

Isobolographic additivity among lacosamide, lamotrigine and phenobarbital in a mouse tonic-clonic seizure model

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899-5276 (print), ISSN 2451-2680 (online)

Adv Clin Exp Med. 2018;27(7):881–886

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Funding sources

None declared

Conflict of interest

Prof. J.J. Łuszczki has been involved in the design and development of new antiepileptics and CNS drugs. He has received, within the last 5 years, an unrestricted research grant from GlaxoSmith-Kline (Brentford, UK). Dr. M.W. Kondrat-Wróbel has no conflict of interest to disclose.

Acknowledgements

This study was supported by grants (DS474/2012-2014 and DS474/2015-2016) from the Medical University of Lublin (Poland).

Received on July 14, 2016

Reviewed on December 28, 2016

Accepted on February 23, 2017

Abstract

Background. Epilepsy is a serious neurological disease affecting about 1% of people worldwide (65 million). Seizures are controllable with antiepileptic drugs (AEDs) in about 70% of epilepsy patients, however, there remains about 30% of patients inadequately medicated with these AEDs, who need a satisfactory control of their seizure attacks. For these patients, one of the treatment options is administration of 2 or 3 AEDs in combination.

Objectives. To determine the anticonvulsant effects of a combination of 3 selected AEDs (i.e., lacosamide – LCM, lamotrigine – LTG and phenobarbital – PB) at the fixed-ratio of 1:1:1 in a mouse maximal electroshock-induced (tonic-clonic) seizure model by using isobolographic analysis.

Material and methods. Seizure activity was evoked in adult male albino Swiss mice by a current (sine-wave, 25 mA, 500 V, 50 Hz, 0.2 s stimulus duration) delivered via auricular electrodes. Type I isobolographic analysis was used to detect interaction for the 3-drug combination.

Results. With type I isobolographic analysis, the combination of LCM, LTG and PB (at the fixed-ratio of 1:1:1) exerted additive interaction in the mouse maximal electroshock-induced (tonic-clonic) seizure model.

Conclusions. The combination of LCM with LTG and PB produced additive interaction in the mouse tonic-clonic seizure model, despite various molecular mechanisms of action of the tested AEDs.

Key words: antiepileptic drugs, isobolographic analysis, maximal electroshock, 3-drug combination

DOI

10.17219/acem/69132

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Introduction

Pharmacotherapy in epilepsy is still a challenge for clinicians and epileptologists because about one third of patients with epilepsy are not sufficiently treated with licensed antiepileptic drugs (AEDs) in monotherapy.¹ To reduce both seizure frequency and seizure activity in patients with refractory epilepsy, some combinations of 2 or 3 various AEDs should be considered as the most appropriate treatment regimen.^{2–5} Nowadays, some combinations of AEDs are recommended to refractory patients in 3 consecutive monotherapies with the established first-line AEDs.^{2,3} Generally, the combined application of AEDs possessing different anticonvulsant modes of action is clinically recommended to potentiate the anticonvulsive properties of AEDs.^{5,6}

Direct clinical assessment of the interactions among 3 drugs in epilepsy patients is extremely difficult due to ethical restrictions and methodological limitations. However, preclinical studies in animal *in vivo* models of epilepsy provide us with plausible proof on the accurate type of interactions occurring among the selected 3 AEDs. There is no doubt that in preclinical *in vivo* experiments based on isobolographic analysis of interaction, it is easily to confirm whether the theoretically selected AED combinations are beneficial or not.⁷ This is the reason to conduct experiments on animals with isobolography and subsequently translate the most favorable combinations into clinical settings.

Lacosamide (LCM) belongs to the 3rd generation of AEDs. Its unique molecular mechanisms of anticonvulsant action rely mainly on the enhancement of slow inactivation in voltage-gated sodium channels, preventing the sodium channels from opening and terminating the action potentials in hyperactive (epileptically changed) neurons for an extended time.^{8,9} Thus, LCM inhibits repetitive neuronal firing and stabilizes hyperexcitable neuronal membranes without affecting the physiological functions of the neurons.^{8,9} At present, LCM is licensed as an added drug prescribed to adult patients with partial-onset seizures that undergo generalization.^{10,11}

