INTRODUCTION

Relatively recently, two novel antiepileptic drugs (AEDs, cenobamate and perampanel, have been licensed and approved as add-on drugs to clinical practice by FDA in USA and EMA in the EU [1, 2]. At present, physicians can prescribe for epileptic patients approx. 25 various AEDs; however, these drugs can offer seizure freedom in only approx. 70% of epileptic patients, while the rest of the patients remain refractory to standard treatment [3, 4]. To help the patients in eliminating seizure attacks, physicians have to take into consideration the application of some novel AEDs and/or various combinations of AEDs [5]. At present, only a few combinations of AEDs are favourable in epilepsy patients [5]. The choice of AEDs in the respective combinations is still a challenging issue for doctors, who must have comprehensive knowledge on AEDs and clinical experience in treating patients with AEDs [5]. Taking into account an ample number of available AEDs (25 or more), it is difficult to properly choose the AEDs for combinations. In such cases, preclinical studies based on isobolographic analysis of interaction can readily help clinicians in their choice and selection of AEDs [6–8].

Accumulating experimental evidence indicates that some triple combinations of AEDs may produce not only synergistic, additive, but also antagonistic interaction [9–11]. Therefore, it is not possible to theoretically predict the exact types of interactions for various three-drug combinations of AEDs in animals.

Three-drug combination of lacosamide, phenobarbital and valproate exerts additive interaction in the tonic-clonic seizure model in mice


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Abstract

Introduction. Triple-therapy with antiepileptic drugs (AEDs) is usually prescribed for epilepsy patients, whose seizures are not fully controlled with standard medications. Although 25 various AEDs are currently licensed for treating epilepsy, no algorithms allowing for the proper combination of AEDs are available.

Objective. The aim of the study is to isobolographically assess the type of interaction among three AEDs (lacosamide [LCM], phenobarbital [PB] and valproate [VPA]), in the model of tonic-clonic seizures in mice.

Materials and Method. The electrically-evoked (25 mA, 500 V, 50 Hz, 0.2 s of stimulus duration) tonic-clonic seizures in male albino Swiss mice allowed determination of the anticonvulsant action of the three-drug mixture of LCM, PB and VPA combined in a dose ratio of 1:1:1 by means of type I isobolographic analysis of interaction.

Results. The experimentally-determined ED50 exp value for the three-drug mixture was 112.04 mg/kg and did not differ from the theoretically calculated ED50 add value, which was 112.36 mg/kg. Lack of statistical significance confirmed that the mixture of LCM, PB and VPA in a dose-ratio of 1:1:1 exerted additive interaction in the mouse tonic-clonic seizure model.

Conclusions. Although the three-drug combination of LCM, PB and VPA produced additive interaction in the mouse tonic-clonic seizure model, the three-drug combination could be recommended for epilepsy patients whose seizures are refractory to the standard medication.

Key words

valproate, drug interactions, phenobarbital, isobolographic analysis, lacosamide, Tonic-clonic seizures
OBJECTIVE

The aim of this study was to determine the exact type of interaction among three AEDs: lacosamide (LCM), phenobarbital (PB), and valproate (VPA) by means of isobolographic analysis of interaction, as recommended elsewhere [9, 10, 12]. Due to various molecular mechanisms of action of the studied AEDs, the three-drug combination of LCM+PB+VPA should theoretically exert supra-additivity (synergy) in the mouse tonic-clonic seizure model. Each beneficial combination of AEDs, verified in preclinical studies, could become a new method of treatment, providing the epileptic patients with a state of seizure freedom.

MATERIALS AND METHOD

Animals. After a week of acclimatization to laboratory conditions, 32 adult male albino Swiss mice (weighing 22–26 g) were subjected to experimental protocol, approved by the Second Local Ethics Committee at the University of Life Sciences in Lublin, Poland. Experiments were performed in strict accordance with the ARRIVE guidelines [13].

Drugs. LCM (Vimpat, UCB Pharma SA, Brussels, Belgium) and PB (Sigma-Aldrich, Poznań, Poland) were suspended in a 1% aqueous solution of Tween 80 (Sigma-Aldrich, Poznań, Poland). Only VPA (sodium salt – Sigma-Aldrich, Poznań, Poland) was dissolved in sterile distilled water. The drugs were injected intraperitoneally: LCM at 15 min, PB – at 60 min and VPA – at 30 min, before the electrically-evoked tonic-clonic seizures, as described elsewhere [9, 14, 15].

Tonic-clonic seizure model in mice. Tonic-clonic seizures in 32 mice were evoked electrically using alternating current (25 mA, 500 V, 50 Hz, 0.2 s stimulus duration) delivered via auricular electrodes. The animals were randomly divided into 4 groups (8 mice in each group), which were given injections of LCM, PB and VPA in a constant combination of dose ratio of 1:1:1. After the respective pretreatment times, the animals were subjected to electroconvulsions. The number of the animals protected from tonic-clonic seizures in each experimental group, along with the increasing doses of the AEDs in combination, were linearly related according to the log-probit method [16]. The protective effects of the mixture of LCM, PB and VPA (when administered in the three-drug mixture in a dose ratio of 1:1:1) from tonic-clonic seizures were expressed as the ED$_{50}$mix (in mg/kg), as presented elsewhere [6, 17, 18].

