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ORIGINAL ARTICLE

Jarogniew J. Luszczki · Stanislaw J. Czuczwar

Isobolographic characterisation of interactions among selected newer antiepileptic drugs in the mouse pentylenetetrazole-induced seizure model

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Abstract The aim of this study was to characterise the types of interactions between gabapentin (GBP), tiagabine (TGB) and three second-generation antiepileptic drugs (AEDs) with different mechanisms of action (felbamate [FBM], loreclezole [LCZ], and oxcarbazepine [OXC]) by isobolographic analysis. Anticonvulsant and acute neurotoxic adverse effect profiles of combinations of GBP and TGB with other AEDs at fixed ratios of 1:3, 1:1 and 3:1 were investigated in pentylenetetrazole (PTZ)-induced seizures and the chimney test (as a measure of motor impairment) in mice so as to identify optimal combinations. Protective indices (PIs) and benefit indices (BIs) were calculated for each combination in order to properly classify the investigated interactions. Isobolographic analysis revealed that only the combination of GBP with OXC at the fixed ratio of 1:1 exerted supra-additive (synergistic) interaction ($P<0.05$) against PTZ-induced seizures. The other combinations tested between GBP and OXC (1:3 and 3:1), as well as all combinations of GBP with FBM or LCZ (1:3, 1:1 and 3:1) were additive in the PTZ test. Similarly, all combinations of TGB with FBM LCZ, and OXC (at the fixed ratios of 1:3, 1:1 and 3:1) were associated with additive interactions against PTZ-induced seizures in mice. In the chimney test, the isobolographic analysis revealed that the combinations of GBP and OXC (at the fixed ratios of 1:3 and 1:1), GBP and LCZ (at 1:1), as well as TGB and OXC (at 1:3 and 1:1) were sub-additive (antagonistic; $P<0.05$ and $P<0.01$). In contrast, only one combination tested (TGB and LCZ at the fixed ratio of 1:1) was supra-additive (synergistic; $P<0.05$) in the chimney test, whereas the other combinations of GBP and TGB with OXC, FBM, and LCZ displayed barely additivity. Based upon the current preclinical data, GBP and OXC appear to be a particularly favourable combination. Also, the combinations of GBP with FBM, GBP with LCZ, and TGB with OXC are beneficial. In contrast, during the combining of TGB with FBM, or TGB with LCZ, the utmost caution is advised because of their unfavourable profiles in this preclinical study.

Keywords Tiagabine · Gabapentin · Drug interactions · Felbamate · Loreclezole · Oxcarbazepine · Pentylenetetrazole · Isobolographic analysis

Abbreviations AED: Antiepileptic drug · BI: Benefit index · CNS: Central nervous system · ED_{50}: Median effective dose · FBM: Felbamate · GABA: γ-Aminobutyric acid · GBP: Gabapentin · LCZ: Loreclezole · OXC: Oxcarbazepine · PI: Protective (therapeutic) index · PTZ: Pentylenetetrazole · TD_{50}: Median toxic dose · TGB: Tiagabine · VGB: Vigabatrin

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J. J. Luszczki (✉) · S. J. Czuczwar
Department of Pathophysiology,
Medical University of Lublin,
Jaczewskiego 8,
20-090 Lublin, Poland
e-mail: jluszczki@yahoo.com
Tel.: +48-81-7425837
Fax: +48-81-7425828

S. J. Czuczwar
Department of Physiopathology,
Institute of Agricultural Medicine,
Jaczewskiego 2,
20-950 Lublin, Poland

Introduction

Despite advanced knowledge of the pathophysiological processes involved in seizure initiation, amplification and propagation in the brain, as well as the introduction of several novel (second- and third-generation) antiepileptic drugs (AEDs) into the therapy of epilepsy, there are still ~30% of patients unsuccessfully medicated with currently available AEDs in monotherapy (Kwan and Brodie 2000a,b). Refractory epilepsy in ~30% of sufferers is still a significant clinical problem and a challenging issue for clinicians and researchers. Once the drug of choice has

failed, the primary concern is to find a highly efficacious combination of two or more AEDs in an attempt to enhance seizure control (Arroyo et al. 2002). Generally, the addition of a second or a third AED may provide enhanced seizure control in ~14% of these patients (Kwan and Brodie 2000a,b). Because of the ethical and methodological difficulties during the evaluation of AED combinations in the clinical setting, preclinical studies in animals can provide invaluable information allowing the preselection of useful AED combinations. The aim of such studies is to identify AED combinations whose anticonvulsant effects offer optimal protection against seizures and, simultaneously, are devoid of any serious neurotoxic side effects (Löscher and Wauquier 1996).

From a pharmacological viewpoint, a combination of two or more drugs may exert supra-additivity (synergy), additivity or sub-additivity (antagonism). Synergy between AEDs is observed when the final antiseizure effect of the AED mixture is stronger than expected, based on the sum of individual anticonvulsant effects of the component drugs. Additivity is present if the anticonvulsant effect of the drug mixture is the sum of the partial protective effects of individual component AEDs. Antagonism between AEDs is observed when the experimentally-derived protective effect of the drug mixture is less than the expected effect resulting from the component AEDs (Berenbaum 1989; Greco et al. 1995; Perucca 1995). Undoubtedly, the AED combinations exerting synergy with respect to seizure control and/or antagonism in terms of acute neurotoxic adverse effects possess great clinical relevance (Perucca 1995).

Generally, no theoretical presumptions, rules and guidelines exist that would allow preselection of synergistic interactions between AEDs as to the seizure suppression and/or antagonistic interactions with respect to acute neurotoxic adverse effects. The combining of two AEDs with similar mechanisms of action, in experimental conditions, may result either in sub-additivity (antagonism—for the combinations of oxcarbazepine [OXC] with lamotrigine or phenytoin; Luszczki et al. 2003a; Luszczki and Czuczwar 2004b) or in additivity (for the combinations of OXC with carbamazepine, Luszczki et al. 2003a). Likewise, the AEDs with diverse complementary mechanisms of action may exert either supra-additive (for the combinations of topiramate with OXC and felbamate [FBM], Luszczki and Czuczwar 2004b, as well as the combination of tiagabine [TGB] with gabapentin [GBP], Luszczki and Czuczwar 2004a) or sub-additive interactions (for the combination of vigabatrin [VGB] with carbamazepine; unpublished data). Thus, only experimental evaluation of AED combinations in preclinical studies on animals may provide reliable and convincing evidence of advantageous interactions between AEDs, which could be beneficial in patients with refractory epilepsy.

On the other hand, there is no consensus, as yet, whether to combine the AEDs affecting the same neurotransmitter system and reach a stronger therapeutic effect or choose the AEDs influencing diverse neurotransmitter systems for rational polytherapy (Deckers et al. 2000). To date, few experimental and clinical data exist reporting the combined treatment with AEDs that selectively activates the GABA-ergic inhibitory system within CNS, despite GABA being a well-known endogenous inhibitory agent that potently reduces seizure activity in the brain (Meldrum 1995; Treiman 2001; Olsen and Avoli 1997). It has been found experimentally that some combinations of two drugs, influencing exclusively the GABA-ergic neurotransmitter system (through diverse mechanisms), may result in synergistic interactions with respect to seizure suppression. Among them, the synergistic combinations of TGB with valproate (VPA), GBP or VGB, as well as the combinations of GBP with VPA and VGB are of importance for further clinical setting (Leach and Brodie 1994; Stringer and Aribi 2002; Kohling et al. 2002; Luszczki et al. 2003c; Luszczki and Czuczwar 2004a).

The objective of this study was to characterise the pharmacological profiles of interactions among TGB, GBP and some other second-generation AEDs (FBM, loreclezole [LCZ], and OXC) with respect to their anticonvulsant activities against pentylenetetrazole (PTZ)-induced seizures in mice. As for the PTZ test in rodents, the seizures induced by PTZ are thought to be a model of myoclonic seizures in humans (Löscher and Schmidt 1988; Löscher et al. 1991). Additionally, the acute neurotoxic adverse effect profiles of the different AED combinations were investigated in relation to motor impairment in the chimney test. Pharmacological characterisation of interactions in both PTZ and chimney tests was performed by using isobolographic analysis for three various fixed drug–dose ratio combinations of 1:3, 1:1 and 3:1, so that a ranking in relation to advantageous combination could be ascertained using paradigms of protective indices (PI) and benefit indices (BI). To date, isobolography is considered as the optimal method that allows the classification of AED interactions in experimental models of epilepsy as follows: additive, supra-additive, sub-additive, indifferent or infra-additive (Berenbaum 1989; Tallarida 2000; Greco et al. 1995).

