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Interactions of retigabine with topiramate in the mouse tonic-clonic seizure model and chimney test – an isobolographic analysis

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Abstract

Introduction and objectives. Nowadays, one of the treatment options for patients with refractory epilepsy is polytherapy with two or more antiepileptic drugs (AEDs). Retigabine (RTG) is a novel third-generation AED with unique molecular mechanisms of action that has recently been approved as an add-on drug for the treatment of tonic-clonic seizures. To characterize types of interactions between RTG and topiramate (TPM – a second-generation AED), the maximal electro-shock-induced seizure model (MES) and chimney test in mice were used.

Materials and method. In the MES model, the anticonvulsant effects of the drugs in terms of suppression of tonic-clonic seizures in male albino Swiss mice were assessed. In the chimney test, the acute neurotoxic effects of the drugs with respect to impairment of motor coordination were determined. Type I isobolographic analysis for the combination of RTG and TPM was applied to assess the anticonvulsant and neurotoxic effects in both the MES and chimney tests. Total brain concentrations of RTG and TPM were measured to exclude any pharmacokinetic interaction between drugs.

Results. The type I isobolographic analysis of interaction revealed that the combination of RTG with TPM produced additive interaction in the MES test and additivity, with a slight tendency towards antagonism in terms of acute neurotoxic effects in the chimney test. Neither RTG nor TPM mutually affected total brain concentrations in the experimental animals.

Conclusions. The isobolographically analyzed combination of RTG with TPM is favourable and may be recommended to some patients with refractory epilepsy.

Key words

maximal electro-shock, drug interaction, isobolographic analysis, Retigabine, Topiramate

INTRODUCTION

Nowadays, the efficacious treatment of patients with epilepsy is a challenging issue for clinicians because of the various types of seizures and availability of dozens of antiepileptic drugs (AEDs). 30% of epilepsy patients are still inadequately treated with the currently available AEDs [1], and therefore, these patients need the therapeutic application of two or more AEDs [2]. It is obvious that every combination of AEDs produces pharmacodynamic, pharmacokinetic or both interactions. To terminate seizures in epileptic patients, clinicians are sometimes obliged to combine various AEDs without any previous information about the type of interactions between the combined drugs. To provide clinicians with necessary information about the perfect selection of AEDs in combination, experiments carried out on animals can properly assess the types of interactions between the AEDs [3]. Overwhelming evidence indicates that several two-drug combinations of AEDs displayed favourable profiles in both preclinical studies on animals and epileptic patients [2, 4]. The most favourable combinations of AEDs are those reported in preclinical studies of synergistic interaction with respect to seizure suppression and/or antagonistic interaction with respect to side (neurotoxic) effects [5, 6].

Retigabine (RTG – a third-generation AED) has been approved for clinical use as an add-on drug in partial onset seizures in adult patients with epilepsy, where other AED combinations proved to be insufficient to terminate seizures [7]. RTG has a unique molecular mechanisms of action owing to properties of a selective M-current potassium channel opener and a subtype selective modulator of $GABA_A$ receptors [8, 9].

In previous preclinical studies, the combination of RTG with valproate (VPA) exerted supra-additive (synergistic) interaction in the mouse maximal electro-shock-induced seizure (MES) model [10]. The combinations of RTG with carbamazepine (CBZ), lamotrigine (LTG), oxcarbazepine (OXC) and phenytoin (PHT) exerted additive interaction in the mouse MES model [10–12]. The combination of RTG with levetiracetam (LEV) exerted both additive and supra-additive interactions in the mouse MES model [13]. Additionally, the combinations of RTG with CBZ, LEV, LTG and VPA

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have recently proved to be clinically effective as the add-on therapies in patients with partial-onset seizures [14].

The aim of this study was to perform experiments on animals to characterize the preclinical profile of the combination of RTG and topiramate (TPM – a second-generation AED with multiple molecular mechanisms of action) in the MES-induced seizure model and chimney test in mice by the use of type I isobolographic analysis. The choice of TPM and RTG for the combination study was based on the theoretical supposition that their various molecular mechanisms of action can be mutually effective. Furthermore, TPM is clinically used in patients with tonic-clonic seizures and partial onset seizures [15].

It is widely accepted that the MES model is considered to be an experimental model of tonic-clonic seizures and partial onset of convulsions in humans [16]. The chimney test is considered to be a model assessing acute adverse (neurotoxic) effects in experimental animals with respect to the impairment of motor coordination [17]. To exclude any pharmacokinetic interactions, total brain concentrations of both, RTG and TPM were determined.

MATERIALS AND METHODS

Animals. Adult male albino Swiss mice (weighing 22 – 26 g) were used in this study. All experimental procedures described complied with the ARRIVE guidelines, approved by the local Ethics Committee at the Medical University in Lublin, and conformed to the *Guide for the Care and Use of Laboratory Animals* (License No.: 28/2007). Each experimental group consisted of 8 mice.

