

Isobolographic analysis of interaction between oxcarbazepine and valproate in pentylenetetrazole-induced seizures in mice

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Abstract: The objective of this study was to assess the characteristics of interaction between oxcarbazepine (OXC) and valproate (VPA) in pentylenetetrazole (PTZ)-induced clonic seizures in mice. The anticonvulsant effects produced by OXC and VPA in two-drug mixture at fixed-ratios of 1:3, 1:1 and 3:1, were determined and statistically analyzed with type I isobolographic analysis for parallel dose-response relationship lines. Total brain concentrations of VPA were measured in order to ascertain any pharmacokinetic contribution to the pharmacodynamic interaction. Moreover, the acute adverse-effect profile for the combination of OXC with VPA was determined in the chimney test (motor coordination), step-through passive avoidance task (long-term memory), and grip-strength test (skeletal muscular strength) in mice. Results indicated that OXC combined with VPA at 3 fixed-ratios of 1:3, 1:1 and 3:1 exerted additive interaction in PTZ-induced clonic seizures in mice, although the combination of OXC and VPA at the fixed-ratio of 1:1 displayed a tendency towards supra-additivity (synergy) in the PTZ test in mice. Pharmacokinetic estimation of total brain antiepileptic (AED) concentrations revealed that OXC did not significantly affect total brain VPA concentrations; therefore, the observed interaction in the PTZ test was pharmacodynamic in nature. Evaluation of acute adverse effects for the combination of OXC with VPA revealed that neither drug had any impact on motor coordination, long-term memory, and skeletal muscular strength in mice. Based on this preclinical study, one can ascertain that the additive interaction between OXC and VPA against PTZ-induced seizures associated with lack of acute adverse effects and no pharmacokinetic interactions between drugs, deserve more attention from a clinical point of view.

Key words: oxcarbazepine, valproate, isobolographic analysis, pentylenetetrazole-induced seizures, pharmacodynamic interaction

INTRODUCTION

To date, there are still approx. 30% of patients with epilepsy who are refractory to currently available first-line antiepileptic drugs (AEDs) when used in monotherapy [1]. Consequently, in these patients polytherapy with 2 or more AEDs is undertaken to enhance seizure control [1]. At present, with 25 AEDs available and licensed for the treatment of epileptic patients, one can theoretically create numbers of two-drug combinations, but only some of them are clinically favorable and offer the patients the status of freedom from seizure [2]. However, the direct testing of anticonvulsant efficacy of all two-drug combinations in patients with refractory epilepsy is not possible, either for ethical reasons and/or methodological limitations [3]. Nonetheless, such combinations may be more readily identified and selected in preclinical studies on animals, and only those whose anticonvulsant effects offer optimal protection against seizures and, simultaneously, which are devoid of any serious neurotoxic side effects [4], can be further investigated and verified in clinical settings.

There has appeared quite recently a trend to characterize interactions between drugs in preclinical studies by using isobolographic analysis. This method is considered as a 'gold standard' for the evaluation of interactions between drugs, which allows their classification as: supra-additive (synergistic), additive, sub-additive (antagonistic) and indifferent (neutral) [5-7].

The aim of this study was to assess the exact characteristic of interaction between oxcarbazepine (OXC—a second-generation AED) and valproate (VPA) in the mouse pentylenetetrazole (PTZ)-induced clonic seizure model. The PTZ-induced seizures are thought to be an animal experimental model of myoclonic convulsions in human [8]. Evaluation of the exact characteristics of interactions between OXC and VPA was performed with type I isobolographic analysis for 3 fixed-ratio combinations of 1:3, 1:1 and 3:1. Previously, it has been documented that OXC produced supra-additive interactions with gabapentin, and simultaneously exerted additive interactions with felbamate, tiagabine and loreclezole in the PTZ test in mice [9]. Therefore, it was of pivotal importance to isobolographically evaluate the characteristic of interactions between OXC and VPA against PTZ-induced clonic seizures in mice. Additionally, in order to determine a preclinical pharmacological profile of interaction between OXC and VPA, the AED combination at the fixed-ratio of 1:1 from the PTZ test was evaluated in the chimney (motor

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performance), step-through passive avoidance (long-term memory), and grip-strength (skeletal muscular strength) tests. Furthermore, total brain VPA concentrations were estimated to confirm or exclude the existence of any pharmacokinetic events which might affect the observed interactions between OXC and VPA.

