ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXIV, N 1, 4, 2009 SECTIO D DOI: 10.2478/v10079-008-0067-2

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Additive interaction of tiagabine with phenobarbital against pentylenetetrazole-induced clonic seizures: an isobolographic analysis for non-parallel dose-response relationship curves

The isobolographic analysis of interaction is a valuable experimental method allowing the precise classification of exact types of interactions among drugs, comprehensively evaluating their nature as: supra-additive (synergistic), sub-additive (relatively antagonistic), infra-additive (absolutely antagonistic), indifferent or additive (1–8). At present, one can distinguish two types of isobolographic analysis: type I – used if all examined drugs are fully active, and type II – applied if one of the drugs produces no effect and is considered as virtually ineffective in an experimental model (1, 9). Quite recently, a novel variant of isobolography for non-parallel dose-response relationship curves (DRRCs) of drugs administered separately, has been introduced into experimental studies (10–14). The isobolographic analysis has been successfully applied in experimental epileptology to characterize interactions among antiepileptic drugs (AEDs) in preclinical studies (6, 15–21).

Previously, it has been documented that tiagabine (TGB – a second-generation AED) had its DRRC parallel to those of oxcarbazepine, loreclezole, felbamate and gabapentin in the pentylenetetrazole (PTZ)-induced seizure test in mice and thus, produced additive interaction with type I isobolographic analysis for parallel DRRCs (18, 20). Moreover, TGB had its DRRC parallel to that of vigabatrin in the PTZ-induced seizure test in mice and the combination produced supra-additive (synergistic) interaction with type I isobolographic analysis for parallel DRRCs (21). In contrast, TGB had its DRRC non-parallel to that of ethosuximide in the PTZ-induced seizure test in mice and the drugs produced additive interaction with the isobolographic analysis for non-parallel DRRCs (12).

Therefore, it was of pivotal importance to characterize the interaction profile between TGB and phenobarbital (PB – a conventional AED), prescribed for partial, tonic-clonic and myoclonic seizures (22). It is widely accepted that the PTZ-induced seizure test is considered as an experimental model of myoclonic seizures and, to a certain extent, of absence seizures in man (23). Hence, it was considered appropriate to use this test for assessing the characteristic of interaction between TGB and PB in this study. To ascertain whether the observed anticonvulsant effects for the combination of TGB with PB were consequent to a pharmacodynamic and/or a pharmacokinetic interaction, total brain concentrations of PB and TGB were evaluated using fluorescence polarization immunoassay (FPIA) and high performance liquid chromatography (HPLC).

MATERIAL AND METHODS

Animals and experimental conditions. All experiments were performed on adult male albino 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, ambient temperature of 22 ± 1°C, relative humidity of 55 ± 5%). 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 09.00 and 15.00 hrs. Procedures involving animals and their care were conducted in accordance with current European Community and Polish law on the experimentation and protection of animals. 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 were approved by the Local Ethics Committee at the Medical University of Lublin (License no. 425/2003/451/2003) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs. The following AEDs were used in this study: TGB (Gabitril®, Sanofi Winthrop, Gentilly, France) and PB (Polfa, Kraków, Poland). Both AEDs were suspended in 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally (i.p.), as two separate injections, in a volume of 5 ml/kg body weight. The control animals received adequate volume of vehicle (1% solution of Tween 80 in distilled water). Fresh drug solutions were prepared on each day of experimentation and administered as follows: TGB – 15 min, and PB – 60 min. prior to the PTZ and before the brain sampling for the measurement of AED concentrations. The route of systemic (i.p.) administration and pretreatment times before testing of the AEDs were based on information about their biological activity from the literature and our previous experiments (24). PTZ (Sigma, St. Louis, MO, USA) was dissolved in sterile saline and administered subcutaneously (s.c.) into a loose fold of skin in the midline of the neck in a volume of 5 ml/kg body weight.

