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Interactions of C-11 with selected antiseizure medications in the mouse 6 Hz psychomotor seizure model – an isobolographic analysis

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Abstract

Introduction and Objective. Currently, polytherapy with two or more antiseizure medications (ASMs) remains one of the therapeutic options for patients with drug-resistant epilepsy. C-11, a pyrrolidine-2,5-dione derivative with previously demonstrated antiseizure activity, has been proposed as a potential adjunct compound. The aim of the present study is to characterize the type of interactions between C-11 and two clinically used ASMs, lacosamide (LCM) and valproate (VPA), in the mouse psychomotor (6 Hz, 32 mA) seizure model.

Materials and Method. In the 6 Hz seizure model, anticonvulsant activity was evaluated in male albino Swiss mice by assessing protection against psychomotor seizures. Dose–response relationships were established using log–probit analysis, and median effective doses (ED50 ± SEM) were calculated. Type I isobolographic analysis was applied to determine the interactions between C-11 and LCM or VPA administered in a fixed-ratio combination (1:1).

Results. Log–probit analysis demonstrated that C-11, LCM, and VPA administered separately produced clear anticonvulsant effects in the 6 Hz seizure model in mice, and linear regression confirmed parallel dose–response relationships for C-11 in combination with selected ASMs. Type I isobolographic analysis demonstrated that C-11 combined with LCM or VPA (fixed-ratio of 1:1) produced additive interactions in the 6 Hz model.

Conclusions. The study shows that mixtures of C-11 with LCM or VPA, evaluated by isobolographic analysis, produce additive interactions in the 6 Hz (32 mA) seizure test in mice. Further studies are required to clarify the mechanisms underlying these effects and to determine their potential clinical relevance.

Key words

psychomotor seizure model, drug interaction, isobolographic analysis, hybrid C-11, lacosamide, valproate

INTRODUCTION

Epilepsy is the second most prevalent neurological disease after stroke, affecting about 1% of the worldwide population [1]. Although there are more than forty antiseizure medications (ASMs) available, more than 30% of patients with epilepsy are resistant to one of the available monotherapy drugs [2]. Patients with drug-resistant epilepsy are at increased risk of reduced quality of life, psychosocial disorders, injury, and premature death, making the development of more effective therapies an urgent clinical need [3].

Failure of monotherapy with two successive agents from the same class provides a rationale for implementing polytherapy with two or more ASMs, ideally characterized by distinct mechanisms of action [4]. This underscores the need to develop novel, non-toxic compounds that exhibit both antiseizure and neuroprotective activity. In recent years, our group has searched for new natural and synthetic compounds that possess these qualities and could improve the effectiveness of existing antiepileptic drugs [5–7].

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One such substance is the 2-(2,5-dioxopyrrolidin-1-yl) propaneamide derivative C-11 (formerly KA-11), which is a hybrid substance created by combining fragments of ethosuximide, levetiracetam, and lacosamide [8]. The combination of three compounds with distinct mechanisms of action resulted in the development of a hybrid C-11 compound, demonstrating significant antiseizure activity in acute mouse seizure models: the maximal electroshock (MES) test, seizures induced by subcutaneous administration of pentylenetetrazol, pilocarpine, and the psychomotor seizure model [8, 9]. Additionally, in a mouse kindling model of epilepsy, C-11 suppresses seizures after repeated administration of PTZ [10]. Furthermore, our recent studies using C-11 in combination with four selected ASMs in the MES test in mice indicated a significant pharmacodynamic effect of C-11 on the anticonvulsant activity of two drugs: lacosamide and valproate [9].

In light of the reported anticonvulsant and neuroprotective effects of C-11, further investigation is warranted, particularly in an additional experimental epilepsy model. Therefore, the present study extends the research to the 6 Hz (32 mA) corneal stimulation model, which reflects focal (partial) seizures observed in patients with epilepsy [11, 12]. We decided to conduct studies to evaluate the effect of C-11 on

two ASMs, namely, lacosamide (LCM) and valproic acid (VPA), in the mouse 6-Hz (32 mA) psychomotor seizure model, in order to determine whether their interaction is synergistic, antagonistic or neutral.