MATERIAL AND METHODS

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, under standardized housing conditions (12 h of a light-dark cycle, temperature: $21 \pm 1^\circ\text{C}$). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 mice. Each mouse participated only in one experiment. All tests were performed between 09:00 – 14:00 to minimize confounding effects of circadian rhythms. Procedures involving animals and their care were conducted in accordance 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 approved by the Local Ethics Committee at the Medical University in Lublin.

Drugs. The following AEDs were used in this study: OXC (Trileptal, Novartis Pharma AG, suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in sterile saline, whereas VPA was directly dissolved in sterile saline. The drugs were administered by intraperitoneal (i.p.) injection in a volume of 5 $\mu\text{l/g}$ body weight, at 30 min. before seizures and behavioural tests as well as before brain sampling for the measurement of AED concentrations. These pretreatment times were chosen based upon information about their biological activity from the literature and own previous studies [10]. PTZ (Sigma, St. Louis, MO, USA) was dissolved in distilled water and administered subcutaneously (s.c.) into a loose fold of skin in the midline of the neck in a volume of 5 $\mu\text{l/g}$ body weight.

Pentylenetetrazole-induced convulsions. The anticonvulsant activities of OXC and VPA against PTZ-induced clonic seizures were determined after s.c. administration of PTZ at its CD_{97} (convulsive dose 97, i.e., the dose of PTZ that produced clonic seizures in 97% of mice, which in this study was 100 mg/kg). Following PTZ administration, the mice were placed separately into transparent Plexiglas cages (25×15×10 cm) and observed for 30 min. for the occurrence of clonic seizures. Clonic seizure activity was defined as clonus of the whole body lasting for over 3 s, with an accompanying loss of righting reflex. The number of animals convulsing out of the total number of mice tested was noted for each treatment regimen. The animals were administered with increasing doses of the AEDs, and the anticonvulsant activity of each drug was evaluated as the ED_{50} (median effective dose of an AED, protecting 50% of mice against clonic convulsions). At least 4 groups of animals were used to estimate each ED_{50} value calculated from the respective log-probit dose-response relationship line according to Litchfield and Wilcoxon [11]. The anticonvulsant

activity of OXC alone was studied at doses of 16, 20, 24, and 28 mg/kg, whereas that of VPA alone at doses of 100, 125, and 150 mg/kg against the clonic phase of PTZ-induced seizures in mice. Similarly, the anticonvulsant activity of a mixture of OXC with VPA was evaluated and expressed as $\text{ED}_{50\text{mix}}$, corresponding to the dose of a mixture of both drugs required to protect 50% of animals tested against PTZ-induced clonic convulsions. This experimental procedure has been described in more detail in earlier studies [10, 12].

Isobolographic analysis of interactions. Interactions between OXC and VPA against PTZ-induced seizures were analyzed according to the methodology previously detailed in earlier studies, where the precise descriptions of the theoretical background with the respective equations showing how to undertake isobolographic calculations have been presented [7, 10, 12, 13].

In the present study, the isobolographic analysis comprised of 6 stages, as follows:

1. Determination of ED_{50} values for OXC and VPA (administered singly) by means of log-probit linear regression analysis according to Litchfield and Wilcoxon [11].
2. Calculation of purely additive $\text{ED}_{50\text{add}}$ values \pm S.E.M. for a mixture of the examined AEDs, which is associated with the choice of at least three fixed drug-dose ratio combinations (usually, 1:3, 1:1 and 3:1). The $\text{ED}_{50\text{add}}$ represents a total additive dose of the drugs in the mixture, providing theoretically a 50% protection against PTZ-induced seizures.
3. Experimental determination of the $\text{ED}_{50\text{mix}}$ values \pm S.E.M. for the corresponding fixed-ratio AED combinations. $\text{ED}_{50\text{mix}}$ is an experimentally determined total dose of a mixture of two component drugs, administered at a fixed-ratio combination sufficient for 50% protective effect against PTZ-induced seizures. To determine the $\text{ED}_{50\text{mix}}$ value, both drugs in the mixture (at proportionally raised doses) were administered to the mice and a dose-response relationship for the mixture (at the fixed-ratio) was denoted using the log-probit method according to Litchfield and Wilcoxon [11].
4. Statistical comparison of the experimentally-derived $\text{ED}_{50\text{mix}}$ values with their corresponding theoretically additive $\text{ED}_{50\text{add}}$ values was undertaken by use of the unpaired Student's *t*-test, according to Porreca et al. [14] and Tallarida [6].
5. Graphical illustration of the examined interactions as isobolograms, which are a simple form of visualization of interactions.
6. Finally, to determine the separate doses of OXC and VPA in the mixture, the $\text{ED}_{50\text{mix}}$ values were multiplied by the respective proportions of AEDs (denoted for purely additive mixture).

