

# Synergy among oxcarbazepine, pregabalin and topiramate in the mouse maximal electroshock-induced seizure test – an isobolographic analysis

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## Abstract

**Introduction.** Assessment of interactions among antiepileptic drugs (AEDs) during polytherapy is still a challenging issue for physicians and epileptologists worldwide. In spite of 25 currently licensed AEDs, there are no algorithms allowing a proper choice of these drugs to create combinations which would offer epileptic patients an efficacious therapy in the case of seizures refractory to monotherapeutic use of the AEDs. To characterize a type of interaction for a three-drug mixture of oxcarbazepine (OXC), pregabalin (PGB) and topiramate (TPM) in an experimental model of tonic-clonic seizures, an isobolographic analysis of interaction was applied.

**Materials and Method.** The anticonvulsant effects of the three-drug mixture of OXC, PGB and TPM with respect to suppression of tonic-clonic seizures in mice were assessed in the mouse maximal electroshock-induced seizure model. Type I isobolographic analysis was used to characterize the type of interactions among three AEDs. Potential acute adverse effects were evaluated in the chimney, passive avoidance and grip-strength tests.

**Results.** The three-drug mixture of OXC, PGB and TPM exerted supra-additive (synergistic) interaction in the mouse maximal electroshock-induced seizure model. The combination of OXC, PGB and TPM did not produce any acute adverse effects in mice in the chimney, passive avoidance and grip-strength tests.

**Conclusions.** The isobolographic synergy observed experimentally for the combination of OXC, PGB and TPM could be recommended to patients with drug-resistant epilepsy, if the results of this study were translated to clinical settings.

## Key words

drug interaction, isobolographic analysis, oxcarbazepine, pregabalin, topiramate, antiepileptic drugs

## INTRODUCTION

In spite of advanced knowledge about antiepileptic drugs (AEDs) and their application in the treatment of epileptic patients, there are still a number of patients inappropriately treated with these AEDs [1]. Each year, several novel compounds are tested in both preclinical conditions and clinical settings in the hope of discovering the most promising drugs that would be able to suppress seizure activity in epileptic patients [2–4]. When the treatment with the first current frontline AED failed, clinicians were obliged to try another AED, fully effective against specific seizure types. However, when three consecutive monotherapies with AEDs failed, doctors were forced to classify the seizures as drug-resistant and, in consequence, start polytherapy with two or more AEDs to either reduce seizure activity or stop seizures in these patients [5, 6].

Every combination of AEDs exerts synergistic, additive or antagonistic interactions with respect to seizure suppression. The types of interactions among AEDs depend on various factors, of which the most important seem to be their

molecular mechanisms of action [7]. About two decades ago, there appeared a suggestion that AEDs with various molecular mechanisms of action are expected to produce synergistic interaction when the AEDs are combined together [8]. Experiments conducted on mice in the model of tonic-clonic seizures, confirmed, at least in part, that some AEDs (whose molecular mechanisms considerably differ) produce synergistic interaction. However, the other AED combinations occurred as additive or even antagonistic in nature.

At present, it is difficult to unequivocally predict which of the AED combinations would be synergistic or antagonistic. However, to correctly classify the interactions as synergistic, additive or antagonistic, researchers should conduct their experiments using isobolographic analysis of interaction that is thought to be a golden standard in the evaluation of types of interaction occurring between drugs [9, 10]. With isobolographic analysis one can classify interactions for two-drug or three-drug mixtures. Generally, the two-drug mixtures and their interactions were extensively examined in preclinical studies. However, scarce information is available from medical literature about types of interactions occurring among three AEDs, even though the three-drug combinations are prescribed for patients with drug-resistant seizures [11, 12].

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## OBJECTIVE

The main goal in this study was to assess a type of interaction among OXC, PGB and TPM in the tonic-clonic seizure model in mice using the type I isobolographic analysis of interaction.