The combined administration of LCM with lamotrigine (LTG) and phenobarbital (PB), 2 commonly prescribed AEDs for patients suffering from tonic-clonic and partial onset seizures,¹² should produce a favorable 3-drug combination, offering protection from tonic-clonic seizures in patients with epilepsy who are resistant to monotherapeutic use of these AEDs.¹³ Furthermore, the combined treatment with LCM, LTG and PB theoretically fulfils the general principles of a perfect AED combination, giving a maximum of therapeutic effect and a minimum of side effects, due to diverse molecular mechanisms of action.⁶ However, this hypothetically ideal combination requires experimental confirmation in a maximal electroshock-induced seizure (MES) test in mice. Of note, the MES test is considered an animal test reflecting generalized tonic-clonic seizures and partial convulsions in humans.¹⁴

This study was aimed at characterizing the interactions among LCM, LTG and PB in the MES test in mice. We used type I isobolographic analysis to evaluate the interaction among the fully effective drugs (i.e., LCM, LTG and PB) because this method is designed to investigate interaction in preclinical experiments.¹⁵

Material and methods

In all experimental tests, we used 4-month-old male albino Swiss mice. The local Ethics Committee (University of Life Sciences, Lublin, Poland) approved all of the described protocols and procedures (License No. 45/2014).

LCM (UCB Pharmaceuticals, Brussels, Belgium), LTG (GlaxoSmithKline, Brentford, UK) and PB (Polfa, Warszawa, Poland) were dispersed in an aqueous solution of Tween 80 (Sigma-Aldrich, St. Louis, USA). The AEDs were injected intraperitoneally (*i.p.*) to animals at the pretreatment times as follows: LCM – 30 min, LTG and PB – 60 min, respectively.

Alternating current (25 mA, 0.2 s stimulus duration) generated by rodent shocker (Hugo Sachs Elektronik, March, Germany) was used to evoke maximal electroconvulsions (seizure activity) in mice, as described earlier.^{16,17} The animals, after receiving different drug doses, were subjected to the MES test and protection from maximal electroconvulsions was noted so as to construct dose-effect functions for the investigated AEDs (LCM, LTG and PB) when injected separately, as described earlier.^{16,18} The anticonvulsant properties of LCM, LTG and PB, when injected alone and combined at a fixed-ratio of 1:1:1, were presented in the form of median effective doses (i.e., ED₅₀ and ED_{50 exp} values).

Type I isobolographic analysis assessed the nature of the interactions among the 3 AEDs in combination.^{15,19} Of note, isobolography is a mathematical method that makes it possible to precisely determine the kind of interactions occurring when drugs are combined together. Isobolographic analysis distinguishes 5 classes of drug interactions: synergy (supra-additivity), additivity, indifference, relative antagonism (sub-additivity), and absolute antagonism (infra-additivity).^{20,21} Subsequently, a linear regression analysis based on logarithms and probits made it possible to construct dose-effect functions for the studied AEDs (LCM, LTG and PB).²² To establish the proper class of interactions, the investigated AEDs are usually administered together in fixed drug dose ratio combinations. However, to properly select the fixed-ratio combinations, one should verify whether the dose-effect functions of the studied AEDs if used alone are collateral. If the drugs have their dose-response effect curves parallel to each other, at least 3 fixed-ratios (i.e., 3:1, 1:1 and 1:3) are experimentally investigated with isobolography.²³ Of note, many more fixed-ratio combinations are sometimes required to establish the exact types of interactions for particular drug mixtures.²¹ On the other hand, for drugs

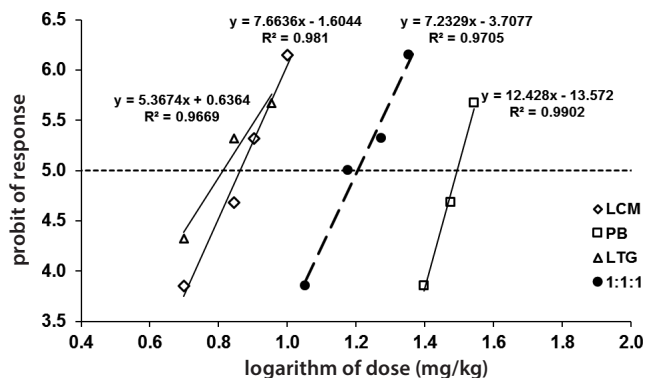


Fig. 1. Dose-effect functions of lacosamide, lamotrigine and phenobarbital injected separately and in combination (1:1:1) against maximal electroconvulsions in mice