In isobolography, it is accepted that half of the effective dose of a first drug plus half of the effective dose of a second drug, should be as therapeutically effective as a full dose of each drug administered separately. This concept of adding fractions of the effective doses of AEDs is the prime, fundamental, and crucial rule underlying the isobolographic analysis [5, 6, 15]. To simplify the notation and nomenclature of interactions in isobolography, the drug doses were administered in fixed-ratio combinations (e.g. 1:3, 1:1, and 3:1). The additive doses of drugs tested in various combinations were calculated from the 'equation of additivity' presented by Loewe [15] as follows:

$x/X + y/Y = 1$; where x and y are respectively the doses of a first and a second drug, co-administered in the mixture, exerting a 50% protection against PTZ-induced seizures; X and Y , respectively, are the doses of the drugs administered separately in order to obtain the same effect (50% suppression of PTZ-induced seizures). The fixed drug dose ratios are usually presented in the form of natural numbers (1:3, 1:1, 3:1) and reflect fractions of ED_{50} values denoted for the drugs used separately. Further details regarding these concepts have been published elsewhere [7, 10, 12, 13].

Chimney test. The effects of combinations of OXC with VPA (at the fixed-ratios of 1:3, 1:1 and 3:1 from the PTZ test) on motor coordination impairment were quantified with the chimney test of Boissier et al. [16]. The pretreatment time for OXC and VPA in the chimney test was identical to that as in the PTZ test. In the chimney test, the mice had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated by the inability of the animals to climb backward up the transparent tube within 60 s. Data were presented as a percentage of mice that failed to perform the chimney test. This experimental procedure has been described in detail in our earlier studies [10, 17].

Light-dark, step-through passive avoidance task. Each mouse was administered OXC in combination with VPA at the fixed-ratios of 1:3, 1:1 and 3:1 from the PTZ test on the first day before training. The time before the commencement of the training session (after drug administration) was identical to that for the PTZ test. Subsequently, the mice were placed in an illuminated box (10×13×15 cm) connected to a larger dark box (25×20×15 cm) equipped with an electric grid floor. Entry of mice into the dark box was punished by an adequate electric foot shock (0.6 mA for 2 s). The animals that did not enter the dark compartment were excluded from subsequent experimentation. On the following day (24 h later), the pre-trained animals were placed again into the illuminated box and observed for up to 180 s. Mice that avoided the dark compartment for 180 s were considered to remember the task. The time that the mice took to enter the dark box was noted and the median latencies (retention times) with 25th and 75th percentiles were calculated. The step-through passive avoidance task provides information concerning the ability to acquire the task (learning) and to recall the task (retrieval). Therefore, it may be regarded as a measure of long-term memory [18]. This experimental procedure has been described in detail in earlier studies [17, 19].

Grip-strength test. The effects of combinations of OXC with VPA (at the fixed-ratios of 1:3, 1:1 and 3:1 from the PTZ test) on muscular strength in mice were quantified by the grip-strength test. The time before the commencement of the grip-strength test (after drug administration) was identical to that for the PTZ test. The grip-strength apparatus (BioSeb, Chaville, France) comprised of a wire grid (8×8 cm) connected to an isometric force transducer (dynamometer). The mice were lifted by the tails so that their forepaws could grasp the grid. The mice were then gently pulled backward by the tail until the grid was released. The maximal force exerted by the mouse before losing grip was recorded. The mean of three measurements for each mouse was calculated, and subsequently the mean maximal force of eight mice per group was determined. The skeletal muscular strength in

mice was expressed in N (Newtons) as means \pm SEM. of eight determinations. This experimental procedure has been described in detail in our earlier study [20].

