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ORIGINAL PAPER

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Additive interaction for three-drug combination of carbamazepine, lacosamide and lamotrigine against maximal electroshock-induced seizures – a type I isobolographic analysis

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ABSTRACT

Introduction. Treatment of epilepsy patients with one antiepileptic drug often fails and then the insufficiently medicated patients need two or three antiepileptic drugs combined together to stop their seizures. However, polytherapy is always associated with drug-drug interactions whose nature may or may not be favorable for epilepsy patients. Preclinical studies on animals can help to select beneficial combinations of antiepileptic drugs that could be used in further clinical settings.

Aim. To isobolographically characterize anticonvulsant effects of a combination of three antiepileptic drugs (carbamazepine, lacosamide and lamotrigine) at the fixed drug dose ratio of 1:1:1 in the mouse maximal electroshock-induced seizure test.

Material and methods. Maximal electroconvulsions were evoked in male Swiss mice by a current (25 mA, 500 V, 0.2 s stimulus duration) delivered *via* auricular electrodes. Type I isobolographic analysis was applied to assess the interaction among carbamazepine, lacosamide and lamotrigine.

Results. Isobolographic analysis revealed that the combination of carbamazepine, lacosamide and lamotrigine produced additive interaction in the mouse maximal electroshock-induced seizure test.

Conclusions. Additivity among carbamazepine, lacosamide and lamotrigine in this preclinical study can be translated to clinical settings and this three-drug combination can be recommended as a treatment option for epilepsy patients who are resistant to standard treatment regimens.

Keywords. antiepileptic drugs, isobolographic analysis, maximal electroshock, three-drug combination

Introduction

Despite our advanced knowledge about epileptogenesis and progress in epilepsy treatment, there is still a number of epilepsy patients who need efficacious treatment.[1,2] Monotherapy with current frontline antiepileptic drugs is the preferred option for these patients. Unfortunately, if three consecutive monotherapies fail, clinicians are obliged to start rational polytherapy with two or more antiepileptic drugs.[3-5] To date, many dozens of two-drug combinations were tested in preclinical studies, however, only a few three-drug combinations were experimentally investigated.[6-9] Generally, evalu-

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ation of the effectiveness of combined treatment with two or three antiepileptic drugs in preclinical studies is usually performed using an isobolographic analysis of interaction.[10-12]

Available preclinical evidence indicates that the three-drug combinations of lacosamide + carbamazepine + phenobarbital and lacosamide + lamotrigine + phenobarbital exerted additive interaction in the mouse maximal electroshock-induced seizure model.[6,9] Only the combination of carbamazepine + phenobarbital + topiramate synergistically protected the mice from maximal electroshock-induced seizures.[7]

The selection of antiepileptic drugs for combination into three-drug mixtures is based on theoretical presumptions concerning diverse molecular mechanisms of anticonvulsant action of the selected drugs. It is widely accepted that drugs whose molecular mechanisms of anticonvulsant activity differ, may offer a wide range of protection from seizures, and simultaneously, the drugs do not produce side effects.[13] This is the main reason to combine together the drugs with different mechanisms of action to avoid harmful and/or intolerable adverse effects accompanied polytherapy with antiepileptic drugs.

The purpose of this study was to determine the anticonvulsant effects for the combination of three (the first-, second- and third-generation) antiepileptic drugs in the mouse model of tonic-clonic seizures using the type I isobolographic analysis. In this study, we combined carbamazepine (a first-generation antiepileptic drug), lamotrigine (a second-generation antiepileptic drug) and lacosamide (a third-generation antiepileptic drug) in a mixture at the fixed drug dose ratio of 1:1:1. The three-drug mixture was administered to mice and the animals were subjected to the maximal electroshock-induced seizures that are thought to be a model of tonic-clonic seizures in humans.[14] Additionally, due to the isobolographic analysis we characterized a type of interactions occurring among carbamazepine, lamotrigine and lacosamide in order to try to translate the results from this preclinical study to clinical conditions.