Materials and methods

Animal 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 ad libitum, under standardised housing conditions (12 h of a light–dark cycle, temperature was 21±1°C). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of eight mice. Each mouse participated in only one experiment. 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 current European Community and Polish legislation on animal experimentation. Additionally, all efforts were made to minimise 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 Commit-

tee at the Medical University of Lublin and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC)—(License no: 425/2003/451/03).

Drugs The following AEDs were used in this study: TGB (Gabitril; Sanofi Winthrop, Gentilly, France), GBP (Neurontin; Parke-Davis, Freiburg, Germany), FBM (Taloxa; Schering Plough, Levallois Perret, France), LCZ (Janssen Research Foundation, Beerse, Belgium) and OXC (Trileptal; Novartis Pharma AG, Basel, Switzerland). All drugs 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^{-1} body weight. Fresh drug solutions were prepared *ex tempore* on each day of experimentation and administered as follows: TGB—15 min; OXC—30 min; GBP, LCZ and FBM—60 min before PTZ-induced seizures and motor coordination evaluation. These pretreatment times were chosen based upon information about their biological activity from the literature and our previous studies (Borowicz et al. 2002; Luszczki et al. 2003d, 2005).

Pentylenetetrazole (Sigma, St. Louis, MO, USA) was dissolved in distilled water and administered subcutaneously (s.c.) into a loose fold of skin in the midline of the neck with a volume of 0.005 ml g^{-1} body weight.

Pentylenetetrazole-induced convulsions Clonic convulsions were induced in mice by the s.c. administration of PTZ at doses that ranged between 70 and 120 mg kg^{-1}. Following PTZ administration, mice were placed separately into transparent Plexiglas cages (25×15×10 cm) and observed for 30 min for the occurrence of clonic seizures. To classify the seizure activity in mice we used a scale for clonic seizures adapted from Löscher et al. (1991). This scale comprises five stages:

1. One or more generalised myoclonic twitches of the whole body
2. Repeated clonic seizures of fore- and hindlimbs without loss of righting reflexes
3. Generalised clonic seizures lasting for over 3 s with loss of righting reflexes, whereby the animals fall onto their side during the generalised clonus
4. Loss of righting reflexes followed by tonic forelimb seizure
5. Loss of righting reflexes with tonic fore- and hindlimb seizure

In our study, the first generalised clonic seizures accompanied by loss of righting reflexes (stage 3) were used as the endpoint. The number of animals convulsing out of the total number of mice tested was noted for each treatment regimen. The convulsive action of PTZ was evaluated as the CD_{97} (convulsive dose 97, i.e., the dose of PTZ that produced clonic seizures in 97% of animals). In order to determine the CD_{97}, four or five different doses of PTZ were administered to animals (eight mice per group). Subsequently, a dose–response relationship line was calculated from the percentage of mice convulsing according to the log probit method described by Litchfield and Wilcoxon (1949).

The anticonvulsant activities of GBP, TGB, FBM, LCZ and OXC against the clonic PTZ-induced seizures were determined after s.c. administration of PTZ at its CD_{97} (100 mg kg^{-1}). The animals were administered with 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 four groups of animals were used to estimate each ED_{50} value calculated from the respective dose–response relationship line according to Litchfield and Wilcoxon (1949). Similarly, the anticonvulsant activity of mixtures of GBP or TGB with FBM, LCZ and OXC was evaluated and expressed as $ED_{50\ mix}$, corresponding to the dose of a two-drug mixture required to protect 50% of animals tested for PTZ-induced clonic convulsions. This experimental procedure was described in more detail in our earlier study (Luszczki and Czuczwar 2004a).

Chimney test The acute neurotoxic adverse effects of newer AEDs (GBP, TGB, OXC, FBM and LCZ) on motor performance impairment were quantified with the chimney test of Boissier et al. (1960). In this test, animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm in length), and motor impairment was indicated by the inability of the animals to do so within 60 s. The acute neurotoxic (adverse) effects of AEDs alone were expressed as their median toxic doses (TD_{50} in mg kg^{-1}), representing the doses at which the respective AEDs impaired motor coordination in 50% of the tested animals. To evaluate each TD_{50} value, at least four groups of animals (each group consisted of eight mice) injected with various doses of the drugs were challenged with the chimney test. A dose–response relationship line was calculated on the

Table 1 Anticonvulsant and neurotoxic effects of newer antiepileptic drugs (AEDs) evaluated in pentylenetetrazole (PTZ)-induced seizures and the chimney test in mice

Drug	ED_{50} (mg kg^{-1})	TD_{50} (mg kg^{-1})	PI
FBM	26.0 (16.5–41.0)	259.4 (222.7–302.1)	9.98
GBP	205.0 (116.4–360.9)	1136.7 (930.8–1388.0)	5.54
LCZ	33.1 (23.4–46.8)	152.0 (130.2–177.4)	4.59
OXC	24.7 (20.8–29.3)	62.1 (54.6–70.5)	2.51
TGB	0.9 (0.5–1.7)	11.4 (9.2–14.1)	12.67

Anticonvulsant activities of newer (second-generation) AEDs are expressed as median effective doses (ED_{50} values ± 95% confidence limits), protecting 50% of animals tested against clonic PTZ-induced seizures. Neurotoxic effects of AEDs are presented as median toxic doses (TD_{50} values ± 95% confidence limits), inducing motor impairment in 50% of animals tested in the chimney test. PI, as a ratio of TD_{50} and ED_{50} values, describes the margin of safety of therapeutic use of each AED. The AEDs were administered i.p., as follows: GBP, FBM and LCZ—60 min, OXC—30 min, and TGB—15 min before PTZ-induced seizures and the chimney test. The clonic PTZ-induced seizures were produced by the s.c. injection of PTZ at its CD_{97} (100 mg kg^{-1})
FBM felbamate, *GBP* gabapentin, *LCZ* loreclezole, *OXC* oxcarbazepine, *TGB* tiagabine, *ED_{50}* median effective dose, *TD_{50}* median toxic dose, *PI* protective index