The rationale was to determine the nature of interaction occurring for the three-drug mixture comprising oxcarbazepine (OXC), pregabalin (PGB) and topiramate (TPM). From a theoretical viewpoint, when considering molecular mechanisms of action of these AEDs, the combination of OXC, PGB and TPM should produce synergy in mice subjected to the tonic-clonic seizure model. To verify and confirm such assumption, experiments on mice that received the combination of OXC, PGB and TPM should be performed. No doubt exists that another synergistic combination of AEDs (confirmed in preclinical conditions), can substantially help doctors to offer some epileptic patients an efficacious treatment against seizures. It is believed that synergistic interaction inferred from experimental animal model of epilepsy will be also synergistic in epilepsy patients [13].

## MATERIALS AND METHOD

**Animals and experimental conditions.** All experimental procedures on animals were performed in strict accordance with the ARRIVE guidelines and EU Directive 2010/63/EU for animal experiments. Experimental protocols described in this study were approved by the Second Local Ethics Committee at the University of Life Sciences in Lublin, Poland. Adult male albino Swiss outbred mice (weighing 22–26 g), after acclimatization to laboratory conditions, were randomly assigned to experimental groups comprising 8 mice each. The total number of mice used in this study was 136, including, 120 animals used in the tonic-clonic seizure model and 16 mice in 3 behavioural (chimney, passive avoidance and grip-strength) tests.

**Drugs.** OXC (Trileptal<sup>®</sup>, Novartis Pharma AG, Basel, Switzerland), PGB (Lyrica<sup>®</sup>, Pfizer Ltd., Sandwich, Kent, UK), and TPM (Topamax<sup>®</sup>, Cilag AG, Schaffhausen, Switzerland) were dispersed in an aqueous 1% solution of Tween 80 (Sigma-Aldrich, Poznan, Poland). All AEDs were administered intraperitoneally (i.p.) as follows: PGB at 120 min, TPM at 60 min, and OXC at 30 min, before the maximal electroshock-induced seizures and all behavioural tests, as recommended elsewhere [14, 15].

**Maximal electroshock-induced seizures.** The anticonvulsant potency of OXC, PGB and TPM, when administered separately, were expressed as the median effective doses ( $ED_{50}$  in mg/kg). Maximal electroshock-induced seizures were generated by a Rodent Shocker (Freiburg, Germany) producing alternating current (25 mA, 500 V, 50 Hz, 0.2 s stimulus duration) delivered via ear-clip electrodes. The respective groups of mice, after receiving different drug doses (either separately or in combination) were exposed to maximal electroconvulsions and a percentage of the mice protected from tonic-clonic seizures contributed to the construction of dose-response effect curves, as recommended earlier [16]. The anticonvulsant potency of the three-drug

mixture of OXC, PGB and TPM at the fixed-ratio of 1:1:1 was expressed as the experimental median effective dose ( $ED_{50\text{ exp}}$ ).

**Isobolographic analysis of interaction.** The interaction among AEDs administered in the mixture at the fixed-ratio of 1:1:1 was analyzed isobolographically, as described earlier [9, 17, 18]. After determination of  $ED_{50}$  values for OXC, PGB and TPM (when administered separately), their dose-response effect curves were tested for parallelism, as recommended elsewhere [19, 20]. Simultaneously, {Luszczki, 2006 #57;Luszczki, 2005 #3;Luszczki, 2006 #40;Luszczki, 2009 #33}the median additive dose ( $ED_{50\text{ add}}$ ) for the three-drug mixture (at the fixed-ratio combination of 1:1:1) was calculated, as presented elsewhere [15, 17]. Subsequently, the median experimental dose ( $ED_{50\text{ exp}}$ ) was determined from doses of the three-drug mixtures that protected the mice from tonic-clonic seizures, as presented elsewhere [21, 22]. Finally, to assess the strength of interaction among the studied AEDs, the interaction index value ( $\alpha$ ), as a ratio of  $ED_{50\text{ exp}}$  and  $ED_{50\text{ add}}$  values, was calculated, as presented elsewhere [9, 23–25].

**Grip-strength test.** Potential acute adverse effects of the three-drug mixture (administered in a dose that corresponded to the  $ED_{50\text{ exp}}$  value from the maximal electroshock-induced seizure test) with respect to changes in skeletal muscular strength in mice were quantified with the grip-strength test, as described elsewhere [26]. In this test, each mouse was lifted by the tail and, after grasping the grid with the forepaws, was pulled backward until the grid was released. The grid was connected to a dynamometer, which recorded a maximal force exerted by a mouse before losing its grip the grid. The skeletal muscular strength in the mice was expressed in N (newtons) as means  $\pm$  S.E.M. of at least 8 determinations, as described elsewhere [21, 27].

