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Interaction of pregabalin with carbamazepine in the mouse maximal electroshock-induced seizure model: a type I isobolographic analysis for non-parallel dose-response relationship curves

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ABSTRACT

Purpose: To characterize the anticonvulsant effects of pregabalin (PGB – a third-generation antiepileptic drug) in combination with carbamazepine (CBZ – a classical antiepileptic drug) in the mouse maximal electroshock (MES)-induced seizure model by using the type I isobolographic analysis for non-parallel dose-response relationship curves (DRRCs).

Material/Methods: Tonic hind limb extension (seizure activity) was evoked in adult male albino Swiss mice by a current (sine-wave, 25mA, 500V, 50Hz, 0.2s stimulus duration) delivered via auricular electrodes. Potential adverse-effect profiles of interaction of PGB with CBZ at the fixed-ratio of 1:1 in the MES test with respect to motor performance, long-term memory, skeletal muscular strength and antinociceptive activity were measured along with total brain CBZ concentrations.

Results: In the mouse MES model, PGB administered singly had its DRRC non-parallel to that for CBZ. With type I isobolographic analysis for non-parallel DRRCs, the combination of PGB with CBZ at the fixed-ratio of 1:1 exerted additive interaction. In the combination, neither motor coordination, long-term memory nor muscular strength were affected. PGB administered alone and in combination with CBZ exerted antinociceptive effects, whereas CBZ administered alone produced no antinociceptive activity in mice subjected to the acute thermal pain model. Pharmacokinetic estimation of total brain antiepileptic drug concentrations revealed that PGB had no impact on total brain concentrations of CBZ in experimental animals.

Conclusions: In conclusion, the additive interaction between PGB and CBZ is worthy of consideration while extrapolating the results from this study to clinical settings.

Key words: pregabalin, carbamazepine, isobolographic analysis, maximal electroshock, pharmacodynamic/pharmacokinetic interaction

INTRODUCTION

Although many new (second-generation) antiepileptic drugs (AEDs) have been introduced in the last decade, there is still a clear need for AEDs with improved efficacy and tolerability that are also easy to use in clinical practice. At present, less than half of all patients become seizure-free with the first AED tried, and approx. 30% remain uncontrolled on either their first or second AED [1]. The remaining patients are difficult to control from the beginning and will still experience regular seizures even when receiving a combination of currently available AEDs. Therefore, some novel (third-generation) AEDs with improved efficacy and novel mechanisms of action are urgently needed to provide effective combination treatment for patients with epilepsy.

Pregabalin (PGB; (*S*)-(+)-3-(aminomethyl)-5-methylhexanoic acid or (*S*)-(+)-3-isobutyl GABA) is a third-generation AED recently licensed as an adjunct therapy for partial (simple and complex) seizures with or without secondary generalization in patients over 18 years of age [2-4].

Although PGB is a substituted analogue of γ-aminobutyric acid (GABA) (*Fig. 1*), the drug is inactive at GABA receptors, including $GABA_A$, benzodiazepine, and $GABA_B$ radioligand binding sites [5]. PGB does not alter GABA concentration in brain tissue [6]. The drug does not have any direct action at sodium channels, however, it binds with high affinity and specificity to the α2δ subunit of P/Q-type voltage-gated calcium channels, which decreases Ca^{2+} influx at nerve terminals, and thus, the drug reduces the release of excitatory neurotransmitters [7-9].

Experimental evidence indicates that PGB exhibits anticonvulsant activity in the maximal electroshock (MES)-induced tonic seizure and pentylenetetrazole (PTZ)-induced clonic seizure models in rodents [10]. PGB provided a partial protection against seizures induced by picrotoxin or bicuculline, but did not prevent strychnine-induced seizures [10]. In hippocampal kindled rats, PGB prevents both behavioral and electrographic seizures [10]. PGB reduced the incidence of seizures in DBA/2 audiogenic mice, but the drug did not reduce the incidence of spontaneous absence seizures in genetically susceptible rats (GAERS) [10].

The aim of this study was to determine the interaction profile of PGB (a third-generation AED) in combination with carbamazepine (CBZ – a classical AED used in patients with generalized tonic-clonic seizures and partial onset seizures) in the mouse MES model. Generally, the mouse MES model is considered as an animal model of tonic-clonic seizures and motor partial seizures with or without secondary generalization in humans [11,12]. Thus, it was appropriate to determine the interaction profile of PGB with CBZ in the mouse MES model.

Additionally, the chimney test (a measure of motor performance impairment), the step-through passive avoidance task (a measure of long-term memory deficits), the grip-strength test (a measure of skeletal muscular strength impairment), and the hot-plate test (a measure of antinociceptive activity against acute thermal pain) were used to determine the acute adverse-effect potential for the combination of PGB with CBZ. Finally, to ascertain whether the observed interaction was pharmacodynamic in nature or that pharmacokinetic interaction also contributed, total brain CBZ concentrations were measured with fluorescence polarization immunoassay.

MATERIAL AND METHODS

Animals and experimental conditions

All experiments were performed on adult male albino Swiss mice (weighing 22-26 g, six-week-old) purchased from licensed breeder (Dr. T. Gorzkowska, Warszawa, Poland). The mice were kept in colony cages with free access to food and tap water under standardized housing conditions (natural light-dark cycle, temperature of 21 ± 1ºC, relative humidity of 55 ± 3%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 mice. Each mouse was used only once. All tests were performed between 9.00 a.m. and 3.00 p.m. Procedures involving animals and their care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this manuscript were approved by the Local Ethics Committee at the Medical University of Lublin (License no.: 21/2007).

Drugs

The following AEDs were used in this study: PGB (Lyrica®, Pfizer Ltd., Sandwich, Kent, UK) and CBZ (a kind gift from Polfa, Starogard Gdanski, Poland). The AEDs were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in saline and administered by intraperitoneal (i.p.) injection in a volume of 0.005 ml/g body weight. Fresh drug solutions were prepared on each day of experimentation and administered as follows: PGB – 60 min and CBZ – 30 min before seizures and behavioral tests as well as before brain sampling for the measurement of AED concentrations. The times to the peak of maximum anticonvulsant effects for all AEDs were used as the reference times in all behavioral tests. The route of systemic (i.p.) administration and these pretreatment times were chosen based upon information about the biological activity of the AEDs from the literature [9] and pilot studies.

Maximal electroshock seizure test

The protective activities of PGB and CBZ administered separately were evaluated and expressed as their median effective doses (ED_{50} in mg/kg), protecting 50% of mice against MES-induced seizures (fixed current intensity of 25 mA, maximum stimulation voltage of 500 V). Electroconvulsions were produced by a current (0.2 s stimulus duration) delivered via standard auricular electrodes by a Hugo Sachs generator (Rodent Shocker, Type 221, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hindlimb extension. The animals were administered with different drug doses so as to obtain a variable percentage of protection against MES-induced seizures, allowing the construction of a dose-response relationship curve (DRRC) for PGB and CBZ administered alone, according to Litchfield and Wilcoxon [13]. The anticonvulsant activity of the mixture of PGB with CBZ at the fixed-ratio of 1:1 was evaluated and expressed as median effective doses ($ED_{50\ mix}$ values) against MES-induced seizures. This experimental procedure has been described in detail elsewhere [14-16].

Isobolographic analysis of interactions

The percent protection of animals against MES-induced seizures per dose of an AED administered alone and the DRRC for each investigated AED in the mouse MES model were fitted using log-probit linear regression analysis