Equations for the anticonvulsant effects of LCM, LTG, PB and their combination are presented graphically; R² – coefficient of determination.

having their dose-effect functions mutually nonparallel, only a fixed-ratio of 1:1 is analyzed with isobolography.²⁴ To the best of our knowledge, calculations of doses for particular drugs with their nonparallel dose-effect functions, applied in fixed-ratio combinations more diverse than 1:1 (i.e., 1:3 and 3:1), are not precise because the effects produced by the drug doses may be either over- or underestimated, ultimately providing improper information on the interaction among the drugs.^{15,24} The isobolographic rule, concerning parallelism of dose-response effect curves for the tested AEDs when they are used alone, is obligatory not only for 2-drug combinations, but also for 3- and 4-drug combinations.²⁴ After verifying the parallelism of dose-effect functions of LCM, LTG and PB, the median additive dose of the mixture of these 3 AEDs (ED_{50 add}) at a fixed-ratio combination of 1:1:1 was calculated according to the methods described earlier.^{16,17} Admittedly, LCM displayed parallelism to PB and LTG. However, these latter 2 AEDs, when compared to each other, had their dose-effect functions nonparallel. Therefore, only 1 fixed-ratio (1:1:1) underwent investigation in this study because, as mentioned earlier, other tested fixed-ratios (i.e., 1:1:3, 1:3:1, 3:1:1, 3:3:1, 3:1:3 or 1:3:3) could provide false classification of interaction.^{15,24–26} The anticonvulsant protection offered by the 3 AEDs in mixture 1:1:1 was presented in the form of ED_{50 exp} value, reflecting a dose of 3 drugs in mixture that protected 50% of the mice from MES-induced seizures.^{16,17}

The unpaired Student's t-test was used to statistically compare the ED_{50 exp} and ED_{50 add} values.

Results

LCM, LTG and PB injected separately protected the mice from maximal electroconvulsions. From log-probit equations of dose-effect functions, the ED₅₀ values for LCM, LTG and PB were calculated (Fig. 1), amounting to 7.27 ± 0.77 mg/kg (LCM), 6.50 ± 0.80 mg/kg (LTG) and 31.21 ± 2.04 mg/kg (PB), respectively (Table 1).

Parallelism of dose-effect functions was observed between LCM and LTG, as well as between LCM and PB (Table 1, Fig. 1). In contrast, dose-effect functions between LTG and PB were non-collateral (Table 1).

LCM, PB and LTG combined together at a fixed-ratio of 1:1:1 produced an anti-electroshock effect and the ED_{50 exp} value was 15.99 ± 1.80 mg/kg. With type I isobolographic analysis, the mixture of LCM, LTG and PB at a fixed-ratio of 1:1:1 produced additivity in mice subjected to the MES test (Fig. 2 A–C). The ED_{50 exp} value for the mixture of LCM, LTG and PB was 15.99 ± 1.80 mg/kg and displayed no significant difference from the ED_{50 add} value, which amounted to 14.99 ± 0.71 mg/kg.

Neither LCM, LTG and PB administered singly (in doses reflecting their ED₅₀ values from the maximal electroconvulsions), nor the combination of LCM, LTG and PB (1:1:1) disturbed memory processes in mice challenged with the passive avoidance task, changed skeletal muscular strength in mice in the grip-strength test, and impaired motor coordination in mice subjected to the chimney test (Table 2).

Discussion

It was found experimentally that the combination of LCM, LTG and PB (1:1:1) produced additivity in the mouse MES-induced seizure test. The illustrated additivity for the combination of the 3 AEDs (LCM, LTG and PB) can be compared to the effects reported from experiments conducted for the combinations of 2 AEDs (i.e., PB + LTG, LCM + LTG, and LCM + PB) in the same seizure test. Previously, we have reported supra-additivity (synergy) for the combination of PB with LTG at a fixed-ratio of 1:1 against

Table 1. Anticonvulsant properties of lamotrigine, lacosamide and phenobarbital when administered separately in the MES-induced seizure test in mice

Drug	ED ₅₀	n	Combination	#Parallelism
LTG	6.50 ± 0.80	24	LCM vs LTG	parallel
LCM	7.27 ± 0.77	16	LCM vs PB	parallel
PB	31.21 ± 2.04	16	LTG vs PB	nonparallel

Results are median effective doses (ED₅₀ values [mg/kg] ± SEM, where SEM is standard error of the mean) of LCM, LTG and PB administered separately in the MES-induced seizure test in mice. The drugs were administered systemically (i.p.), as follows: LCM – 30 min, LTG and PB – 60 min before the MES-induced seizures; n – total number of animals used at doses whose expected anticonvulsant effects ranged between the 4th and 6th probit; # test for parallelism was performed according to Litchfield and Wilcoxon.²² For more details see Fig. 1.