Measurement of total brain VPA concentrations. Brain VPA concentrations were determined only in mice that were administered OXC+VPA at the fixed-ratio combination of 1:1. This fixed-ratio combination was chosen because it comprised of both AEDs being present at maximally equi-effective doses. Mice were killed by decapitation at times chosen to coincide with that scheduled for the PTZ test, and whole brains were removed from skulls, weighed, harvested and homogenized using Abbott buffer (2:1 vol/weight; Abbott Laboratories, North Chicago, IL, USA) in an Ultra-Turrax T8 homogenizer (IKA-Werke, Staufen, Germany). The homogenates were centrifuged at 10,000 \times g for 10 min and the supernatant samples (75 μ l) analyzed by fluorescence polarization immunoassay (FPIA) using a TDx analyzer and reagents as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA). Total brain concentrations were expressed in μ g/ml of brain supernatants as means \pm SD of eight separate brain preparations.

Statistics. The percent protection of mice against PTZ-induced clonic seizures per dose of the AEDs and subsequently, dose-response relationship lines were fitted using log-probit linear regression analysis according to Litchfield and Wilcoxon [11]. The ED_{50} values with their 95% confidence limits were calculated by computer-assisted log-probit analysis according to Litchfield and Wilcoxon [11]. To precisely analyze the experimental data, the test for homogeneity of data points creating the dose-response relationship lines and the test for parallelism of the dose-response relationship lines for OXC and VPA were presented as indispensable conditions for testing and characterizing AED interactions with isobolography. Because the dose-response relationship lines for OXC and VPA were parallel in this study, the type I isobolographic analysis for parallel dose-response relationship lines was used. Otherwise, one should apply the type I isobolographic analysis for non-parallel dose-response curves, as presented earlier [21, 22]. The obtained 95% confidence limits were transformed to standard errors of the means (S.E.M.) as described previously [10, 13]. Statistical evaluation of isobolographic interactions was performed by the use of Student's t -test in order to detect the differences between the experimentally derived ($ED_{50\text{ mix}}$) and theoretical additive ($ED_{50\text{ add}}$) values, according to Porreca et al. [14] and Tallarida [6]. Qualitative variables from the chimney test were compared by use of the Fisher's exact probability test. The results from the step-through passive avoidance task were statistically analyzed using Kruskal-Wallis nonparametric ANOVA, followed by the *post-hoc* Dunn's test. The data from the grip-strength test were statistically analyzed using one-way ANOVA followed by the *post-hoc* Bonferroni's test. Total brain VPA concentrations were statistically analyzed using the unpaired Student's t -test. All statistical tests were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). Differences among values were considered statistically significant if $P < 0.05$.

RESULTS

Log-probit dose-response relationship line analysis for OXC and VPA against PTZ-induced clonic seizures in mice. In the PTZ test, OXC was administered separately at the increasing doses of 16, 20, 24, and 28 mg/kg, and the percent protection offered by these drug doses was 12.5, 50, 75 and 87.5, respectively. Subsequently, log-probit transformation of the data allowed the determination of the equation of dose-response relationship for OXC administered alone ($y = 9.4648x - 7.4493$ [$r^2 = 0.9863$]; where y – probit of response, x – logarithm of the drug dose to the base 10, and r^2 – coefficient of determination). To analyze homogeneity of data, the test of the line for ‘goodness of fit’ was conducted using Chi-squared analysis according to Litchfield and Wilcoxon [11]. In this test, the χ^2 [(Chi)²] value for experimentally-derived points for OXC was 0.1685, whereas the χ^2 tabular (for two degrees of freedom at the 95% level of significance for $P < 0.05$) was 5.99. Because the experimentally-derived χ^2 value was lower than the tabular χ^2 value, data points comprising the line were not heterogeneous, i.e., the log-probit line for OXC was good-to-fit according to Litchfield and Wilcoxon [11]. Thus, the ED_{50} value for OXC was 20.67 (17.46 – 24.46) mg/kg.

With respect to VPA, the drug was given at doses of 100, 125, and 150 mg/kg, respectively, and the protection (in %) against PTZ-induced clonic seizures was 12.5, 37.5, and 75%, respectively. The equation of dose-response relationship for VPA was $y = 10.297x - 16.796$ [$r^2 = 0.9881$]. The experimentally denoted χ^2 for VPA was 0.0895, whereas the tabular χ^2 (for 1 degree of freedom at the 95% level of significance for $P < 0.05$) was 3.84. Since the experimental χ^2 value was lower than the tabular χ^2 value, data points were not heterogeneous; thus, the log-probit line for VPA was good-to-fit according to Litchfield and Wilcoxon [11]. The ED_{50} for VPA was 130.83 (112.06 – 152.74) mg/kg.