**Step-through passive avoidance task.** Potential acute adverse effects of the three-drug mixture (administered in a dose that corresponded to the  $ED_{50\text{ exp}}$  value from the maximal electroshock-induced seizure test) with respect to changes in long-term memory in mice were quantified with the step-through passive avoidance task, as described elsewhere [28]. On the first experimental day, each mouse, after receiving the three-drug mixture, was placed in an illuminated compartment connected to a dark compartment equipped with an electric grid floor. After exploring the illuminated compartment, the mouse crossed to the dark compartment and was punished by an electric footshock (0.6 mA for 6 s), as described elsewhere [24, 29]. On the second experimental day, the pre-trained mice were placed separately into the illuminated compartment and observed up for to 180 s. The animals that avoided passing into the dark compartment for 180 s had remembered the task. Any changes in the time, necessary for each animal to stay in the illuminated compartment, were noted, and the median latencies as retention times (with 25th and 75th percentiles) were calculated, as described elsewhere [29, 30].

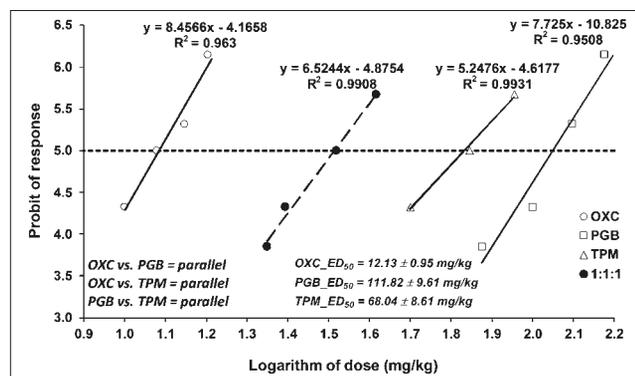
**Chimney test.** Potential acute adverse effects of the three-drug mixture (administered in a dose that corresponded to the  $ED_{50\text{ exp}}$  value from the maximal electroshock-induced seizure test) with respect to impairment of motor coordination in

mice were quantified with the chimney test, as recommended elsewhere [31]. The number of the animals unable to climb backwards up the plastic transparent tube within 1 min. per total number of the mice in the experimental group was noted. The impairment of motor coordination was evident if the mouse could not escape from the tube within 60 s, as described elsewhere [32].

**Statistics.** To calculate the  $ED_{50}$  and  $ED_{50\text{ exp}}$  values, log-probit analysis was used, as recommended elsewhere [16]. To statistically compare the  $ED_{50\text{ exp}}$  with  $ED_{50\text{ add}}$  values for the three-drug mixture, the unpaired Student's *t*-test was used, as described elsewhere [21, 33]. Results from the chimney test were statistically compared by using the Fisher's exact probability test. Median retention times from the passive avoidance task were statistically evaluated with Mann-Whitney U-test. The results from the grip-strength test were statistically verified with the unpaired Student's *t*-test. Level of statistical significance was established at  $P < 0.05$ . All data were statistically analyzed with GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA, USA).

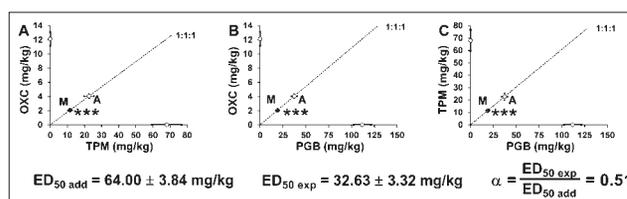
## RESULTS

**Anticonvulsant potencies of OXC, PGB and TPM together with isobolographic analysis of interaction.** All three AEDs: OXC, PGB and TPM, when administered alone produced a clear-cut anticonvulsant effect in the mouse tonic-clonic seizure model; their median effective doses ( $ED_{50}$  values) were 12.13 mg/kg for OXC, 111.82 mg/kg for PGB and 68.04 mg/kg for TPM, respectively (Fig. 1). Linear regression analysis revealed that log-probit dose-response effect curves of OXC, PGB and TPM were mutually parallel (Fig. 1).