Pentylenetetrazole-induced convulsions. The anticonvulsant activities of TGB and PB against PTZ-induced clonic seizures were determined after s.c. administration of PTZ at its CD97 (convulsive dose 97, i.e., the dose of PTZ that produced clonic seizures in 97% of mice, which in this study was 100 mg/kg). Following PTZ administration, mice were placed separately into trans¬parent Plexiglas cages (25×15×10 cm) and observed for 30 min for the occurrence of clonic seizures. Clonic seizure activity was defined as clonus of whole body lasting for over 3 s, with an accompanying loss of righting reflex. The number of animals convulsing out of the total number of mice tested was noted for each treatment regimen. The animals were administered increasing doses of the AEDs, and the anticonvulsant activity of each drug was evaluated as the ED_{50} (median effective dose of an AED, protecting 50% of mice against clonic convulsions). At least 4 groups of animals were used to estimate each ED_{50} value calculated from the respective log-probit DRRC according to Litchfield and Wilcoxon (25). The anticonvulsant activity of TGB administered alone was studied at doses of 0.5, 1, 2 and 3 mg/kg, whereas that of PB administered alone at doses of 11, 13, 16, 19 mg/kg against the clonic phase of PTZ-induced seizures in mice. Similarly, the anticonvulsant activity of the mixture of TGB with PB was evaluated and expressed as $ED_{50\,mix}$, corresponding to the dose of the mixture of both drugs required to protect 50% of animals tested against PTZ-induced clonic convulsions. This experimental procedure has been described in more detail in our earlier studies (18, 20, 21).

Measurement of total brain AED concentrations. The animals were administered PB + vehicle, TGB + vehicle or the combination of PB + TGB, at doses corresponding to the $ED_{50\,mix}$ value at the fixed ratio of 1:1 from the PTZ test. Mice were killed by decapitation at times chosen to coincide with that scheduled for the PTZ test and the whole brains of mice were removed from skulls,

weighed, harvested and homogenized with Abbott buffer (1:2 weight/vol) in an Ultra-Turrax T8 homogenizer (IKA Werke, Staufen, Germany). The brain homogenates were centrifuged at 10,000 g for 10 min and the supernatant samples (100 μl) were analyzed for PB content by FPIA using a TDx analyzer and reagents exactly as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA). Simultaneously, the identically prepared supernatant samples (200 μl) containing TGB were analyzed using an automated Gilson HPLC system (Anachem Ltd., Bedfordshire, UK), consisting of Gilson 234 autosampler and Gilson 306 pumps. The mobile phase comprised of methanol (20 mmol), acetonitrile and citric buffer (pH = 3) in a ratio of 25:30:40 (vol:vol:vol). Chromatographic separation was achieved using a Hypersil BDS-2-C18 5 lm column (Agilent Technologies Inc., Santa Clara, CA, USA). Brain supernatant samples were prepared for analysis, as follows: 200 μl of brain supernatant was pipetted into a C8 column conditioned with methanol (2×1 ml) and water (2×1 ml) and eluted with methanol (2×0.2 ml) and 0.4 ml of water. Subsequently, samples were centrifuged for 5 min, and 200 μl of eluant was transferred into the HPLC column. The concentrations of PB were expressed in μg/ml, while those of TGB were expressed in ng/ml of brain supernatant as means ± S.D. of at least 8 determinations (separate brain preparations).

