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FULL-LENGTH ORIGINAL RESEARCH

# Synergism of lacosamide with established antiepileptic drugs in the 6-Hz seizure model in mice

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## Summary

**Purpose: Lacosamide (LCM, Vimpat) is an anticonvulsant with a unique mode of action. This provides lacosamide with the potential to act additively or even synergistically with other antiepileptic drugs (AEDs). The objective of this study was to determine the presence of such interactions by isobolographic analysis.**

**Methods: The anticonvulsant effect of LCM in combination with other AEDs including carbamazepine (CBZ), phenytoin (PHT), valproate (VPA), lamotrigine (LTG), topiramate (TPM), gabapentin (GBP), and levetiracetam (LEV) at fixed dose ratios of 1:3, 1:1, and 3:1, was evaluated in the 6-Hz–induced seizure model in mice. In addition, the impact of the combinations of LCM with the other AEDs on motor coordination was assessed in the rotarod test. Finally, AED concentrations were measured in blood and brain to evaluate potential pharmacokinetic drug interactions.**

**Key Findings: All studied AEDs produced dose-dependent anticonvulsant effects against 6-Hz–induced seizures. Combinations of LCM with CBZ, LTG, TPM, GBP, or LEV were synergistic. All other LCM/AED combinations displayed additive effects with a tendency toward synergism. Furthermore, no enhanced adverse effects were observed in the rotarod test by combining LCM with other AEDs. No pharmacokinetic interactions were seen on brain AED concentrations. Coadministration of LCM and TPM led to an increase in plasma levels of LCM, whereas the plasma concentration of PHT was increased by coadministration of LCM.**

**Significance: The synergistic anticonvulsant interaction of LCM with various AEDs, without exacerbation of adverse motor effects, highlights promising properties of LCM as add-on therapy for drug refractory epilepsy.**

**KEY WORDS: Pharmacodynamic drug interactions, Pharmacokinetic drug interactions, Motor impairment.**

Given that approximately one third of patients with epilepsy are prescribed polytherapy regimens in an attempt to control their seizures (Perucca, 1995; Genton & Roger, 1997; Kwan and Brodie, 2000), the search to identify optimal combinations of antiepileptic drugs (AEDs) has been an important and long-standing one. Assessment of AED combinations in the clinical setting is fraught with difficulties, not only due to the sheer number of potential combinations, but also due to the variation in the pharmacokinetics and pharmacodynamics of AEDs currently used in clinical practice (Stafstrom, 2010). Consequently, preclinical models are used as an alternative for the evaluation of pharmacodynamic drug interactions. To analyze the possible interaction between two different agents in a particular assay, various approaches can be used (Loewe, 1953, 1957; Wallin et al., 1970). According to Deckers et al. (2000) and Jonker et al. (2007), an isobolographic method used to evaluate interactions among AEDs is considered to be the optimal method for detecting synergy, additivity, or antagonism among AEDs in animal models of epilepsy. Of interest, Jonker et al. (2007) performed a post hoc analysis of several hundred experiments addressing potential anticonvulsant drug interactions, and found that synergism is less likely to be reported when isobolography is used compared with other methods, especially when potential pharmacokinetic interactions including those in the brain were investigated in parallel. Therefore, isobolography coupled with pharmacokinetic analysis of drug concentrations in plasma and brain appears to be the most stringent approach for determining synergism and was consequently adopted for the current experiments.

Lacosamide (LCM, Vimpat [Brussels, Belgium], R-2-acetamido-*N*-benzyl-3-methoxypropionamide) is a member of a series of functionalized amino acids that was synthesized as potential anticonvulsant compounds at the University of Houston (Conley & Kohn, 1987; Kohn et al., 1990, 1991). Upon testing in the National Institutes of Health (NIH) Anticonvulsant Screening Program it was found to

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have potential anticonvulsant effects in many animal models of epilepsy including maximal electroshock seizure (MES), the 6-Hz refractory seizure model, and sound-induced seizures in Frings mice (Hovinga, 2003; Stöhr et al., 2007). LCM also demonstrated antiepileptogenic properties against kindling acquisition (Brandt et al., 2006) and is active against refractory self-sustaining status epilepticus (Wasterlain et al., 2011). In contrast to its activity in electrically induced seizures, LCM displays little efficacy against chemoconvulsant-induced seizures (pentylenetetrazole, bicuculline, and picrotoxin) but is effective against cobalt-homocysteine– and lithium-pilocarpine–induced status epilepticus (Stöhr et al., 2007).

In initial radioligand-binding studies, neither LCM nor its metabolites showed interaction with >100 different neurotransmitter receptors or ion channels (Errington et al., 2006). Subsequent studies demonstrated that by enhancing slow inactivation of voltage-gated $Na^+$ channels, LCM has a unique mode of action (Errington et al., 2008), a finding corroborated in a recent comparative in vitro electrophysiology study (Niespodziany et al., 2012).

