Epilepsy Research (2007) **75**, 112—121

journal homepage: www.elsevier.com/locate/epilepsyres

Influence of levetiracetam on the anticonvulsant efficacy of conventional antiepileptic drugs against audiogenic seizures in DBA/2 mice

Eugenio Donato Di Paola[a], Pietro Gareri[a], Alessandro Davoli[a], Santo Gratteri[a], Francesca Scicchitano[a], Clara Naccari[b], Giovambattista De Sarro[a,*]

[a] *Section of Pharmacology, Department of Experimental and Clinical Medicine, Faculty of Medicine and Surgery, University of Catanzaro, Catanzaro, Italy*
[b] *Department Farmaco-Biologico, School of Pharmacy, University of Messina, Villaggio SS Annunziata, Contr. Annunziata, 98168 Messina, Italy*

Received 8 February 2007; received in revised form 22 April 2007; accepted 24 April 2007
Available online 5 June 2007

KEYWORDS
Epilepsy;
Levetiracetam;
Antiepileptic drug;
Carbamazepine;
Audiogenic seizure;
DBA/2 mouse

Summary Levetiracetam (LEV, [S]-alpha-ethyl-2-oxo-1-pyrrolidine acetamide) is a new antiepileptic that has been used as adjunctive therapy to treat patients with intractable epilepsy. Systemic administration of levetiracetam (2.5—30 mg/kg, intraperitoneally (i.p.)) was able to produce a dose-dependent decrease in DBA/2 audiogenic seizure severity score. In combination with conventional antiepileptic drugs, levetiracetam, 5 mg/kg, i.p., which per se did not significantly affect the occurrence of audiogenic seizures in DBA/2 mice, potentiated the anticonvulsant activity of some antiepileptic drugs studied against sound-induced seizures in DBA/2 mice. The degree of potentiation induced by levetiracetam was greater, approximately twice, for carbamazepine, diazepam, felbamate, topiramate, gabapentin, and valproate, less for lamotrigine, phenobarbital and phenytoin. This increase was associated with a comparable impairment in motor activity; however, the therapeutic index of combined treatment of antiepileptic drugs with levetiracetam was more favourable than the combination with saline with the exception of lamotrigine, phenytoin and phenobarbital. Since levetiracetam did not significantly influence the total and free plasma and the brain levels of antiepileptics studied. In addition, levetiracetam did not significantly affect the hypothermic effects of the anticonvulsants tested. In conclusion, levetiracetam showed an additive anticonvulsant effect when administered in combination with some classical anticonvulsants, most notably carbamazepine, diazepam, felbamate, gabapentin, topiramate and valproate, implicating a possible therapeutic relevance of such drug combinations.

* Corresponding author at: Department of Experimental and Clinical Medicine, School of Medicine at Catanzaro, Policlinico Mater Domini, Via T. Campanella 115, 88100 Catanzaro, Italy. Tel.: +39 0961 712323; fax: +39 0961 774424.
E-mail address: desarro@unicz.it (G. De Sarro).

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doi:10.1016/j.eplepsyres.2007.04.008

Introduction

Monotherapy is actually considered as the optimal treatment of epilepsy. However, around 30% of patients may experience seizures resistant to the currently available anticonvulsant drugs (Kwan and Brodie, 2000a,b). Actually, polytherapy with two or more antiepileptic drugs remains the treatment of choice in patients who do not respond adequately to antiepileptic monotherapy (Perucca, 2006). Furthermore, these patients experience numerous problems, including Central Nervous System (CNS) side effects and idiosyncratic reactions, which are exacerbated by pharmacokinetic and/or pharmacodynamic interactions between drugs (Patsalos and Perucca, 2003a,b). With the recent introduction of 10 new antiepileptics, the number of possible antiepileptic combinations has exponentially increased. Actually, there are no rational two-drug combinations to choose from, although through clinical experience several drug antiepileptic combinations have been identified as being efficacious against specific seizure types (Stephen and Brodie, 2002). Since the direct clinical evaluation of the anticonvulsant efficacy of drug combinations in epileptic patients is ethically and methodologically very difficult, potential combinations may be readily pre-selected in preclinical studies on animals, and only those, displaying synergistic interactions in terms of seizure suppression could be considered for further clinical evaluation.