Isobolographic analysis of interactions. The isobolographic analysis has been recommended as the method of choice for evaluation of characteristics of interactions for various fixed drug dose ratio combinations (usually, at three fixed-ratios of 1:3, 1:1 and 3:1). However, the original isobolographic analysis has a fundamental presumption requiring the parallelism of two DRRCs of the investigated drugs administered separately. Recently, a novel isobolographic approach has been developed to analyze the interactions between drugs whose DRRCs are not parallel (3–5, 10–14, 26). The isobolographic analysis of interactions between TGB and PB was performed for drugs with non-parallel DRRCs. The percent protection of animals against PTZ-induced clonic seizures per dose of an AED administered alone and the DRRC for each investigated AED in the PTZ-induced clonic seizure model were fitted using log-probit linear regression analysis according to Litchfield and Wilcoxon (25). Subsequently, from the respective linear equations the median effective doses (ED_{50}s) of AEDs administered alone were calculated. Subsequently, based upon these ED_{50} values, median additive doses of the mixture of TGB with PB – i.e., doses of the mixture, which theoretically should protect 50% of the animals tested against PTZ-induced clonic seizures ($ED_{50\ add}$) were calculated from two equations of additivity presented by Tallarida (13, 14). For the lower line of additivity the equation at a 50% effect is: $y = ED_{50\_PB} - [ED_{50\_PB} / (ED_{50\_TGB} / x)^{q/p}]$; where y – is the dose of PB; x – is the dose of TGB; q and p – are curve-fitting parameters (Hill coefficients) for PB and TGB, respectively. Similarly, for the upper line of additivity the equation at a 50% effect is: $y = ED_{50\_PB} (ED_{50\_PB} - x/ ED_{50\_TGB})^{q/p}$. To calculate the curve-fitting parameters (p and q), probits of response for PB and TGB administered alone were transformed to % effect. It is important to note that when two drugs produce maximal effect but are "heterodynamic" (i.e., have non-parallel DRRCs), the additivity is represented as an area bounded by two defined curves (lower and upper isoboles of additivity). The experimentally-derived ED_{50} values are statistically different if their points are placed outside this region. For supra-additivity (synergy), the experimentally-derived $ED_{50\ mix}$ points are placed below the area bounded by the lower and upper isoboles of additivity, and for sub-additivity (antagonism) – above this region (13, 14). In isobolography, it is accepted that half the ED_{50} of TGB plus half the ED_{50} of PB drug should be as effective therapeutically as a full dose of either drug administered separately. This concept of adding fractions of the ED_{50} values of drugs is a basic principle of isobolography (1, 27). Subsequently, proportions of TGB and PB in the mixture were calculated for the fixed-ratio combination of 1:1, and the mixture of TGB with PB was 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

PTZ-induced seizures in mice. Statistical comparison of the experimentally-derived $ED_{50\ mix}$ value with its corresponding theoretically additive $ED_{50\ add}$ values was undertaken by the use of the unpaired Student's t-test, according to Tallarida (8). Finally, to determine the separate doses of TGB and PB in the mixture, the $ED_{50\ mix}$ value was multiplied by the respective proportions of AEDs (denoted for purely additive mixture). Further details regarding these concepts have been published elsewhere (3–5, 10–14, 26).

Statistics. The percent protection of animals against PTZ-induced clonic seizures per dose of the AEDs and the DRRCs for PB and TGB administered alone and in combination at the fixed-ratio of 1:1 were fitted using log-probit linear regression analysis according to Litchfield and Wilcoxon (25). The ED50 values with their 95% confidence limits were calculated by computer-assisted log-probit analysis according to Litchfield and Wilcoxon (25). To precisely analyze the experimental data, the test for parallelism of the DRRCs for TGB and ETS was presented as indispensable conditions for testing AED interactions with isobolography. The obtained 95% confidence limits were transformed to S.E.M. as described previously (7). Statistical evaluation of isobolographic interactions was performed by the use of Student's t-test in order to detect the differences between the experimentally-derived (ED50 mix) and theoretical additive (ED50 add) values, according to Tallarida (8). Total brain concentrations of PB and TGB were statistically analyzed using the unpaired Student's t-test. Differences among values were considered statistically significant if $P<0.05$.

Software used. Microsoft's Excel spreadsheet was used to perform calculations and to graphically illustrate the results in form of isobolograms. This spreadsheet was programmed to compute all calculations automatically and determine the DRRCs of the AEDs administered alone and in combination from the log-probit linear regression analysis according to Litchfield and Wilcoxon (25). The theoretically additive ED50 add values and their S.E.M. at the fixed-ratio combination of 1:1 were also calculated with this programmed spreadsheet. All statistical tests were performed using commercially available GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

EFFECTS OF TGB AND PB ADMINISTERED ALONE ON PTZ-INDUCED CLONIC SEIZURES

The anticonvulsant effects of TGB, expressed as the percentage of protection against PTZ-induced clonic seizures in mice ranged between 25% for TGB at the dose of 0.5 mg/kg and 87.5% for TGB at the dose of 3 mg/kg. The log-probit analysis of DRRC for TGB allowed to calculate its ED_{50} value, which was 0.99 (0.57 – 1.73) mg/kg (Table 1, Figure 1). Similarly, PB exerted clear-cut anticonvulsant effects in the PTZ test in mice and its ED_{50} value, as denoted from the log-probit method was 15.29 (13.22 – 17.69) mg/kg (Table 1, Fig. 1). In this case, PB at the dose of 13 mg/kg produced a 25% protection against PTZ-induced clonic seizures and PB at the highest tested dose of 19 mg/kg offered a 87.5% anticonvulsant effect in the PTZ test in mice. The test of parallelism of two DRRCs for TGB and PB administered alone in the PTZ test revealed that both AEDs had their DRRCs non-parallel to one another (Table 1, Fig. 1).