The aim of this study was to investigate potential pharmacodynamic interactions between LCM and conventional AEDs, that is, phenytoin (PHT), carbamazepine (CBZ), and valproate (VPA), lamotrigine (LTG), and also with the newer AEDs, topiramate (TPM), gabapentin (GBP), and levetiracetam (LEV) in the 6-Hz seizure model in mice using isobolographic analysis. The adverse effects of such combinations were evaluated in the rotarod test. In order to assess whether potential interactions in the 6-Hz seizure model were of pharmacodynamic or pharmacokinetic nature, brain and blood AED concentrations were determined at dose combinations for each AED with LCM that gave the highest level of synergism.

## Methods

### Animals

Experiments were performed on adult male CBA mice weighing between 20 and 28 g (Biomodel Service, Kyiv, Ukraine and Charles River, Margate, United Kingdom). The mice were kept in colony cages with free access to food and water, under standard laboratory conditions with natural light–dark cycle. After 1-week adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 10 mice for the pharmacodynamic experiments and 6 mice for the pharmacokinetic analysis. Each mouse was used only once. All experiments were performed between 9 a.m. and 4 p.m. Procedures involving animals and their care were conducted in accordance with current European Community regulations.

### Drugs

LCM, LEV, and GBP were synthesized in the chemical laboratories of UCB. CBZ and VPA were obtained from Sigma-Aldrich (Munich, Germany), PHT from BIOS CHEM (Pamiers, France), LTG from Glaxo Wellcome (Middlesex, United Kingdom), and TPM from RW Johnson Pharmaceutical Research Institute (Raritan, NJ, U.S.A.). All drugs were dissolved in 0.5% methylcellulose and administered intraperitoneally (i.p.) in a volume of 0.2 ml/20 g body weight (CBZ, VPA 15 min before either the 6-Hz seizure or the rotarod test; LCM, LTG 30 min; LEV, GBP 60 min; PHT, TPM 120 min). These pretreatment times before testing of AEDs were based on information from literature (Barton et al., 2001; Luszczki et al., 2006). Drug solutions were freshly prepared on each day of experimentation.

### 6-Hz seizure test

Psychomotor seizures were induced via corneal stimulation (6 Hz, 32 mA, 0.2 msec rectangular pulse width, 3 s duration) using a Grass S48 stimulator (Barton et al., 2001).

At the time of drug administration, a drop of 0.5% tetracaine was applied to the eyes of all animals. A drop of 0.9% saline was also placed on the eyes prior to the placement of corneal electrodes. Animals were manually restrained and released immediately following the stimulation and observed for the presence or absence of seizure activity, being characterized by stun, forelimb clonus twitching of the vibrissae, and Straub-tail. Protection was defined as the absence of a seizure (Barton et al., 2001). In control groups (with vehicle injection) all animals exhibited seizures. The protective efficacy of AEDs was determined as their ability to protect 50% of mice against 6-Hz seizures and expressed as respective median effective dose ($ED_{50}$) values. To evaluate each $ED_{50}$ value, at least four groups of 10 mice, after receiving progressive doses of an AED, were challenged with 6-Hz seizures. $ED_{50}$ values (with 95% confidence interval, CI) were calculated by computer probit analysis (Litchfield & Wilcoxon, 1949) and subsequently transformed into standard errors of mean (SEMs). After determination of each individual $ED_{50}$ for every AED, drug combinations were assessed in different dose ratios (1:3, 1:1, and 3:1) in order to determine the $ED_{50}$ of combinations (see "Pharmacodynamic/isobolographic analysis").

### Rotarod test

Impaired motor function was quantified by the rotarod test in mice according to Dunham and Miya (1957). The rotarod test was undertaken by use of a rod of 3-cm diameter, rotating at constant speed of 6 rpm. In this test, an acute neurologic deficit (adverse effects produced by AEDs) was indicated by the inability of the animals to maintain their equilibrium for at least 120 s on the rotating rod. The dose ratio assessed in this model was always 1:1 (i.e., corresponding to equi-effective doses).

### Pharmacokinetic analysis

To evaluate possible pharmacokinetic interactions of LCM coadministered with other AEDs (in a fixed

AED/LCM ratio of 3:1), separate groups of mice (n = 6/drug combination) were administered AEDs and LCM in combination and separately under the same conditions as for the 6-Hz test.

At time of sacrifice, that is, the timepoint of 6-Hz testing in the pharmacodynamic groups, blood (approximately 1 ml) and brain were collected. Brain samples were rinsed, weighed, and stored at −20°C until analysis. Blood samples were centrifuged and plasma collected and frozen at −20°C until analysis.