Materials and Methods

Animals

Experiments on animals were conducted in a strict accordance with the Guide for the Care and Use of Laboratory Animals (NIH, USA), the ARRIVE guidelines and EU Directive 2010/63/EU for animal experiments. All experimental procedures were approved by the Second Local Ethics Committee at the University of Life Sciences in Lublin, Poland. Adult male albino Swiss mice (six-week-old, weighing 22-26 g), after 4 days of acclimatization to laboratory conditions, were randomly assigned to experimental groups consisting of 8 mice. Total number of animals used in this study was 256.

Drugs

In this study we used: carbamazepine (Sigma-Aldrich, Poznan, Poland), lacosamide (Vimpat®, UCB Pharma, Brussels, Belgium), and lamotrigine (Lamictal®, Glaxo Wellcome, Greenford, Middlesex, UK). All drugs were suspended in an aqueous 1% solution of Tween 80 (Sigma-Aldrich, Poznan, Poland) and administered intraperitoneally (i.p.) as follows: carbamazepine and lacosamide at 30 min, and lamotrigine at 60 min, prior to the maximal electroshock-induced seizures as documented earlier.[6,7]

Maximal electroshock-induced seizures

The antielectroshock activities of carbamazepine, lacosamide, lamotrigine (administered singly) and their mixture at the fixed drug dose ratio of 1:1:1 were expressed as the median effective doses (ED_{50} in mg/kg). Maximal electroconvulsions (seizure activity) were produced by a current (25 mA, 500 V, 50 Hz, 0.2 s stimulus duration) delivered *via* auricular electrodes from a Hugo Sachs generator (Rodent Shocker, Freiburg, Germany). The animals after receiving different drug doses were subjected to the maximal electroshock-induced seizures and percentage of the mice protected from tonic-clonic seizures allowed us to construct dose-response effect lines for the studied antiepileptic drugs.[15] The anticonvulsant activity of the mixture of carbamazepine, lacosamide and lamotrigine was expressed as the experimental median effective dose ($ED_{50\ exp}$) against maximal electroshock-induced seizures.

Type I isobolographic analysis of interaction

Interactions among drugs combined together in a mixture are usually analyzed with isobolographic analysis as described earlier.[10,16,17] Percentage of animals protected from tonic-clonic seizures per doses of carbamazepine, lacosamide and lamotrigine administered alone were fitted using log-probit linear regression analysis.[15] The ED_{50} values for carbamazepine, lacosamide and lamotrigine were calculated from the respective equations. The test for parallelism of log-probit dose-response effect lines was used, as described in more detail elsewhere.[18] Meanwhile, the median additive dose ($ED_{50\ add}$) of the mixture of carbamazepine, lacosamide and lamotrigine for the fixed-ratio combination of 1:1:1 was calculated, as presented elsewhere.[16] Subsequently, mass quantities of carbamazepine, lacosamide and lamotrigine in the purely additive mixture for the fixed drug dose ratio combination of 1:1:1 were theoretically calculated and the respective mixtures of the studied drugs were administered to animals. The experimentally-derived $ED_{50\ exp}$ at the fixed-ratio of 1:1:1 was calculated from doses of the mixture protecting the tested animals from tonic-clonic seizures. Ultimately, to calculate doses of particular antiepileptic drugs in the mixture, the $ED_{50\ exp}$ value was multiplied by the respective proportions of carbamazepine, lacosamide and lamotrigine (denoted earlier for purely additive mixture), as presented elsewhere.[6,19]

Statistical analysis of data

Log-probit analysis was used to calculate the ED_{50} and $ED_{50\,exp}$ values for the tested antiepileptic drugs administered separately and combined together at the fixed-ratio of 1:1:1.[15] The unpaired Student's *t*-test was used to statistically compare the experimentally-derived $ED_{50\,exp}$ value with the theoretical additive $ED_{50\,add}$ value for the three-drug mixture, as described earlier.[6,20]

Results

Antielectroshock activities of carbamazepine, lacosamide and lamotrigine along with isobolographic analysis of interaction among the antiepileptic drugs

Carbamazepine, lacosamide and lamotrigine administered separately exerted, in a dose-dependent manner, the anticonvulsant effects in the mouse maximal electroshock-induced seizure test. The experimentally determined equations of log-probit dose-response effects for carbamazepine, lacosamide and lamotrigine (Figure 1), allowed us to calculate the median effective doses (ED_{50} values) for the studied drugs (Table 1).