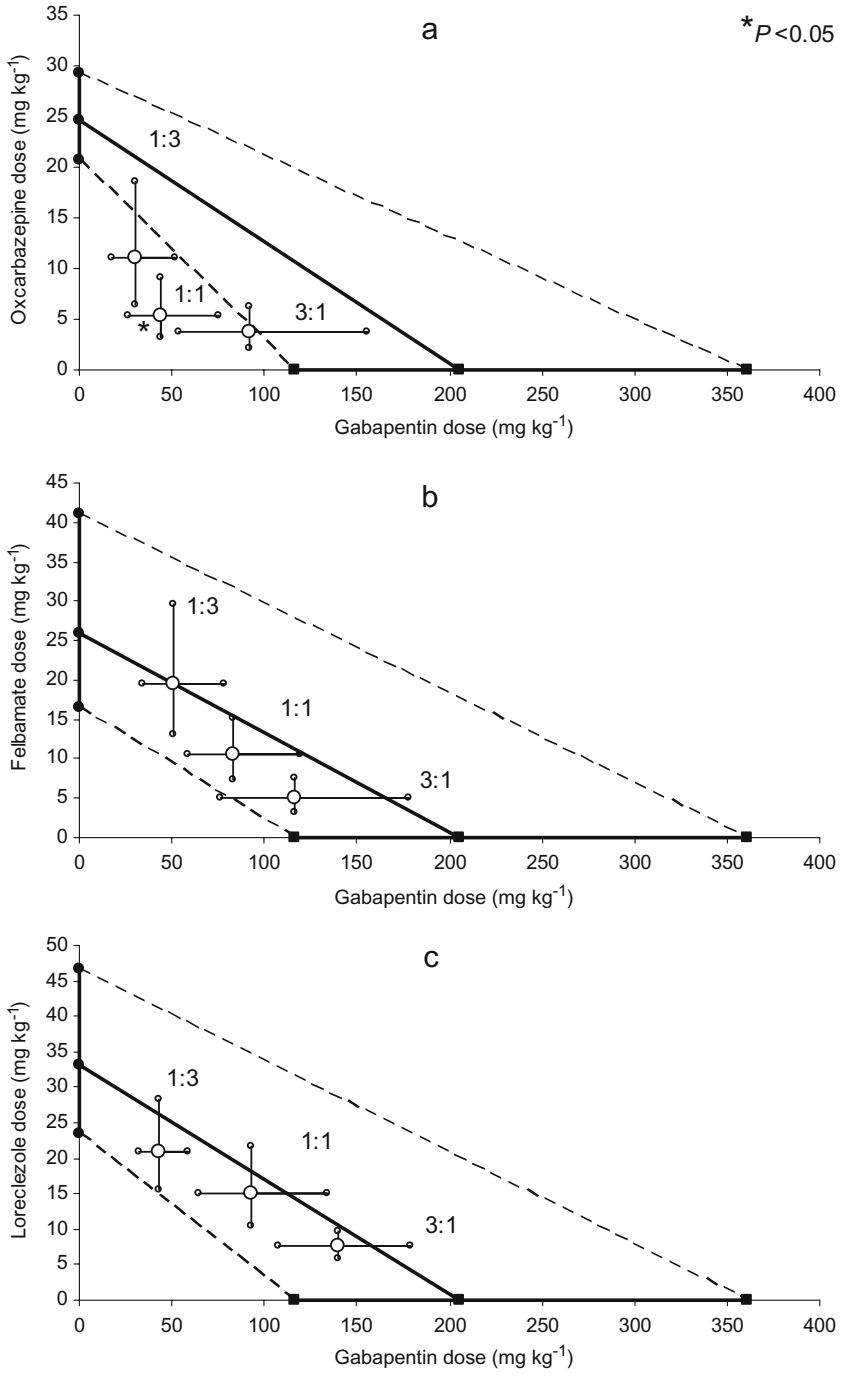

Fig. 1 Isobolograms illustrating interactions between gabapentin and oxcarbazepine, felbamate and loreclezole in the pentylenetetrazole (PTZ) test in mice. The median effective dose (ED_{50}) for gabapentin (GBP) is plotted graphically on the X-axis, whereas the ED_{50} of the AEDs examined (oxcarbazepine [OXC], felbamate [FBM] or loreclezole [LCZ]) is placed on the Y-axis (**a**–**c**). The *solid lines* on the X and Y axes represent the 95% confidence limits (CLs) for the AEDs administered alone. The *straight line* connecting these two ED_{50} values on each graph represents the theoretical line of additivity for a continuum of different fixed dose ratios, whereas the *dashed lines* represent on each isobologram the theoretical additive 95% CLs of the $ED_{50\ add}$ values. The *open circles* depict the experimentally-derived $ED_{50\ mix}$ values (with 95% CLs as the error bars) for total dose expressed as the proportion of GBP and an AED that produced a 50% anticonvulsant effect. **a** Interactions between GBP and OXC. The experimental $ED_{50\ mix}$ of the mixture of GBP+OXC for the fixed ratio of 1:1 is significantly below the theoretical line of additivity, indicating supra-additive interaction at $*P<0.05$. The $ED_{50\ mix}$ values for the fixed ratios of 1:3 and 3:1 are close to the line of additivity and thus display a tendency towards supra-additivity. **b** Interactions between GBP and FBM. The experimental $ED_{50\ mix}$ values of the mixture of GBP+FBM, for the fixed ratios of 3:1, 1:1 and 3:1 are close to the theoretical line of additivity, indicating additive interaction and a tendency towards supra-additivity in the PTZ test. **c** Interactions between GBP and LCZ. The experimental $ED_{50\ mix}$ values are near to the line of additivity and thus display almost pure additive interactions

basis of the percentage of mice showing motor deficits by means of the log-probit method according to Litchfield and Wilcoxon (1949). Similarly, the combinations of GBP or TGB with AEDs were challenged with the chimney test in order to determine $TD_{50\ mix}$ values, corresponding to the doses of mixtures of AEDs necessary to produce motor impairment in 50% of animals tested.

Isobolographic analysis of interactions It is widely accepted that isobolography allows the determination of equieffective doses of AEDs and the classification of observed interactions as: supra-additive (synergistic), sub-additive (antagonistic), indifferent or additive (Berenbaum 1989; Greco et al. 1995; Tallarida 2000). Interactions between GBP or TGB and FBM, LCZ and OXC against PTZ-induced seizures and in the chimney test were analysed according to the methodology previously detailed in our earlier studies, where the precise descriptions of theoretical background with the respective equations showing how to undertake isobolographic calculations have been presented (Luszczki et al. 2003a,b; Luszczki and Czuczwar 2003a, 2004a).

Briefly, with isobolographic analysis, both purely additive $ED_{50\ add}$ (±SE) and $TD_{50\ add}$ (±SE) values for mixtures of the examined AEDs at three fixed drug–dose ratio combinations (1:3, 1:1 and 3:1) were calculated. The $ED_{50\ add}$ represents a total additive dose of the drugs in the mixture, theoretically providing a 50% protection against PTZ-induced seizures, whereas the $TD_{50\ add}$ corresponds to a total dose of the drugs in the mixture, which theoretically impairs motor performance in 50% of animals challenged with the chimney test. Subsequently, using the log-probit method we determined the $ED_{50\ mix}$ (±SE) and $TD_{50\ mix}$ (±SE) values for the corresponding fixed ratio AED combinations. The $ED_{50\ mix}$ is an experimentally-derived total dose of a mixture of two component drugs sufficient for 50% protective effect against PTZ-induced seizures. Analogously, the $TD_{50\ mix}$ is an experimentally determined total dose of a mixture of two drugs sufficient for 50% impairment of motor coordination in the chimney test in mice. Statistical comparison of the experimentally-derived $ED_{50\ mix}$ or $TD_{50\ mix}$ values with their corresponding theoretically additive $ED_{50\ add}$ or $TD_{50\ add}$ values was performed by the use of the unpaired Student's *t* test, according to Porreca et al. (1990) and Tallarida (2000). Finally, to visualise the interactions between AEDs, the experimentally denoted $ED_{50\ mix}$ and $TD_{50\ mix}$ values were plotted graphically into the Cartesian plot system as isobolograms.

Moreover, in this study, the protective index (PI) for each AED was calculated by dividing a given TD_{50} value, evaluated in the chimney test, by the respective ED_{50} value determined in the PTZ test. The PI is considered an index of the margin of safety and tolerability between anticonvulsant doses and doses of AEDs exerting acute adverse effects (e.g., sedation, ataxia, impairment of motor coordination or other neurotoxic manifestations) in preclinical studies (Löscher and Nolting 1991). Subsequently, the isobolographic parameter benefit index (BI) was calculated for all combinations examined as a quotient of PI_{mix} and PI_{add} of respective fixed ratio combinations. PI_{mix} is experimentally determined from the corresponding $TD_{50\ mix}$ and $ED_{50\ mix}$ values, whereas PI_{add} is theoretically calculated from the equation of additivity for the PTZ and chimney tests as a ratio of $TD_{50\ add}$ and $ED_{50\ add}$ values. The BI provides the rationale for combining the respective AEDs in clinical practice, if its value greatly exceeds the value of 1.3, whereas BI lower than 0.7 indicates unfavourable combinations of AEDs (Luszczki et al. 2003a,b).

Statistical evaluation of data The ED_{50}s and TD_{50}s with their 95% confidence limits were calculated by computer log-probit analysis (Litchfield and Wilcoxon 1949). The 95% confidence limits were transformed to standard errors (SEs) as described previously (Luszczki et al. 2003a,b). Statistical evaluation of the isobolographic interactions was performed by the use of the Student's *t* test to detect the differences between experimental ($ED_{50\ mix}$ or $TD_{50\ mix}$) and theoretical additive ($ED_{50\ add}$ or $TD_{50\ add}$) values, according to Porreca et al. (1990) and Tallarida (2000).

Results

Anticonvulsant and neurotoxic profile of AEDs administered separately in mice

All five AEDs (FBM, GBP, LCZ, OXC and TGB) administered alone exhibited a clear-cut anticonvulsant activity against clonic PTZ-induced seizures in mice. The ED_{50} values for AEDs are presented in Table 1. Similarly, in the

Table 2 Isobolographic analysis of interactions between gabapentin and some newer AEDs against the clonic PTZ-induced seizures in mice

Combination	FR	$ED_{50\ mix}$ (mg kg^{-1})	n_{mix}	$ED_{50\ add}$ (mg kg^{-1})	n_{add}	I
GBP+OXC	1:3	41.48±11.15	16	69.75±16.40	52	O
	1:1	46.65±13.33*	16	114.84±30.64	52	S
	3:1	95.42±25.69	16	159.92±44.89	52	O
GBP+FBM	1:3	70.71±15.23	16	70.73±19.31	52	O
	1:1	94.19±17.10	24	115.49±32.58	52	O
	3:1	121.76±26.27	16	160.24±45.86	52	O
GBP+LCZ	1:3	64.00±9.81	16	76.06±19.17	52	O
	1:1	108.36±20.02	16	119.04±32.49	52	O
	3:1	147.35±16.66	16	162.02±45.81	52	O

Results are presented as median effective doses (ED_{50}s±SE) for drug mixtures, determined either experimentally ($ED_{50\ mix}$) or theoretically calculated ($ED_{50\ add}$) from the equation of additivity. Statistical evaluation of data was performed using the unpaired Student's *t* test according to Porreca et al. (1990) and Tallarida (2000).