All AEDs were quantified in plasma using two validated liquid chromatography/electrospray ionization/mass spectrometry methods. One method quantified LCM, PHT, CBZ, LTG, TPM, GBP, and LEV, whereas a second method was used for VPA quantification. Both methods contain a sample preparation step using solid phase extraction (Waters OASIS HLB, Milford, MA, U.S.A.) followed by a reversed phase chromatographic separation using an ACE C18 chromatography column in the first case and a Pursuit PFP (Agilent Technologies, Santa Clara, CA, U.S.A.) column for VPA. Samples were subsequently analyzed by electrospray mass spectrometry using a Quattro Ultima (Micromass, Waters) mass spectrometer in the case of VPA analysis or an API3000 (Applied Biosystems, Foster City, CA, U.S.A.) mass spectrometer for the other compounds. Both methods were validated in accordance with the recommendations of the U.S. Food and Drug Administration (FDA) Guidance for Industry "Bioanalytical Method Validation"; May 2001), Shah et al. (2000), and Viswanathan et al. (2007). The methods were extended to the assay of these analytes in brain samples; these were diluted and homogenized in water (4 volumes of water to 1 volume of brain tissue) by ultrasonification before undergoing the same treatment as plasma samples. QC (quality control) samples prepared in the sample matrix were added to each analysis to check the validity of the obtained results.

### Pharmacodynamic/isobolographic analysis

The isobolographic analysis is based on a comparison of equieffective drug doses. In the present study, interactions between drugs, with respect to their anticonvulsant efficacy in the 6-Hz seizure test, were evaluated isobolographically according to the procedure elaborated by Porreca et al. (1990), Tallarida (1992), and Luszczki et al. (2006). The experimental ($ED_{mix}$) and theoretical additive ($ED_{add}$) were determined from the dose–response curves of combined drugs (Tallarida et al., 1997). $ED_{50}$ is defined as a dose of a drug protecting 50% of the animals against 6-Hz–induced seizures. $ED_{50mix}$ is an experimentally determined total dose of the mixture of two component drugs, which were administered in the fixed-ratio combination sufficient for a 50% protective effect. Conversely, $ED_{50add}$ represents a total additive dose of two drugs (calculated from the line of additivity), theoretically providing 50% protection against seizures. The respective 95% CIs of $ED_{mix}$ were calculated according to Litchfield and Wilcoxon (1949), and those of $ED_{add}$ according to Tallarida and Murray (1987), and subsequently transformed to SEM, according to a procedure described in detail by Luszczki et al. (2003).

To estimate the types of interactions, three fixed equieffective dose ratios of the drugs were examined (i.e., 1:3, 1:1, and 3:1) in the 6-Hz seizure model. To visualize the types of interactions between LCM and AEDs studied, the isoboles were drawn by plotting the points reflecting the respective doses of LCM (on the Y-axis) and doses of an AED (on the X-axis). The straight line connecting $ED_{50}$ values for the two tested drugs administered alone against 6-Hz–induced seizures, represents the theoretic isobole for additivity. If experimentally determined data points, reflecting the combinations of various fixed ratios, lie on this line, the drug effects are additive (no interaction). If the points fall significantly below the additive line, the two component drugs act synergistically. Conversely, antagonism may be recognized if these points are localized above the additive isobole.

Moreover, an interaction index for various fixed-ratio combinations of two AEDs in the 6-Hz test was calculated as a ratio $ED_{50mix}/ED_{50add}$. This ratio appears to describe the strength of interaction between two AEDs in isobolographic analyses quite well (Berenbaum, 1989; Tallarida, 2001, 2002). An index smaller than 0.7 indicates a synergistic effect and an index larger than 1.3 indicates an antagonistic effect, whereas an index in between these two values indicates purely additive interaction (Luszczki et al., 2003).

AED levels as determined in the pharmacokinetic experiments are expressed as microgram drug per milliliter plasma and milligram tissue for blood and brain, respectively. The evaluation for potential pharmacokinetic interactions was performed by comparing drug levels following coadministration with vehicle versus drug levels following co-administration of an AED or LCM. Relative levels outside the bioequivalence range (i.e., below 80% or above 125%) were considered clinically significant.

## Results

### Anticonvulsant effects of AEDs against 6-Hz–induced seizures in mice

All studied AEDs (LCM, LTG, VPA, CBZ, PHT, LEV, TPM, and GBP) produced dose-dependent anticonvulsant effects against 6-Hz seizures in mice. The $ED_{50}$ values for the drugs administered alone are presented in Table 1. Among the AEDs tested, LCM displayed the highest potency (i.e., lowest $ED_{50}$ value).

### Isobolographic analysis of interactions between LCM and various AEDs

Based on $ED_{50}$ values determined for each AED individually, a theoretical additive $ED_{50}$ for drug combinations ($ED_{50add}$ values) was calculated for three fixed ratios (1:3, 1:1, and 3:1). Subsequently, the experimental $ED_{50mix}$

**Table 1. Effects of LCM and other antiepileptic drugs against 6-Hz seizures in mice**

| Drug | $ED_{50}$ (mg/kg, i.p.)[a] |
|---|---|
| Lacosamide | 10.1 (4.5–19.8) |
| Lamotrigine | 85.0 (48.0–145.2) |
| Valproate | 132.0 (78.7–205.6) |
| Carbamazepine | 48.1 (27.4–81.5) |
| Phenytoin | 67.0 (39.6–111.6) |
| Levetiracetam | 22.8 (9.97–48.74) |
| Topiramate | 271.7 (143.0–493.0) |
| Gabapentin | 224.0 (108.0–428.0) |

i.p., intraperitoneal.
[a]Confidence intervals in brackets.