ISOBOLOGRAPHIC ASSESSMENT OF INTERACTION BETWEEN TGB AND PB

The mixture of TGB and PB at the fixed ratio of 1:1 exerted additive interaction in the PTZ test in mice. The $ED_{50\ mix}$ for this fixed ratio combination was 8.15 mg/kg, whereas the corresponding ED50 add values were 3.43 mg/kg (for the lower $ED_{50\ add}$) and 12.53 mg/kg (for the upper $ED_{50\ add}$; Table 2). In this case, the $ED_{50\ mix}$ (i.e., protecting a 50% of animals against PTZ-induced clonic

seizures) did not significantly differ from the $ED_{50\ add}$ values and thus, indicating additive interaction between drugs (Table 2, Fig. 2).

Table 1. Anticonvulsant effects of tiagabine (TGB) and phenobarbital (PB) administered singly against pentylenetetrazole (PTZ)-induced clonic seizures in mice

Drug	ED50	n	SEM	CFP	q/p
TGB	0.99 (0.57 – 1.73)	24	0.283	1.229 (p)	-
PB	15.29 (13.22 – 17.69)	16	0.920	7.818 (q) 6.361	
Test for parallelism: TGB vs. PB		S.R. = 2.183		f ratio S.R. = 1.422	
S.R. > f ratio S.R., the examined two DRRCs are not parallel (25).					

Results are presented as median effective doses (ED_{50} values in mg/kg; with 95% confidence limits in parentheses) of TGB and PB administered singly against PTZ-induced clonic seizures in mice. The AEDs were administered systemically (i.p.), as follows: TGB – 15 min and PB – 60 min before the PTZ seizure initiation. n – total number of animals used at doses whose expected anticonvulsant effects ranged between 4 and 6 probits (16% and 84%); SEM – standard error of the mean of ED_{50}; CFP – (q and p) curve-fitting parameters; q/p – ratio of q and p values; S.R. – slope function ratio for the combination (i.e., S_{TGB}/S_{PB}); f ratio S.R. – factor for slope function ratio for the combination. Test for parallelism of two DRRCs was performed according to Litchfield and Wilcoxon (25). 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 (19)

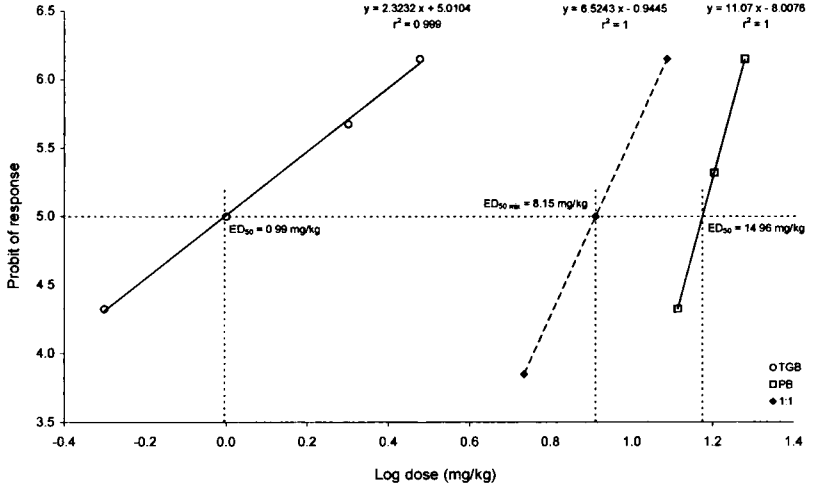

Fig. 1. Log-probit analysis and dose-response relationship curves (DRRCs) for tiagabine (TGB) and phenobarbital (PB) administered alone and their combination at the fixed-ratio of 1:1 in the pentylenetetrazole (PTZ)-induced seizure test in mice

