ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXII, N 4, 4 SECTIO DDD 2009

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Additive interaction of pregabalin with phenytoin in the mouse maximal electroshock-induced seizure model: an isobolographic analysis

Addytywna interakcja pregabaliny z fenytoiną
w modelu maksymalnego wstrząsu elektrycznego u myszy: analiza izobolograficzna

Pregabalin (PGB; (*S*)-(+)-3-(aminomethyl)-5-methylhexanoic acid or (*S*)-(+)-3-isobutyl GABA) 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 [3, 5].

Accumulating evidence indicates that PGB exhibits anticonvulsant activity in the maximal electroshock (MES)-induced tonic seizure test and pentylenetetrazole (PTZ)-induced clonic seizure model in rodents [16]. In hippocampal kindled rats, PGB prevents both behavioral and electrographic seizures and the drug reduces seizures in DBA/2 audiogenic mice [16]. Moreover, PGB has no impact on the incidence of spontaneous absence seizures in genetically susceptible rats from Strasbourg (GAERS) [16].

The aim of this study was to determine the interaction of PGB with phenytoin (PHT – 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 [8]. Thus, it was appropriate to determine the interaction profile of PGB with PHT in the mouse MES model. Additionally, to ascertain whether the observed interaction was pharmacodynamic in nature or that pharmacokinetic interaction also contributed, total brain PHT concentrations were measured with fluorescence polarization immunoassay.

MATERIAL AND METHODS

Animals and experimental conditions. All experiments were performed on adult male albino Swiss mice (weighing 22–26 g) purchased from a 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).

Drugs. The following AEDs were used in this study: PGB (Lyrica®, Pfizer Ltd., Sandwich, Kent, UK) and PHT (Polfa, Warszawa, Poland). The AEDs were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in saline and administered by intraperitoneal (i.p.) injection in a volume of 0.005 ml/g body weight. The AEDs were administered as follows: PGB – 60 min and PHT – 120 min before seizures and brain sampling for the measurement of AED concentrations. The route of systemic (i.p.) administration and these pretreatment times were chosen based upon information about their biological activity from the literature [15] and the previous study [12].

Maximal electroshock seizure test. The protective activities of PGB and PHT 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 hindlimb 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 PHT administered alone, according to Litchfield and Wilcoxon [7]. The anticonvulsant activity of the mixture of PGB with PHT 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 was described in detail earlier [6, 12].

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 [7]. 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 PHT based on the log-probit analysis was used [10, 11]. The test for parallelism was performed according to Litchfield and Wilcoxon (1949), as previously described in detail [9]. In this test PGB had its DRRC non-parallel to that of PHT. Therefore, the interactions between PGB and PHT against MES-induced seizures were analyzed according to the methodology described by Tallarida [14], and Łuszczki [11]. Based upon the ED_{50} values denoted previously for the AEDs administered alone, median additive doses of the mixture of PGB with PHT – 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 [14]. For the lower line of additivity the equation at a 50% effect for the combination of PGB with PHT is as follows: $y = ED_{50\_PHT} - [ED_{50\_PHT} / (ED_{50\_PGB} / x)^{q/p}]$; where y – is the dose of PHT; x – is the dose of PGB; p and q – are curve-fitting parameters (Hill coefficients) for PHT and PGB, respectively. Similarly, for the upper line of additivity the equation at a 50% effect for the combination of PGB with PHT is: $y = ED_{50\_PHT}\ [(ED_{50\_PGB} - x)/ ED_{50\_PGB}]^{q/p}$. To calculate the curve-fitting parameters (p and q), probits of response for PHT and PGB administered alone were transformed to % effect. Proportions of PGB and PHT in the mixture were calculated only for the fixed-ratio combination of 1:1, as recommended earlier [11, 12], and the mixtures of PGB with PHT 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 PHT 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 S.E.M. for $ED_{50\ add}$ values have been published elsewhere [11, 12, 14].

Measurement of total brain antiepileptic drug concentrations. Total brain concentrations of PHT were determined in mice that were administered PGB + PHT 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 (PHT) exactly as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA). Total brain concentrations of PHT were expressed in µg/ml of brain supernatants as means ± S.D. of at least eight separate brain preparations.

Statistics. The ED_{50} and $ED_{50\ mix}$ values (with their respective 95% confidence limits) for PGB and PHT administered alone or 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 [7]. In the isobolographic analysis for non-parallel DRRCs, the experimentally derived $ED_{50\ mix}$ value for the mixture of PGB with PHT at the fixed-ratio of 1:1 was statistically compared with their 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. Differences among values were considered statistically significant if $P<0.05$.

