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Pharmacodynamic and/or pharmacokinetic characteristics of interactions between loreclezole and four conventional antiepileptic drugs in pentylenetetrazole-induced seizures in mice: An isobolographic analysis ☆

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Abstract

Isobolographic analysis was used to characterize the interactions between loreclezole (LCZ) and clonazepam (CZP), ethosuximide (ETS), phenobarbital (PB), and valproate (VPA) in suppressing pentylenetetrazole (PTZ)-induced seizures and in producing acute neurotoxic adverse effects in the chimney test in mice so as to identify optimum combinations. Moreover, protective indices (PIs) and benefit indices (BIs) were calculated so that a ranking in relation to advantageous combination could be established. Any pharmacokinetic contribution was ascertained by measurement of brain antiepileptic drug (AED) concentrations.

All AED combinations comprising LCZ and CZP, ETS, PB, and VPA (at the fixed ratios of 1:3, 1:1, and 3:1) were additive in their seizure suppression. However, these interactions were complicated by changes in brain AED concentrations consequent to pharmacokinetic interactions. Thus, LCZ significantly increased total brain ETS concentrations (VPA, CZP, and PB concentrations were unaffected), and ETS decreased, and VPA increased, total brain LCZ concentrations. Only combinations of LCZ with CZP and PB were completely free of any pharmacokinetic interaction. Furthermore, in the chimney test, isobolographic analysis showed that the combination of LCZ and CZP, at the fixed ratio of 1:1, was supra-additive (synergistic, $P < 0.05$), whereas LCZ and ETS at fixed ratios of 1:3 and 1:1 were subadditive (antagonistic, $P < 0.05$). The remaining combinations of LCZ with CZP (1:3 and 3:1), ETS (3:1), PB (all fixed ratios of 1:3, 1:1, and 3:1), and VPA (at the fixed ratios of 1:3, 1:1, and 3:1) barely displayed additivity.

In conclusion, BI, which is a measure of the margin of safety and tolerability of drugs in combination and comprises anticonvulsant and neurotoxic measures, was favorable for only one combination (LCZ and ETS at a fixed ratio of 1:3) with a value of 1.39. In contrast, LCZ and CZP constitute an unfavorable combination (BI = 0.61–1.01). The combinations of LCZ with PB or VPA do not offer any advantage as assessed by the parameters (BI range: 0.75–0.91) used in this study. However, these conclusions are confounded by the fact that LCZ is associated with significant pharmacokinetic interactions.

Keywords: Loreclezole; Antiepileptic drugs; Pharmacodynamic interactions; Pharmacokinetic interactions; Pentylenetetrazole; Isobolographic analysis

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1. Introduction

Approximately 30% of patients with epilepsy are refractory to first-line antiepileptic drugs (AEDs) [1,2]. Furthermore, even though 10 new AEDs have been licensed for clinical use during the last decade, these drugs have had little impact on the prognosis of intractable epilepsy. Consequently the chances of seizure freedom with AED monotherapy for these patients are low, and invariably they are prescribed polytherapy (two or more AEDs) in an attempt to enhance seizure control. Indeed, the addition of a second or a third AED may provide enhanced seizure control in ~14% of these patients [1,2]. However, polytherapy can be associated with problematic pharmacokinetic interactions which may result in adverse CNS side effects [3–5], and there are few data to guide clinicians on strategies for combining AEDs. With advances in our understanding of the modes of AED action and of the pathophysiology of seizure initiation and propagation, the scope for rational polytherapy is increasing. Because of the difficulties in evaluating AED combinations systematically in clinical practice, preclinical studies in animals can provide invaluable information so as to allow preselection of useful combinations. The aim of such studies is to identify AED combinations whose anticonvulsant effects offer optimal protection against seizures and, simultaneously, are devoid of any serious neurotoxic side effects [6].

Loreclezole {LCZ; (*Z*)-1-[2-chloro-2-(2,4-dichlorophenyl)ethenyl]-1H-1,2,4-triazole} is a novel broad-spectrum AED acting specifically at two separate allosteric regulatory sites on $GABA_A$ receptors. LCZ potentiates $GABA_A$ receptor-mediated Cl^- currents through a site present on the β2 and β3 (but not β1) subunits of $GABA_A$ receptors [7]. It has been observed that LCZ's affinity for receptors containing β2 or β3 subunits is >300-fold higher than its affinity for those containing β1 subunits [8]. LCZ also acts in an inhibitory manner, increasing the rate and degree of apparent desensitization of $GABA_A$ receptor-mediated currents. This negative modulation is independent of β subunit subtype and occurs via a novel site independent of the benzodiazepine and picrotoxin binding sites [9]. Moreover, LCZ dose-dependently inhibits ionic currents elicited by GABA in homomeric ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes [10]. In biochemical and electrophysiological studies, it has been shown that low doses of LCZ potentiate GABA receptor currents, increasing inhibitory neurotransmission, whereas at high concentrations, the drug attenuates the effectiveness of inhibitory neurotransmission by reducing the duration of postsynaptic GABA receptor activity [9].

LCZ has been shown to be effective against generalized absence seizures in WAG/Rij rats [11], amygdala-kindled seizures [12], and direct cortical stimulation-evoked clonic movements of the forelimbs in rats [13]. LCZ also attenuated clonic seizures induced by cocaine [14] and pentylenetetrazole (PTZ) [15] in mice, as well as suppressed both tonic and clonic seizures induced by PTZ at various developmental stages in rats [16]. Additionally, the drug showed efficacy against tonic–clonic convulsions in the maximal electroshock seizure test in mice [17]. Moreover, in clinical trials, LCZ was associated with significant efficacy in patients with refractory partial epilepsy [18–20].

Considering the anticonvulsant activity of LCZ, we sought to characterize the mechanism of interaction between LCZ and four conventional AEDs [clonazepam (CZP), ethosuximide (ETS), phenobarbital (PB), and valproate (VPA)] against PTZ-induced seizures in mice using isobolographic analysis. To date, isobolography is the only method that allows the characterization of AED interactions in experimental models of epilepsy. It is widely accepted that isobolography allows the determination of equieffective doses of AEDs and the classification of observed interactions as: supra-additive (synergistic), subadditive (antagonistic), indifferent, or additive [21–25]. As for the PTZ test in rodents, the seizures induced by PTZ are thought to be a model of myoclonic seizures in humans [26,27]. Additionally, the adverse effect profiles of the different AED combinations were investigated in relation to motor impairment in the chimney test so that a ranking in relation to advantageous combination could be ascertained in relation to paradigms of protective indices (PIs) and benefit indices (BIs). Finally, to ascertain any pharmacokinetic contribution to the pharmacodynamic paradigms, brain AED concentrations were determined.

2. Material 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 ad libitum, under standardized housing conditions (12 h light–dark cycle, temperature 21 ± 1 °C). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of eight mice. Each mouse participated in only one experiment. All tests were performed between 9.00 AM and 2.00 PM. Procedures involving animals and their care were conducted in conformity 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 article were ap-