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Pharmacodynamic and pharmacokinetic interaction profiles of levetiracetam in combination with gabapentin, tiagabine and vigabatrin in the mouse pentylenetetrazole-induced seizure model: An isobolographic analysis

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ABSTRACT

To characterize the interactions between levetiracetam and the antiepileptic drugs gabapentin, tiagabine, and vigabatrin in suppressing pentylenetetrazole-induced clonic seizures in mice, type II isobolographic analysis was used.

Clonic seizures were evoked in Albino Swiss mice by subcutaneous injection of pentylenetetrazole at its CD_{97} (98 mg/kg). Adverse-effect profiles with respect to motor performance, long-term memory and skeletal muscular strength were measured along with total brain antiepileptic drug concentrations.

The combination of gabapentin with levetiracetam at the fixed-ratios of 2:1, 1:1, 1:2, and 1:4 were supra-additive (synergistic) in terms of seizure suppression whilst the combination at the fixed-ratio of 4:1 was additive. Tiagabine with levetiracetam and vigabatrin with levetiracetam at the fixed-ratios of 1:25, 1:50, 1:100, 1:200, and 1:400 and at 2:1, 3:1, 4:1, 6:1, 8:1, and 16:1 were additive, respectively. No acute adverse effects were observed. Measurement of total brain antiepileptic drug concentrations revealed that levetiracetam in combination with gabapentin at the fixed-ratio of 1:4 significantly elevated (21%) total brain gabapentin concentrations. In contrast, levetiracetam was without affect on tiagabine or vigabatrin concentrations and co-administration with gabapentin, tiagabine or vigabatrin had no effect on levetiracetam brain concentrations, indicating the pharmacodynamic nature of interaction between these antiepileptic drugs in the mouse pentylenetetrazole model.

The combination of gabapentin with levetiracetam at the fixed-ratios of 2:1, 1:1, 1:2, and 1:4 appears to be particularly favorable combination exerting supra-additive interaction in suppressing pentylenetetrazole-induced seizures, although there is a pharmacokinetic contribution to the interaction between levetiracetam and gabapentin at the fixed-ratio of 1:4. Levetiracetam in combination with tiagabine and vigabatrin appear to be neutral combinations producing only additivity in the mouse pentylenetetrazole model.

1. Introduction

Levetiracetam ([S]-alpha-ethyl-2-oxo-1-pyrrolidine acetamide) is a second-generation antiepileptic drug that is licensed for clinical use as monotherapy or adjunctive therapy to treat patients with intractable partial-onset seizures with or without secondary generalization (Cereghino et al., 2000; Grant and Shorvon, 2000). In the clinical setting, levetiracetam has a broad spectrum of anticonvulsant activity, showing efficacy in suppressing juvenile myoclonic epilepsy (Specchio et al., 2006); tonic–clonic, absence and myoclonic epilepsy (Frucht et al., 2001); photosensitive epilepsy (Kasteleijn-Nolst Trenite et al., 1996); and atypical absence or atonic seizures (Khurana et al., 2007). Additionally, the antiepileptic drug is efficacious in children aged 10 years and younger with partial-onset seizures and in those with myoclonic seizures (Tan and Appleton, 2004).

In preclinical studies, it has been found that levetiracetam is virtually ineffective in acute models of epilepsy (i.e., maximal electroshock- and pentylenetetrazole-induced seizures), which are routinely used to screen for potential new antiepileptic drugs (Löscher et al., 1991). In contrast, the antiepileptic drug 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). Levetiracetam has also shown protective activity against seizures induced by 6 Hz electrical

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stimulation (a model of psychomotor seizures) (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). Levetiracetam is also associated with antiepileptogenic effect after a single administration because pretreatment with the drug retards the acquisition of audiogenic seizures in Krushinsky–Molodkina rats (Vinogradova and van Rijn, 2008). Moreover, chronic (21 days) administration of levetiracetam completely inhibits the development of hippocampal hyperexcitability following pilocarpine-induced status epilepticus in rats (Margineanu et al., in press).

