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Isobolographic interaction between AO-294, an enantiomer of losigamone, and phenobarbital in the mouse model of maximal electroshock

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Abstract: The objective of the present study was to determine the exact type of interaction between AO-294, the less active enantiomer of a novel antiepileptic drug losigamone and phenobarbital in the model of maximal electroshock-induced convulsions in mice. Isobolographic analysis of obtained data show that the 2 drugs interact additively when administered in proportions of 1:3 and 1:1. Antagonism was found at the dose ratio of 3:1. This may suggest that AO-294 is not a good candidate for 2-drug therapy with phenobarbital, especially when administered at relatively high doses.

Key words: AO-294, phenobarbital, drug interactions

INTRODUCTION

Losigamone is a new generation antiepileptic drug, effective both in experimental epileptology [1, 2] and complex partial seizures in humans [3]. From the chemical point of view the drug is a racemic mixture consisting of 2 enantiomers: AO-242 and AO-294. Electrophysiological and biochemical studies *in vivo* and *in vitro* have demonstrated that it possesses several mechanisms of action which contribute to its anticonvulsant activity. The drug inhibits the glutamatergic neurotransmission, opens Cl^- channels within the $GABA_A$ receptor complex, and changes permeability of Na^+ and K^+ channels [3]. Studies conducted on mouse cortex slices revealed that only AO-242 (not AO-294) reduced the release of glutamate and aspartate to the synaptic cleft [4]. On the other hand, in *in vitro* studies on AO-294 was a less effective modulator of GABA-ergic neurotransmission [5] and inhibitor of picrotoxin-evoked discharges in hippocampal slices [3].

Since up to 30% of epileptic patients remain resistant to conventional antiepileptic therapy, new therapeutic strategies are being investigated, including the search for new medicines with significant anticonvulsant properties [1]. On the other hand, the efficacy two-drug antiepileptic treatment usually depends on the type of interaction between the component drugs. The aim of the present study was to estimate an interaction profile between AO-294, the less active enantiomer of losigamone, and phenobarbital.

MATERIALS AND METHODS

Animals. All experiments were performed on male Swiss mice, kept in colony cages, with free access to food and tap water, under standardized housing conditions. The animals were randomly assigned to experimental groups consisting of 8 mice. Each mouse participated only in 1 experiment. All tests were performed between 09:00-14:00 to minimize confusing effects of circadian rhythms. All experimental procedures were approved by the Local Ethics Committee at the Medical University in Lublin.

Drugs. AO-294 (a gift from Dr. S. S. Chatterjee, Dr. Willmar Schwabe Company, Karlsruhe, Germany) and phenobarbital (Polfa, Kraków, Poland) were suspended in 1% solution of Tween 80 (Sigma St. Louis, MO, USA). Both drugs were administered *i.p.*, AO-294 30 min, and phenobarbital 60 min before electroconvulsions.

Electroconvulsions. Electroconvulsions were induced with the use of alternating current (50 Hz) produced by a Rodent Shocker Generator (Ataner, Lublin, Poland), and delivered by ear-clip electrodes. The stimulus duration was 0.2 s. The median effective dose (ED_{50}) is the drug dose (in mg/kg) providing a protective effect in 50% of the animals tested against maximal electroshock (25 mA). In order to evaluate ED_{50} values, at least 4 groups of 8 mice were used. Subsequently, intensity-response and dose-response curves, respectively, were constructed on the percentage of mice convulsing.

Isobolographic analysis and statistics. The ED_{50}s of AO-294 and phenobarbital administered alone in the MES-test were determined using log-probit analysis according to Litchfield and Wilcoxon [6]. Subsequently, based upon these ED_{50} values, the median additive doses of mixtures of AO-242 with phenobarbital ($ED_{50\,add}$s), for 3 fixed-ratio combinations of 1:3, 1:1 and 3:1, were calculated from the equation of additivity described by Loewe [7]. The evaluation of experimental drug doses in mixtures ($ED_{50\,mix}$s) for 3 fixed-ratios of 1:3, 1:1 and 3:1 was based on antiepileptic drug doses protecting 50% of animals tested against MES-induced seizures [8].

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Statistical comparison of experimentally-derived $ED_{50\ mix}$ with theoretically calculated $ED_{50\ add}$ allowed determination of the exact type of interactions between the AO-294 and phenobarbital [9]. If the $ED_{50\ mix}$ is not different from the respective $ED_{50\ add}$, then the effect of the drug combination is additive. If the $ED_{50\ mix}$ is statistically lower than the $ED_{50\ add}$, a synergistic interaction between drugs is evident. Otherwise, when the $ED_{50\ mix}$ is statistically higher than the respective $ED_{50\ add}$, an antagonism may be postulated.

RESULTS

ED_{50} values of AO-294 and phenobarbital were 89.9 and 18.1 mg/kg (Table 1). The isobolographic analysis of obtained data revealed additive interactions between drugs administered at dose ratios of 1:3 and 1:1, while antagonism was found when AO-294 was combined with phenobarbital at the ratio of 3:1 (Table 2).

Table 1 Effect of AO-294 and phenobarbital (PB) on the maximal electroshock-induced seizures in mice. ED_{50} (in mg/kg) is the median effective dose protecting 50% of animals against seizures. AO-294 was administered 30 min after, while PB 60 min before electroconvulsions

Treatment (mg/kg)	ED_{50} (mg/kg)
AO-294	89.9 (66.5-108.6)
PB	18.1 (14.1-23.1)

Table 2 Isobolographic analysis of interaction between AO-294 and phenobarbital (PB). ED_{50mix} (in mg/kg) is the experimentally determined median effective dose for the mixture; ED_{50add} (in mg/kg) is the theoretically calculated median effective dose for the mixture. AO-294 was administered 30 min, while phenobarbital (PB) 60 min before the test

Treatment (mg/kg)	ED_{50mix} ± SEM	ED_{50add} ± SEM	Type of interaction
AO-294 + PB (1:3)	36.9 ± 3.4	29.2 ± 3.2	Additivity
AO-294 + PB (1:1)	41.2 ± 4.2	40.2 ± 3.7	Additivity
AO-294 + PB (3:1)	66.5 ± 3.5	51.3 ± 3.7	Antagonism

DISCUSSION

AO-294, a less active enantiomer of losigamone, interacts additively with phenobarbital at dose ratios of 1:3, 1:1. This type of interaction is positive from the clinical point of view. However, at the proportion 3:1, the 2 drugs interacted antagonistically. In contrast to synergism and additivity, antagonism is not profitable from the clinical point of view. It is interesting that similar results were obtained with a combination of losigamone (the racemic mixture) with phenobarbital [1]. It is also interesting that the more active enantiomer of losigamone, AO-242, interacted additively with phenobarbital (unpublished data). This suggests that AO-294 is responsible for the antagonistic interaction between losigamone and phenobarbital (3:1).

In clinical conditions, losigamone is an effective and safe drug employed in the treatment of highly refractory partial seizures (with or without secondary generalized seizures) [3]. AO-294, similar to losigamone, does not seem to be a profitable auxiliary drug in the 2-drug therapy with phenobarbital.

CONCLUSIONS

1. AO-294 is effective against maximal electroshock in mice.
2. AO-294 interacts additively (at dose ratios of 1:3 and 1:1) or antagonistically (3:1) with phenobarbital.
3. AO-294 is not a good drug candidate for concomitant treatment with phenobarbital.

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