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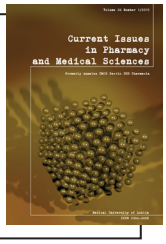

Synergistic interaction of levetiracetam with gabapentin in the mouse 6 Hz psychomotor seizure model – a type II isobolographic analysis

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ABSTRACT

This study was aimed at characterizing the anticonvulsant effects of levetiracetam in combination with gabapentin, in the mouse 6 Hz psychomotor seizure model. Herein, psychomotor seizures were evoked in male albino Swiss mice by a current (32 mA, 6 Hz, 3 s stimulus duration) delivered via ocular electrodes. Type II isobolographic analysis was used to characterize the anticonvulsant interactions between the drugs in combination, for fixed-ratios of 1:1, 1:2, 1:5 and 1:10. The type II isobolographic analysis revealed that the combinations of levetiracetam with gabapentin for the fixed-ratios of 1:5 and 1:10 were supra-additive (synergistic; $P<0.05$) in terms of seizure suppression, while the combinations for the fixed-ratios of 1:1 and 1:2 were additive in the mouse 6 Hz psychomotor seizure model. We conclude that, as the combinations of levetiracetam with gabapentin for the fixed-ratios of 1:5 and 1:10 exerted supra-additive (synergistic) interaction in the mouse 6 Hz psychomotor seizure model, this may be considered as particularly favorable combinations in further clinical practice.

INTRODUCTION

In medical practice, temporal lobe epilepsy is the most commonly seen form of partial seizures, and it accounts for approx. 60% of all patients with epilepsy [5]. Temporal lobe epilepsy is characterized by recurrent, unprovoked seizures consisting of simple and complex partial seizures. This form of epilepsy is frequently resistant to medications [5,7].

Generally, monotherapy with one of the available antiepileptic drugs is thought to be a treatment of choice for approx. 70% of epileptic patients [7]. Unfortunately, there is still a population of 30% of epileptic patients who need the application of two or more antiepileptic drugs to reduce their seizure attacks [2,7]. Thus, polytherapy with antiepileptic drugs with diverse molecular mechanisms of action seems to be a favorable treatment option for these epileptic patients [2,14]. From a theoretical point of view, the most beneficial antiepileptic two-drug combination is that offering synergy (supra-additivity) in relation to its therapeutic (anticonvulsant) activity, and with concomitant antagonism (infra-additivity) with respect to its adverse effects [2,9].

Experimental evidence indicates that levetiracetam combined with lacosamide and phenobarbital exerted supra-additive (synergistic) interactions in mice challenged with the 6 Hz psychomotor seizure model [15,17]. Moreover, isobolographic analysis revealed both supra-additive and additive interactions between levetiracetam and carbamazepine, ethosuximide, phenytoin, topiramate and vigabatrin in the mouse 6 Hz model [6,11]. Additionally, the combinations of levetiracetam with clonazepam, clobazam, lamotrigine, oxcarbazepine, tiagabine and valproate exerted additive interaction in the mouse 6 Hz model [10,12,17]. It is widely accepted that the mouse 6Hz model (low frequency (6 Hz) long-duration (3 s) corneal electrical stimulation) is an experimental animal model of psychomotor or limbic seizures in humans [1].

The aim of this study was to determine the type of interaction between levetiracetam and gabapentin in the mouse 6 Hz psychomotor seizure model. Generally, antiepileptic drugs in combination should possess diverse and complementary molecular mechanisms of action with respect to

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their anticonvulsant effects. This is the reason for levetiracetam in combination with gabapentin being tested in the present study.

MATERIALS AND METHODS

Animals and experimental conditions

Experiments were conducted on adult male albino Swiss mice weighing 22-26 g, purchased from a licensed breeder (J. Kolacz, Warsaw, Poland). The animals were kept in colony cages with free access to food and tap water under standardized housing conditions. The animals were randomly assigned to experimental groups comprising 8 mice per group. The experimental protocols and procedures listed were approved by the Local Ethics Committee at the Medical University of Lublin, and conformed to the *Guide for the Care and Use of Laboratory Animals*.

Drug administration

Levetiracetam (UCB Pharma, Braine-l'Alleud, Belgium) and gabapentin (Parke-Davis, Berlin, Germany) were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and were administered intraperitoneally (i.p.) as a single injection in a volume of 5 ml/kg body weight. Both antiepileptic drugs were administered 60 min before initiation of psychomotor seizures evoked by 6 Hz corneal electrical stimulation. The pretreatment time before testing of these antiepileptic drugs was based on information from the literature and our previous experiments [4].

