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Isobolographic characterization of the anticonvulsant interaction profiles of levetiracetam in combination with clonazepam, ethosuximide, phenobarbital and valproate in the mouse pentylenetetrazole-induced seizure model

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ABSTRACT

This study was designed so as to characterize the interactions between levetiracetam (LEV) and the conventional antiepileptic drugs (AEDs) clonazepam (CZP), ethosuximide (ETS), phenobarbital (PB), and valproate (VPA) in suppressing pentylenetetrazole (PTZ)-induced clonic seizures in mice by use of type II isobolographic analysis. Adverse-effect profiles of the drugs in combination were determined and brain AED concentrations were measured. The combinations of VPA and ETS with LEV at the fixed-ratio of 1:2, CZP with LEV (1:20,000), and PB with LEV (1:20) were supra-additive (synergistic) in suppressing seizures. In contrast, VPA and ETS with LEV (1:1, 2:1, and 4:1), CZP with LEV (1:1000, 1:5000, and 1:10,000), and PB with LEV (1:1, 1:5, and 1:10) were additive. No adverse effects were observed. ETS significantly reduced brain LEV concentrations but no other pharmacokinetic changes were observed. The combinations of CZP with LEV (1:20,000); VPA and ETS with LEV (1:2); and PB with LEV (1:20) appear to be favorable combinations exerting supra-additive interactions in suppressing PTZ-induced seizures.

1. Introduction

Approximately 70% of patients with epilepsy can be satisfactory treated with a monotherapy antiepileptic drug (AED) treatment.[1–3] However, for the remaining ~30% of patients that are refractory[1,2,4] there is a need to prescribe two or more (polytherapy) AEDs in an attempt to control their seizures.[1,2] Additionally, polytherapy is prescribed to patients who may suffer from multiple seizure types and usually require different AEDs in order to control their heterogeneous seizures.[5] However, AED polytherapy can be associated with numerous problems, including acute and chronic CNS side effects and idiosyncratic reactions, which can be exacerbated by adverse pharmacokinetic and/or pharmacodynamic interactions.[6–8] Although there are no randomized clinical trials to ascertain which AED combinations are most suited for a particular seizure type, anecdotal evidence and clinical experience has highlighted some useful combinations.[9] From a theoretical point of view, the most advantageous AED combination is that between two AEDs that are synergistic in relation to their therapeutic (anticonvulsant) activity and thus supra-additive in seizure suppression and with concomitant infra-additivity (antagonism) in relation to their adverse effects.[5,10–12]

Levetiracetam (LEV, [S]-alpha-ethyl-2-oxo-1-pyrrolidine acetamide) is a new AED that is licensed for clinical use as monotherapy and adjunctive treatment of patients with intractable partial-onset seizures with or without secondary generalization,[13–19] and for adjunctive therapy of myoclonic seizures[20,21] and primary generalised tonic clonic seizures.[21–23] In the clinical setting, LEV has also been shown to be efficacious in photosensitive epilepsy,[24] and in children from 4 years and older with partial-onset seizures.[25–27]

In preclinical studies, it has been observed that LEV is virtually ineffective in acute seizure models (i.e., maximal electroshock (MES)- and pentylenetetrazole (PTZ)-induced seizures), which are routinely used to screen for potential new AEDs.[27] In contrast, LEV increased the threshold for electroconvulsions and suppressed seizures in kindled and genetically epileptic animals.[28–33] LEV has

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also shown protective activity against seizures induced by 6 Hz electrical stimulation—a model of psychomotor seizures,[34] attenuates spike-and-wave discharges in DBA/2J mice (an animal model of absence epilepsy),[33] and is effective against kindled audiogenic seizures in Krushinsky–Molodkina rats (a strain of rats selected for susceptibility to audiogenic seizures).[35] LEV produces also antiepileptogenic effect: it retards the acquisition of audiogenic kindling in Krushinsky–Molodkina rats[35] and inhibits the development of hippocampal hyperexcitability following pilocarpine-induced status epilepticus in rats.[36]

The precise mechanism of action of LEV has not been fully elucidated. It reduces voltage-operated K^+ current and inhibits the delayed rectifier K^+ current in neurons,[37] reduces N-type and partially P/Q-type high-voltage-activated Ca^{2+} currents,[38,39] but not low-voltage-activated Ca^{2+} currents,[40] suppresses the inhibitory action of zinc and β-carbolines on $GABA_A$- and glycine-gated currents,[41] blocks $GABA_A$ receptor run-down in neocortex and thus, increases GABA-ergic inhibitory neurotransmission in the brain,[42] inhibits ryanodine receptor (RyR) and inositol 1,4,5-triphosphate receptor (IP_3R) mediated calcium-induced calcium release (CICR) in hippocampal neurons in culture,[43] and thus, LEV by inhibiting Ca^{2+} release through both RyR and IP_3R, affects a major second messenger system in neurons[43] and activates renal outer medullary potassium (ROMK1) channels through a protein kinase A (PKA)-mediated phosphorylation.[44] The major physiological function of ROMK1 channels is to maintain the resting membrane potential during cellular excitation, therefore, LEV is capable of reducing neuronal excitability.[44] Molecular studies involving transgenic mice suggest that LEV binds to a synaptic vesicle protein 2A (SV2A), which is involved in vesicle neurotransmitter exocytosis, and that the affinity of binding to SV2A significantly correlates with anticonvulsant potency by a series of LEV derivatives.[45]