Doses of TGB and PB administered alone were transformed to logarithms (log), whereas the protective effects offered by the AEDs against PTZ-induced clonic seizures were transformed to probits (25). Linear regression equations of DRRCs are presented on the graph; where y – is the probit of response, and x – is the logarithm (to the base 10) of a drug dose. The dotted lines represent on the graph median effective doses (ED_{50} values) for TGB and PB administered alone and their combination at the fixed ratio of 1:1. The dashed line reflects the DRRC for the mixture of TGB with PB at the fixed ratio of 1:1. The test for parallelism of DRRCs (for TGB and PB) revealed that both lines are not parallel to one another (25). For more details see the legends to Tables 1 and 2

Table 2. Isobolographic analysis of interaction between tiagabine (TGB) and phenobarbital (PB) at the fixed-ratio of 1:1 against pentylenetetrazole (PTZ)-induced clonic seizures in mice

$ED_{50\ mix}$	n_{mix}	TGB	PB	$^{\#}ED_{50\ add}$	n_{add}	TGB	PB	$^{\&}ED_{50\ add}$	n_{add}	TGB	PB
8.15 ± 1.44	8	0.5	7.65	3.43 ± 1.45	36	0.21	3.22	12.53 ± 3.06	36	0.78	11.75

Results are presented as median effective doses (ED_{50} values in mg/kg ± SEM) for two-drug mixtures, determined either experimentally ($ED_{50\ mix}$) or theoretically calculated ($ED_{50\ add}$) from the equations of additivity (13, 14), protecting 50% of the animals against PTZ-induced clonic seizures. The actual doses of TGB and PB 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. TGB – dose of TGB in the mixture; PB – dose of PB in the mixture; n_{mix} – total number of animals used at those doses whose expected anticonvulsant effects ranged between 16% and 84% (i.e., 4 and 6 probits) for the experimental mixture; n_{add} – total number of animals calculated for the additive mixture of the drugs examined ($n_{add} = n_{-TGB} + n_{-PB} - 4$); $^{\#}$ – $ED_{50\ add}$ 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 (8, 9)

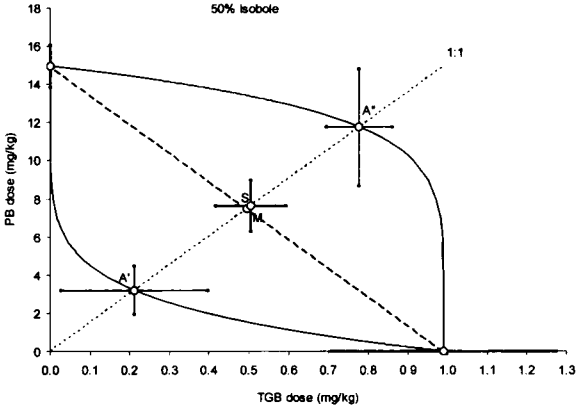

Fig. 2. Isobologram showing additive interaction between tiagabine (TGB) and phenobarbital (PB) against pentylenetetrazole (PTZ)-induced clonic seizures in mice

The median effective doses (ED50) for TGB and PB are shown plotted graphically on the X- and Y-axes, respectively. The solid lines on the X and Y axes represent the SEM for the ED_{50}s of AEDs administered alone. The lower and upper isoboles of additivity represent the curves connecting the ED_{50} values for TGB and PB administered alone. The dotted line starting from the point (0,0) corresponds to the fixed ratio of 1:1 for the combination of TGB with PB. 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 TGB and PB that produced 50% anticonvulsant effects in the PTZ test in mice. The point S on the graph reflects the $ED_{50\ add}$ value denoted theoretically from the Loewe's equation for the fixedratio combination of 1:1. On the graph, the SEM values are presented as horizontal and vertical error bars for every ED_{50} value. The $ED_{50\ mix}$ value is placed close to the point S and within the area bounded by two isoboles of additivity, indicating additive interaction between TGB and PB in the PTZ test in mice. The sum of X and Y coordinates, for each point placed on the isobologram (M, A', A", S), corresponds to the respective ED_{50} values that are as follows: A' (0.21; 3.22), A" (0.78; 11.75), S (0.49; 7.65), and M (0.50; 7.65)