**Figure 1.** Linearly related dose-response effects for oxcarbazepine (OXC), pregabalin (PGB) and topiramate (TPM) administered separately and combined together at the fixed-ratio of 1:1:1 in the tonic-clonic seizure model in mice. Legend to Figure 1. Doses of particular AEDs (after transformation to logarithms) were plotted on X-axis, whereas their anticonvulsant effects (after transformation to probits) were placed on Y-axis, according to log-probit method [22]. Equations for linearly related dose-response functions for OXC, PGB and TPM along with their  $ED_{50}$  values are presented on the graph

Similarly, the mixture of OXC, PGB and TPM at the fixed-ratio combination of 1:1:1 also exerted a clear-cut anticonvulsant effect in the tonic-clonic seizure model in mice; its experimentally-derived median effective dose ( $ED_{50\text{ exp}}$  values) amounted to 32.63 mg/kg (Figure 2A-C). The isobolographic analysis of interaction revealed that the  $ED_{50\text{ exp}}$  value for the three-drug mixture of OXC, PGB and TPM considerably differed from the additively calculated



**Figure 2A-C.** Isobolograms with synergistic interaction among oxcarbazepine (OXC), pregabalin (PGB) and topiramate (TPM) in the tonic-clonic seizure model in mice.

Legend to Figure 2A-C. The  $ED_{50}$  values ( $\pm$  S.E.M.) of OXC, PGB and TPM from the tonic-clonic seizure model are plotted on X- and Y-axes, respectively. Points A and M (on each graph) indicate the  $ED_{50\text{ add}}$  and  $ED_{50\text{ exp}}$  values ( $\pm$  S.E.M.), respectively. The  $ED_{50\text{ exp}}$  value (point M) of the three-drug mixture is placed significantly below the  $ED_{50\text{ add}}$  value (point A), indicating the supra-additive (synergistic) interaction (\*\* $P < 0.001$ ); the unpaired Student's *t*-test) among OXC, PGB and TPM in the mouse tonic-clonic seizure model.  $\alpha$  – interaction index.

$ED_{50\text{ add}}$  value ( $P < 0.001$ ) (Fig. 2A-C), indicating supra-additive (synergistic) interaction among the studied AEDs.

**Acute adverse effects of the mixture of OXC, PGB and TPM.** Determination of potential acute adverse effects produced by the mixture of 3 AEDs (in a dose reflecting the  $ED_{50\text{ exp}}$  value from the tonic-clonic seizure model) in behavioural tests revealed that the combination of OXC, PGB and TPM neither disturbed long-term memory, nor altered skeletal muscular strength in the mice (Tab. 1). Similarly, no impairment in motor coordination was observed in the animals, receiving the mixture of OXC, PGB and TPM and challenged with the chimney test (Tab. 1).

**Table 1.** Assessment of potential acute adverse effects of the mixture of oxcarbazepine (OXC), pregabalin (PGB) and topiramate (TPM) in the grip-strength, passive avoidance and chimney tests in mice

Treatment (mg/kg)	Muscular strength (N)	Retention time (s)	Deficits in motor performance
Vehicle + vehicle + vehicle	$0.964 \pm 0.079$	180 (180; 180)	0/8
OXC (2.06) + PGB (19.0) + TPM (11.56)	$0.947 \pm 0.081$	175.0 (150.5; 180)	1/8

Columns indicate: (1) mean muscular strengths (in newtons [N]  $\pm$  S.E.M.) in mice from the grip-strength test; (2) median retention times (with 25<sup>th</sup> and 75<sup>th</sup> percentiles in parentheses) of the mice from the passive avoidance task; and (3) animals with impairment of motor coordination per total number of animals used in the chimney test. Doses of particular drugs correspond to the  $ED_{50\text{ exp}}$  value from the tonic-clonic seizure model.