RESULTS

ANTICONVULSANT EFFECTS OF PREGABALIN AND PHENYTOIN ADMINISTERED SEPARATELY AND IN COMBINATION IN THE MOUSE MES MODEL

Table 1. Anticonvulsant effects of pregabalin (PGB) and phenytoin (PHT) 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*)	-
PHT	9.87 ± 0.86	24	4.097 (*q*)	3.026
#Test for parallelism: PGB vs. PHT S.R. = 1.847 f ratio S.R. = 1.349 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.M.) of PGB and PHT administered singly against MES-induced seizures in mice. The drugs were administered systemically (i.p.), as follows: PGB – 60 min and PHT – 120 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_{PHT}), where: S_{PHT} 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 [7]. #All detailed calculations required to perform the test for parallelism of two DRRCs were presented in the Appendix to the paper by Łuszczki and Czuczwar [9], and Łuszczki et al. [12]

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, PHT administered singly (i.p., 120 min. before the test) produced a definite antiseizure activity in the mouse MES model and the ED_{50} value for PHT amounted to 9.87 ± 0.86 mg/kg (Table 1). The test for parallelism of DRRCs between PGB and PHT revealed that the AEDs had their DRRCs non-parallel to one another (Table 1; Figure 1). The combination of PGB with PHT at the fixed-ratio of 1:1 exerted an antiseizure effect in the MES test and the experimentally derived $ED_{50\ mix}$ value from the DRRC for the mixture of both AEDs was 53.34 ± 5.81 mg/kg (Table 2).

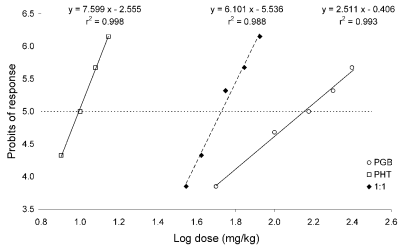

Fig. 1. Log-probit dose-response relationship curve (DRRC) analysis of pregabalin (PGB) and phenytoin (PHT) administered alone and in combination against maximal electroshock (MES)-induced seizures in mice

Doses of PGB and PHT 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 [7]. Linear regression equations of DRRCs for PGB and PHT 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 PHT; and r^2 – coefficient of determination. Test for parallelism revealed that the experimentally determined DRRC for PGB was non-parallel to that for PHT when administered alone. For more details see Table 1.

ISOBOLOGRAPHIC ANALYSIS OF INTERACTION BETWEEN PREGABALIN AND PHENYTOIN IN THE MOUSE MES MODEL

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

AED combination		ED_{50}	*n*	PGB	PHT
PGB + PHT	$ED_{50\ mix}$	53.34 ± 5.81	24	49.88	3.46
	$^{\#}ED_{50\ add}$	48.10 ± 23.52	52	44.97	3.12
	$^{\&}ED_{50\ add}$	103.92 ± 14.91	52	97.18	6.75

Data are presented as median effective doses (ED_{50} values in mg/kg ± S.E.M.) for two-drug mixtures, determined either experimentally ($ED_{50\ mix}$) or theoretically calculated ($ED_{50\ add}$) from the equations of additivity [14], protecting 50% of the animals against MES-induced seizures. The actual doses of PGB and PHT 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; PHT – dose of PHT 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). The total number of animals were determined either experimentally (n_{mix}) or theoretically from the equation of additivity ($n_{add} = n_{\_PGB} + n_{\_PHT} - 4$); $^{\#}$ – $ED_{50\ add}$ 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

The isobolographic analysis of interaction for non-parallel DRRCs revealed that the mixture of PGB with PHT 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 53.34 ± 5.81 mg/kg, whereas the additively calculated $ED_{50\ add}$ values were 48.10 ± 23.52 mg/kg (for the lower $ED_{50\ add}$) and 103.92 ± 14.91 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, Figure 2).