TOTAL BRAIN AED CONCENTRATIONS

Total brain AED concentrations were evaluated for PB and TGB administered at doses corresponding to the $ED_{50\ mix}$ at the fixed ratio of 1:1 from the PTZ test. With FPIA technique, total brain concentration of PB for the mixture of PB (7.65 mg/kg) with TGB (0.50 mg/kg) did not

considerably differ from that evaluated for PB administered singly at 7.65 mg/kg (Table 3). Similarly, with HPLC technique, the total brain concentration of TGB, administered singly at 0.50 mg/kg did not significantly differ from that evaluated for the mixture of TGB (0.50 mg/kg) with PB (7.65 mg/kg) (Table 3).

Table 3. Total brain concentrations of the studied AEDs when administered singly or in combination

Treatment [mg/kg]	Total brain AED concentrations a [μg/ml]	[ng/ml]
TGB (0.50) + vehicle		62.04 ± 9.25
TGB (0.50) + PB (7.65)		64.62 ± 9.99
PB (7.65) + vehicle	3.19 ± 0.48	
PB (7.65) + TGB (0.50)	3.35 ± 0.43	

Data are presented as means (± S.D.) of at least 8 determinations. aConcentrations relate to that AED shown first in the respective treatment column. Statistical evaluation of data was performed by the use of the unpaired Student's t-test. The AEDs were administered systemically (i.p.), as follows: TGB – 15 min and PB – 60 min before the brain sampling for the measurement of AED concentrations

DISCUSSION

Results presented herein indicate that the combination of TGB with PB at the fixed-ratio of 1:1 exerted additive interaction against PTZ-induced clonic seizures in mice. It is important to note that the isobolographic analysis of interaction was performed in this study only for the fixed-ratio of 1:1 because of the lack of parallelism of two DRRCs for TGB and PB administered alone. Other fixed-ratio combinations, such as: 1:3 and 3:1, etc., could not be evaluated appropriately with this method due to changing proportions of the drugs in the mixture along with the increase in AED doses (11–14). Generally, the proportions of drugs in the mixture are calculated from the Loewe's equation based on fixed fractions of drugs present in the mixture (27). In case of two parallel DRRCs, the proportions of drugs in the mixture are constant for every effect ranging between 16% and 84% (or 4 and 6 probits). In contrast, if two drugs have their DRRCs non-parallel, the proportions of drugs in the mixture change (11–14). This is why, only the fixed-ratio combination of 1:1 for the 50% effect (i.e., when both drugs were present in the mixture at equi-effective doses), was examined in this study.

It should be stressed that the observed additive interaction for the combination of TGB with PB against PTZ-induced clonic seizures, is consistent with that reported earlier for the combinations of TGB with oxcarbazepine, loreclezole, felbamate, ethosuximide and gabapentin in the PTZ test in mice (18, 20, 26). Moreover, with respect to the combination of TGB with PB, it has been documented that the two-drug mixture produced additive interaction in the amygdala-kindled rats (28), and maximal electroshock-induced seizures in mice (24).

The pharmacokinetic evaluation of total brain TGB and PB concentrations in this study revealed that neither TGB nor PB significantly altered total brain AED concentrations in the experimental animals. Thus, the observed additive interaction between TGB and PB in the PTZ test was pharmacodynamic in nature. It is worthy of mentioning that the bidirectional estimation of total brain AED concentrations allowed the proper classification of the interaction from the PTZ test. For more details see discussion in (24).

The combination of PB with TGB in the PTZ test has some clinical limitations due to the fact that TGB is not a drug used clinically in patients with myoclonic seizures (22). It has been documented that TGB can aggravate myoclonic seizures in epileptic patients or even, it can evoke

non-convulsive status epilepticus (22). Although the PTZ test in rodents is considered as an animal model of myoclonic seizures in humans (23), the combination of TGB with PB cannot be recommended for patients with myoclonic seizures, unless, for patients with several epileptic syndromes and/or various complex seizure types for which the combination of PB with TGB might appear favorable. On the other hand, the additive interaction between TGB with PB could occur favorably in clinical settings because this combination could offer epileptic patients a substantial reduction of acute adverse effects associated with the treatment of patients with an AED at high effective, but poorly tolerated doses. Therefore, the application of two-drug combination may be beneficial due to the reduction of adverse effects and better tolerance of the applied AEDs in epileptic patients without losing the anticonvulsant effects (6, 15).

