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Isobolographic characterization of interactions of levetiracetam with the various antiepileptic drugs in the mouse 6 Hz psychomotor seizure model

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Summary The aim of this study was to characterize the anticonvulsant effects of levetiracetam (LEV) in combination with the various antiepileptic drugs (clonazepam [CZP], oxcarbazepine [OXC], phenobarbital [PB], tiagabine [TGB], and valproate [VPA]), in the mouse 6 Hz psychomotor seizure model.

Limbic (psychomotor) seizure activity was evoked in albino Swiss mice by a current (32 mA, 6 Hz, 3 s stimulus duration) delivered via ocular electrodes and isobolographic analysis for parallel and non-parallel dose—response effects was used to characterize the consequent anticonvulsant interactions between the various drug combinations. Potential concurrent adverse-effect profiles of interactions between LEV and CZP, OXC, PB, TGB, and VPA at the fixed-ratio of 1:1 were evaluated in the chimney (motor performance), passive avoidance (long-term memory), and grip-strength (muscular strength) tests.

LEV administered singly was associated with a dose—response relationship curve (DRRC) that was parallel to that for CZP and non-parallel to that for OXC, PB, TGB and VPA. With isobolography for parallel DRRCs, the combination of LEV with CZP at three fixed-ratios of 1:3, 1:1 and 3:1 was additive in nature. With isobolography for non-parallel DRRCs the combinations of LEV with OXC, TGB and VPA at the fixed-ratio of 1:1 were also additive. In contrast, the isobolography for non-parallel DRRCs revealed that the interaction for the combination of LEV with PB at the fixed-ratio of 1:1 was supra-additive (synergistic). None of the combinations were associated with any concurrent adverse effects with regards to motor coordination, long-term memory or muscular strength.

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LEV is associated with favorable anticonvulsant synergism with PB and is additive with regards to CZP, OXC, TGB and VPA in the mouse 6 Hz psychomotor seizure model.

Introduction

Levetiracetam (LEV) is a unique second-generation antiepileptic drug (AED) that, in preclinical studies, is virtually ineffective in acute models of epilepsy i.e., the maximal electroshock (MES)- and pentylenetetrazole (PTZ)-induced seizures (Gower et al., 1992, 1995; Löscher and Hönack, 1993; Klitgaard et al., 1998; Löscher et al., 1998), which are routinely used to screen for potential new AEDs (Löscher et al., 1991a,b). In contrast, LEV increased the threshold for electroconvulsions and suppressed seizures in kindled and genetically epileptic animals (Gower et al., 1992, 1995; Löscher and Hönack, 1993; Klitgaard et al., 1998; Löscher et al., 1998; Luszczki and Czuczwar, 2005). LEV has also shown protective activity against acute seizures induced by low frequency (6 Hz), long-duration (3 s), corneal electrical stimulation (a model of psychomotor or limbic seizures) (Toman, 1951; Toman et al., 1952; Brown et al., 1953; Barton et al., 2001). Moreover, the drug attenuates spike-and-wave discharges in DBA/2J mice (an animal model of absence epilepsy) (Marrosu et al., 2007), and it demonstrates potent anticonvulsant effects against audiogenic seizures in Krushinsky—Molodkina rats (a strain of rats selected for susceptibility to audiogenic seizures) (Vinogradova and van Rijn, 2008). Moreover, chronic (21 days) administration of LEV inhibits the development of hippocampal hyperexcitability following pilocarpine-induced status epilepticus in rats (Margineanu et al., 2008).

Accumulating evidence indicates that LEV is associated with favorable anticonvulsant pharmacodynamic interactions with numerous AEDs in various animal models including: topiramate (Sills et al., 2004; Luszczki et al., 2006a; Donato Di Paola et al., 2007; Kaminski et al., 2009), oxcarbazepine (OXC), carbamazepine (Luszczki et al., 2006a; Donato Di Paola et al., 2007; Kaminski et al., 2009), diazepam (Mazarati et al., 2004; Donato Di Paola et al., 2007; Kaminski et al., 2009), felbamate (Donato Di Paola et al., 2007; Luszczki et al., 2007), clonazepam (CZP) and valproate (VPA) (Donato Di Paola et al., 2007; Kaminski et al., 2009; Dudra-Jastrzebska et al., in press), phenobarbital (PB) (Dudra-Jastrzebska et al., in press; Kaminski et al., 2009), gabapentin (Donato Di Paola et al., 2007; Dudra-Jastrzebska et al., 2009), and also, with lamotrigine, vigabatrin, phenytoin, chlordiazepoxide, dizocilpine (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) (Kaminski et al., 2009). With regards to LEV and felbamate in combination, anticonvulsant synergism between the drugs was complicated by a pharmacokinetic increase in total brain LEV concentrations (Luszczki et al., 2007). Similarly a pharmacokinetic increase in total brain gabapentin concentrations was observed when gabapentin was administered in combination with LEV (Dudra-Jastrzebska et al., 2009). A further confounding factor with topiramate and carbamazepine has been a pharmacodynamic potentiation of acute neurotoxic effects, as assessed by the rotarod test in mice, by LEV (Luszczki et al., 2005a), and these later observations concur with the adverse pharmacodynamic interactions that have been reported in patients prescribed LEV in combination with carbamazepine (Sisodiya et al., 2002) and with topiramate (Glauser et al., 2002).

Considering the fact that LEV is virtually ineffective in experimental models of acutely evoked seizures (e.g., MES and PTZ) except for the low frequency long-duration corneal stimulation model (6 Hz psychomotor seizures), it was of pivotal importance to determine the interaction profile for LEV in combination with other classical and second-generation AEDs that were also effective against 6Hz-induced psychomotor seizures in mice. The 6 Hz psychomotor seizures were reported to involve a minimal, clonic phase followed by stereotyped and automatistic behaviors that were reminiscent of aura of patients with partial or limbic epilepsy (Toman, 1951; Toman et al., 1952; Brown et al., 1953; Barton et al., 2001). At present, the 6 Hz psychomotor seizure model is used for the early identification of anticonvulsant activity of new compounds effective against therapy-resistant epilepsy (Barton et al., 2001, 2003). Therefore, the objective of this study was to evaluate potential interaction of LEV in combination with CZP, OXC, PB, tiagabine (TGB) and VPA in this model and to use type I isobolographic analysis for parallel and non-parallel dose—response relationship curves (DRRCs). Additionally, in order 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.

Materials and methods

Animals

All experiments were performed on adult male 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 (natural light-dark cycle, temperature of $21 \pm 1\,^{\circ}C$, relative humidity of $55 \pm 5\%$). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 mice per group. 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 conformity with current European Communities Council Directive of 24 November 1986 (86/609/EEC) and Polish legislation on animal experimentation.