MATERIALS AND METHOD

Animals. The study was conducted in adult male albino Swiss mice (22–26 g), with each experimental group consisting of eight mice.

All experimental procedures were approved by the Local Ethics Committee in Lublin, Poland (Approval No. 73/2018) and carried out in compliance with EU regulations (Directive 2010/63/EU) on the protection of animals used for scientific purposes.

Drugs administration. Pyrrolidine-2,5-dione derivatives (C-11), lacosamide (LCM, Vimpat®, UCB Pharma, Brussels, Belgium), valproate (VPA, sodium salt — Sigma-Aldrich, Poznań, Poland) were suspended in a 1% solution of Tween 80 (Sigma-Aldrich, Poznań, Poland) in distilled water and administered intraperitoneally (*i.p.*) in a volume of 10 ml/kg body weight. Compound C-11 was synthesized at the Department of Medicinal Chemistry, Jagiellonian University Medical College in Kraków, Poland, as previously described [8]. Each compound was administered 30 min prior to testing.

Psychomotor (6 Hz) seizure model. Seizures were induced in mice by corneal electrical stimulation (6 Hz, 0.2 ms pulse width, 32 mA, 3 s) using an S48 stimulator with a CCU1 constant current unit (Grass Technologies, West Warwick (RI), USA). After topical ocular anaesthesia, animals were stimulated and subsequently placed individually in Plexiglas cages to assess psychomotor seizure activity [13,14]. Median effective doses (ED_{50}) were determined after administration of ASMs within the following dose ranges: C-11 (10–50 mg/kg), LCM (2–12 mg/kg), and VPA (75–150 mg/kg).

Isobolographic analysis of interactions. Interactions between C-11 and LCM or VPA in the 6 Hz seizure model were evaluated using isobolographic analysis, as previously described [15,16]. Following determination of the ED_{50} values for C-11, LCM, and VPA administered individually, the theoretical median additive doses ($ED_{50\,add}$) for combinations of C-11 with LCM or VPA were calculated. Subsequently, parallelism of the dose–response curves for C-11, LCM, and VPA was assessed in the 6 Hz (32 mA) test [17, 18].

Statistical analysis. ED_{50} and $ED_{50\,exp}$ values (± S.E.M.) for C-11, LCM, and VPA, administered alone or in combination, were calculated using log–probit analysis in the 6 Hz (32 mA) test [19]. Differences between $ED_{50\,exp}$ and the corresponding $ED_{50\,add}$ values were analyzed using the unpaired Student's t-test [20]. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Anticonvulsant effects of C-11, LCM, VPA and combination C-11 with chosen ASMs in the psychomotor (6 Hz) seizure model in mice. Log–probit analysis demonstrated that C-11, LCM, and VPA, when administered individually, produced evident anticonvulsant effects in the 6 Hz seizure test in mice (Fig. 1 A-B). The experimentally determined ED_{50} values from the 6Hz test are presented on Figure 1 A-B. Linear regression analysis confirmed that the dose-response lines of C-11 and LCM (Fig. 1A) or VPA (Fig. 1B) in the 6Hz test were parallel to one another.

Type I isobolographic analysis of interactions between C-11 and chosen ASMs in the psychomotor (6 Hz) seizure model in mice. Isobolographic assessment (Type I) of parallel dose–response curves showed that combinations of C-11 with LCM (Fig. 2A) or VPA (Fig. 2B) at a fixed 1:1 ratio produced additive effects in the 6 Hz test in mice (Tab. 1). The experimentally determined ED_{50exp} values did not significantly differ from the theoretically calculated ED_{50add} values derived from the line of additivity (Tab. 1; Fig. 2 A-B).

DISCUSSION

Isobolographic analysis showed that the combination of C-11 with LCM or VPA in a 1:1 ratio caused an additive interaction in the psychomotor (6 Hz) seizure model in mice. Experimentally determined ED_{50} values for the combination were close to the theoretical additivity line, indicating that the observed anticonvulsant effect resulted from the summation of the actions of the individual components rather than from synergism or antagonism.

In contrast to these results, previous studies conducted in another experimental seizure model – the MES test – showed that compound C-11 (30 mg/kg, i.p.) significantly enhanced the antiseizure effects of LCM ($p < 0.001$) and VPA ($p < 0.05$). At the same time, no effect of C-11 on the efficacy of carbamazepine or lamotrigine was observed in this model [9].