The test for parallelism of two log-probit dose-response relationship lines revealed that the slope function ratio for these lines (SR = 1.0199) was lower than the factor for the slope function ratio (f ratio SR = 1.1317), indicating that the lines were parallel to one another (Figure 1). For more details concerning the calculation of SR and f ratio SR see Appendix in [13].

Isobolographic characteristic of interaction between oxcarbazepine and valproate in pentylenetetrazole-induced seizures in mice. With isobolographic analysis it was observed that all combinations tested between OXC and

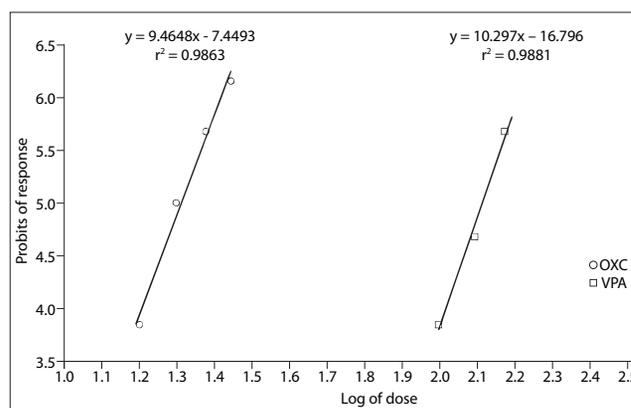


Figure 1 Log-probit dose-response relationship lines for oxcarbazepine and valproate administered alone in the mouse pentylenetetrazole-induced clonic seizure model

Doses of oxcarbazepine (OXC) and valproate (VPA) were transformed in logarithms, whereas the protective effects offered by the AEDs administered alone against PTZ-induced seizures were transformed in probits of response according to Litchfield and Wilcoxon [11]. The equations of dose-response-relationship lines for OXC and VPA are presented on the graph, where y – probit of response, and x – logarithm to the base 10 of drug doses. The test for parallelism of two dose-response relationship lines revealed that both dose-response-relationship lines were parallel.

VPA displayed additive interactions against PTZ-induced seizures in mice (Table 1; Figure 2). The mixture of OXC with VPA at the fixed-ratio of 1:1 exerted a tendency towards supra-additive (synergistic) interaction, because the experimentally denoted $ED_{50\text{mix}}$ (59.3 mg/kg) was lower than the theoretically calculated $ED_{50\text{add}}$ (75.8 mg/kg) (Table 1; Figure 1). The experimental $ED_{50\text{mix}}$ at the fixed-ratio of 1:3 for the mixture of OXC and VPA was 111.2 mg/kg, while the theoretically calculated $ED_{50\text{add}}$ was 103.3 mg/kg (Table 1; Figure 1). Therefore, this interaction was additive in nature. The last combination comprising of OXC and VPA at the fixed-ratio of 3:1 was associated with additivity in the PTZ test (Figure 1). The $ED_{50\text{mix}}$ for the fixed-ratio of 1:3 was 47.6 mg/kg, whereas the $ED_{50\text{add}}$ was 48.2 mg/kg (Table 1; Figure 1).

Effect of oxcarbazepine administered alone and in combinations with valproate on long-term memory, motor coordination and skeletal muscular strength in mice. When OXC was co-administered with VPA at the fixed-ratios of 1:3, 1:1, and 3:1, the motor coordination in the mice was unaffected (Table 2). Furthermore, none of the studied combinations impaired long-term memory as determined in the passive avoidance task, the median retention times

Table 1 Isobolographic characterization of interactions between oxcarbazepine (OXC) and valproate (VPA) in the mouse PTZ-induced clonic seizure model

AED-combination	FR	OXC	+	VPA	=	$ED_{50\text{mix}}$ (mg/kg)	n_{mix}	$ED_{50\text{add}}$ (mg/kg)	=	OXC	+	VPA	n_{add}
OXC + VPA	1:3	5.56		105.64		111.2 ± 11.12	32	103.3 ± 8.19		5.17		98.12	28
OXC + VPA	1:1	8.09		51.21		59.3 ± 6.41	24	75.8 ± 6.05		10.34		65.42	28
OXC + VPA	3:1	15.31		32.29		47.6 ± 4.16	24	48.2 ± 3.92		15.50		32.71	28

Data are presented as median effective doses (ED_{50} values ± S.E.M.) protecting 50% of animals tested against PTZ-induced clonic seizures. The clonic phase of PTZ-induced seizures was produced by the s.c.-injection of PTZ at its CD_{50} (100 mg/kg). The ED_{50} s were either experimentally determined from the mixture of two AEDs ($ED_{50\text{mix}}$) or theoretically calculated from the equation of additivity ($ED_{50\text{add}}$). Additionally, the actual doses of OXC and VPA that comprised the mixtures at all fixed-ratio combinations, and for both $ED_{50\text{mix}}$ and $ED_{50\text{add}}$ values, are presented in separate columns. Statistical evaluation of the data was performed by using unpaired Student’s t -test according to Porecca et al. [14] and Tallarida [6].