Isobolographic analysis and statistics. Isobolographic analysis is the method of choice when classifying pharmacodynamic interaction among the tested drugs. This method allows comparison of the experimentally derived effects for the drugs in mixture with the theoretically calculated pure additive effects offered by these drugs [19]. Statistical comparison of experimentally-derived values with purely additive values allowed differentiating the interaction from additivity. If the effects are higher than the additive effect, then synergy (supra-additivity) is reported [19]. If the observed effects are lower than the additive effect, antagonism (sub-additivity) is documented [19]. In the current study, isobolographic analysis was based on calculation of the ED$_{50}$ values, i.e., median experimental dose of three-drug mixture that protected 50% of the animals tested from tonic-clonic seizures. The median additive dose of the drugs in the mixture that protected 50% of the animals from seizures, was calculated from the equation of additivity for three-drug mixture [20], as follows:

$$ED_{50\ add} = 1/3 ED_{50}^L + 1/3 ED_{50}^P + 1/3 ED_{50}^V$$

In this case, the drug ratio for the three-drug mixture was constant and amounted to 1:1:1. With isobolographic analysis, the experimentally-derived ED$_{50\ exp}$ value (in mg/kg) for the three-drug mixture of LCM, PB and VPA (in a dose ratio of 1:1:1) was statistically compared to the theoretically calculated ED$_{50\ add}$ value (in mg/kg), as recommended elsewhere [8,19]. The unpaired Student’s t-test was used to perform statistical comparison between ED$_{50\ exp}$ Values for the three-drug mixture of LCM, PB and VPA. Statistical significance was set up at P<0.05.

RESULTS

Isobolographic analysis of protective effects of the three-drug mixture from tonic-clonic seizures in animals. To calculate the ED$_{50\ add}$ value for the three-drug mixture of LCM, PB and VPA, the ED$_{50}$ values of the AEDs was used when administered alone that amounted to 7.27 ± 0.77 mg/kg for LCM; 31.21 ± 2.04 mg/kg for PB and 298.63 ± 15.15 mg/kg for VPA (Figure 1).

In such a situation, the theoretically calculated ED$_{50\ add}$ value was 112.36 ± 5.10 mg/kg. The three-drug combination of LCM, PB and VPA, in a dose-dependent manner, protected the animals from electrically-evoked tonic-clonic seizures; the experimentally-derived ED$_{50\ exp}$ value was 112.04 ± 9.43 mg/kg (Tab. 1).

Statistical comparison of both, ED$_{50\ exp}$ and ED$_{50\ add}$ values revealed no significance (Fig. 2A-C). Isobolographic analysis of interaction revealed that the three-drug mixture of LCM, PB and VPA exerted additive interaction in the tonic-clonic seizure model in mice (Fig. 2A-C).
DISCUSSION

Results obtained in this study indicate that the combination of the three AEDs produced purely additive interaction in the mouse tonic-clonic seizure model (Table 1). This combination of LCM+PB+VPA is worthy of being recommended for epilepsy patients whose seizures are refractory to the standard treatment by means of AEDs. No doubt exists that when three consecutive monotherapies with different current frontline AEDs failed, clinicians prescribe their patients polytherapy with two or more AEDs [21, 22]. The first presumption when choosing AEDs to combine them together is molecular mechanisms of action of the tested drugs. The main rule is to combine AEDs with diverse mechanisms of action so that the threedrug combination has several potential target sites; thus, the drugs could really have the potential to suppress seizures [22]. Multi-targeted antiseizure activity exerted by the triple AED combination minimizes the risk of ineffective treatment in epileptic patients. The second presumption when selecting the tripledose for the combination is their low (or no) potential to evoke adverse effects, especially, if they are combined together [23, 24]. This can be achieved by reduction of drug doses used to suppress seizures. In the current study, both presumptions were fulfilled. The third presumption taken into account by physicians when choosing the AEDs for a combination is the favourable pharmacokinetic profiles exerted by the AEDs used in combination [25, 26]. In such a case, the reduction of drug doses should also minimize or even eliminate pharmacokinetic drug-drug interactions among LCM, PB and VPA.

From a pharmacological viewpoint, LCM potentiates slow inactivation of voltage-gated sodium channels while simultaneously having no impact on the fast inactivation of these channels; thus, LCM inhibits repetitive neuronal firing and stabilizes hyperexcitable neuronal membranes that finally suppresses seizure generation in the pathologically-changed brain tissue [27]. PB potentiates neuronal inhibition through the GABA\(_{\text{A}}\) receptors [28]. Regarding VPA, the drug blocks T-type calcium channels, increases GABA content in the brain, reduces NMDA-mediated excitatory neurotransmission and blocks high voltage activated sodium channels in neurons [29]. All the above-mentioned mechanisms can be involved in suppression of various forms of epilepsy. Besides, it is not clear whether one single mechanism of VPA can sufficiently diminish seizure activity in the epileptic brain, or several various VPA mechanisms must be simultaneously activated to terminate seizure attacks in the epileptic brain. Theoretically, bearing in mind all the molecular mechanisms of action produced by LCM, PB and VPA, there is no doubt that the combination comprising these three AEDs should exert favorable interaction in experimental animals.