Accumulating experimental evidence indicates that levetiracetam is associated with favorable pharmacodynamic interactions with numerous antiepileptic drugs in various animal models including: topiramate (Sills et al., 2004; Luszczki et al., 2006), oxcarbazepine, carbamazepine (Luszczki et al., 2006), diazepam (Mazarati et al., 2004), felbamate (Luszczki et al., 2007), valproate and clonazepam (Kaminski et al., in press). In the case of the combination of levetiracetam with felbamate, a synergistic interaction between drugs in terms of suppression of maximal electroshock-induced seizures was additionally complicated by a pharmacokinetic increase in total brain levetiracetam concentrations (Luszczki et al., 2007). Levetiracetam also potentiated the anticonvulsant activity of carbamazepine, diazepam, felbamate, topiramate, gabapentin, and valproate in sound-induced seizures in DBA/2 mice (Donato Di Paola et al., 2007). Additionally, levetiracetam enhanced the anticonvulsant activity of valproate, clonazepam, diazepam, phenobarbital, lamotrigine, carbamazepine, 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) in the mouse audiogenic seizure model (Kaminski et al., in press). Levetiracetam also potentiated the anticonvulsant activity of clonazepam, valproate, carbamazepine and phenobarbital in the rat amygdala kindling model (Kaminski et al., in press). However, in addition levetiracetam can pharmacodynamically potentiate the acute neurotoxic effects of topiramate and carbamazepine in the rotarod test in mice (Luszczki et al., 2005), and in the clinical setting, adverse pharmacodynamic interactions have been reported in patients prescribed levetiracetam in combination with carbamazepine (Sisodiya et al., 2002) and with topiramate (Glauser et al., 2002).

The objective of this study was to evaluate potential interaction of levetiracetam in combination with that of some selected second-generation antiepileptic drugs: gabapentin, tiagabine and vigabatrin. Anticonvulsant effects of the antiepileptic drug combinations were determined in the mouse pentylenetetrazole-induced clonic seizure test, a model of myoclonic seizures in humans (Löscher et al., 1991), and the data were analyzed by type II isobolographic analysis. 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 levetiracetam, gabapentin, tiagabine and vigabatrin concentrations were measured.

2. Materials and methods

2.1. Animals and experimental conditions

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 (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) 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 antiepileptic drugs were used in this study: levetiracetam (UCB Pharma, Braine-l'Alleud, Belgium), gabapentin (Parke-Davis GmbH, Freiburg, Germany), tiagabine (Sanofi Winthrop, Gentilly, France), and vigabatrin (Marion Merrell S.A., Puteaux, France). All drugs 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. The doses of drugs refer to the free drug forms. Fresh drug solutions were prepared on each day of experimentation and administered as follows: levetiracetam and gabapentin — 60 min, tiagabine — 15 min, and vigabatrin — 240 min before seizures and behavioral tests as well as before brain sampling for the measurement of antiepileptic drug concentrations. These pretreatment times were chosen based upon information about their biological activity from the literature and our previous studies (Luszczki et al., 2005, 2006, 2008). The control animals received an equivalent volume of vehicle (1% Tween 80). Pentylenetetrazole (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.

2.3. Pentylenetetrazole-induced clonic convulsions

The anticonvulsant activities of gabapentin, tiagabine, and vigabatrin against the clonic phase of pentylenetetrazole-induced seizures were determined after s.c. administration of pentylenetetrazole at its CD_{97} (convulsive dose 97, i.e., the dose of pentylenetetrazole that produced clonic seizures in 97% of mice, which in this study was 98 mg/kg). In order to unequivocally assess and classify seizure activity we used a scale for clonic seizures adapted from that described by Löscher et al. (1991). This scale comprises of 5 stages, as follows: 1) one or more generalized myoclonic twitches of the whole body of animals; 2) repeated clonic seizures of fore- and hindlimbs without loss of righting reflexes; 3) generalized clonic seizures lasting for over 3 s with loss of righting reflexes, where the animals fall onto their side during the generalized clonus; 4) loss of righting reflexes followed by tonic forelimb seizure; and 5) loss of righting reflexes with tonic fore- and hindlimb seizure.

The endpoint was that of the first generalized clonic seizures with loss of righting reflexes (stage 3) and the number of animals convulsing out of the total number of mice tested was noted for each treatment regimen. The animals were administered with increasing doses of the antiepileptic drugs, and the anticonvulsant activity of each drug was evaluated as the ED_{50} (median effective dose of an antiepileptic drug, protecting 50% of mice against clonic convulsions). At least four groups of animals were used to estimate each ED_{50} value calculated from the respective log-probit dose–response relationship line according to Litchfield and Wilcoxon (1949). Similarly, the anticonvulsant activity of a mixture of