FR – fixed-ratio of drug dose combinations

n – total number of animals used at those doses whose expected anticonvulsant effects were ranged between 4 - 6 probits, denoted for the experimental mixture of drugs (n_{mix}) and theoretically calculated (n_{add}) from the equation of additivity.

Table 2 Effects of oxcarbazepine (OXC), valproate (VPA), and their combinations on long-term memory, skeletal muscular strength and motor coordination in mice

Treatment (mg/kg)	FR	Retention time (s)	Grip-strength (N)	Motor coordination impairment (%)
Control	-	180 (180; 180)	89.98 ± 6.02	0
OXC (20.67) + vehicle	-	180 (180; 180)	91.90 ± 5.79	0
VPA (130.83) + vehicle	-	180 (180; 180)	92.02 ± 5.95	0
OXC (5.56) + VPA (105.64)	1:3	180 (180; 180)	88.97 ± 5.83	0
OXC (8.09) + VPA (51.21)	1:1	180 (180; 180)	90.73 ± 6.07	0
OXC (15.31) + VPA (32.29)	3:1	180 (180; 180)	89.49 ± 5.91	0

Results are presented as:

- 1) median retention times (in seconds; with 25th and 75th percentiles in parentheses) from the passive avoidance task, assessing long-term memory in mice;
- 2) mean grip-strengths (in Newtons ± S.E.M.) from the grip-strength test, assessing muscular strength in mice;
- 3) percentage of animals showing motor coordination impairment in the chimney test in mice.

Each experimental group consisted of 8 animals. Statistical analysis of data from the passive avoidance task was performed with nonparametric Kruskal-Wallis ANOVA test followed by *post-hoc* Dunn's test, whereas those from the grip-strength test were analyzed with one-way ANOVA followed by *post-hoc* Bonferroni's test. The Fisher's exact probability test was used to analyze the results from the chimney test. All drugs were administered i.p. at times scheduled from the PTZ test and at doses corresponding to the ED₅₀ values against PTZ-induced seizures (for more detail see the legend to Table 1).

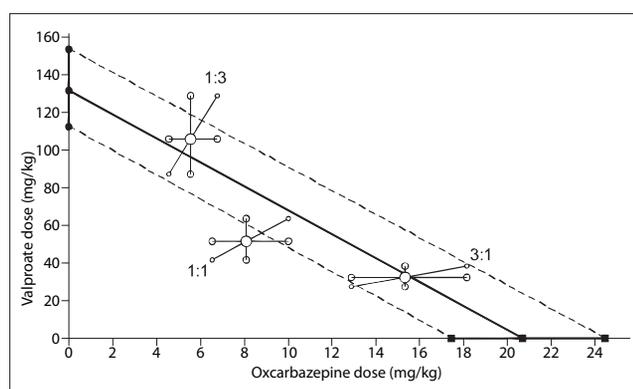


Figure 2 Isobologram showing interaction between oxcarbazepine and valproate against pentylenetetrazole-induced clonic seizures in mice. The median effective dose (ED₅₀) for oxcarbazepine (OXC) is plotted graphically on the X-axis, whereas the ED₅₀ value of valproate (VPA) is plotted on the Y-axis. The solid lines on the X and Y axes represent the 95% confidence limits (CLs) for the AEDs administered alone. The straight line connecting these two ED₅₀ values on the graph represents the theoretical line of additivity for a continuum of different fixed dose ratios, whereas the dashed lines represent on each isobologram the theoretical additive 95% CLs of ED_{50add} values. The open circles (o) depict the experimentally derived ED_{50mix} values (with 95% CLs as error bars) for the total dose expressed as the proportion of OXC and VPA that produced a 50% anticonvulsant effect. Alternatively, all 95% CLs of the experimental ED_{50mix} values are presented horizontally and vertically in the shape of a cross. The experimental ED_{50mix} values of the mixture of OXC+VPA for the fixed-ratio of 1:3 and 3:1 are placed above the theoretical line of additivity, indicating the tendency towards sub-additivity. In contrast, the experimental ED_{50mix} for the fixed-ratio of 1:1 is placed below the line of additivity, indicating a tendency towards supra-additivity.

being 180 s (Table 2). Similarly these combinations had no effect on muscular strength, as assessed by the grip-strength test (Table 2). Moreover, OXC and VPA administered alone at doses of 20.67 mg/kg and 130.83 mg/kg, being their ED₅₀ values from the PTZ test, did not significantly affect long-term memory, muscular strength and motor performance in the mice (Table 2).