## DISCUSSION

The results obtained confirm the authors' research hypothesis that the AEDs with various molecular mechanisms of action produced synergistic interaction in the mouse maximal electroshock-induced seizure model. Two indicators of proper classification of interaction were applied. The first one was based on statistical analysis of data with Student's *t*-test, as recommended elsewhere [34, 35], and the second – calculation of the interaction index, which was a ratio of  $ED_{50\text{ mix}}$  and  $ED_{50\text{ add}}$  values. The interaction index value for the combination of OXC, PGB and TPM amounted to 0.51, indicating synergistic interaction in the mouse tonic-clonic seizure model. Of note, the interaction index characterizes the strength of interaction occurring among drugs. However, its border values were arbitrary accepted as: synergistic – if they were lower than 0.7; antagonistic – if they were higher than 1.3; and additive – if they range between 0.7 – 1.3 [9]. Although both methods are independent and used by various

authors, the statistical analysis of data seems to be more powerful and superior to the interaction index, whose values classifying interactions are arbitrary selected.

Synergistic interaction observed for the combination of OXC, PGB and TPM is quite similar to that reported earlier for the combinations of PB, PHT and PGB [30], and CBZ, PB, and TPM [36] in the mouse model of tonic-clonic seizures. Previously, it was been reported that the combinations of LCM with CBZ and PB or LTG exerted additive interaction [21, 37]. Only the three-drug mixture of LCM with CBZ and VPA displayed antagonistic interaction in the mouse tonic-clonic seizure model [20].

Analyzing the results from this study and comparing them with those published earlier for two-drug mixtures, it can be ascertained that the two-drug combinations of PGB with OXC and PGB with TPM exerted only additive interaction with a tendency towards supra-additivity (synergy) in the mouse model of tonic-clonic seizures [38]. Only the two-drug combination of OXC with TPM produced synergistic interactions for three tested fixed-ratios of 1:3, 1:1 and 3:1 in the mouse model of tonic-clonic seizures [39]. Noticeably, the rationale in this study was to confirm the suggestion that three various AEDs, whose molecular mechanisms of action substantially differ from one another, are able to synergistically cooperate in terms of suppression of tonic-clonic seizures in the experimental animals.

Considering the chemical structures of OXC, PGB and TPM (Fig. 3), it is possible to create a new molecule possessing the anticonvulsant properties of all three drugs. Medicinal chemistry techniques allow for modification of the structures of the examined AEDs, and the creation of a novel compound having active centres that will interact specifically with receptors, ion channels and/or protein targets, in a similar manner to the studied AEDs. More specifically, PGB is a drug that interacts with the  $\alpha_2\delta$  subunit of calcium channels [40, 41]. OXC blocks the sodium and calcium channels [42], whereas TPM affects AMPA and NMDA receptors, blocking the sodium and calcium channels [42–45]. The concept of creating and designing of novel drugs seems to fulfil the to-date unmet needs associated with reduction and/or elimination of seizures in epileptic patients.

At present, isobolographic analysis provides information about the proportions of the drugs used in the experimental mixture that consists of AEDs administered in particular doses exerting the similar and comparable effects. Of note, in this study all three AEDs exerted anticonvulsant properties in the mouse maximal electroshock-induced seizure model, offering the animals protection from tonic-clonic seizures. The experimental approach in this study was based on the evaluation of  $ED_{50}$  values for the AEDs administered alone and in combination at the fixed-ratio of 1:1:1.

The choice of OXC, PGB and TPM to test these AEDs in combination in the mouse maximal electroshock-induced seizure model was not incidental. These AEDs belong to the

second and third generations of AEDs, which are generally characterized by high therapeutic index, low toxic profile and low inclination to evoke adverse effects in epileptic patients. All the AEDs are prescribed to patients as add-on drugs to treat tonic-clonic seizures and partial convulsions in humans. Considering the facts that OXC, PGB and TPM possess various molecular mechanisms of anticonvulsant action, and the AEDs offer suppression of tonic-clonic seizures, it was not surprising that their interaction in the mouse maximal electroshock-induced seizure model was synergistic.