values were determined for the same fixed-ratio combinations in the 6-Hz seizure test (Table 2). The isobolographic analysis demonstrated pure additive interactions between LCM + PHT (Fig. 1A) and LCM + VPA (Fig. 1B) in all fixed-ratio combinations. The combinations of LCM with LTG (Fig. 1C), TPM (Fig. 1D), and GBP (Fig. 1E) exerted additive interactions for low doses of the test AED combined with high doses of LCM (i.e., at a fixed ratio of 1:3). For the 1:1 ratios, synergistic effects were observed between LCM and LTG, TPM, or GBP, respectively. Similarly, synergistic interactions were noted for high doses of LTG, TPM, or GBP, respectively, combined with a low dose of LCM (i.e., fixed ratio of 3:1; Table 2). Interaction between LCM + CBZ (Fig. 1G) and LCM + LEV (Fig. 1F) were synergistic across all ratios (Table 2), since interaction indices for these combinations were lower than 0.7 (Table 2).

Table 2 summarizes the types of interactions observed between seven drug pairs with respect to the 6-Hz seizure test.

### Rotarod test

LEV, LCM, and VPA induced the least rotarod impairment at the $ED_{50}$ in the 6-Hz test, whereas LTG, CBZ, and PHT induced the greatest impairment (≥50% rotarod impairment at the $ED_{50}$ in the 6 Hz test, Table 3). A 1:1 combination of LCM and the other AEDs at the theoretical calculated $ED_{50}$ for the combination in the 6-Hz test (i.e., ½ $ED_{50}$ for LCM and ½ $ED_{50}$ for the other AED) induced equal or less motor impairment when compared to that induced by the $ED_{50}$ in the 6-Hz test for each of the other AEDs administered alone (Table 3).

### Pharmacokinetic analysis

Plasma and brain concentrations of LCM and the other AEDs, administered at $ED_{50}$ dose ratios of 3:1 are presented in Table 4. None of the relative brain concentrations of either AEDs or LCM fell outside the bioequivalence range. In plasma, LCM slightly increased PHT concentrations (131%), whereas TPM increased LCM levels (131%).

Minor pharmacokinetic interactions (i.e., within bioequivalence range but statistically significant) were seen between PHT and LCM in the brain, and between GBP and LCM in the plasma, resulting in increased concentrations for both AEDs; LCM levels in brain and blood were also slightly but significantly increased by TPM and PHT, respectively.

## Discussion

This study demonstrates that LCM fully protected mice from 6-Hz psychomotor seizures with an $ED_{50}$ of 10.1 mg/kg. This dose corresponds well with the $ED_{50}$ (9.9 mg/kg) determined in the anticonvulsant drug screening program of National Institute of Neurological Disorders and Stroke (NINDS) but is 2–3 times higher than the $ED_{50}$ needed for protection of MES in mice and rats (Stöhr et al., 2007). Furthermore, the antiseizure effect of various AEDs (LTG, VPA, CBZ, PHT, TPM, and GBP; Table 1) in this study are in agreement with those reported by Barton et al. (2001). LCM is the drug with the highest potency in this model when compared with the other tested AEDs. In contrast to the sodium channel blocking AEDs, PHT, LTG, and CBZ, there was a clear separation between the doses providing antiseizure activity in the 6-Hz model compared with those inducing motor impairment in the rotarod test of LCM.

The 6-Hz test is regarded as a model for treatment-resistant seizures based on the observation that LEV, a highly clinically effective AED provides complete protection in this model despite being inactive in a variety of other models (Gower et al., 1992; Löscher and Honack, 1993; Klitgaard et al., 1998; Patsalos, 2004). Data presented herein confirm the differences in the pharmacologic profile of the MES and 6-Hz seizure models. Barton et al. (2001) used the immediate early gene c-Fos as a marker of seizure-induced neuronal activation and showed that 6-Hz–induced seizures result in a clearly different pattern of neuronal activation than that observed following maximal electroshock or PTZ-induced seizures. Using the 2-deoxyglucose technique, Duncan and Kohn (2005) demonstrated that LCM attenuated this specific pattern of neuronal activation but had no effect on basal patterns. This is fully in line with the present finding of antiseizure effects of LCM at doses inducing minimal motor deficits.

The isobolographic analysis revealed that LCM acts synergistically with LEV and CBZ across all examined fixed ratios (Fig. 1F,G). LTG, TPM, and GBP in combination with LCM (at the fixed ratios of 1:1 and 3:1) were similarly associated with synergistic interactions and showed tendency towards synergistic interactions at fixed ratios of 1:3 (Fig. 1C–E). Additionally, it was found that the interactions between LCM and VPA or PHT were additive with a tendency toward synergism for protection against 6-Hz–induced seizures (Fig. 1A,B).