Brain valproate concentrations. Total brain VPA concentrations were determined only in mice that were administered the combination of OXC with VPA at the fixed-ratio of 1:1 at doses from the PTZ test. With FPIA technique, the total brain concentration of VPA administered alone (at a dose of 51.2 mg/kg) was 44.48 ± 6.76 µg/ml, and did not differ significantly from that for the combination of VPA (51.2 mg/kg) with OXC (8.1 mg/kg), which amounted to 49.32 ± 5.92 µg/ml (results not shown).

DISCUSSION

Results presented here indicate that the combination of OXC with VPA in the PTZ test produced additive interaction for all the fixed-ratios tested, although the two-drug mixture at the fixed-ratio of 1:1 exerted a tendency towards supra-additivity. Previously, it has been found that OXC synergistically (supra-additively) interacted with gabapentin in the PTZ test [9]. Simultaneously, OXC produced additive interactions with felbamate, tiagabine, and loreclezole in the PTZ-induced seizures in mice [9]. In the maximal electroshock seizure (MES) test, OXC produced additive interactions with VPA, phenobarbital, carbamazepine, and loreclezole [9, 10, 23]. In contrast, OXC exerted antagonistic (sub-additive) interactions with phenytoin and lamotrigine in the MES test [10, 24]. Moreover, OXC exerted a biphasic characteristic of interactions between OXC and clonazepam in the MES test in mice [7]. It was found that the mixture of OXC and clonazepam produced antagonistic interactions when the anticonvulsant effect offered by clonazepam prevailed over that for OXC. On the contrary, the mixture of OXC with clonazepam exerted supra-additive (synergistic) interactions in the MES test, if the effect produced by OXC prevailed over that for clonazepam [7]. With isobolographic analysis of interactions, it has been documented that OXC supra-additively (synergistically) interacted with topiramate, felbamate and gabapentin in the MES test [24, 25]. It should be noted that the evaluation of the anti-seizure effect of OXC with conventional and newer AEDs was performed with isobolographic analysis, which is thought to be the most appropriate method for investigating and classifying types of interactions between drugs in pre-clinical studies on animals [26].

Another fact is worth mentioning while interpreting the results from this study is that OXC – in contrast to its maternal drug: carbamazepine – produced a clear-cut anti-seizure action in PTZ-induced seizures in mice. The experimentally-derived ED₅₀ value for OXC in PTZ test was 20.1 mg/kg, i.e., twice higher than that denoted experimentally in the MES test in mice, which was 9.5 mg/kg [10]. Experimental evidence indicates that OXC suppresses seizures in both MES and PTZ tests, whereas carbamazepine is only active against MES-induced seizures in mice. The explanation of such a difference in the anti-seizure activity between OXC and CBZ is unknown at present. However, more advanced neurochemical and electrophysiological studies are required to elucidate this phenomenon between drugs.

Evaluation of acute adverse-effect profile for the combination of OXC with VPA at the fixed-ratio of 1:1 (in which both drugs were present in equi-effective doses), revealed that neither OXC or VPA administered alone nor in combination affected motor coordination, long-term memory and skeletal muscular strength in mice challenged with the chimney test, step-through passive avoidance task, and grip-strength test, respectively. Similarly, pharmacokinetic assessment of VPA concentrations with fluorescence polarization immunoassay technique revealed that OXC had no impact on total brain VPA concentration, suggesting a pharmacodynamic characteristic of interaction between OXC and VPA in mice.

Based on this pre-clinical study, one can ascertain that the observed additive interaction between OXC and VPA against PTZ-induced seizures, especially the combination at the fixed-ratio of 1:1, offers a favourable pharmacological profile, lack of acute adverse effects, and no pharmacokinetic interactions between drugs, deserves more attention from a clinical point of view.

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