C-11 binds relatively effectively to voltage-dependent neuronal Na^+ channels, indicating that its anti-seizure mechanism is most likely related to its effect on voltage-gated sodium channels. Furthermore, in studies of binding to voltage-dependent Ca^{2+} channels, it was found that C-11 has a marked affinity for L-type calcium channels, while showing no interaction with N-type channels [8].

LCM acts mainly by enhancing slow inactivation of sodium channels, without affecting their fast inactivation, unlike drugs such as carbamazepine or lamotrigine [21]. This mechanism leads to the inhibition of long-term pathological excitation of neurons and stabilization of their cell membranes. In addition, LCM modulates the CRMP-2 protein, limiting the formation of abnormal neuronal connections in the brain [22]. VPA, on the other hand, acts primarily by modulating the GABAergic system. It also changes nerve transmission by blocking calcium and sodium channels in nerve cells, although this has not yet been fully confirmed [23, 24].

Taking into account the mechanisms of action of both compound C-11 and the antiepileptic drugs studied, it seems most likely that C-11, by inhibiting sodium and/or calcium channels, enhances the anticonvulsant effects of LCM and VPA in the MES test by acting on neurophysiological pathways that are not fully utilized by these drugs when used alone. Despite the observed increase in anticonvulsant activity, no synergy between C-11 and the ASMs studied

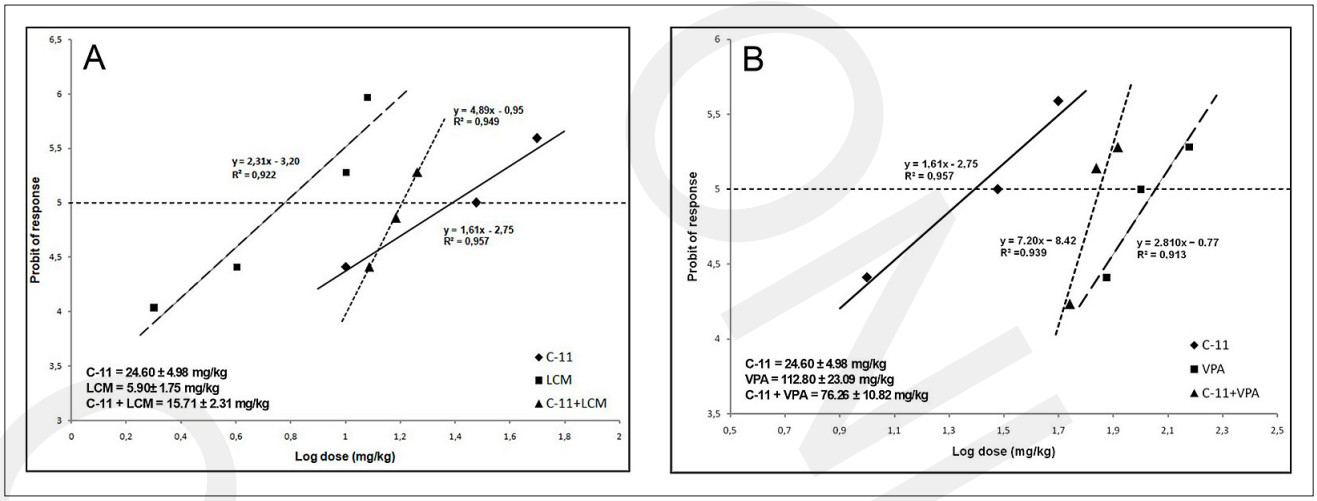

Figure 1 A-B. Log–probit analysis of dose–response effects of C-11 administered alone and in combination with lacosamide (LCM) or valproate (VPA) in the 6 Hz (32 mA) seizure model in mice.
Doses of C-11, LCM, VPA and their fixed-ratio combinations were transformed into logarithms to the base 10. The anticonvulsant effects produced by the drugs against seizures in the 6 Hz (32 mA) test in mice were transformed into classical probits. Each point represents a group of 8 mice. Linear regression equations characterizing the dose–response relationships for C-11, LCM, VPA administered alone, as well as for the fixed-ratio combinations of C-11+LCM (Fig. 1A) and C-11+VPA (Fig. 1B), are shown on the graphs. The experimentally determined median effective doses (ED_{50}) for C-11, LCM, VPA and their combinations were calculated from the regression equations and are indicated on the graphs