Finally, based on this preclinical study, one can ascertain that the combination of TGB with PB exerted additive interaction in the mouse PTZ model and the nature of this interaction was pharmacodynamic.

Acknowledgements. This study was supported by a grant (DS 345/2003-2005) from the Medical University of Lublin. The authors express thanks to Dr. G. Raszewski (Institute of Agricultural Medicine, Lublin, Poland) for the skilful determination of the brain concentrations of tiagabine with HPLC.

Disclosure of conflicts of interest. The authors have no conflicts of interest to disclose.

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SUMMARY

To characterize the interaction between tiagabine (TGB) and phenobarbital (PB) – two antiepileptic drugs in the pentylenetetrazole (PTZ)-induced clonic seizure test in mice, type I isobolographic analysis for non-parallel dose-response relationship curves (DRRCs) was used. Clonic seizures were evoked in Albino Swiss mice by subcutaneous injection of pentylenetetrazole

at its CD_{97} (100 mg/kg). To ascertain the nature of interaction between TGB and PB administered in combination, total brain concentrations of TGB and PB were estimated by using high-performance liquid chromatography (HPLC) and fluorescence polarization immunoassay (FPIA). TGB and PB produced clear-cut anticonvulsant effects against PTZ-induced clonic seizures in mice and their DRRCs were not parallel to one another. The type I isobolographic analysis for non-parallel DRRCs revealed that the combination of TGB with PB at the fixed-ratio of 1:1 exerted additive interaction against PTZ-induced clonic seizures in mice. With FPIA technique, it was found that TGB did not affect total brain PB concentrations in experimental animals. Moreover, PB had no significant impact on total brain concentrations of TGB in mice, as measured with HPLC technique. The additive interaction between TGB and PB at the fixed-ratio of 1:1 in the mouse PTZ model is pharmacodynamic in nature.

Addytywna interakcja tiagabiny z fenobarbitalem przeciw drgawkom klonicznym wywoływanym pentetrazolem: analiza izobolograficzna dla nierównoległych krzywych zależności dawka-efekt

Aby scharakteryzować interakcję pomiędzy tiagabiną (TGB) i fenobarbitalem (PB) – dwoma lekami przeciwpadaczkowymi w teście drgawek klonicznych wywoływanych penetetrazolem (PTZ) u myszy – użyto typu I analizy izobolograficznej dla nierównoległych krzywych zależności dawka-efekt (DRRCs). Drgawki kloniczne wywoływano u myszy Albino Swiss, podając podskórnie iniekcję z PTZ w dawce CD_{97} (100 mg/kg). Aby stwierdzić naturę interakcji pomiędzy TGB i PB podawanymi w kombinacji, całkowite stężenia mózgowe TGB i PB oceniano przy użyciu wysokosprawnej chromatografii cieczowej (HPLC) i immunofluorescencji (FPIA). TGB i PB wywierały znaczące działanie przeciwko drgawkom wywoływanym PTZ u myszy i ich DRRCs były nierównoległe jedna względem drugiej. Typ I analizy izobolograficznej dla nierównoległych DRRCs ujawnił, że kombinacja TGB z PB w stałej proporcji dawek 1:1 wywierała interakcję addytywną przeciw drgawkom klonicznym wywoływanym PTZ u myszy. Metodą FPIA stwierdzono, że TGB nie wpływała na całkowite stężenia mózgowe PB u zwierząt doświadczalnych. Ponadto PB nie miał istotnego wpływu na całkowite stężenia mózgowe TGB u myszy, co zmierzono metodą HPLC. Zatem addytywna interakcja pomiędzy TGB a PB dla stałej proporcji dawek 1:1 w teście PTZ u myszy jest farmakodynamiczna w naturze.