The test for parallelism of log-probit dose-response effects revealed that carbamazepine had its log-probit dose-response line non-parallel to that of lamotrigine and lacosamide (Table 1; Figure 1). In contrast, log-probit dose-response lines of lacosamide and lamotrigine were parallel to each other (Table 1; Figure 1). The mixture of carbamazepine, lacosamide and lamotrigine at the fixed drug dose ratio combination of 1:1:1 protected, in a dose-dependent manner, the mice from tonic-clonic seizures and the experimentally derived $ED_{50\,exp}$ value in the mouse maximal electroshock-induced seizure test amounted to 9.83 ± 1.29 mg/kg (Table 2).

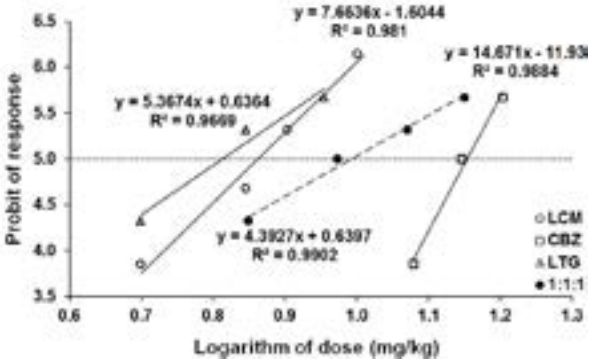

Figure 1. Log-probit dose-response effect analysis of lacosamide (LCM), carbamazepine (CBZ), and lamotrigine (LTG) administered separately and combined together at the fixed-ratio of 1:1:1 in the mouse maximal electroshock-induced seizure model

Doses of carbamazepine, lacosamide and lamotrigine administered separately and in combination at the fixed drug dose ratio of 1:1:1 were transformed to logarithms, whereas the anticonvulsant (protective) effects of the drugs in the mouse tonic-clonic model were transformed to probits. Linear regression equations for carbamazepine, lacosamide and lamotrigine and their combination are presented on the graph; where y – is the probit of response; x – is the logarithm (to the base 10) of a drug dose or a three-drug mixture dose; and R^2 – is the coefficient of determination. Points constructing lines reflect numbers of groups of animals (8 mice per group) used in this study.

With type I isobolographic analysis of interaction it was found that the $ED_{50\,exp}$ value for the combination of carbamazepine, lacosamide and lamotrigine did not significantly differ from the corresponding additively cal-

Table 1. Effects of carbamazepine, lacosamide and lamotrigine administered separately in the mouse tonic-clonic seizure model

Drug	ED_{50}	n	Test for parallelism	S.R.	f ratio S.R.	
Carbamazepine	14.25 ± 0.79	16	Carbamazepine vs. Lacosamide	1.154	1.126	non-parallel
Lacosamide	7.27 ± 0.77	16	Carbamazepine vs. Lamotrigine	1.313	1.228	non-parallel
Lamotrigine	6.50 ± 0.80	24	Lacosamide vs. Lamotrigine	1.137	1.244	parallel

Data are median effective doses (ED_{50} values in mg/kg ± S.E.M.) of the antiepileptic drugs administered systemically (i.p.) in the maximal electroshock-induced seizure test in mice. n – total number of animals used at those doses whose expected anticonvulsant effects ranged between 4 and 6 probits (16% and 84%); S.R. – slope function ratio for the respective two-drug combinations; f ratio S.R. – factor for slope function ratio for the respective two-drug combinations. Test for parallelism was performed according to Litchfield and Wilcoxon[15], as described in more detail earlier.[33]