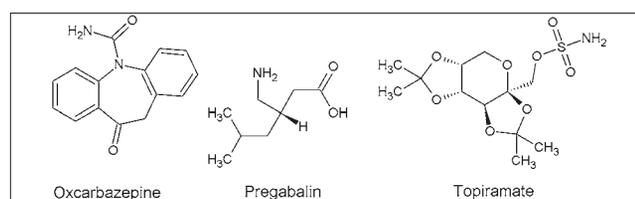
Evaluation of the potential of AEDs (when administered in combination) to produce acute adverse effects, confirmed that the three-drug combination of OXC, TPM and PGB (at the fixed-ratio of 1:1:1) was devoid of any acute side effects. Experiments conducted on animals subjected to the passive avoidance task revealed that the AEDs when combined together did not produce any significant changes in remembering and learning acquisition processes in mice. Moreover, no changes in mean skeletal muscular strength were observed in the mice subjected to the grip-strength test.

The last behavioural test conducted in this study confirmed that the combination of three AEDs did not affect motor coordination in mice challenged with the chimney test. It should be stressed that the three-drug mixture comprised doses of AEDs that corresponded to the  $ED_{50\text{exp}}$  value from the maximal electroshock-induced seizure test. Thus, it was documented that the synergistic interaction in the mouse maximal electroshock-induced seizure model was also free from acute adverse effects when tested in this preclinical study.

The results from this study are in strict agreement with those reported earlier in the same behavioural tests in mice receiving various three-drug combinations of AEDs, where it was found that the combinations of PB + PHT + PGB [30], CBZ + PB + TPM [36], LCM + CBZ + PB [21], LCM + CBZ + LTG [27], LCM + CBZ + VPA [20] and LCM + LTG + PB [27], were devoid of any acute adverse effects in mice.

When interpreting the results from this study, another fact deserves more attention. It should be stressed that total brain AED concentrations were not verified in this study; therefore, it could not be excluded that the observed synergistic interaction in the mouse model of tonic-clonic seizures had a pharmacokinetic nature. Nevertheless, it should be mentioned that the doses of particular drugs used in the mixture for the combination of OXC, PGB and TPM, were low enough to be capable of interacting pharmacokinetically with other co-administered AEDs. Moreover, some earlier reports confirmed that none of the AEDs studied (OXC, PGB and TPM) interacted pharmacokinetically when combined together. For instance, it was found that the two-drug combinations of OXC with TPM, OXC with PGB and PGB with TPM did not produce pharmacokinetic interaction in mice [38, 39]. Since three two-drug combinations did not affect pharmacokinetic parameters of co-administered AEDs, it is highly likely that the AEDs did not mutually alter their total brain concentrations when the AEDs would be combined in one three-drug combination.

When translating the results from this preclinical study to clinical settings, the main limitation seems to be the experimental model used in animals. Generally, the maximal electroshock-induced seizure test is thought to be a model of tonic-clonic seizures [46]. However, in this model, the seizure activity is evoked acutely in healthy naïve animals. In



**Figure 3.** Chemical structure of oxcarbazepine, pregabalin and topiramate

contrast, pharmaco-resistant epilepsy in humans is defined if at least three various monotherapies failed and polytherapy with two or three AEDs is prescribed by physicians or neurologists to suppress seizures in patients. In such a situation, patients with seizures are obliged to chronically receive AEDs, while the animals in this study received only one (single) treatment with three AEDs. In conclusion, the combination of OXC, TPM and PGB produced a synergistic interaction that is worthy of recommendation for further clinical settings.

Considering the molecular mechanisms of action of these three AEDs, it can be stated that AEDs with different mechanisms can offer suppression from tonic-clonic seizures in experimental animals. It is possible that these AEDs (OXC, PGB and TPM), when combined together, would be able to suppress seizures in epilepsy patients. Thus, the observed synergy in mice can also be expected in patients with epilepsy because the molecular mechanisms of action of these AEDs are constant and do not change in mammals.

## CONCLUSIONS

1. The mixture of OXC, PGB and TPM produced synergistic (supra-additive) interaction in the mouse model of tonic-clonic seizure.
2. When translating the results of this study to clinical conditions, a special recommendation is advised for physicians: to treat the epilepsy patients with the combination of OXC, PGB and TPM.

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