*$P<0.05$ vs. the respective $ED_{50\ add}$

FR fixed ratio combination, n_{mix} total number of animals used at those doses whose expected anticonvulsant effects ranged between four and six probits for the experimental mixture, n_{add} total number of animals calculated for the additive mixture of the drugs examined, *I* isobolographic characteristic of interaction, *O* additivity, *S* supra-additivity (synergy)

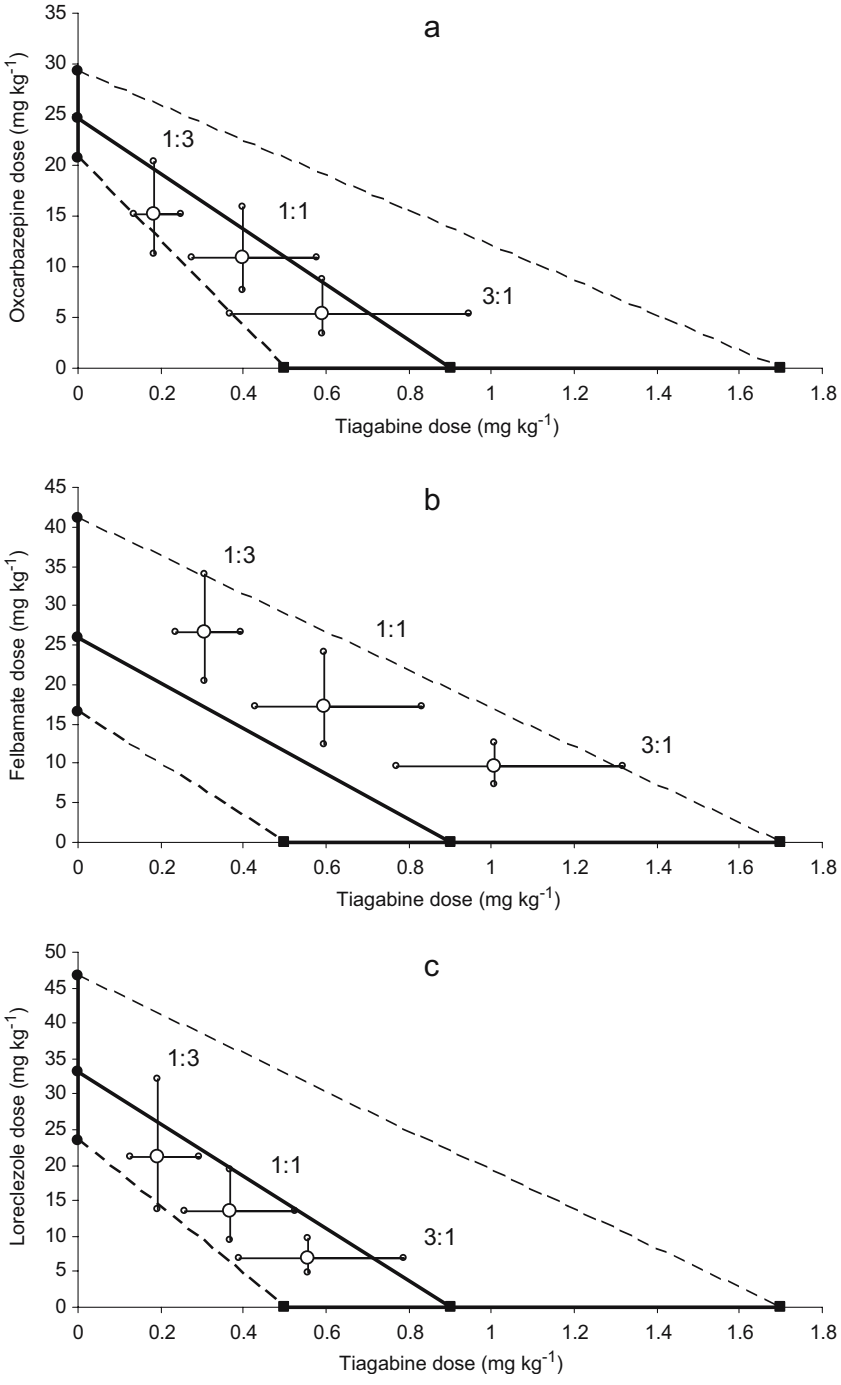

Fig. 2 Isobolograms showing interactions between tiagabine (TGB) and OXC, FBM and LCZ against PTZ-induced seizures in mice. The median effective dose (ED_{50}) for TGB is plotted graphically on the X-axis, whereas the ED_{50} of the AEDs examined (OXC, FBM or LCZ) is placed on the Y-axis (**a**–**c**). For more details see the legend to Fig. 1. **a** Interactions between TGB and OXC. The experimental $ED_{50\ mix}$ values of the mixture of GBP+OXC for the fixed ratios of 3:1, 1:1 and 3:1 are close to the theoretical line of additivity, indicating additive interactions in the PTZ test, although a tendency towards supra-additivity is observed. **b** Interactions between TGB and FBM. The experimental $ED_{50\ mix}$ values of the mixture of GBP+FBM, for all fixed ratios of 3:1, 1:1 and 3:1 are above the line of additivity, although they did not statistically differ from the $ED_{50\ add}$ values. A tendency towards sub-additivity (antagonism) was displayed for all fixed ratios investigated in the PTZ test. **c** Interactions between TGB and LCZ. The experimental $ED_{50\ mix}$ values are near to the line of additivity, displaying additive interactions with a tendency towards supra-additivity in the PTZ test

chimney test, it was possible to determine TD_{50} values for all the AEDs (Table 1). Simultaneous evaluation of TD_{50} and ED_{50} values for all AEDs allowed the determination of their PIs, describing the safety and tolerability profile of AEDs administered singly (Table 1). The highest PI was associated with TGB (12.67), whereas OXC had the lowest value (2.51). LCZ, FBM and GBP had PIs that ranged between 4.59 and 9.98 (Table 1).

Isobolographic assessment of interactions between GBP, TGB and FBM, LCZ and OXC against the clonic PTZ-induced seizures

Gabapentin co-administered with OXC at the fixed ratio of 1:1 exerted a supra-additive (synergistic) interaction (at $P<0.05$; Fig. 1a; Table 2). In contrast, in the fixed ratios of 1:3 and 3:1 GBP and OXC in combination showed ad-

ditivity in isobolography, although a tendency towards supra-additivity was observed (Fig. 1a; Table 2). All fixed ratio combinations of GBP with FBM or LCZ examined were additive in the PTZ test (Fig. 1b,c; Table 2). The difference between the observed and the theoretical ED_{50} values was not statistically significant, displaying only a tendency towards supra-additivity (Fig. 1b,c).

In the cases of the combinations of TGB with LCZ and OXC, it was found that all the fixed ratio combinations tested (1:3, 1:1 and 3:1) showed additivity in the PTZ test, although a tendency towards supra-additivity was observed (Fig. 2a, c; Table 3). Moreover, the combination between TGB and FBM (at all fixed ratios examined) exerted additivity in the PTZ test; however, the interactions displayed a tendency towards sub-additivity (Fig. 2b; Table 3).

Isobolographic analysis of interactions among GBP, TGB and FBM, LCZ and OXC in the chimney test

It was observed that GBP combined with OXC at the fixed ratios of 1:3 and 1:1 exerted sub-additive (antagonistic) interactions in the chimney test (at $P<0.05$; Fig. 3a; Table 4). Similarly, the interaction between GBP and LCZ at the fixed ratio of 1:1 was sub-additive (antagonistic) in the chimney test (at $P<0.05$; Fig. 3c; Table 4). The remaining combinations of GBP and OXC at the fixed ratio of 3:1, as well as GBP and LCZ at the fixed ratios of 1:3 and 3:1 displayed additivity in the chimney test, although a tendency towards sub-additivity was observed (Fig. 3a, c). Moreover, all combinations of GBP with FBM (at the fixed ratios of 1:3, 1:1 and 3:1) were additive, showing a tendency to sub-additivity (Fig. 3b).