Table 2. Type I isobolographic analysis of interaction among carbamazepine (CBZ), lacosamide (LCM) and lamotrigine (LTG) at the fixed drug dose ratio of 1:1:1 in the mouse maximal electroshock-induced seizure model

$ED_{50\,exp}$	n_{exp}	CBZ_{exp}	LCM_{exp}	LTG_{exp}	$ED_{50\,add}$	n_{add}	CBZ_{add}	LCM_{add}	LTG_{add}
9.83 ± 1.29	32	5.00	2.55	2.28	9.34 ± 0.38	50	4.75	2.42	2.17

Data are median effective doses (ED_{50} values in mg/kg ± S.E.M.) protecting 50% of animals tested against maximal electroshock-induced seizures. The $ED_{50\,exp}$ value was determined experimentally whereas the $ED_{50\,add}$ was theoretically calculated from the equation of additivity.[16] Doses of particular drugs that comprised the mixture (at the fixed drug dose ratio of 1:1:1) for both, $ED_{50\,exp}$ and $ED_{50\,add}$ values are presented separately. The unpaired Student's t-test was used to statistically analyze the data. n_{exp} and n_{add} are total numbers of animals used at those doses whose expected anticonvulsant effects ranged between 4 and 6 probits.

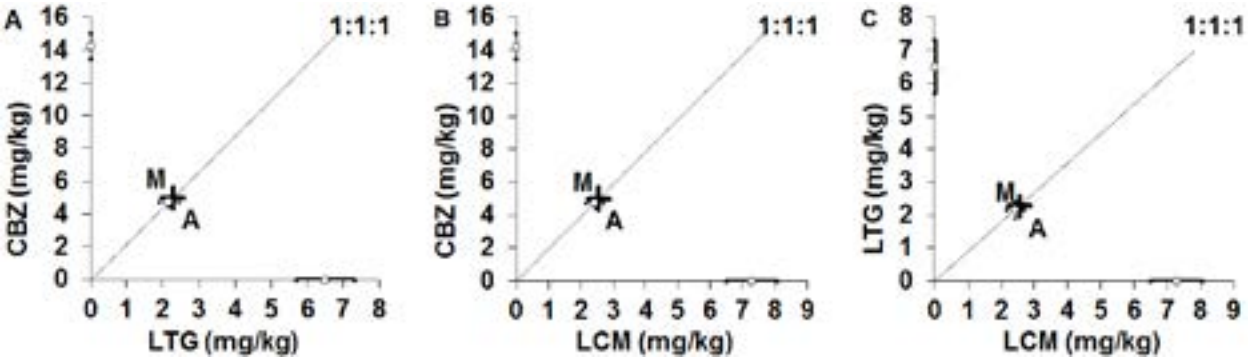

Figure 2A-C. Additive interactions for three-drug mixture of lacosamide (LCM), carbamazepine (CBZ) and lamotrigine (LTG) in the tonic-clonic seizure model in mice

Median effective doses (ED_{50} values ± S.E.M.) for lacosamide, carbamazepine and lamotrigine, that protected 50% of animals from maximal electroshock-induced seizure-induced seizures, are plotted on X-axis and Y-axis. Points M and A on each graph correspond to the experimentally-derived $ED_{50\ exp}$ (± S.E.M.) and theoretically calculated $ED_{50\ add}$ (± S.E.M.) values, respectively. The point M on each graph, reflecting the $ED_{50\ exp}$ value for the mixture of lacosamide, carbamazepine and lamotrigine for the fixed-ratio of 1:1:1, is plotted close to the point A, indicating additive interaction among drugs.

culated $ED_{50\ add}$ value (Table 2; Figure 2A-C), and thus, the interaction among the studied antiepileptic drugs was additive.

