywność drgawkowa) był wywoływany u dorosłych samców myszy albino Swiss poprzez prąd (25mA, 500V, 50Hz, 0,2s czas trwania impulsu) doprowadzony przez elektrody uszne. W teście MES u myszy, PGB podawana osobno miała swoją DRRC nierównoległą do tej dla VPA. Według typu I analizy izobolograficznej dla nierównoległych DRRCs, kombinacja PGB z VPA dla stałej proporcji dawek 1:1 wywierała addytywną interakcję. Farmakokinetyczne badania ujawniły, że PGB nie miała żadnego wpływu na całkowite mózgowe stężenia VPA u zwierząt doświadczalnych. Ponadto, PGB, VPA i ich kombinacja w stałej proporcji dawek 1:1 nie zmieniały koordynacji ruchowej, pamięci długotrwałej i siły mięśni szkieletowych u badanych zwierząt. W podsumowaniu, addytywna interakcja pomiędzy PGB a VPA jest warta rozważenia podczas ekstrapolacji wyników z tego badania przedklinicznego do badań klinicznych.

Słowa kluczowe: Pregabalina; walproinian; analiza izobolograficzna; maksymalny wstrząs elektryczny; farmakodynamiczna / farmakokinetyczna interakcja.