Levetiracetam ([S]-alpha-ethyl-2-oxo-1-pyrrolidine acetamide) is a new antiepileptic that has been licensed for clinical use as adjunctive therapy to treat patients with intractable partial-onset seizures with or without secondary generalization (Betts et al., 2000; Cereghino et al., 2000; Shorvon et al., 2000; Siniscalchi et al., 2005). In the clinical setting, levetiracetam has demonstrated a broad spectrum of anticonvulsant activity, showing efficacy in suppressing juvenile myoclonic epilepsy (Kumar and Smith, 2004); tonic–clonic, absence and myoclonic epilepsy (Genton and Gelisse, 2000; Frucht et al., 2001; Krauss et al., 2003); atypical absence or atonic seizures and photosensitive epilepsy (Kasteleijn-Nolst Trenite et al., 1996). Additionally, levetiracetam is efficacious in children with partial-onset seizures and in those with myoclonic seizures (Tan and Appleton, 2004). Levetiracetam has been successfully used in patients with refractory partial epilepsy converted to monotherapy (Ben-Menachem and Falter, 2000; Alsaadi et al., 2002, 2004; Alsaadi and Thieman, 2003).

In preclinical studies, it has been found that levetiracetam is virtually ineffective in acute models of epilepsy (i.e. the maximal electroshock (MES)- and pentylenetetrazole (PTZ)-induced seizures), which are routinely used to screen for potential new antiepileptics (Löscher and Schmidt, 1988). In contrast, levetiracetam 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). The precise mechanism of action of levetiracetam has not been fully understood. It reduces voltage-operated K^+ current and inhibits the delayed rectifier K^+ current in neurons (Madeja et al., 2003), reduces N-type and partially P/Q type high-voltage activated (HVA) Ca^{2+} currents (Niespodziany et al., 2001; Lukyanetz et al., 2002; Pisani et al., 2004) but not low-voltage-activated Ca^{2+} currents (Zona et al., 2001). Indeed, levetiracetam suppresses the inhibitory action of zinc and β-carbolines on $GABA_A$- and glycine-gated currents (Rigo et al., 2002). Recent molecular studies involving transgenic mice suggest that levetiracetam 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 levetiracetam derivatives (Lynch et al., 2004).

There is increasing evidence that levetiracetam is associated with favourable pharmacodynamic interaction with numerous antiepileptics in various animal models including: diazepam (Mazarati et al., 2004), topiramate (Sills et al., 2004; Luszczki et al., 2005, 2006) oxcarbazepine and carbamazepine (Luszczki et al., 2007).

Consequently, the aim of the present study was to investigate the efficacy of conventional antiepileptic drugs such as carbamazepine, diazepam, felbamate, phenytoin, gabapentin, lamotrigine, phenobarbital, topiramate and valproate administered together with levetiracetam against audiogenic seizures in DBA/2 mice. Additionally, we investigated the antiepileptic combinations in relation to motor impairment by the use of the rotarod test. Finally, brain levetiracetam and other antiepileptics concentrations were measured in order to ascertain whether any observed effects were consequent to a pharmacodynamic and/or a pharmacokinetic interaction.

Materials and methods

Animals

The experiments were carried out on DBA/2 mice, weighing 6–12 g (22–26 days old) or 20–28 g (48–56 days old). Animals were purchased from Harlan Italy (Correzzana, Milan, Italy) and housed in groups of 8–10 in colony cages at room temperature, under a 12-h light/12-h dark cycle (lights on at 7:00 a.m.). The animals were housed with free access to food pellets and tap water with food and water available ad libitum. Experimental groups, consisting of 10 animals, were assigned according to a randomised schedule, and each mouse was used only once. Control animals were always tested on the same day as the respective experimental groups. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Procedures involving animals and their care were conducted in conformity with international and national law and policies (European Communities Council Directive of 24th November 1986, 86/609EEC).

Experimental protocols

DBA/2 mice were exposed to auditory stimulation, 30, 60, 90 or 120 min following intraperitoneal (i.p.) administration of levetiracetam (2.5–30 mg/kg) or saline and 30, 45, 60 or 120 min following i.p. injection of some antiepileptics. Each mouse was placed under a hemispheric perspex dome (diameter 58 cm) and 1 min was allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 1 min or until tonic extension occurred. The seizure response, as previously reported (De Sarro et al., 1984), was assessed using the following scale: 0: no response, 1: wild running, 2: clonus, 3: tonus, 4: respiratory arrest. The maximum response was recorded for each animal. Rectal temperature was recorded immediately prior to auditory testing using an Elektrolaboratoriet thermometer type T.E.3. Behavioural