Discussion

In this study we found that the combination of three antiepileptic drugs (carbamazepine + lacosamide + lamotrigine) exerted additive interaction in the mouse maximal electroshock-induced seizure model. The obtained results are quite similar to those published earlier and documenting that two other three-drug combinations of lacosamide + carbamazepine + phenobarbital and lacosamide + lamotrigine + phenobarbital offered additive interactions in the mouse maximal electroshock-induced seizure model.[6,9] Only the combination of carbamazepine + phenobarbital + topiramate exerted supra-additive (synergistic) interaction in the mouse maximal electroshock-induced seizure model.[7] Additionally, the results observed in this study for the three-drug combination of carbamazepine + lacosamide + lamotrigine can be compared to those reported earlier for three two-drug combinations of carbamazepine + lamotrigine, lacosamide + carbamazepine and lacosamide + lamotrigine) in the same experimental seizure model. Available evidence indicates that the combination of carbamazepine with lamotrigine exerted sub-additive (antagonistic) interaction in the mouse tonic-clonic seizure model.[21] Unfortunately, no experimental evidence exists providing information on types of interactions for the combinations of lacosamide + lamotrigine and lacosamide + carbamazepine in the mouse maximal electroshock-induced seizure model. On the other hand, the combinations of lacosamide with lamotrigine and lacosamide with carbamazepine produced synergistic interactions in the mouse 6 Hz-induced (psychomotor) seizure model – another experimental model of seizures in mice.[22] However, despite evident differences between the maximal electroshock- and psychomotor 6 Hz-induced seizure models in mice (with respect to the clinical types of seizures), the synergistic interactions of lacosamide + lamotrigine and lacosamide + carbamazepine observed in the 6 Hz model, cannot be compared with our results because of some methodological problems.[22] More specifically, a crucial problem is associated with the anticonvulsant activities of the tested antiepileptic drugs, i.e., lamotrigine and carbamazepine in the 6 Hz model. It is widely accepted that conventional sodium channel blockers, including carbamazepine, phenytoin, lamotrigine are virtually ineffective (inactive) in the mouse 6 Hz model.[23] In contrast, Shandra and coworkers have determined the median effective doses (ED_{50} values) for lamotrigine, carbamazepine and phenytoin in the mouse 6 Hz model, which were 85 mg/kg, 48.1 mg/kg and 67 mg/kg, respectively.[22] However, these ED_{50} values were high enough to simultaneously produce impairment of motor coordination (acute adverse effects) in mice when subjected to the rotarod test.[22] It was found that lamotrigine in a dose of 85 mg/kg impaired motor coordination in 95% of the animals tested. Likewise, carbamazepine at a dose of 48.1 mg/kg and phenytoin in a dose of 67 mg/kg impaired motor coordination in 50% of the mice subjected to the rotarod test.[22] Of note, the experimentally-derived median toxic doses (TD_{50} values) for lamotrigine, carbamazepine and phenytoin, as denoted in our previous study in the rotarod test in mice were 31.8 mg/kg, 53.6 mg/kg and 61.7 mg/kg, respectively.[24] In the chimney test – another animal model assessing ataxia and impairment of motor functions in mice, the TD_{50} values for lamotrigine, carbamazepine and phenytoin were 28.7 mg/

kg, 53.3 mg/kg and 85.0 mg/kg, respectively.[21] Since the ED_{50} values for lamotrigine and carbamazepine determined by Shandra et al. in the 6 Hz test are higher than and/or similar to the TD_{50} values as documented in the rotarod and chimney tests, we should not compare them together. Thus, considering the above-mentioned facts, the question arises whether the observed protection from 6 Hz-induced seizures in mice receiving sodium channel blockers (lamotrigine, carbamazepine and phenytoin) in high doses (as documented by Shandra et al.) was mediated by the anticonvulsant mechanisms of action of the drugs or was related to the acute adverse effects produced by the drugs. Obviously, ataxia, flaccidity and any types of impairment of motor functions in animals may mimic the antiepileptic drugs-mediated anticonvulsant response to the 6 Hz-induced stimulation. Thus, the synergistic combinations of lacosamide with carbamazepine and lacosamide with lamotrigine observed in mice in the 6 Hz test, cannot be directly translated to the results observed in the maximal electroshock-induced seizure model. This was the reason not to take into account the results obtained by Shandra et al., when comparing them with our data.