The current study proves that the combination of LCM+PB+VPA is beneficial, although its pharmacodynamic interaction was additive in the mouse tonic-clonic seizure model. This combination is also a good example demonstrating that clinicians can combine drugs belonging to the first (PB and VPA) and third (LCM) generations of AEDs. Considering PB and its anticonvulsant profile in both humans and animals, the authors are aware of the fact that application of PB is clinically restricted to situations related with termination of neonatal and childhood seizures [28]. The drug is seldom prescribed to adult patients, except for drug-resistant convulsive and non-convulsive status epilepticus [28], but in the presented study, PB was combined together with LCM and VPA, due to its unique molecular mechanisms action linked to GABA\(_{\text{A}}\)-receptor-mediated inhibition of neurotransmission in the brain.

Preclinical verification of interaction for the combination of LCM with PB and VPA confirmed the existence of pure additivity in terms of suppression of tonic-clonic seizures in animals, and the results are quite similar to those already published in the same seizure model for the three-drug combinations containing LCM (i.e., LCM+CBZ+LTG, LCM+CBZ+PB, LCM+LTG+PB (Tab. 2) [30–32]. On the other hand, some three-drug combinations comprising LCM occurred antagonistic (i.e., LCM+CBZ+VPA and LCM+LTG+VPA) in the mouse tonic-clonic seizure model (Tab. 2) [9,10]. At present, it is not clear why some combinations of three AEDs produce additivity while the others exert antagonistic interaction.

Of note, the supra-additive (synergistic) interactions among the three AEDs can be readily recommended for testing in clinical conditions. Also, the additive interactions among AEDs can be worthy of consideration when testing them in clinical settings. In the case of sub-additive (antagonistic)
interactions among three AEDs, a special caution is advised for clinicians when combining these AEDs during the treatment of refractory patients in order not to expose them to ineffective treatment regimens [23]. It should be highlighted that in the presented study only isobolographic analysis of the interaction for the mixture of three AEDs injected singly in an acute model of experimental seizures was performed. No chronic administration of drugs in mixture was conducted in this study. Since the drugs were injected singly, there were no pharmacokinetic interactions associated with induction and/or inhibition of the drugs’ metabolism pathways. It is noteworthy that both PB and VPA when administered chronically, mutually affect some liver CYP isoenzymes, contributing to substantial changes in drugs concentrations [29]. Besides, chronic administration of PB is associated with the development of tolerance to the drug dose in patients, which requires a substantial adjustment of drug doses to reach the same anticonvulsant effects [28].

The main limitation in this study was lack of determination of acute adverse effects produced by the three-drug mixture. However, results from previously published studies revealed that the doses of AEDs used in three-drug mixtures were generally too low to exert any acute adverse effects in experimental animals [10, 30–32]. Previously, it was found that none of the studied AED combinations produced impairment in motor coordination in rodents or disturbed long-term memory in experimental animals. Additionally, skeletal muscular strength was also unchanged in experimental animals receiving three-drug combinations in doses reflecting the ED$_{50}$exp values from the mouse tonic-clonic seizure model [10, 30–32]. Thus, the three-drug combination of LCM+PB+VPA seems to be safe enough, and therefore can be recommended to clinical practice for epileptic patients.

Another fact needs discussion and explanation while transferring the results from this study to clinical conditions. The isobolographic protocol for evaluating AED interactions refers to the activity of one AED because the equation of additivity is based on one fully active drug [20]. In contrast, in clinical conditions, epilepsy patients receive two or three AEDs in a full dose range each, providing them with duo-therapies or triple-therapies [5, 36, 37]. Of note, the ED$_{50}$ values for LCM, PB and VPA when used alone were determined in previous studies [10, 30, 35] that allowed for calculating the ED$_{50}$add$_{50}$ value for the three-drug mixture. This was the reason that in the current study only 32 animals were used in the mouse tonic-clonic seizure model for determining the ED$_{50}$exp value. Such a drastic reduction of tested animals was in strict accordance with the 3Rs rule (Reduction, Refinement and Replacement) of laboratory animals during the in vivo study [13]. The total number of animals used was reduced to a minimum in order to determine the ED$_{50}$exp value for the combination of LCM+PB+VPA in a constant dose-ratio of 1:1:1. It should be stressed that the main limitations in this study were both the route of drug administration and single application of the drugs. In clinical conditions, the AEDs are usually taken by the patients orally and chronically for a long period of time [36, 37]. In this study, the AEDs were injected i.p. and only as single injections of LCM, PB and VPA.

CONCLUSIONS

1. LCM combined with PB and VPA produced additivity in the mouse model of tonic-clonic seizures.
2. LCM might be combined with PB and VPA for patients with refractory epilepsy uncontrolled with monotherapy.

REFERENCES