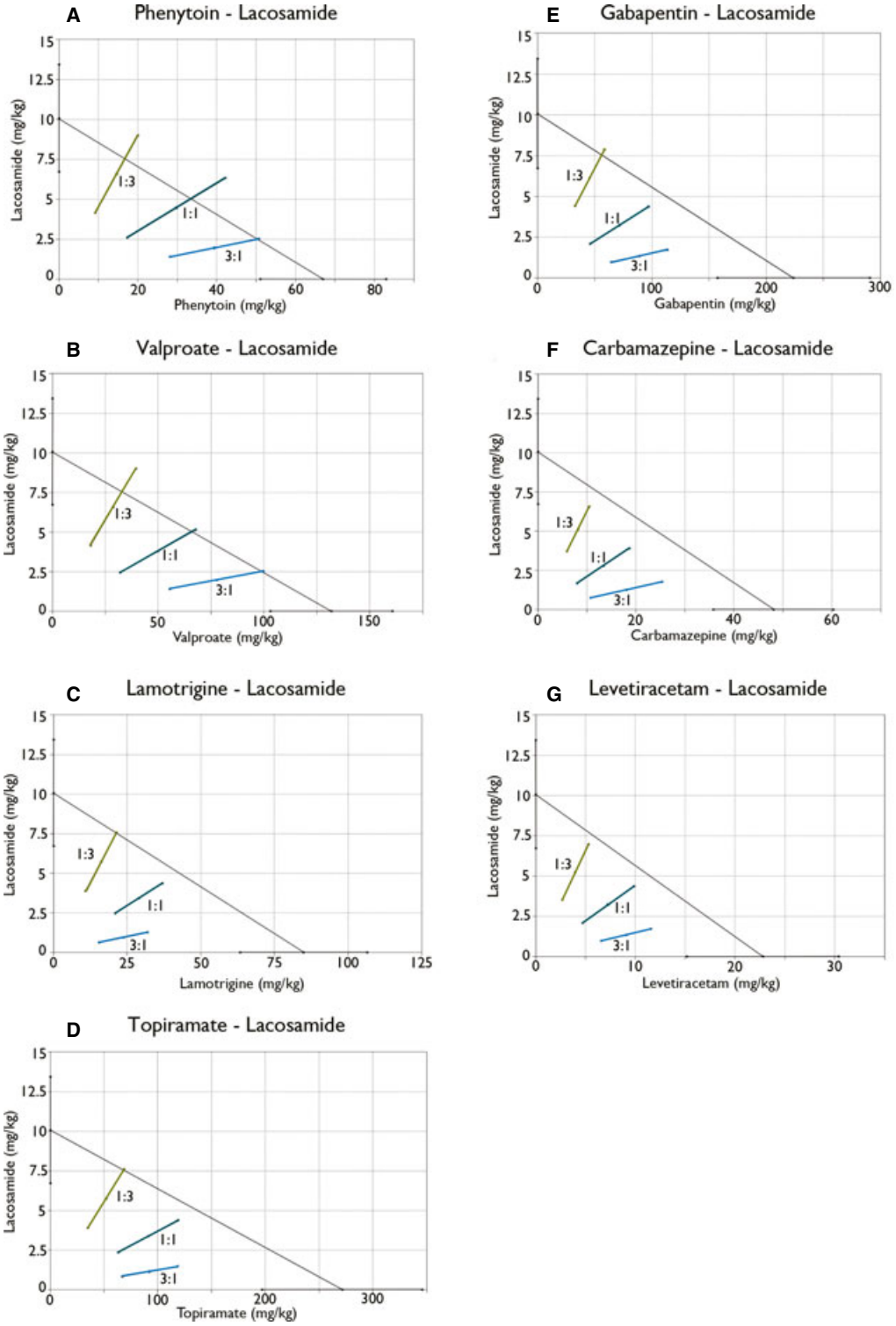


**Figure 1.**
Isobologram showing interactions between AEDs and lacosamide for three fixed-ratio combinations in the 6-Hz–induced seizure model in mice. Median effective dose ($ED_{50}$) values for the tested AEDs and LCM are placed on the X- and Y-axes, respectively. The straight line connecting these both $ED_{50}$ values represents the theoretic line of additivity for a continuum of different fixed-dose ratios. The solid points depict the experimentally derived $ED_{50mix}$ values (with 95% confidence limits as the error bars) for total dose expressed as the proportion of AED and LCM that produce a 50% effect.