Accumulating experimental evidence indicates that LEV is associated with favourable pharmacodynamic interaction with numerous AEDs in various animal models including: topiramate (TPM),[10,46] oxcarbazepine (OXC), carbamazepine (CBZ),[10] diazepam (DZP),[47] felbamate (FBM),[12] gabapentin (GBP),[48] valproate (VPA) and clonazepam (CZP).[49] In the case of the combination of LEV with FBM, a synergistic interaction in terms of suppression of MES-induced seizures was additionally complicated by a pharmacokinetic increase in total brain LEV concentrations.[12] Similarly, the combination of LEV with GBP, exerting a synergistic interaction in terms of suppression of PTZ-induced clonic seizures, was associated with a pharmacokinetic increase in total brain GBP concentrations.[48] LEV also potentiated the anticonvulsant activity of CBZ, DZP, FBM, TPM, GBP, and VPA in sound-induced seizures in DBA/2 mice.[50] Additionally, LEV enhanced the anticonvulsant activity of VPA, CZP, DZP, phenobarbital (PB), lamotrigine (LTG), CBZ, vigabatrin (VGB), phenytoin (PHT), chlordiazepoxide, MK-801 (an NMDA receptor antagonist), NBQX (an AMPA/kainate receptor antagonist), NO-711 (a GABA transporter inhibitor), allopregnenolone (a positive allosteric modulator of $GABA_A$ receptors), bretazenil (a partial agonist of the benzodiazepine receptors), propranolol (a β-adrenergic receptor blocker) and flunarizine (a calcium channel blocker) in the mouse audiogenic seizure model.[49] LEV also potentiated the anticonvulsant activity of CZP, VPA, CBZ and PB in the rat amygdala kindling model.[49] Moreover, it has been documented that LEV can pharmacodynamically potentiate the acute neurotoxic effects of TPM and CBZ in the rotarod test in mice.[11] Clinically, a similar antiepileptic and adverse pharmacodynamic interaction profile has been reported in patients receiving LEV and CBZ[51] and TPM.[52]

Consequently, it can be considered appropriate to evaluate a preclinical profile of LEV in combination with four conventional AEDs that are commonly used in the management of generalized seizures namely: CZP, ethosuximide (ETS), PB, and VPA. In the present study the anticonvulsant effects of the AED combinations were determined in the mouse PTZ-induced clonic seizure test, a model of myoclonic seizures in humans and the data analyzed by use of type II isobolographic analysis.[27,53] Additionally, to determine the acute adverse-effect profiles for the various combinations, the chimney test (a measure of motor performance impairment), the step-through passive avoidance task (a measure of long-term memory deficits), and the grip-strength test (a measure of skeletal muscular strength impairment) were used. Finally, to ascertain whether the observed interactions were purely pharmacodynamic in nature or that pharmacokinetic interactions also contributed, brain LEV, CZP, ETS, PB and VPA concentrations were measured.

2. Materials and methods

2.1. Animals and experimental conditions

All experiments were performed on adult male (4-week-old) Swiss mice weighing 22–26 g. The mice were kept in colony cages with free access to food and tap water, under standardized housing conditions (12 h of a light–dark cycle, temperature was 21 ± 1 °C). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups comprising of 8 mice per group. Each mouse participated only in one experiment and all tests were performed between 9.00 a.m. and 2.00 p.m. to minimize confounding effects of circadian rhythms. Procedures involving animals and their care were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985), and Polish legislation on animal experimentation. 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.: 547/2005/589/2005).

2.2. Drugs

The following AEDs were used in this study: LEV (UCB Pharma, Braine-l'Alleud, Belgium), CZP (Polfa, Warszawa, Poland), ETS (Sigma, St. Louis, MO, USA), PB (Polfa, Krakow, Poland) and VPA (magnesium salt, ICN-Polfa S.A., Rzeszow, Poland). All drugs, except for VPA, were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in saline, whereas VPA was directly dissolved in saline. All drugs were 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: LEV and PB—60 min; ETS—45 min; VPA—30 min, and CZP—15 min before PTZ administration and behavioral tests as well as before brain sampling for the measurement of AED concentrations. These pretreatment times were chosen based upon information about their biological activity from the literature and our previous studies.[10–12] PTZ (Sigma, St. Louis, MO, USA) was dissolved in distilled water and administered subcutaneously (s.c.) into a loose fold of skin in the midline of the neck in a volume of 0.005 ml/g body weight. Since anesthetic and/or analgesic drugs may interfere with brain concentrations of AEDs, such drugs were not used in our study. In order to minimize the variability of animal behavioral response to the mild stress produced by handling and i.p. injections, each animal was subjected to the same experimental conditions. Thus, each mouse was given two consecutive injections of vehicle (1% solution of Tween 80 in saline) or respective AEDs. For the