The combinations of TGB and OXC at the fixed ratios of 1:3 and 1:1 exerted sub-additive interactions in the chimney test (at $P<0.05$ and $P<0.01$; Fig. 4a; Table 5). Only, the combination of TGB with OXC at the fixed ratio of 3:1 was additive, displaying a tendency towards sub-additivity (Fig. 4a). In contrast, the co-administration of TGB with LCZ at the fixed ratio of 1:1 resulted in a supra-additive (synergistic) interaction in the chimney test ($P<0.05$; Fig. 4c; Table 5). The remaining combinations of TGB with LCZ (at the fixed ratios of 1:3 and 3:1) were additive, although a tendency towards synergy was observed for these combinations (Fig. 4c). The combinations of TGB and FBM examined in the present study (at the fixed ratios of 1:3, 1:1 and 3:1) were associated with additivity in the chimney test, although the tendency towards sub-additivity was observed for all combinations (Fig. 4b; Table 5).

Table 3 Isobolographic analysis of the interactions between TGB and some newer AEDs against clonic PTZ-induced seizures

Combination	FR	$ED_{50\ mix}$ (mg kg^{-1})	n_{mix}	$ED_{50\ add}$ (mg kg^{-1})	n_{add}	I
TGB+OXC	1:3	15.34±2.33	16	18.72±1.69	44	O
	1:1	11.38±2.12	16	12.77±1.22	44	O
	3:1	5.98±1.45	8	6.82±0.75	44	O
TGB+FBM	1:3	26.90±3.34	24	19.70±4.60	44	O
	1:1	17.86±3.01	16	13.42±3.16	44	O
	3:1	10.7±1.47	16	7.15±1.72	44	O
TGB+LCZ	1:3	21.33±4.59	24	25.0±4.46	44	O
	1:1	13.89±2.55	24	16.97±3.07	44	O
	3:1	7.36±1.32	24	8.92±1.68	44	O

Data are presented as median effective doses (ED_{50} values±SE) for drug mixtures, determined either experimentally ($ED_{50\ mix}$) or theoretically calculated ($ED_{50\ add}$) from the equation of additivity. Statistical evaluation of data was performed using the unpaired Student's *t* test according to Porreca et al. (1990) and Tallarida (2000). For more details see the Table 2

Isobolographic parameter evaluation

In the present study, 10 out of the 18 fixed ratio combinations investigated could be considered to be favourable, having BI values higher than the border value of 1.3. The highest BI value for the combination of GBP and OXC was associated with the fixed ratio combination of 1:1 and was 3.31, whereas the BI values for GBP with OXC at the fixed ratios of 3:1 and 1:3 were 2.16 and 2.08 respectively (Table 6). In these cases, the supra-additivity (synergy) in the PTZ test associated with sub-additivity (antagonism) in terms of acute neurotoxic effects in the chimney test fulfilled a criterion of the most advantageous AED combination. Similarly, the combinations of GBP with LCZ or FBM may also be considered advantageous since their BIs were higher than the border value of 1.3 (Table 6).

The combination of TGB with OXC at all fixed ratios may also be considered as advantageous because their BI values ranged between 1.32 and 1.5 (Table 6), due to the existence of a tendency towards supra-additivity against PTZ-induced seizures and sub-additivity in the chimney test. In contrast, in the present study, the combinations of TGB with FBM were unfavourable, as their BI values were near to or lower than 0.7 (Table 6). Furthermore, the BI values calculated for the combination of TGB with LCZ ranged between 0.93 and 1.13, indicating that these combinations were at best neutral (Table 6).

Discussion

The results presented herein indicate that only one combination tested between GBP and OXC at the fixed ratio of 1:1 exerted a supra-additive (synergistic) interaction in the PTZ test. The remaining fixed ratio combinations of GBP and OXC (i.e., 1:3 and 3:1) and all fixed ratios for the combinations of GBP with LCZ or FBM, as well as the combinations of TGB with FBM, LCZ or OXC, had merely additive effects on PTZ-induced seizures in mice. In the chimney test, evaluating the acute neurotoxic adverse effects, it was found that the combinations of GBP with OXC and LCZ, as well as the combinations of TGB with OXC, produced sub-additive (antagonistic) interactions. Only one combination of TGB and LCZ at the fixed ratio of 1:1 was supra-additive in the chimney test, whereas the other combinations tested were additive.

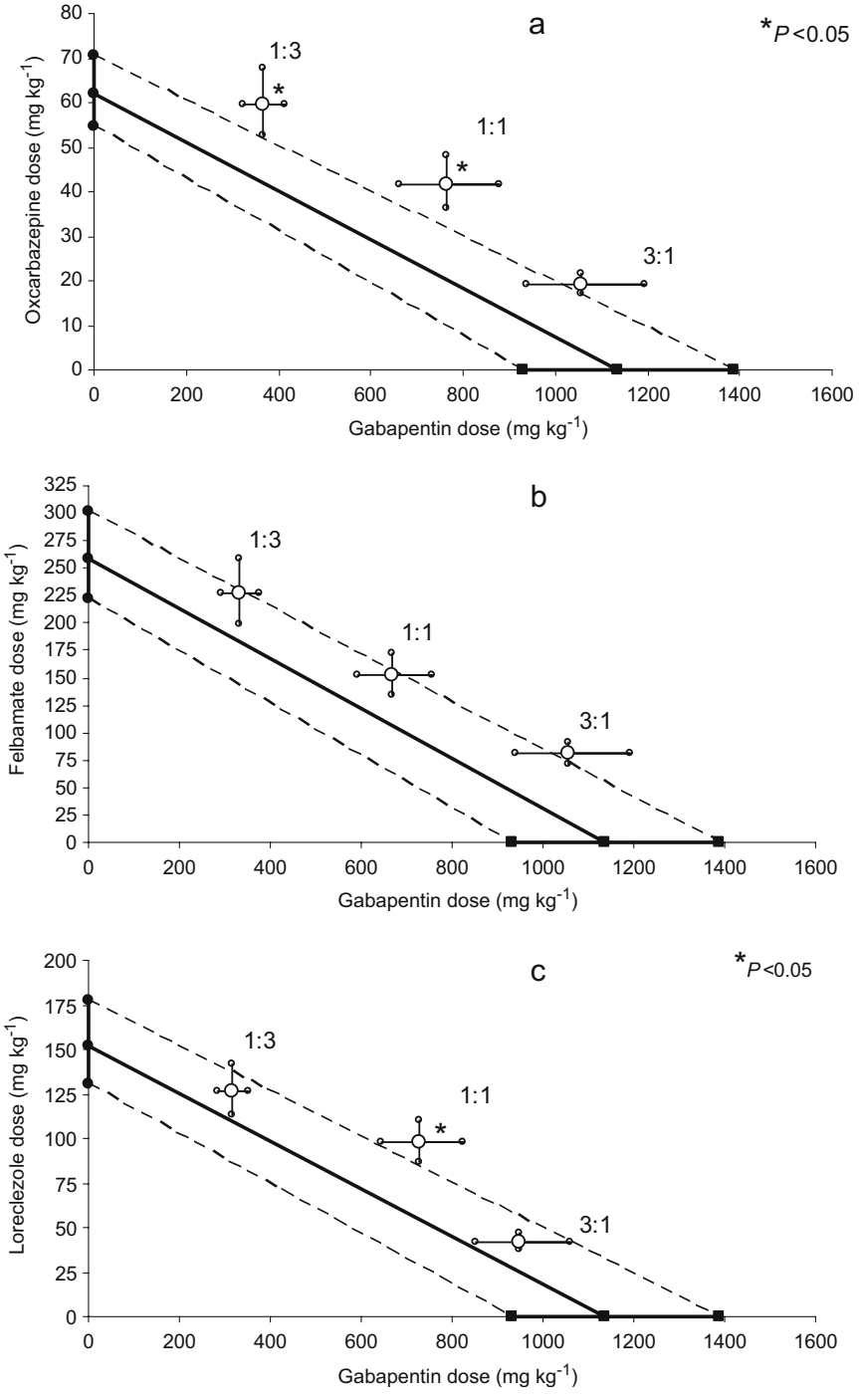

Fig. 3 Isobolograms illustrating interactions among GBP and OXC, FBM and LCZ in the chimney test in mice. The median toxic dose (TD_{50}) for GBP is plotted graphically on the X-axis, whereas the TD_{50} of the AED examined (OXC, FBM or LCZ) is placed on the Y-axis (**a**–**c**). For more details see the legend to Fig. 1. **a** Interactions between GBP and OXC. The experimental $TD_{50\ mix}$ values of the mixture of GBP+OXC for the fixed ratios of 1:3 and 1:1 are significantly above the theoretical line of additivity, indicating sub-additive (antagonistic) interactions at $^*P<0.05$. The $TD_{50\ mix}$ for the fixed ratio of 3:1 is close to the line of additivity and thus displays a tendency towards sub-additivity. **b** Interactions between GBP and FBM. The experimental $TD_{50\ mix}$ values of the mixture of GBP +FBM, for the fixed ratios of 3:1, 1:1 and 3:1 are close to the theoretical line of additivity, indicating additive interactions in the chimney test, although a tendency towards sub-additivity is observed. **c** Interactions between GBP and LCZ. The experimental $TD_{50\ mix}$ of the of GBP+LCZ for the fixed ratio of 1:1 is significantly above the theoretical line of additivity, indicating sub-additive interaction at $^*P<0.05$. The other $TD_{50\ mix}$ values for the fixed ratios of 1:3 and 3:1 are close to the line of additivity and thus display a tendency towards sub-additivity