On the other hand, lacosamide dose-dependently protected the animals from electrically-evoked seizures (i.e., maximal electroshock- and 6 Hz-induced seizures) after single exposition of the mice to electric current with different parameters of amplitude and intensity. It is worth mentioning that lacosamide, despite its sodium channel blocker properties protected the animals from 6 Hz seizures, while lamotrigine, carbamazepine or phenytoin did not. The ED_{50} value for lacosamide, as denoted in the 6 Hz model, ranged from 10.1 mg/kg [22] to 6.5 mg/kg [25], while in the mouse maximal electroshock-induced seizure model the ED_{50} value for lacosamide was 9.4 mg/kg.[26] Of note, the ED_{50} of lacosamide in both acute experimental models of epilepsy did not differ significantly. Simultaneously, the TD_{50} value for lacosamide as determined in our previous study was 33.77 mg/kg (i.e., 3–5-times higher than the ED_{50} value).[26] Finally, it may be concluded that the addition of the third drug lacosamide to the two-drug mixture comprised lamotrigine and carbamazepine can ameliorate the antagonistic effects exerted by the mixture of lamotrigine and carbamazepine in the mouse maximal electroshock-induced seizure model.

It should be stressed that two main reasons prompted us to select the combination of carbamazepine, lacosamide and lamotrigine to this isobolographic analysis. First, all the studied antiepileptic drugs in combination (carbamazepine, lamotrigine and lacosamide) are clinically efficacious against generalized tonic-clonic seizures and partial-onset convulsions in epilepsy patients.[27] Second, the general criteria of rational selection of antiepileptic drugs for combination, based on different molecular mechanisms of anticonvulsant action of the investigated drugs, were fulfilled.[13] As regards carbamazepine and lamotrigine, the drugs inhibit use-dependent voltage-gated sodium channels.[28] In contrast, lacosamide affects the slow inactivation of voltage-gated sodium channels without changing their fast inactivation.[28]

Generally, all the three-drug combinations tested in the mouse maximal electroshock-induced seizure model, including the combination of carbamazepine + lacosamide + lamotrigine, were associated with a substantial reduction of doses of particular drugs in mixture. The reduction of drug doses is essential from a clinical perspective because it allows to diminish side effects accompanied these multi-drug therapies.[13] Adverse effects occurring in epilepsy patients are usually the principal cause of withdrawal and discontinuation of antiepileptic drugs.[29] In this study we found that lacosamide, lamotrigine and carbamazepine combined together in doses corresponding to their $ED_{50\ exp}$ produced no side effects in animals challenged with the grip-strength test (assessing skeletal muscular strength in animals), passive avoidance task (evaluating acquisition and remembering processes in mice) and chimney test (assessing motor coordination in mice) (results not shown). All three behavioral tests used in this study firmly confirmed that the triple therapy did not produce any harmful adverse effects and the combination could be used clinically without any additional worries about patients' lives. On the other hand, all three used behavioral tests were sensitive enough to detect any side effects reported in animals receiving the antiepileptic drugs in combinations. For instance, we have documented earlier that the combination of tiagabine with valproate significantly impaired motor coordination in the chimney test.[30] Additionally, the combinations of vigabatrin with clonazepam or valproate significantly disturbed long-term memory in mice subjected to the standard variant of passive avoidance task.[31] As regards the grip-strength test, it was found that some antiepileptic drugs (in a dose-dependent manner) reduced skeletal muscular strength in mice.[32]

Finally, it can be concluded that the combination of carbamazepine + lacosamide + lamotrigine was expected to additively inhibit tonic-clonic seizures in experimental animals subjected to the maximal electroshock-induced seizure test. If the results from this study were translated to clinical conditions, the epilepsy patients inadequately treated with the antiepileptic drugs in monotherapy would profit from the combination of carbamazepine + lacosamide + lamotrigine.

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Conflicts of interest statement

The author has no conflicts of interest to disclose.

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