**Table 2. Isobolographic characterization of the interaction between lacosamide and various AEDs in the 6-Hz seizures tests in mice**

| FR | $ED_{50add}$ ± SEM | $ED_{50mix}$ ± SEM | α | Interpretation |
|---|---|---|---|---|
| Lacosamide + phenytoin | | | | |
| 3:1 | 24.3 ± 6.5 | 21.2 ± 7.9 | 0.87 | Additivity |
| 1:1 | 38.5 ± 9.7 | 34.2 ± 14.3 | 0.89 | Additivity |
| 1:3 | 52.8 ± 12.8 | 41.4 ± 11.7 | 0.78 | Additivity |
| Lacosamide + valproate | | | | |
| 3:1 | 40.5 ± 9.7 | 35.4 ± 13.1 | 0.87 | Additivity |
| 1:1 | 71.0 ± 16.1 | 53.7 ± 19.3 | 0.76 | Additivity |
| 1:3 | 101.5 ± 22.4 | 79.6 ± 22.5 | 0.78 | Additivity |
| Lacosamide + lamotrigine | | | | |
| 3:1 | 28.8 ± 7.9 | 21.9 ± 7.0 | 0.76 | Additivity |
| 1:1 | 47.5 ± 12.4 | 32.3 ± 8.9 | 0.68 | Synergism |
| 1:3 | 66.2 ± 17.0 | 24.7 ± 8.6 | 0.37 | Synergism |
| Lacosamide + topiramate | | | | |
| 3:1 | 75.5 ± 21.2 | 57.7 ± 18.7 | 0.76 | Additivity |
| 1:1 | 140.9 ± 38.9 | 94.4 ± 28.9 | 0.67 | Synergism |
| 1:3 | 206.3 ± 56.7 | 93.7 ± 25.8 | 0.45 | Synergism |
| Lacosamide + gabapentin | | | | |
| 3:1 | 63.6 ± 19.2 | 51.8 ± 14.5 | 0.82 | Additivity |
| 1:1 | 117.1 ± 35.0 | 74.8 ± 26.5 | 0.64 | Synergism |
| 1:3 | 170.6 ± 50.9 | 90.4 ± 25.0 | 0.53 | Synergism |
| Lacosamide + carbamazepine | | | | |
| 3:1 | 19.6 ± 5.6 | 13.3 ± 3.7 | 0.68 | Synergism |
| 1:1 | 29.1 ± 7.8 | 16.2 ± 6.5 | 0.56 | Synergism |
| 1:3 | 38.6 ± 10.0 | 19.3 ± 7.8 | 0.50 | Synergism |
| Lacosamide + levetiracetam | | | | |
| 3:1 | 13.2 ± 4.4 | 9.2 ± 3.0 | 0.69 | Synergism |
| 1:1 | 16.4 ± 5.5 | 10.5 ± 3.7 | 0.64 | Synergism |
| 1:3 | 19.6 ± 6.6 | 10.4 ± 2.9 | 0.53 | Synergism |

FR, fixed ratio of drug dose combinations; α, interaction index for fixed ratio combinations calculated as the ratio of the experimental and theoretical additive $ED_{50}$ ($[ED_{50mix}]/[ED_{50add}]$). An index <0.7 indicates a synergistic effect and an index >1.3 indicates an antagonistic effect, whereas an index between the two values indicates purely additive interaction.

None of the drug combinations studied exhibited infraadditive effects (antagonism between drugs for antiseizure efficacy) or potentiation of motor incoordination. This is, of course, a desirable interaction for any drug combination, since the result is an improved therapeutic index.

It is of interest to note that, in general, a combination of low-dose LCM with a high dose of another AED yielded higher levels of synergism as the converse. This, and the observation that LCM acted at least additively with all other tested AEDs makes it an ideal add-on drug for patients with treatment-resistant seizures.

Pharmacokinetic analysis of the dose combinations with highest levels of synergism revealed no brain concentration of either anticonvulsant drug increased beyond bioequivalence levels by coadministration of LCM or vice versa. In plasma, LCM increased the concentration of PHT while its levels were increased by coadministration of TPM. This increase detected in mice was a relatively small and just outside the bioequivalence range of ±25%. In contrast, a similar interaction was not seen in any of the pivotal clinical trials (Ben-Menachem et al., 2007; Halasz et al. 2009, Chung et al., 2010). TPM inhibits the cytochrome P450 (CYP) enzyme 2C19 (Anderson, 1998), whereas LCM is metabolized by this enzyme to its pharmacologically inactive desmethyl metabolite. However, a clinical trial investigating the effect of the prototypical CYP2C19 inhibitor omeprazole on LCM pharmacokinetics yielded no effect (Lacosamide SPC, 2008). Moreover, no differences in LCM exposure were observed in extensive and poor metabolizers of CYP2C19. Therefore, the observed interaction between TPM and LCM in this experiment is most likely not clinically relevant. LCM did not affect metabolizing enzymes in vitro; therefore, the mechanism for increased PHT plasma levels by LCM remains unclear (Lacosamide SPC, 2008).