As already mentioned, from a preclinical point of view, the most favourable combination is that exerting supra-additivity with respect to seizure suppression and simultaneously sub-additivity in terms of neurotoxic adverse effects. Theoretically, such a combination provides full antiseizure protection and is devoid of acute neurotoxic side effects. Based on the classification of interactions in preclinical study, the combination of GBP with OXC, showing synergy in seizure suppression and antagonism with respect to neurotoxic adverse effects, may be considered to be the best AED combination (Luszczki et al. 2003b). In contrast, the combination of TGB with LCZ that produced synergy in the chimney test and additivity in the PTZ test was classified as unfavourable. The remaining combinations between GBP and FBM or LCZ, as well as those between TGB and OXC, were classified as neutral combinations, displaying additivity in both antiseizure and neurotoxic adverse effects (Luszczki et al. 2003b). It is worth noting that BI values provided us with more subtle information about the final pharmacological profile of AED combinations with regard to their antiseizure and acute neurotoxic effects. Based on BI values, the combi-

Table 4 Isobolographic analysis of the interactions between GBP and newer AEDs in the chimney test in mice

Combination	FR	$TD_{50\ mix}$ (mg kg^{-1})	n_{mix}	$TD_{50\ add}$ (mg kg^{-1})	n_{add}	I
GBP+OXC	1:3	424.4±26.6*	24	330.7±32.0	60	A
	1:1	805.1±59.9*	24	599.4±59.9	60	A
	3:1	1,077.1±65.9	16	868.0±87.9	60	O
GBP+FBM	1:3	558.4±37.4	24	478.7±44.1	60	O
	1:1	819.9±51.1	24	698.0±68.0	60	O
	3:1	1,138.4±69.6	16	917.3±91.9	60	O
GBP+LCZ	1:3	443.5±24.9	24	398.2±37.9	60	O
	1:1	826.6±51.8*	24	644.3±63.9	60	A
	3:1	991.9±55.6	24	890.5±89.9	60	O

Results are presented as median toxic doses (TD_{50}±SE) of a drug mixture, determined either experimentally ($TD_{50\ mix}$) or theoretically calculated ($TD_{50\ add}$) from the line of additivity. The AEDs were administered at times corresponding to the peak of maximum anticonvulsant effects for these AEDs, as follows: GBP, FBM, LCZ—60 min, OXC—30 min, and TGB—15 min. Statistical analysis of data was performed using the unpaired Student's *t* test according to Porreca et al. (1990) and Tallarida (2000)

*$P<0.05$ vs. the respective $TD_{50\ add}$ value. For more details see the Table 2

A sub-additivity (antagonism)

nations of TGB with FBM were unfavourable, whereas those between TGB and LCZ were neutral from a preclinical point of view.

We can attempt to explain the observed synergy between GBP and OXC in the PTZ test on the basis of their molecular mechanisms of action. It is worth mentioning that the exact mechanisms of action of GBP are still unknown; however, several potential mechanisms may account for its anticonvulsant activity. Despite its chemical similarity to GABA, GBP inhibits Ca^{2+} voltage-gated channels through the interaction with the $alpha_2delta$ subunit (Gee et al. 1996). The drug activates glutamate decarboxylase (GAD) and weakly inhibits GABA transaminase (GABA-T)—contributing to the increment in GABA concentration, as well as activating glutamate dehydrogenase (GDH) and potently inhibiting branched-chain aminotransferase (BCAA-T)—decreasing glutamate concentration (Goldlust et al. 1995). GBP competes with the transport of branched-chain amino acids (L-leucine, L-valine, L-phenylalanine), and thus the drug affects cytosolic concentrations of endogenous amino acids in neurons (Taylor et al. 1998). Moreover, the drug increases the conductance of hyperpolarisation-activated cation currents (I_h) contributing to the protection of neurons against excessive synaptic or intrinsic activity, and stabilising the neuronal network within the hippocampus (Surges et al. 2003). More recently, it has been found that GBP selectively activates presynaptic $GABA_B$ heteroreceptors (but not $GABA_B$ autoreceptors) decreasing the release of neurotransmitters by reducing Ca^{2+} conductance in neurons of the CNS (Ng et al. 2001; Parker et al. 2004).

With regard to the mechanisms of action of OXC, compelling evidence indicates that the drug and its rapidly formed 10-monohydroxy derivative (MHD), at therapeutically relevant concentrations: reduce high-frequency repetitive firing of neurons by an action on voltage-dependent Na^+ channels; decrease the frequency of penicillin-induced epileptiform spike discharges in neurons suggesting an effect on K^+ currents; and inhibit high-voltage-activated N-type Ca^{2+} channels (Schmutz et al. 1994; Stefani et al. 1995). Considering the mechanisms of action of both AEDs, the synergistic interaction between GBP and OXC at the fixed ratio of 1:1 in the PTZ test seems to be closely related to Ca^{2+} channel blockade, mediated by both AEDs. Nevertheless, all the above-mentioned mechanisms of anticonvulsant activity of the two AEDs could contribute to their synergistic interaction against PTZ-induced seizures. A similar situation was also documented for the combination of GBP with OXC in maximal electroshock (MES)-induced seizures in mice. Indeed it has been found isobolographically that GBP synergistically potentiated the antiseizure effects of OXC on maximal electroconvulsions in mice (Luszczki et al. 2005).

Relatively recently, it has been found that GBP interacted synergistically with TGB and VGB in the PTZ-test in mice (Luszczki and Czuczwar 2003b, 2004a). Noticeably, TGB is a second-generation AED, acting as a potent GABA re-uptake inhibitor into neurons and glia, which by blocking the GABA transporter 1 (GAT-1) in mice increases GABA concentration into the synaptic clefts of neurons and prolongs the duration of GABA-related inhibitory synaptic potentials (Nielsen et al. 1991; Czuczwar and Patsalos 2001). VGB is also a second-generation AED, whose anticonvulsant action is related with irreversible inhibition of GABA-transaminase, the enzyme responsible for the metabolic degradation of GABA in neurons and glia (Brodie and Schachter 2001). It should be stressed that the anticonvulsant activities of VGB, TGB and GBP are closely related with the increment in GABA content within the brain and thus, the enhancement of GABA-ergic inhibitory neurotransmission (Czuczwar and Patsalos 2001). Surprisingly, in the present study, GBP interacted synergistically only with OXC, but not with LCZ—an AED that exerts its modulatory effect on two separate allosteric regulatory sites on $GABA_A$ receptors (Wingrove et al. 1994; Wafford et al. 1994). Neurochemical studies have revealed that LCZ potentiates $GABA_A$ receptor-mediated Cl^- currents through a site present on the β2 and β3 (but not β1) subunits of $GABA_A$ receptors (Wafford et al. 1994). The drug also acts in an inhibitory manner, increasing the rate and degree of apparent desensitisation of $GABA_A$ receptor-mediated currents via a novel site independent on the benzodiazepine and picrotoxin binding sites (Donnelly and Macdonald 1996).