Psychomotor (6 Hz) seizure model

Psychomotor (limbic) seizures were evoked in mice via corneal stimulation (6 Hz, 0.2 ms rectangular pulse width, 32 mA, 3 s duration) delivered by a S48 Square Pulse Stimulator and CCU1 Constant Current Unit (Grass Technologies, West Warwick, RI, USA). Ocular anaesthetic (0.5% tetracaine) was dropped to the mouse corneas 15 min before stimulation. Immediately following stimulation, mice were placed separately in Plexiglas cages (25 × 15 × 10 cm) and observed for the presence or absence of seizure activity. Protection in the 6 Hz model was defined as the absence of a seizure, and the mice not experiencing seizures exhibited normal exploratory behaviour when placed in the cages [1]. In this study, to determine the $ED_{50\ mix}$ values, the antiepileptic drugs were administered i.p. as follows: gabapentin, 5-80 mg/kg and levetiracetam, 5-20 mg/kg.

Isobolographic analysis of interactions

In the present study, 4 fixed-ratio combinations of 1:1, 1:2, 1:5 and 1:10 were used to correctly assess types of interactions between levetiracetam and gabapentin against psychomotor seizures with type II isobolographic analysis. The experimentally derived $ED_{50\ mix}$ values (± S.E.M.) for the mixture of levetiracetam with gabapentin were determined using the log-probit method [8]. The theoretically additive $ED_{50\ add}$ values (± S.E.M.) were calculated as presented earlier [6,11]. A more detailed description and the theoretical background relating to the type II isobolographic analysis has been presented in our previous studies [6,11].

Statistical analysis

Both ED_{50} and $ED_{50\ mix}$ values for antiepileptic drugs administered alone or in combination for the fixed-ratios of 1:1, 1:2, 1:5 and 1:10 in the mouse 6 Hz-induced seizure test, were calculated by computer-assisted log-probit analysis, according to Litchfield and Wilcoxon [8]. The $ED_{50\ mix}$ and $ED_{50\ add}$ values were statistically compared by the use of the unpaired Student's *t*-test [16]. Results were considered statistically significant if $P < 0.05$.

RESULTS

Type II isobolographic analysis of interactions between levetiracetam and gabapentin in the mouse 6 Hz psychomotor seizure model

Isobolographic analysis revealed that the combination of levetiracetam with gabapentin for the fixed-ratios of 1:5 and 1:10 was supra-additive (synergistic) in the mouse 6Hz-induced psychomotor seizure model ($P<0.05$; Tab. 1; Fig. 1). In contrast, the antiepileptic drug combination for fixed-ratios of 1:1 and 1:2 depicted additive interaction in this seizure model in mice (Tab. 1; Fig. 1).

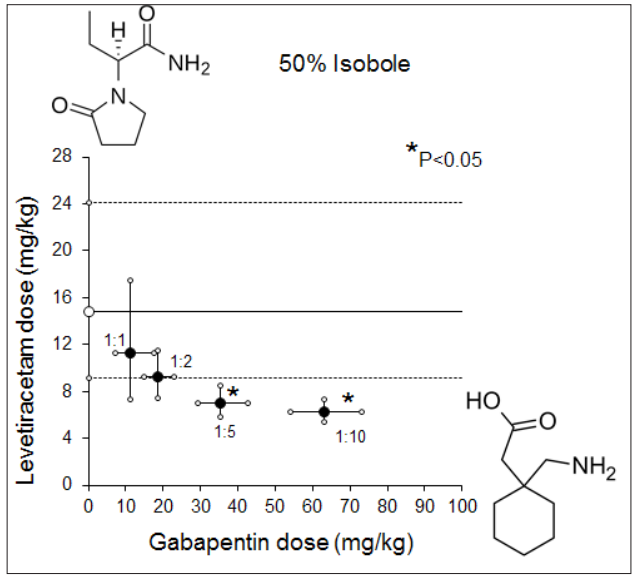

Figure 1. Isobologram illustrating structural formulas and interactions of levetiracetam with gabapentin for the 50% anticonvulsant effect in the mouse 6 Hz psychomotor seizure model

Doses of gabapentin and levetiracetam are plotted graphically on the X- and Y-axis, respectively. The heavy line (parallel to the X-axis) represents the ED_{50} value for levetiracetam administered alone, and defines the theoretical dose-additive line. The dotted lines represent S.E.M. values for levetiracetam administered alone. The closed circles (●) illustrate the experimentally derived $ED_{50\ mix}$ values for total doses of mixtures expressed as proportions of levetiracetam and gabapentin at the fixed-ratios of 1:1, 1:2, 1:5 and 1:10 that produced median anticonvulsant effects. The S.E.M. values for $ED_{50\ mix}$ are presented in form of vertical and horizontal components of the error. The $ED_{50\ mix}$ values for the mixture of levetiracetam with gabapentin for the fixed-ratios of 1:5 and 1:10 are placed significantly below the theoretical line of additivity, indicating supra-additivity (synergy; *$P<0.05$). In contrast, the combinations for the fixed-ratios of 1:1 and 1:2 indicate additive interactions between levetiracetam and gabapentin in the mouse 6 Hz psychomotor seizure model.