Table 2. Effects of lamotrigine, lacosamide and phenobarbital, administered alone and in mixture at a fixed-ratio of 1:1:1 on long-term memory in the passive avoidance task, muscular strength in the grip-strength test and motor performance in the chimney test in mice

Treatment [mg/kg]	Retention time [s]	Muscular strength [N]	Motor coordination impairment [%]
Vehicle + vehicle	180 (180; 180)	0.987 ±0.067	0
LTG (6.50) + vehicle	180 (180; 180)	0.948 ±0.062	0
LCM (7.27) + vehicle	180 (180; 180)	0.949 ±0.065	0
PB (31.21) + vehicle	180 (180; 180)	0.962 ±0.072	0
LCM (2.58) + LTG (2.31) + PB (11.09)	180 (180; 180)	0.952 ±0.071	0

Results are median retention times (with 25th and 75th percentiles in parentheses) from the passive avoidance task, mean muscular strengths ±SEM from the grip-strength test and percentage of animals with impairment of motor coordination from the chimney test. Each experimental group consisted of 8 mice. Doses of particular drugs (in parentheses) correspond to their ED₅₀ values from the MES test.

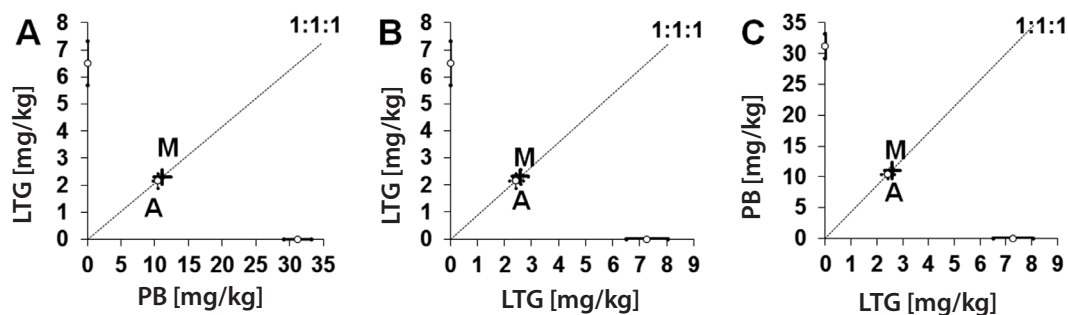


Fig. 2 A–C. Isobolograms illustrating additivity among lacosamide, lamotrigine and phenobarbital in mice subjected to maximal electroconvulsions

The ED_{50_{exp}} and ED_{50_{add}} values ±SEM for the mixture of LCM, LTG and PB (1:1:1) were plotted graphically as points M and A. A – additive interaction of PB with LTG; B – additive interaction of LCM with LTG; C – additive interaction of LCM with PB.

maximal electroconvulsions in mice.²⁷ Unfortunately, no information is available on the type of interactions for the combinations of LCM with PB and LTG against maximal electroconvulsions in mice. Nevertheless, in another experimental model of electrically-induced seizures, a 6 Hz psychomotor seizure test, the combination of LCM with LTG also produced supra-additivity (synergy) in mice.²⁸

The interaction among the 3 AEDs (LCM, LTG and PB) from this study can also be compared to the interactions observed previously for the combinations of LCM, carbamazepine (CBZ) and PB,¹⁶ or topiramate (TPM), CBZ and PB, against maximal electroconvulsions in mice.¹⁷ More specifically, a supra-additivity (synergy) for the combination of TPM, CBZ and PB was found in the MES test in mice.¹⁷ In contrast, the replacement of TPM with LCM in the mixture of LCM, CBZ and PB resulted in additivity with a trend to sub-additivity in the MES test in mice.¹⁶ On the other hand, the replacement of CBZ with LTG produced additivity without any trend to sub-additivity (antagonism) in the MES test, as documented in this study for LCM, LTG and PB. Thus, it can be concluded that the mixture of LCM, LTG and PB is more favorable than that of LCM, CBZ and PB and, simultaneously, less beneficial than that of CBZ, PB and TPM in the MES-induced seizure test.

