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Montelukast potentiates the anticonvulsant effect of phenobarbital in mice: An isobolographic analysis

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Leukotriene D_4 (PubChem CID: 6435286)
Dimethyl sulfoxide (PubChem CID: 679)
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ABSTRACT

Although leukotrienes have been implicated in seizures, no study has systematically investigated whether the blockade of $CysLT_1$ receptors synergistically increases the anticonvulsant action of classic antiepileptics. In this study, behavioral and electroencephalographic methods, as well as isobolographic analysis, are used to show that the $CysLT_1$ inverse agonist montelukast synergistically increases the anticonvulsant action of phenobarbital against pentylenetetrazole-induced seizures. Moreover, it is shown that LTD_4 reverses the effect of montelukast. The experimentally derived ED_{50mix} value for a fixed-ratio combination (1:1 proportion) of montelukast plus phenobarbital was 0.06 ± 0.02 μmol, whereas the additively calculated ED_{50add} value was 0.49 ± 0.03 μmol. The calculated interaction index was 0.12, indicating a synergistic interaction. The association of montelukast significantly decreased the antiseizure ED_{50} for phenobarbital (0.74 and 0.04 μmol in the absence and presence of montelukast, respectively) and, consequently, phenobarbital-induced sedation at equieffective doses. The demonstration of a strong synergism between montelukast and phenobarbital is particularly relevant because both drugs are already used in the clinics, foreseeing an immediate translational application for epileptic patients who have drug-resistant seizures.

Introduction

Accumulating evidence suggests a role for inflammatory mediators in seizures, from early pathogen- and damage-associated molecular pattern recognition to late arachidonic acid derivatives production and signaling [1–9]. While there are several studies suggesting a role for COX-derived arachidonic acid metabolites in seizures, only a few have investigated a role for lipoxygenase (LOX)-derived arachidonic acid metabolites. Leukotrienes levels increase in the brain during kainate-induced seizures. Moreover, phenidone and BW755C, dual inhibitors of LOX/COX pathways, decrease kainic acid-induced seizures, suggesting the involvement of leukotrienes in this effect [10,11]. In line with this view, Rehni and Singh (2011) have shown that montelukast, a $CysLT_1$ receptor inverse agonist [12] and1,2,3,4-tetrahydroisoquinoline, regarded as a LTD_4 synthetic pathway inhibitor, dose-dependently suppress the development of kindled seizures, as well as pilocarpine-induced spontaneous recurrent seizures [13]. Similarly, Liu and colleagues (2014) have shown that zileuton, a 5-LOX inhibitor, decreases spike-wave discharges in pilocarpine epileptic rats [14]. Interestingly, it has been recently shown that while LTD_4 facilitates and montelukast, pranlukast (a $CysLT_1$ antagonist) and Bay-u9773 (a dual $CysLT_1$/$CysLT_2$ antagonist) decrease PTZ-induced seizures and BBB permeability disruption [15]. Notwithstanding, low doses of LTD_4 (0.2 and 2 pmol, i.c.v.) prevent the anticonvulsant, but not the protective effect of MTK on BBB. The dissociation of these effects suggests that BBB maintenance does not determine the anticonvulsant effect of montelukast [15], and that an alternative mechanism

Abbreviations: CysLT1, cysteinyl leukotriene receptor 1; PTZ, pentylenetetrazole; COX, cyclooxygenase; LOX, lipoxygenase; LTD_4, leukotriene D_4; MTK, montelukast; PB, phenobarbital; NaCl, sodium chloride; AED, antiepileptic drug; PGE_2, prostaglandine E_2; BBB, blood–brain barrier; EEG, electroencephalographic.

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to decrease excitability shall exist. In fact, it has been long known that LTD_4 increases the firing rate of Purkinje cells *in vivo* [16], suggesting an excitatory role for this lipid mediator.

Since none of the classic anticonvulsants used in the clinics has the $CysLT_1$ receptor as a target, we hypothesized that the combination of a $CysLT_1$ receptor inverse agonist with a classic anticonvulsant could be advantageous. Therefore, we tested whether the association of montelukast with a classic anticonvulsant, phenobarbital, would result in a synergistic anticonvulsant effect. This is particularly relevant because both drugs are safely used in the clinics, and the demonstration of an increased anticonvulsant efficacy could have an immediate translational application for the approximately one third of the epileptic patients who are considered to have drug-resistant seizures [17,18]. Therefore, in the current investigation we determined, by isobolographic analysis [19], whether the combination of montelukast and phenobarbital results in sub-additive, additive or supra-additive anticonvulsant effects.

Material and methods

Animals

Adult female Swiss mice (25 ± 3 g), housed ten to a cage (35 cm L × 52 cm W × 17 cm H) were used. All animals were kept in colony cages under controlled light and environment (12:12-h light/dark cycle, 24 ± 1 °C, 55% relative humidity) with free access to food (Supra, Santa Maria, Brazil) and water. Animals were obtained from the Animal House of the Federal University of Santa Maria habituated to laboratory conditions 48 h before each experiment. Behavioral tests were conducted during the light phase of the cycle, from 9:00 to 17:00 h in accordance with the national and international legislation (guidelines of Brazilian Council of Animal Experimentation – CONCEA – and of EU Directive 2010/63/EU for animal experiments), with the approval of Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria (authorization number: 084/2013). All protocols were designed aiming to reduce the number of animals used to a minimum, as well as to minimize their suffering. One hundred and nine animals were used in the experiments designed to determine synergism. Twenty-nine animals were used in the experiments designed to determine the effects of montelukast and phenobarbital on electrocorticographic recorded seizures, leukotriene reversal of montelukast effects and locomotor activity.