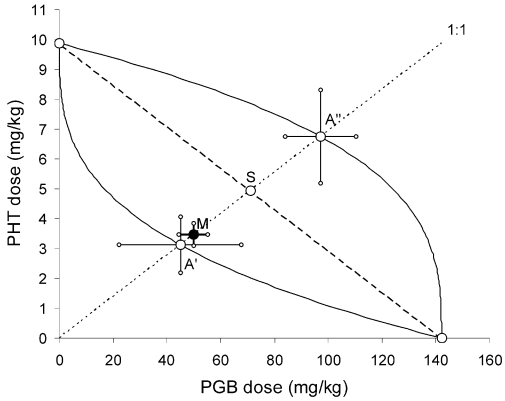

Fig. 2. Isobologram showing additive interaction between pregabalin (PGB) and phenytoin (PHT) against maximal electroshock (MES)-induced seizures in mice

The median effective dose (ED_{50}) for PGB is plotted graphically on X-axis, whereas the ED_{50} of PHT is placed on Y-axis. The lower and upper isoboles of additivity represent the curves connecting the ED_{50} values for PGB and PHT 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 PHT. The diagonal dashed line connects the ED_{50} for PGB and PHT on the X- and Y-axes. The closed circle (•) depicts the experimentally derived $ED_{50\ mix}$ (± S.E.M.), whereas the open circles (○) depict the theoretically calculated $ED_{50\ add}$s (± S.E.M.) for total doses expressed as the proportions of PGB and PHT that produced 50% protection of animals against MES-induced seizures. The S.E.M. values are presented as horizontal and vertical error bars for the $ED_{50\ mix}$s and $ED_{50\ add}$s. 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 PHT 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 PHT in the mouse MES model. The X- and Y-coordinates for all points presented on the isobologram are as follows: A' (44.97; 3.12), A" (97.18; 6.75), S (71.07; 4.94), and M (49.88; 3.46)

BRAIN ANTIEPILEPTIC DRUG CONCENTRATIONS

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

Table 3. Total brain concentration of phenytoin (PHT) administered singly or in combination

ATreatment (mg/kg)	Total brain concentration (μg/ml)
PHT (3.46) + vehicle	0.381 ± 0.046
PHT (3.46) + PGB (49.88)	0.376 ± 0.086

Data are presented as means (± S.D.) and expressed as μg/ml of brain supernatants of eight determinations (n = 8). Estimation of total brain concentrations of PHT was performed with fluorescense 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

DISCUSSION

It was found out that PGB combined with PHT at the fixed-ratio of 1:1 exposed additive interaction in the mouse MES model. Pharmacokinetic verification of total brain AED concentrations revealed that PGB did not alter total brain concentrations of PHT 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 PHT would be able to affect total brain PGB concentrations in experimental animals.

To explain the exact characteristics of interaction between PGB and PHT 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 [15]. As regards the anticonvulsant activity of PHT, the drug blocks frequency-, use- and voltage-dependent neuronal sodium channels, and therefore, it limits repetitive firing of action potentials in neurons [13]. 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 PHT.

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 PHT should be identical to that denoted for the combination of GBP with PHT in the mouse MES test. Experimental studies have revealed that the interaction of GBP with PHT at the fixed-ratios of 1:1, 3:1, 5:1, 7:1, and 10:1 was supra-additive (synergistic) in the mouse MES model [2]. Pharmacokinetic verification of free (non-protein bound) plasma concentrations of PHT in experimental animals revealed that the observed interaction in the mouse MES model was pharmacodynamic in nature [2]. Comparing the interactions of GBP and PGB with PHT, one can ascertain that the combinations of GBP with PHT were superior to that for PGB with PHT in the mouse MES model. The apparent discrepancy between the interaction profiles of PGB and GBP with PHT 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 [4], type II isobolographic analysis of interaction was used to analyze the interaction between GBP and PHT in the mouse MES model [2]. Because types I and II isobolographic analysis considerably differ from each other, the fixed-ratios for the combinations of PGB with PHT and GBP with PHT also differ. This is why the combination of GBP with PHT was investigated at several fixed-ratios

of 1:1, 3:1, 5:1, 7:1, and 10:1, whereas the combination of PGB with PHT was examined only at the fixed-ratio of 1:1. Details concerning the isobolographic background were presented elsewhere [10, 11, 14].

In conclusion, the combination of PGB with PHT 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 PHT would be beneficial for patients remaining refractory to currently available AEDs.

Acknowledgments. This study was supported by a grant (DS 475/09) from the Medical University of Lublin (Lublin, Poland). Professor J.J. Łuszczki is a Recipient of the Fellowship for Leading Young Researchers from the Ministry of Science and Higher Education (Warszawa, Poland).

Disclosure of conflicts of interest. The author has no disclosures to declare.