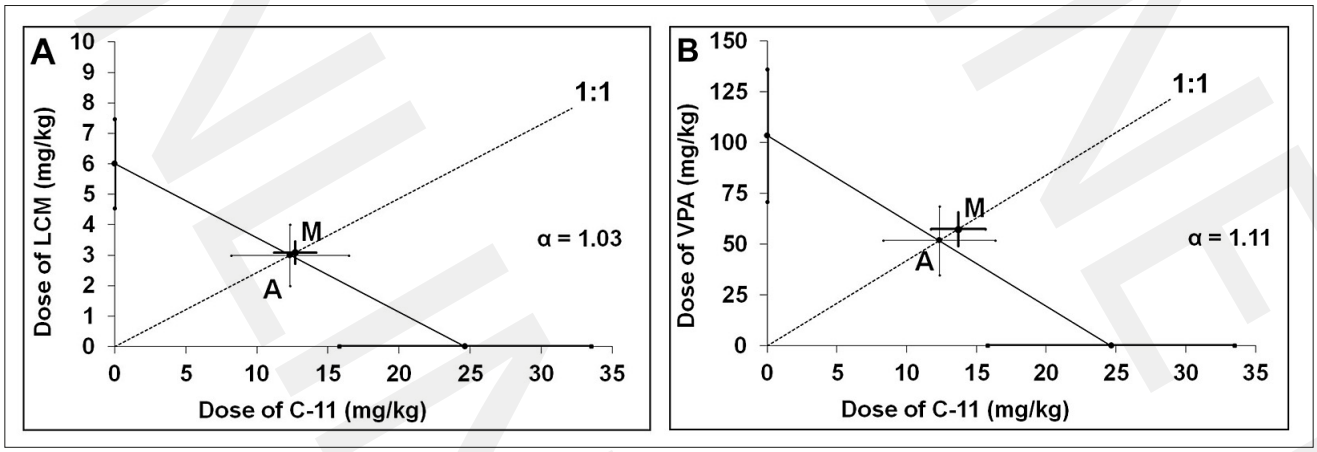

Figure 2 A-B. Isobolographic analysis of the interactions between C-11 and lacosamide (LCM) or valproate (VPA) in the 6 Hz (32 mA) seizure model in mice.
Median effective doses (ED_{50} ± S.E.M.) for C-11 and LCM (Fig. 2A) or C-11 and VPA (Fig. 2B) are plotted on the X- and Y-axes, respectively. The straight line connecting the ED_{50} values of the individual drugs represents the theoretical line of additivity. The point **A** represents the theoretically calculated additive ED_{50} values (ED_{50add}) for the fixed-ratio combination of C-11 with LCM or VPA, derived from the line of additivity. The point **M** represents the experimentally determined ED_{50} values (ED_{50exp}) for the fixed-ratio combination, corresponding to the doses of each drug in the mixture that produced 50% anticonvulsant protection in the 6 Hz (32 mA) seizure test in mice.

was found in this study, this may result from different mechanisms of generation and propagation of neuronal discharges characteristic of the experimental model used, as opposed to the MES test.

The 6 Hz psychomotor seizure model is an acute seizure model in mice [11] and rats [25], widely used as a rapid screening tool for anti-seizure compounds. It is based on low-frequency (6 Hz) corneal electrical stimulation (0.2 ms pulse width, 3 s duration), which induces seizures with limited propagation of epileptic activity. This contrasts with the generalized discharges observed in the classic MES model using high-frequency stimuli (50–60 Hz). Unlike the MES test, which produces generalized tonic–clonic seizures, the 6 Hz paradigm engages more selective neural circuits associated with focal seizure activity [11].

Furthermore, it should be noted that an important feature of the 6 Hz model is its reduced sensitivity to ASMs acting mainly through modulation of sodium channels, such as carbamazepine, phenytoin, or lacosamide, as well as lamotrigine, especially at higher stimulation intensities.