Reagents

PTZ was purchased from Sigma (St. Louis, MO, USA), LTD_4 and montelukast were from Cayman Chemical (Ann Arbor, MI, USA) and phenobarbital was from Cristália Pharmaceutical Co (Sao Paulo, Brazil). PTZ was dissolved in sterile 0.9% NaCl. Phenobarbital and montelukast were dissolved in 0.5% dimethyl sulfoxide and sterile apyrogenic saline containing 10% propylene glycol. Fresh drug solutions were prepared on each day of experimentation.

Surgical procedures

All animals were anesthetized with ketamine/xylazine intraperitoneally (100/10 mg/kg, i.p.), and had a 27-gauge guide cannula implanted 1 mm above the right lateral ventricle, under stereotaxic guidance for intracerebroventricular (i.c.v.) injection. The following stereotaxic coordinates relative to bregma were used: AP 0 mm, ML 0.9 mm, V 1.6 mm from the dura [20]. The cannula was fixed to the skull with dental acrylic cement.

For EEG recording, two screw electrodes were implanted over the frontoparietal cortices (bilaterally) keeping intact both the dura and cortex surface (coordinates in mm: AP −4.5 and L 2.5). An additional stainless steel screw electrode was driven into the skull positioned over the nasal sinus, and served as a reference electrode. The electrodes were connected to a multipin socket and were fixed to the skull with dental acrylic cement. After surgery, all mice were injected subcutaneously with buprenorphine hydrochloride (0.01 mg/kg, s.c.) for amelioration of pain and chloramphenicol (200 mg/kg, i.p.) to prevent infection. The experiments were performed 7 days after surgery.

Determination of ED_{50} for each drug and combination

The criterion of anticonvulsant efficacy was the latency to tonic–clonic seizures, that was transformed to percentage of maximal effect against PTZ-induced tonic–clonic generalized seizures [%ME = measured latency (in s)/1200 × 100]. The ED_{50} for the anticonvulsant effect of phenobarbital and montelukast, alone, was calculated based on dose-effect curves. Phenobarbital was administered alone at adjusted doses of 0.09, 0.18, 0.3, 0.5, 0.9, 1.4, 2.7 and 4.5 μmol/25 g (μmol/animal, i.p.), whereas montelukast was administered at the doses of 0.02, 0.05, 0.07, 0.1, 0.17 and 0.3 μmol/animal (i.c.v.). The percentage of maximal effect was plotted against the logarithm of drug doses and the curves were fitted using linear regression analysis. The ED_{50} was calculated from respective linear equations according to Tallarida (2000) [19]. The estimated ED_{50} for each drug was the basis for the fixed ratio combination of phenobarbital and montelukast used to determine synergism.

Mean additive doses of the mixture of phenobarbital and montelukast that should theoretically afford a 50% protection against PTZ-induced seizures (ED_{50add}) were calculated from general equations of additivity, as follows: $a/A + b/B = 1$; where "*a*" and "*b*" are doses of phenobarbital and montelukast that were co-administered and "*A*" and "*B*", the ED_{50} of drugs administered alone. The anticonvulsant activity of the mixture of phenobarbital with montelukast at the fixed-ratio of 1:1 was evaluated and expressed as ED_{50mix}, that is the calculated dose of the mixture of both drugs that afford a 50% protection against PTZ-induced seizures. The doses of phenobarbital and montelukast in the mixture were determined by multiplying the ED_{50} for each drug by the respective proportions of drugs in the mixture. For instance, the two-drug mixture for the combination of 1:1 consisted of phenobarbital (0.5 × 0.74 μmol = 0.37 μmol) plus montelukast (0.5 × 0.25 μmol = 0.125 μmol). Drugs were combined in equieffective doses, as follows: 0.02 + 0.008; 0.05 + 0.016; 0.09 + 0.031; 0.185 + 0.063; 0.37 + 0.125 and 0.74 + 0.25 μmol for phenobarbital and montelukast, respectively.

Isobolographic analysis

The isobolographic analysis is experimental method in pharmacological determination of drug interactions that is currently accepted as the standard method for detecting drug interactions and provides the understanding the real nature of these interactions, regardless of their mechanism of action or the nature of relations [24].

Isobolographic analysis of interactions between phenobarbital and montelukast was performed according to Tallarida (2000). This analysis is based on a comparison of equieffective drug doses, determining pharmacological interactions. ED_{50} values are placed on the isobologram, which consists of the additive line that intercepts each EC_{50} on the *X*- and *Y*-axes. This line of additivity has Cartesian coordinates that represent all possible combinations of drugs in equieffective doses and represents the theoretical isobole for an additive effect. When the experimentally determined ED_{50} of 1:1 combinations are placed on this line, the two-drug mixture