The mechanistic basis of the additive or synergistic interactions observed between LCM and other AEDs is unlikely to be a pharmacokinetic one. The site of action of AEDs is in the brain, and brain levels of anticonvulsant drugs were not influenced by LCM, and vice versa, at the single time point tested in this study. This finding is in line with in vitro results showing that LCM does not inhibit or induce drug metabolizing enzymes, nor is it metabolized to a significant extent by any one CYP subtype (Beyreuther et al., 2007). In addition, clinical population pharmacokinetic analyses provided no evidence for any effect of LCM on plasma levels

**Table 3. The effects of various AEDs administered alone and in combination with lacosamide on motor coordination in the rotarod test in mice**

| Dose (6-Hz model) | Treatment (mg/kg, i.p.) | Mice impaired (%) |
|---|---|---|
| $ED_{25}$ | Lacosamide (5.0) | 0 |
| $ED_{50}$ | LCM (10) | 20 |
| $ED_{25}$ | Lamotrigine (42.5) | 40 |
| $ED_{50}$ | LTG (85) | 95 |
| $ED_{50add}$ (theoretical) | LCM (5.0) + LTG (42.5) | 50 |
| $ED_{25}$ | Carbamazepine (24.0) | 20 |
| $ED_{50}$ | CBZ (48) | 50 |
| $ED_{50add}$ (theoretical) | LCM (5.0) + CBZ (24.0) | 20 |
| $ED_{25}$ | Valproate (66.0) | 0 |
| $ED_{50}$ | VPA (132) | 25 |
| $ED_{50add}$ (theoretical) | LCM (5.0) + VPA (66.0) | 10 |
| $ED_{25}$ | Phenytoin (33.0) | 30 |
| $ED_{50}$ | PHT (67) | 50 |
| $ED_{50add}$ (theoretical) | LCM (5.0) + PHT (33.0) | 20 |
| $ED_{25}$ | Levetiracetam (11.4) | 0 |
| $ED_{50}$ | LEV (23) | 0 |
| $ED_{50add}$ (theoretical) | LCM (5.0) + LEV (11.4) | 0 |
| $ED_{25}$ | Topiramate (133.9) | 0 |
| $ED_{50}$ | TPM (272) | 35 |
| $ED_{50add}$ (theoretical) | LCM (5.0) + TPM (133.9) | 0 |
| $ED_{25}$ | Gabapentin (112.0) | 10 |
| $ED_{50}$ | GBP (224) | 35 |
| $ED_{50add}$ (theoretical) | LCM (5.0) + GBP (112.0) | 20 |

i.p., intraperitoneal.

Results are expressed as the percentage of animals showing impairment of motor coordination. Each group consisted of at least 10 animals. Fisher's exact test was used for statistical comparisons.

of AEDs or vice versa (Doty et al., 2007; Ben-Menachem, 2008), and there is no evidence for species differences in the metabolism of LCM. Therefore, the interactions found in the present study appear to be purely of pharmacodynamic nature.

As noted, the mode of action of LCM appears to be unique. It displays rapid onset of anticonvulsant and analgesic effects that are thought to be due to its ability to selectively enhance slow inactivation of voltage-gated $Na^+$ channels (Errington et al., 2008; Sheets et al., 2008). Because synergistic interactions are likely between drugs with complementary mechanisms of action (Deckers et al., 2000), such an interaction between LCM and LEV across all ratios would have been anticipated. LEV exerts its therapeutic activity by binding to the synaptic vesicle protein SV2A (Lynch et al., 2004), and therefore it has a completely different mechanism of action from that of LCM.

Of interest, LCM displayed additive to synergistic effects with three AEDs that mainly act via voltage-gated $Na^+$ channels: CBZ, PHT, and LTG. These three AEDs mainly enhance fast inactivation of $Na^+$ channels and are relatively ineffective in the 6-Hz psychomotor seizure test. In contrast, LCM does not affect fast inactivation and is relatively potent in the 6-Hz model. This suggests that the enhancement of fast and slow inactivation represents distinct mechanisms. Moreover, the current experiments indicate that these two mechanisms can complement each other to pro-

**Table 4. Plasma and brain concentrations of antiepileptic drugs administered alone or in combination with lacosamide at 3:1 $ED_{50}$ dose ratios**