In case of FBM, electrophysiological and neurochemical studies have indicated that the drug interferes with voltage-gated Na^+ channels, inhibiting the sustained repetitive neuronal firing, and thereby, preventing the seizure spread (White et al. 1992). Moreover, FBM indirectly antagonises N-methyl-D-aspartate (NMDA) by interfering with the binding of glycine to strychnine-insensitive gly-

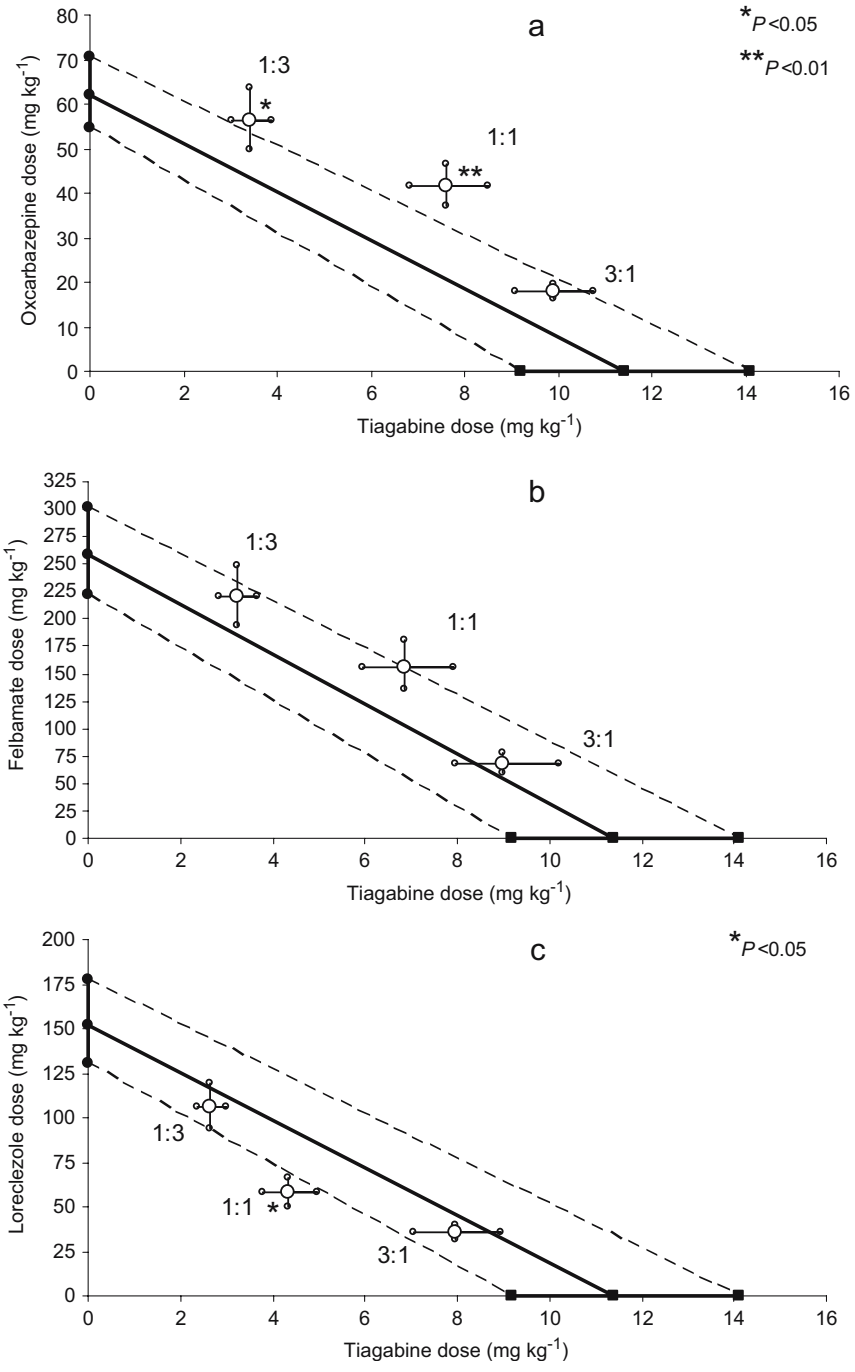

Fig. 4 Isobolograms showing interactions between TGB and OXC, FBM and LCZ in the chimney test in mice. The median toxic dose (TD_{50}) for TGB is plotted graphically on the X-axis, whereas the TD_{50} of the AED examined (OXC, FBM or LCZ) is placed on the Y-axis (**a**–**c**). For more details see the legend to Fig. 1. **a** Interactions between TGB and OXC. The experimental $TD_{50\ mix}$ values of the mixture of TBP+OXC for the fixed ratios of 1:3 and 1:1 are significantly above the theoretical line of additivity, indicating sub-additive (antagonistic) interactions at *P<0.05 and **P<0.01 respectively. Only the $TD_{50\ mix}$ for the fixed ratio of 3:1 is close to the line of additivity and thus displays a tendency towards sub-additivity. **b** Interactions between TGB and FBM. The experimental $TD_{50\ mix}$ values of the mixture of TGB+FBM, for all fixed ratios of 3:1, 1:1 and 3:1, are above the line of additivity, although they did not statistically differ from the $TD_{50\ add}$ values. The tendency towards sub-additivity (antagonism) was displayed for all fixed ratios investigated in the chimney test. **c** Interactions between TGB and LCZ. The experimental $TD_{50\ mix}$ of the mixture of TGB+LCZ for the fixed ratio of 1:1 is significantly below the theoretical line of additivity, indicating supra-additive (synergistic) interaction at *P<0.05. The $TD_{50\ mix}$ values for the fixed ratios of 1:3 and 3:1 are close to the line of additivity and thus display a tendency towards supra-additivity

cine receptors, leading to a reduction of NMDA receptor-modulated cationic conductance (McCabe et al. 1993). The drug suppresses dose-dependently the seizures induced by kainate, alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), quisqualate (De Sarro et al. 1994) and PTZ (Borowicz et al. 2004), as well as, it inhibits voltage-activated Ca^{2+} currents (Stefani et al. 1997). Previously, we have reported a supra-additive interaction between FBM and phenobarbital at the fixed-ratio of 1:3 in the PTZ-test in mice (Borowicz et al. 2004). Moreover, in the PTZ-test, a sub-additive interaction has been observed for the combination of FBM with valproate at the fixed-ratio of 3:1. The other combinations tested between FBM and phenobarbital or valproate, as well as, those between FBM and clonazepam or ethosuximide had an additive effect on PTZ-induced seizures in mice (Borowicz et al. 2004). In this study, it was found that all combinations of FBM and GBP or TGB had an additive effect in the PTZ test in mice.

Surprisingly, TGB did not interact synergistically with LCZ, FBM and OXC. In our previous studies, it was found that TGB synergistically enhanced the anticonvulsant action of GBP and VGB in the PTZ test in mice (Luszczki et al. 2003d; Luszczki and Czuczwar 2003b, 2004a).

Table 5 Isobolographic analysis of the interactions between TGB and newer AEDs in the chimney test in mice

Combination	FR	$TD_{50\ mix}$ (mg kg^{-1})	n_{mix}	$TD_{50\ add}$ (mg kg^{-1})	n_{add}	I
TGB+OXC	1:3	59.6±3.8 *	16	49.4±3.3	52	A
	1:1	49.1±2.8 **	16	36.7±2.6	52	A
	3:1	27.9±1.2	16	24.1±1.9	52	O
TGB+FBM	1:3	223.1±14.3	24	197.4±15.4	52	O
	1:1	163.2±11.8	32	135.4±10.7	52	O
	3:1	73.4±6.0	32	77.3±4.9	52	O
TGB+LCZ	1:3	108.8±6.6	16	116.9±9.3	52	O
	1:1	62.2±4.4*	24	81.7±6.6	52	S
	3:1	43.3±2.6	16	46.5±3.9	52	O

Results are presented as median toxic doses (TD_{50}±SE) of a drug mixture, determined either experimentally ($TD_{50\ mix}$) or theoretically calculated ($TD_{50\ add}$) from the line of additivity. Data were statistically analysed using the unpaired Student's *t* test according to Porreca et al. (1990) and Tallarida (2000)

*P<0.05 and **P<0.01 vs. the respective $TD_{50\ add}$ value. For more details see Table 2 and 4

Moreover, one clinical report exists that documented that the combination of TGB with VGB considerably improved the control of seizure attacks in refractory epileptic patients (Leach and Brodie 1994). Additionally, it has been experimentally confirmed that low doses of VGB co-administered with TGB significantly reduced GABA uptake from synaptic clefts (Leach et al. 1996) resulting in an increase in GABA concentration in the whole mouse neocortex (Leach et al. 1997). Likewise, the drugs applied together (TGB and VGB) drastically and firmly inhibited the experimentally-induced epileptic potentials from hippocampal slices of guinea pigs (Kohling et al. 2002). Thus, the previously documented synergistic interactions for the combinations of TGB with GBP, TGB with VGB and GBP with VGB may suggest that the activation of various mechanisms within the same neurotransmitter system (GABA) may exert advantageous effects offering a significant reduction in seizure activity. In the light of this fact, it is surprising that neither GBP nor TGB interacted synergistically with LCZ, although all AEDs influence the GABA-ergic inhibitory neurotransmission system. Relatively recently, a similar situation was reported in an experimental study by Jonker et al. (2003), who found that the combination of midazolam (an allosteric modulator of the $GABA_A$ receptor) with TGB was additive in nature.

