

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

Kinga K. Borowicz, Bożena Jaszczuk

Experimental Neuropathophysiology Unit, Department of Pathophysiology, Medical University, Lublin, Poland

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 valproate, in the model of maximal electroshock-induced convulsions in mice. Isobolographic analysis of obtained data show that the 2 drugs interact additively. This may suggest that AO-294 may be used in the 2-drug therapy of refractory epilepsy. However, it does not seem to be superior to its maternal antiepileptic, since losigamone (according to previous reports) interacted synergistically with valproate.

Key words: AO-294, valproate, drug interactions

INTRODUCTION

Up to 30% of newly diagnosed epilepsy remains resistant to conventional antiepileptic therapy. This justifies intense investigations of new antiepileptics for the possibility of their application in polytherapy [1]. From the chemical point of view, losigamone, a novel antiepileptic drug, [(±)-5(R,S)-α(S,R)-5-(2-chlorophenylhydroxymethyl)-4-methoxy-2(5H)-furanone], is a racemate consisting of 2 enantiomers, AO-242 [(+)-5(R)-α(S)-5-(2-chlorophenylhydroxymethyl)-4-methoxy-2(5H)-furanone] and AO-294 [(-)-5(S)-α(R)-5-(2-chlorophenylhydroxymethyl)-4-methoxy-2(5H)-furanone]. Experimental evidence indicates that LSG in rodents can inhibit maximal electroshock-induced seizures (MES) test, or those produced by a variety of convulsants, including pentylenetetrazole (PTZ), bicuculline, picrotoxin, nicotine and 4-aminopyridine [2]. Losigamone is also effective against cocaine-induced convulsions in mice [3], and audiogenic seizures in rodents [2, 4]. The effectiveness of losigamone and both of its enantiomers is different in electrically-evoked seizures in mice. In the MES test, the median effective dose of losigamone was estimated as 7.7 mg/kg, while those of AO-242 and AO-294 were 4.9 and 69 mg/kg, respectively [2, 5].

Effectiveness of add-on therapy in resistant epilepsy may depend on the interaction between the component drugs. The aim of the present study was to estimate the type of interaction between AO-294, the less active enantiomer of losigamone, and valproate, a commonly used antiepileptic drug.

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 in only 1 experiment. All tests were performed between 09:00-14:00 to minimize confounding effects of circadian rhythms. All experimental procedures were approved by the Local Ethics Committee at the Medical University in Lublin, Poland.

Drugs. AO-294 (a gift from Dr. S. S. Chatterjee, of the Dr. Willmar Schwabe Company, Karlsruhe, Germany) was suspended in 1% solution of Tween 80 (Sigma St. Louis, MO, USA), while valproate (Polfa, Rzeszów, Poland) was dissolved in a sterile saline. All drugs were administered *i.p.* 30 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 (ED₅₀) 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 ED₅₀ 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 ED₅₀s of AO-242 and valproate administered alone in the MES-test were determined using log-probit analysis according to Litchfield and Wilcoxon [6]. Subsequently, based upon these ED₅₀ values, the median additive doses of mixtures of AO-242 with valproate (ED_{50add}s), for 3 fixed-ratio combinations of 1:3,

Corresponding author: Prof. Kinga K. Borowicz, Department of Pathophysiology, Medical University, Jaczewskiego 8, 20-090 Lublin, Poland.
E-mail: kinga.borowicz@am.lublin.pl

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1:1 and 3:1, were calculated from the equation of additivity described by Loewe [7]. The evaluation of experimental drug doses in mixtures ($ED_{50\text{mix}}$) 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].

Statistical comparison of experimentally-derived $ED_{50\text{mix}}$ with theoretically calculated $ED_{50\text{add}}$ allowed the determination of the exact type of interaction between the AO-242 and valproate [9]. If the $ED_{50\text{mix}}$ is not different from the respective $ED_{50\text{add}}$, then the effect of a drug combination is additive. If the $ED_{50\text{mix}}$ is statistically lower than the $ED_{50\text{add}}$, a synergistic interaction between the drugs is evident. Otherwise, when the $ED_{50\text{mix}}$ is statistically higher than the respective $ED_{50\text{add}}$, an antagonism may be postulated.

RESULTS

ED_{50} values of AO-294 and valproate were 89.9 and 273.3 mg/kg, respectively (Table 1). The isobolographic analysis of obtained data revealed additive interactions between the drugs administered in all 3 proportions (Table 2).

Table 1 Effect of AO-294 and valproate (VPA) on maximal electroshock-induced seizures in mice. ED_{50} (in mg/kg) is a median effective dose protecting 50% of animals against seizures. AO-294 and VPA were administered 30 min before electroconvulsions

Treatment (mg/kg)	ED_{50} (mg/kg)
AO-294	89.9 (66.5-108.6)
VPA	273.3 (250.1-298.6)

Table 2 Isobolographic analysis of interactions between AO-294 and valproate. $ED_{50\text{mix}}$ (in mg/kg) is an experimentally determined median effective dose for the mixture; $ED_{50\text{add}}$ (in mg/kg) is the theoretically calculated median effective dose for the mixture. AO-294 and valproate (VPA) were administered 30 min before the test

Treatment (mg/kg)	$ED_{50\text{mix}} \pm \text{SEM}$	$ED_{50\text{add}} \pm \text{SEM}$	Type of interaction
AO-294 + VPA (1:3)	212.3 \pm 10.3	220.5 \pm 10.4	Additivity
AO-294 + VPA (1:1)	181.1 \pm 10.3	168.4 \pm 8.3	Additivity
AO-294 + VPA (3:1)	113.0 \pm 6.0	115.9 \pm 5.9	Additivity

DISCUSSION

AO-294, a less active enantiomer of losigamone, interacts additively with valproate at all dose ratios (1:3, 1:1, 3:1). This type of interaction is positive from the clinical point of view. It is interesting that a combination of the more active enantiomer of losigamone, AO-242, with valproate (in all 3 proportions)

also resulted in additive interaction (unpublished data). On the other hand, the interaction between racemate, losigamone, and valproate was synergistic [1]. Bidirectional measurement of losigamone and valproate brain concentrations excluded pharmacokinetic interactions [1]. This suggests that the mixture may have a different pattern of interactions from its components.

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 [1]. Racemate seems to be superior to AO-294, at least in respect of interaction with valproate.

CONCLUSIONS

1. AO-294 is effective against maximal electroshock in mice.
2. AO-294 interacts additively with valproate.
3. AO-294 is not as beneficial as losigamone in combination with valproate.

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