From a theoretical viewpoint, the mixture of LCM, LTG and PB should be favorable and of particular relevance for

epileptic patients because of the diverse mechanisms of action of the component drugs.⁶ Of note, the conjunction of LCM, LTG and PB complies with theoretical presumptions related to combining drugs with diverse mechanisms of anticonvulsant action. As is widely known, LTG attaches to inactive voltage-dependent sodium channels and thus, it reduces the sustained repetitive firing in neurons.²⁹ LTG also blocks voltage-activated N- and P/Q-type calcium channels.³⁰ LCM selectively enhances slow inactivation of sodium channels and makes hyperexcitable neurons stable.^{8,31} Additionally, LCM allosterically blocks NMDA receptors containing the NR2B subunit.^{8,31} As regards PB, the drug enhances the effects of GABA_A receptors by attaching to an allosteric regulatory site within the complex of GABA_A-receptor and chloride ionophore.^{32–34} Furthermore, PB inhibits AMPA receptors.³⁵ Thus, taking into account the mentioned mechanisms of action of LCM, LTG and PB, the mixture of these AEDs should supra-additively inhibit generalized tonic-clonic seizures in mice challenged with maximal electroconvulsions because their mechanisms of anticonvulsant action complement each other. Unfortunately, in this study we barely found additivity among LCM, LTG and PB against maximal electroconvulsions in mice.

Much more attention should be paid to the doses of particular AEDs used – we combined LCM, LTG and PB in doses 3 times lower than their respective ED₅₀ values as denoted singly in the MES test in mice. This is the reason

we obtained results reflecting monotherapy with one effective AED, as regards the inhibition of maximal electroconvulsions in mice. Of note, any decrease in drug doses is beneficial due to the limitation of acute adverse effects exerted by AEDs used separately that would occur if the AEDs could be injected in higher doses into the mice.

Here we also determined the potential of acute adverse effects evoked by LCM, LTG and PB in a mixture with respect to their influence on motor performance, memory processes and skeletal muscular strength in the animals subjected to the chimney, passive avoidance and grip-strength tests, respectively. Generally, drugs or their mixtures that significantly affect motor performance in animals disturb coordination or make the animals unable to climb backward up the plastic Plexiglas tube within 60 s.^{36,37} Similarly, in the passive avoidance test, drugs or their mixtures that significantly affect the animals' long-term memory may impair memory acquisition and/or remembering in experimental animals, which manifests by the entrance of the mice into the dark box of the passive avoidance apparatus without a period of staying in the light compartment for up to 180 s.^{38,39} In the grip-strength test, drugs or their mixtures that significantly alleviate skeletal muscular strength induce flaccidity of the animal's bodies or reduce muscle tension in the mice that manifest in a low force in grasping and pulling the squared stainless steel wire mesh connected to the electronic dynamometer.^{40,41} Of note, the mixture of LCM, LTG and PB was administered to animals in doses corresponding to the ED_{50 exp} value obtained in the MES-induced seizure test. In this study, the mixture of LCM, LTG and PB had no significant impact on motor performance in mice since all of the tested animals climbed backward up the tube within 1 min (Table 2). Similarly, the AEDs in combination had no significant effect on memory processes in the animals tested – the mice remained in the light compartment of the apparatus for up to 3 min (Table 2). The combination of LCM, LTG and PB had no significant impact on the animals' skeletal muscular strength because no significant changes in grasping and pulling the wire mesh connected to the dynamometer were observed in the mice (Table 2). Considering the results from these 3 behavioral tests, it can be concluded that LCM, LTG and PB in combination (1:1:1) exerted no acute side effects as compared to the animals from the control group, suggesting that the AEDs in mixture, at doses reflecting the ED_{50 exp} from the MES test, were safe enough to be recommended for application in further clinical settings, especially in epileptic patients treated with these AEDs in monotherapy.

Summing up, it can be concluded that the combined application of LCM, LTG and PB exerted additivity against maximal electroconvulsions in mice. Finally, LCM combined with LTG and PB can modify the interaction among drugs from synergistic to additive, despite their various anticonvulsant modes of action.

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