| | Plasma concentration (μg/ml) | | | | Brain concentration (μg/g) | | | |
|---|---|---|---|---|---|---|---|---|
| | AED | | LCM | | AED | | LCM | |
| VEH/LCM | | | 3.0 ± 0.4 | | | | 1.84 ± 0.21 | |
| CBZ/VEH | 17.5 ± 1.5 | | | | 24.2 ± 2.2 | | | |
| CBZ/LCM | 19.0 ± 1.9 | 109% | 3.0 ± 0.3 | 101% | 25.7 ± 6.6 | 106% | 1.65 ± 0.17 | 90% |
| GBP/VEH | 46.3 ± 8.1 | | | | 28.8 ± 4.2 | | | |
| GBP/LCM | 52.6 ± 3.9 | 114% | 3.4 ± 0.3 | 114% | 31.2 ± 2.9 | 108% | 1.81 ± 0.15 | 99% |
| LTG/VEH | 33.2 ± 2.8 | | | | 53.7 ± 3.9 | | | |
| LTG/LCM | 36.0 ± 4.2 | 109% | 2.9 ± 0.3 | 95% | 54.8 ± 6.0 | 102% | 1.70 ± 0.14 | 92% |
| LEV/VEH | 18.3 ± 1.7 | | | | 10.0 ± 0.7 | | | |
| LEV/LCM | 18.7 ± 1.3 | 102% | 3.1 ± 0.2 | 102% | 9.67 ± 0.88 | 97% | 1.83 ± 0.08 | 99% |
| PHT/VEH | 15.5 ± 2.7 | | | | 21.7 ± 3.4 | | | |
| PHT/LCM | 20.3 ± 1.4 | **131%** | 3.6 ± 0.2 | 121% | 26.1 ± 2.5 | 120% | 2.17 ± 0.06 | 118% |
| TPM/VEH | 161.2 ± 21.3 | | | | 85.5 ± 8.2 | | | |
| TPM/LCM | 164.8 ± 26.4 | 102% | 3.9 ± 0.6 | **131%** | 86.8 ± 13.1 | 102% | 2.24 ± 0.51 | 122% |
| VPA/VEH | 242.5 ± 17.4 | | | | <125.0[a] | | | |
| VPA/LCM | 236.8 ± 12.5 | 98% | 3.2 ± 0.3 | 106% | <125.0[a] | n.a. | 1.91 ± 0.31 | 104% |

AED, antiepileptic drug; LCM, lacosamide; VEH, vehicle; CBZ, carbamazepine; GBP, gabapentin; LTG, lamotrigine; LEV, levetiracetam; PHT, phenytoin; TPM, topiramate; VPA, valproate.

Results are given as absolute concentration (mean ± standard deviation) of the AED and LCM and as relative values comparing drug administered alone and in combination with LCM. Relative values outside the bioequivalence range (80–125%) are highlighted in bold.

N = 6 per drug combination except for VEH/LCM (n = 24).

[a]Levels were below the lower limit of quantification that is, 25 μg/ml.

duce additive or even synergistic effects. Of interest, this possibility was already theoretically anticipated by Staffstrom (2010) who argued that synergism could also arise from modulating a cellular target (e.g., the voltage-gated sodium channel) by two different mechanisms (e.g., enhancing fast and slow inactivation). However, it is important to emphasize that CBZ, LTG, and PHT may also have additional modes of action that may influence their interactions with other AEDs.

It should be emphasized that the dose ratio may be critical for the final outcome of type of an interaction between AEDs. This is evident from the present results that for some dose ratios the interactions were simply additive (e.g., LCM + GBP, 1:3) and in other dose ratios they were synergistic. Results from other studies also point to this finding (Gordon et al., 1993; Borowicz et al., 2000). For instance, Borowicz et al. (2002) by using the MES test in mice, observed that GBP in combination with CBZ showed an additive interaction at a dose ratio of 1:1, but for many other dose ratios it displayed significant synergistic interactions. However, the interpretation of dose ratios other then 1:1 may also be affected by the shape of the individual dose-response curves of the single AEDs.

From the analysis of the adverse activity in the rotarod test it may be postulated that the combinations displaying clear-cut synergy or additivity in the 6-Hz seizure test were not associated with impairment of motor coordination in mice.

Further studies are needed to analyze both anticonvulsant activity and adverse potential of combination of LCM and other AEDs. The limitations of the current experiments are the restriction to acute rather chronic dosing and to using only one experimental model of seizures. With different tests (e.g., MES or PTZ-induced seizures) and chronic dosing other results might have been obtained for the anticonvulsant efficacy of these combinations.

The limitations noted above could further affect the interpretation of the results when extrapolating them to predict efficacy or safety responses in humans. In a post hoc analysis of data pooled from three pivotal trials in patients with focal epilepsy, adjunctive LCM effectively reduced seizures regardless of concomitant AED regimen (Chung et al., 2010). Further analyses suggested that combining LCM with non–$Na^+$-channel blocking AEDs resulted in greater efficacy than a combination of LCM with traditional $Na^+$-channel blocking AEDs (Saké et al., 2010). This observation does not fully reflect the findings of the current experimental studies, in that synergy was observed with both LEV, a non–$Na^+$-channel blocking AED, and CBZ, a traditional $Na^+$-channel blocking AEDs. However, although the combination of LCM and CBZ was associated with slight impairment of motor coordination in mice (20% impairment), the combination of LCM and LEV had no negative impact on motor coordination. Finally, it is also important to note that in the pooled analysis only few patients were on dual AED therapy; indeed most were two to three concomitant AEDs and this would have acted as an important confounding factor.

Although further studies, both experimental and clinical, are needed to fully clarify synergism between LCM and other AEDs, and the clinical implications, results from the experiments reported herein provide some evidence for additive to synergistic anticonvulsant effects of combinations of LCM with marketed AEDs and a concomitant infra-additive effect on adverse effects. It is notable that the results indicate that none of the combinations resulted in infra-additive anticonvulsant efficacy.

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## Disclosure

Alain Matagne is an employee and Thomas Stohr a former employee of UCB Pharma. The remaining authors have no conflicts of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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