REFERENCES

1. Ben-Menachem E.: Pregabalin pharmacology and its relevance to clinical practice. Epilepsia, 45 Suppl. 6, 13, 2004.
2. Borowicz K. K., Świąder M., Łuszczki J. et al.: Effect of gabapentin on the anticonvulsant activity of antiepileptic drugs against electroconvulsions in mice: an isobolographic analysis. Epilepsia, 43, 956, 2002.
3. Brodie M. J.: Pregabalin as adjunctive therapy for partial seizures. Epilepsia, 45 Suppl. 6, 19, 2004.
4. Dalby N. O., Nielsen E. B.: Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin and vigabatrin. Epilepsy Res., 28, 63, 1997.
5. French J. A., Kugler A. R., Robbins J. L. et al.: Dose-response trial of pregabalin adjunctive therapy in patients with partial seizures. Neurology, 60, 1631, 2003.
6. Kozińska J., Sawicka K. M., Zadrożniak A. et al.: Spironolactone potentiates the protective action of some selected antiepileptic drugs against maximal electroshock-induced seizures in mice. Annales UMCS, Sectio DDD, 22, 123, 2009.
7. Litchfield J. T., Wilcoxon F.: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 96, 99, 1949.
8. Löscher W., Fassbender C. P., Nolting B.: The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. Epilepsy Res., 8, 79, 1991.
9. Łuszczki J. J., Czuczwar S. J.: Biphasic characteristic of interactions between stiripentol and carbamazepine in the mouse maximal electroshock-induced seizure model: a three-dimensional isobolographic analysis. Naunyn-Schmiedebergs Arch. Pharmacol., 374, 51, 2006.
10. Łuszczki J. J.: Interaction of oxcarbazepine with phenobarbital in the mouse pentylenetetrazole-induced seizure model: an isobolographic analysis. Annales UMCS, Sectio DDD, 22, 61, 2009.
11. Łuszczki J. J.: Isobolographic analysis of interaction between drugs with nonparallel dose-response relationship curves: a practical application. Naunyn-Schmiedebergs Arch. Pharmacol., 375, 105, 2007.
12. Łuszczki J. J., Antkiewicz-Michaluk L., Czuczwar S. J.: Isobolographic analysis of interactions between 1-methyl-1,2,3,4-tetrahydroisoquinoline and four conventional antiepileptic drugs in the mouse maximal electroshock-induced seizure model. Eur. J. Pharmacol., 602, 298, 2009.

13. Macdonald R. L., Greenfield L. J.: Mechanisms of action of new antiepileptic drugs. Curr. Opin. Neurol., 10, 121, 1997.
14. Tallarida R. J.: An overview of drug combination analysis with isobolograms. J. Pharmacol. Exp. Ther., 319, 1, 2006.
15. Taylor C. P., Angelotti T., Fauman E.: Pharmacology and mechanism of action of pregabalin: the calcium channel alpha2-delta (alpha2-delta) subunit as a target for antiepileptic drug discovery. Epilepsy Res., 73, 137, 2007.
16. Vartanian M. G., Radulovic L. L., Kinsora J. J. et al.: Activity profile of pregabalin in rodent models of epilepsy and ataxia. Epilepsy Res., 68, 189, 2006.
17. Warner G., Figgitt D. P.: Pregabalin: as adjunctive treatment of partial seizures. CNS Drugs, 19, 265, discussion 273, 2005.

SUMMARY

The aim of this study was to characterize the interaction between pregabalin (PGB – a third-generation antiepileptic drug) and phenytoin (PHT – 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 PHT. According to type I isobolographic analysis for non-parallel DRRCs, the combination of PGB with PHT at the fixed-ratio of 1:1 exerted additive interaction. Pharmacokinetic studies revealed that PGB had no impact on total brain concentrations of PHT in experimental animals. In conclusion, the additive interaction between PGB and PHT is worthy of consideration while extrapolating the results from this preclinical study to clinical settings.

STRESZCZENIE

Celem pracy było scharakteryzowanie interakcji pomiędzy pregabaliną (PGB – lekiem przeciwpadaczkowym trzeciej generacji) a fenytoiną (PHT – 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 przez 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 PHT. Według typu I analizy izobolograficznej dla nierównoległych DRRCs kombinacja PGB z PHT dla stałej proporcji dawek 1:1 wywierała addytywną interakcję. Farmakokinetyczne badania ujawniły, że PGB nie miała żadnego wpływu na całkowite mózgowe stężenie PHT u zwierząt doświadczalnych. Addytywna interakcja pomiędzy PGB a PHT jest warta rozważenia podczas ekstrapolacji wyników z tego badania przedklinicznego do badań klinicznych.