Table 1. Isobolographic characterization of interaction between C-11 and chosen ASMs at a fixed ratio of 1:1 in the psychomotor (6 Hz) seizure model in mice

LCM_{add}	$C\text{-}11_{add}$	$ED_{50\ add}$	n_{add}	$ED_{50\ mix}$	LCM_{mix}	$C\text{-}11_{mix}$	n_{mix}
2.95	12.3	15.25	40	15.71	3.04	12.67	24
VPA_{add}	$C\text{-}11_{add}$	$ED_{50\ add}$	n_{mix}	$ED_{50\ mix}$	VPA_{mix}	$C\text{-}11_{mix}$	n_{mix}
56.4	12.3	68.70	48	76.26	62.60	13.65	16

ED_{50mix} – experimentally determined dose of a mixture that increased the anticonvulsant effect by 50%; ED_{50add} – theoretically determined from the equation of additive, dose of a mixture that enhanced the anticonvulsant effect by 50%; $C\text{-}11_{add}$, LCM_{add}, VPA_{add} and $C\text{-}11_{mix}$, LCM_{mix}, VPA_{mix} – the doses of drugs that comprised the mixture, for both $ED_{50\ mix}$ and ED_{50add} values; n – the total number of animals at those doses whose expected anticonvulsant effects ranged between 4 -6 probits, denoted for the experimental mixture of drugs (n_{exp}) and theoretically calculated (n_{add}) from the equation of additivity.

In this model, relatively greater efficacy is demonstrated by certain GABAergic drugs, including clonazepam, phenobarbital, and tiagabine, which further emphasizes the difference in the mechanisms of seizure generation and propagation compared to MES [26].

Given the differences in the nature of neuronal discharges and in the pharmacological response profile of drugs between these experimental seizure models, this may explain the lack of statistically significant potentiation of LCM and VPA by C-11 in the 6 Hz test. Despite the modulation of sodium and calcium channels by C-11, the effect of these mechanisms under 6 Hz stimulation conditions is insufficient to produce a synergistic effect. Further neurochemical and electrophysiological studies are warranted to comprehensively elucidate the molecular and network- level mechanisms underlying the differences in pharmacological responses observed between these seizure models.

In this study, the concentrations of antiepileptic drugs in the blood or in the whole brain tissue were not determined. However, in previous studies, the results of the assessment of the effect of C-11 (30 mg/kg) on the total concentrations of LCM (4.4 mg/kg) and VPA (251.5 mg/kg) in brain tissue did not show a statistically significant increase in the level of either drug in combination with C-11. These finding indicate that, within the MES model, the interaction between the investigated substances is pharmacodynamic in nature, hence, for ethical considerations, concentration measurements were not performed in the current study [9]. Moreover, C-11 does not affect the activity of CYP liver enzymes; even at the highest doses used, this compound showed only a slight inhibitory effect on the CYP3A4 isoenzyme, which is responsible for the metabolism of over 50% of drugs. Additionally, C-11 also has no effect on CYP2D6 activity, considered the second most important cytochrome P450 isoenzyme in terms of potential metabolic interactions [8].

Additionally, Zagaja et al. [9] used three behavioural tests to assess the acute adverse effect profile of the combination of compound C-11 with CBZ, LCM, LTG, and VPA: the chimney test (assessment of motor coordination), the grip strength test (assessment of skeletal muscle strength), and the passive avoidance test (assessment of long-term memory). None of the tests revealed any abnormalities in the animals studied, either after administration of C-11 alone at a dose of 30 mg/kg or after its combination with antiepileptic drugs at doses corresponding to their ED_{50} values determined in the MES test.

CONCLUSIONS

In summary, the conducted studies showed that the hybrid C-11 modulates the antiseizure activity of selected ASMs in the mouse 6-Hz (32 mA) psychomotor seizure model. Combinations of C-11 with LCM or VPA were characterized by additive interaction, as confirmed by isobolographic analysis, and the results obtained indicate that the anticonvulsant effect results from the summation of the actions of individual drugs, rather than their synergistic action. Further research is needed to better understand the causes of the observed interactions and to assess their potential clinical significance.

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