Table 1. Type II isobolographic analysis of interactions between levetiracetam and gabapentin in the mouse 6 Hz psychomotor seizure model.

Antiepileptic drug combination	Fixed-ratio	$ED_{50\ mix}$	n_{mix}	$ED_{50\ add}$	n_{add}
Levetiracetam + gabapentin	1:1	22.6±2.9	24	29.7±7.3	30
Levetiracetam + gabapentin	1:2	27.7±2.7	24	44.5±11.0	30
Levetiracetam + gabapentin	1:5	42.3±3.9 *	24	89.0±22.0	30
Levetiracetam + gabapentin	1:10	69.2±10.2*	16	163.2±40.3	30

Results display median effective doses (ED_{50} in mg/kg ± S.E.M.) of two-drug combinations protecting 50% of animals tested against 6 Hz psychomotor seizures. ED_{50} values were either experimentally determined from the mixture of two antiepileptic drugs ($ED_{50\ mix}$), or theoretically calculated from the equation of additivity ($ED_{50\ add}$). Data were statistically evaluated using the unpaired Student's t-test with Welch's correction; n – total number of animals at those doses of two-drug mixture whose expected anticonvulsant effects ranged between 4 and 6 probits, denoted for the experimental mixture of drugs (n_{mix}) and theoretically calculated (n_{add}) from the equation of additivity. *P<0.05 vs. the respective $ED_{50\ add}$, indicating supra-additive (synergistic) interaction

DISCUSSION

The type II isobolographic analysis revealed that, while the combinations of levetiracetam with gabapentin (for the fixed-ratios of 1:1 and 1:2) were additive, the combinations of the antiepileptic drugs (for the fixed-ratios of 1:5 and 1:10) were supra-additive (synergistic) and potentially useful combinations.

In the present study, it was documented that by adding gabapentin, which is virtually ineffective when given alone, to levocardiogram, a drug that is fully effective in the mouse 6 Hz psychomotor seizure model, the combination of these antiepileptic drugs exerted synergistic interaction and added efficacy when given as add-on. This phenomenon, one can try to explain by taking into account the molecular mechanisms of action of the tested drugs.

In the case of levetiracetam, the drug binds to a synaptic vesicle protein 2A (SV2A) and inhibits vesicle neurotransmitter exocytosis [13]. With respect to gabapentin, the drug binds with high affinity to the $\alpha_2\delta$ type 1 and 2 subunits of voltage-gated calcium channels, and thus, reduces the synaptic release of neurotransmitters. This results in diminished excitation [3]. Hence, in the case of the observed synergistic interactions between levetiracetam and gabapentin in the mouse 6 Hz seizure model, it can be concluded that the calcium channel blocking properties of gabapentin and the levetiracetam-evoked inhibition of the SV2A proteins, as well as the subsequent reduction of glutamate release, contribute to the suppression of psychomotor seizures in mice.

It should be highlighted that neither blood nor total brain antiepileptic drug concentrations were measured in the present study. Previously, it has been documented that levetiracetam (223.1 mg/kg, i.p.) significantly elevated (by 21%) total brain concentrations of gabapentin (55.8 mg/kg, i.p.) in experimental mice challenged with the pentylenetetrazole-induced clonic seizures [4]. In contrast, gabapentin (55.8 mg/kg, i.p.) had no impact on total brain concentrations of levetiracetam (223.1 mg/kg, i.p.) in mice [4]. Since pharmacokinetic interactions were observed earlier in experimental animals, we did not repeat such experiments because of ethical reasons. Nevertheless, in the present study, doses of levetiracetam were substantially low i.e., the combination of levetiracetam with gabapentin for the fixed-ratio of 1:10 comprised levetiracetam at a dose of 6.29 mg/kg and gabapentin at a dose of 62.9 mg/kg. In this situation, it was less probable that levetiracetam 6.29 mg/kg (i.e., in a dose of 35-times lower than in our previous study) would be able to elevate total brain concentrations of gabapentin in mice. However, to confirm this hypothesis, more advanced studies are required.

Of note, the tested combination of levetiracetam with gabapentin for the fixed-ratio of 1:10, neither impaired motor coordination, disturbed learning nor reduced skeletal muscular strength in the animals (results not shown). Such results are in agreement with our earlier studies reporting no acute adverse effects in animals [4].

CONCLUSION

The combination of levetiracetam with gabapentin can potentially offer some epileptic patients with limbic seizures, the favorable combination.

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