Another fact should be discussed here, since the supra-additive interaction between GBP and OXC in the PTZ test is similar in nature to the supra-additivity reported previously in the MES test in mice (Luszczki et al. 2005). Moreover, the additive interactions between TGB and FBM observed in this study confirmed our previous findings, also demonstrating the additivity between these AEDs in the MES test in mice (Luszczki et al. 2003c). Additionally, it has been documented that the combination of TGB with GBP exerted supra-additive interactions in both MES and PTZ tests (Luszczki and Czuczwar 2004a). Thus, considering the isobolographic characteristics of the above-mentioned combinations and their identical interactions in both MES and PTZ tests, the existence of a good correlation between AED interactions and seizure models used in preclinical studies can be ascertained.

As mentioned above, the PTZ-induced seizures are considered to be an experimental animal model of myoclonic convulsions in man (Löscher and Schmidt 1988; Löscher et al. 1991). In the light of this fact, it should be stressed that TGB and GBP, although they protect animals against the clonic seizures induced by PTZ, the drugs are not only ineffective in suppressing myoclonic seizures in patients, but they may even contribute to the aggravation of absence seizures and/or the induction of non-convulsive *status epilepticus* in patients (Genton 2000; Schapel and Chadwick 1996; Mangano et al. 2003). It has also been found experimentally that TGB (administered i.p. at doses of 3 and 10 mg kg^{-1}) enhanced both the number and mean duration of spike-and-wave discharges in WAG/Rij rats (an animal model of generalised non-convulsive absence epilepsy), indicating evidently that the drug aggravated absence seizures in rats (Coenen et al. 1995).

Table 6 Calculations of isobolographic benefit indices (BIs) from the PTZ and chimney tests

Combination	FR	PI_{mix}	PI_{add}	BI
GBP+OXC	1:3	10.23	4.74	2.16
	1:1	17.26	5.22	3.31
	3:1	11.29	5.43	2.08
GBP+FBM	1:3	7.90	6.77	1.17
	1:1	8.70	6.04	1.44
	3:1	9.35	5.72	1.63
GBP+LCZ	1:3	6.93	5.24	1.32
	1:1	7.63	5.41	1.41
	3:1	6.73	5.50	1.22
TGB+OXC	1:3	3.89	2.64	1.47
	1:1	4.31	2.87	1.50
	3:1	4.67	3.53	1.32
TGB+FBM	1:3	8.29	10.02	0.83
	1:1	9.14	10.09	0.91
	3:1	6.86	10.81	0.63
TGB+LCZ	1:3	5.10	4.68	1.09
	1:1	4.48	4.81	0.93
	3:1	5.88	5.21	1.13

Data represent the protective indices (PIs) of AEDs in combinations, determined experimentally (PI_{mix}) and computed theoretically from the equation of additivity (PI_{add}). The PIs are quotients of their respective TD_{50} and ED_{50} values from the chimney and PTZ tests. BI, as a ratio of PI_{mix} and PI_{add}, determines the margin of safety and tolerability of drugs in combinations, considering both the anticonvulsant and neurotoxic effects produced by the mixture of the two drugs. Drug combinations whose BI value is greater than the border value of 1.3, can be considered as favourable combinations, perhaps worthy of further clinical study. Drug combinations whose BIs range between 0.7 and 1.3 are considered to be neutral, whereas those whose BI is lower than the border value of 0.7 are considered unfavourable, from a preclinical point of view (Luszczki et al. 2003a,b)

The question arises whether the combinations of AEDs effective in the MES and/or PTZ tests would be also effective in other epileptic syndromes that have no representation in experimental animal models. For example, to date, there is no firmly established animal model reflecting the Lennox-Gastaut or West syndrome in children. Hence, it is questionable whether the combinations of two AEDs effective in the MES or PTZ tests would be effective in these syndromes. Since a close correlation exists between the results from the MES and PTZ tests in preclinical studies, it can be supposed that some AED combinations may also exert anticonvulsant properties in various epileptic syndromes in humans. Noticeably, there are no preclinical methods, except for isobolography, that allow the adequate examination and pre-selection of the anticonvulsant efficacy of AEDs in combinations.

Recently, numbers of various AEDs in combination with topiramate have been tested in two models of experimental epilepsy (in the MES and PTZ tests), even if they were virtually ineffective in one of these models (Sills et al. 2004). The authors have demonstrated that levetiracetam (50 and 250 mg kg^{-1}), clobazam (1 and 5 mg kg^{-1}) and lamotrigine (2.5 and 12.5 mg kg^{-1}), although when administered singly did not suppress PTZ-induced seizures, when combined with topiramate (5, 25 and 125 mg kg^{-1}), an AED that was also ineffective in the PTZ test, strongly inhibited PTZ-induced seizures in mice, indicating that the AED combinations may have a broader spectrum of antiseizure activity in preclinical study, producing entirely different anticonvulsant properties than the constituent AEDs when administered separately (Sills et al. 2004). With regard to clobazam, experimental evidence indicates that the drug (at a dose range of 1–7.5 mg kg^{-1}) was fully effective in suppressing both minimal clonic seizures and generalised tonic–clonic convulsions induced by PTZ in rats at various developmental stages (18-, 25- and 90-day-old rats; Haugvicova et al. 1999). Hence, the observed difference between the anticonvulsant action of clobazam in the PTZ test in rats and its inactivity in the mouse model of PTZ-induced seizures requires explanation in further neuropharmacological studies.

Moreover, in the study by Sills et al. (2004), TGB (0.2, 1 and 5 mg kg^{-1}), GBP (5, 25 and 125 mg kg^{-1}) and VGB (10, 50 and 250 mg kg^{-1}), the AEDs possessing a high inclination to aggravate myoclonic and absence seizures in patients, were tested in combination with topiramate (5, 25 and 125 mg kg^{-1}), showing no significant effect on the clonic PTZ-induced seizures in mice. On the other hand, it is surprising that the authors have tested combinations of AEDs in the PTZ test that are never prescribed for patients with myoclonic and absence seizures, such as: phenytoin (2, 10 and 50 mg kg^{-1}) and carbamazepine (2, 10 and 50 mg kg^{-1}) with topiramate (5, 25 and 125 mg kg^{-1}). Obviously, no changes in the latency to the clonic PTZ-induced seizures were found for the combinations of these AEDs in mice (Sills et al. 2004). Noticeably, Sills et al. (2004) set a precedent for studying and examining AEDs in the PTZ test in mice, even if the AEDs aggravate seizure in the clinical setting.

Generally, in isobolography, the observed interactions between the AEDs studied represent the final pharmacological effects of AED combinations in vivo, whose nature may be pharmacokinetic, pharmacodynamic or mixed. In this study, we evaluated the final effects of AED combinations against clonic PTZ-induced seizures as well as in the chimney test in mice. Since the isobolographically denoted interactions had barely additive effects on PTZ-induced seizures, except for the combination of GBP with OXC at the fixed ratio of 1:1, the AED combinations were not pharmacokinetically verified in this study. We are fully aware of the possibility of the existence of pharmacokinetic interactions among the AEDs studied in the biophase of experimental animals; however, in this study we determined the isobolographic characteristics of interactions without differentiating their pharmacokinetic or pharmacodynamic nature.

Conclusions

The aim of this study was to investigate the interactions among GBP, TGB and some other newer AEDs with different mechanisms of action to provide evidence of whether or not to combine AEDs with different or similar mechanisms of action. Moreover, this study served as a screening process to preselect favourable combinations with respect to both anticonvulsant and neurotoxic adverse effects in preclinical study. Based on the BI, which is a composite measure of the anticonvulsant effects in the PTZ test and of motor impairment in the chimney test, the combination of GBP and OXC appears to be particularly beneficial. Additionally, the combinations of GBP with LCZ or FBM, as well as TGB with OXC, are also advantageous using these test paradigms. In contrast, the combinations of TGB with FBM or LCZ were unfavourable from a preclinical point of view.

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