Drug administration. RTG (GlaxoSmithKline, Brentford, UK) and TPM (Cilag AG, Schaffhausen, Switzerland) were suspended in a 1% solution of Tween 80 (Sigma-Aldrich, Poznań, Poland) in distilled water and administered intraperitoneally (i.p.) in a volume of 5 ml/kg body weight. RTG was administered 15 min. and TPM 60 min. before all experimental tests.

Maximal electro-shock seizure (MES) test. Maximal electro-convulsions in mice were produced by an alternating current (50 Hz, 25 mA, 500 V, 0.2 s stimulus duration) delivered via ear-clip electrodes, and the tonic hind limb extension (seizure activity) in animals was taken as the endpoint. The anticonvulsant potency of RTG and TPM was determined as their median effective doses (ED_{50} values), i.e., doses of AEDs that suppressed 50% of the animals tested against maximal electro-convulsions, as described in more detail elsewhere [10–12, 18]. The animals received increasing doses of RTG and TPM to obtain an increasing percentage of protection from tonic hind limb extension that allowed the construction of dose-response effect lines for RTG and TPM. The anticonvulsant potency of the mixture of RTG with TPM was determined and expressed as the $ED_{50\ exp}$ value, i.e., the dose of the mixture required to suppress tonic hind limb extension in 50% of the mice. This procedure has been described in more detail elsewhere [11, 12].

Chimney test. Motor coordination impairment in mice was evoked by RTG, TPM and their combination, when the drugs were administered in high (neurotoxic) doses. The mice receiving RTG and TPM in high doses displayed ataxia and impairment of motor performance, which were quantified with the chimney test [17]. In this test, the mice had to climb backwards up a transparent plastic tube (3 cm inner diameter, 30 cm length) within 1 min., as described in more detail elsewhere [11, 12]. The acute neurotoxic effects of RTG and TPM observed in the animals in the chimney test were expressed as their median toxic doses (TD_{50} values), i.e., doses of AEDs that disturbed motor coordination in 50% of the tested animals. The animals received increasing doses of RTG and TPM to obtain an increasing percentage of impairment of motor coordination that allowed the construction of dose-response effect lines for RTG and TPM. Similarly, the neurotoxic (adverse) effects of the mixture of RTG with TPM were determined and expressed as the $TD_{50\ exp}$ value, i.e., the dose of the mixture required to impair motor coordination in 50% of mice subjected to the chimney test. This experimental procedure has been described in more detail elsewhere [11, 12, 19].

Type I isobolographic analysis. Interactions between RTG and TPM in the MES-induced seizure and chimney test were isobolographically analyzed according to the methodology as described elsewhere [10–12, 20]. After determining the ED_{50} and TD_{50} values for RTG and TPM administered alone, median additive doses for the mixture of RTG with TPM (i.e., $ED_{50\ add}$ and $TD_{50\ add}$) were calculated. Subsequently, the test for parallelism of dose-response effects for RTG and TPM was used independently for both the MES and chimney tests [6, 20]. In this study, two parameters were calculated: protective indices (PI) and benefit indices (BI). The PI were ratios of TD_{50} values from the chimney test and the respective ED_{50} values from the MES model. The PI reflected a satisfactory margin of safety between the anticonvulsant and neurotoxic doses of RTG and TPM [21]. The BI were ratios of PI_{exp} values (determined experimentally) and PI_{add} values (theoretically predicted to be additive). The BI reflected advantages for the combination of RTG with TPM, considering both the anticonvulsant and neurotoxic effects exerted by the combination [6, 19]. It is widely accepted that a BI higher than 1.3 indicates a favourable combination that can be recommended to further clinical practice [6, 19].

Measurement of total brain AED concentrations. Measurement of total brain concentrations of RTG and TPM was undertaken at doses of the drugs corresponding to the $ED_{50\ exp}$ value for a two-drug mixture at the fixed-ratio of 1:1 from the MES test. Total brain concentrations of RTG were estimated with high-pressure liquid chromatography (HPLC) and those of TPM with fluorescence polarization immunoassay (FPIA), as described in more detail elsewhere [10–12, 22]. Total brain concentrations of RTG and TPM are expressed in μg/ml of brain supernatants.

Statistical analysis. Log-probit analysis was used to calculate ED_{50}, $ED_{50\ exp}$, TD_{50} and $TD_{50\ exp}$ values (with their S.E.M.) for RTG and TPM administered alone and in combination in the MES and chimney tests [23]. Subsequently, the unpaired Student's ***t***-test was used to statistically compare the $ED_{50\ exp}$ and $TD_{50\ exp}$ values with their respective $ED_{50\ add}$ and $TD_{50\ add}$ values, as reported earlier [24]. Total brain concentrations were statistically analyzed using the unpaired Student's ***t***-test. $P<0.05$ was considered to be statistically significant.

RESULTS

Anticonvulsant and acute neurotoxic effects of RTG, TPM and their combination in the tonic-clonic seizure model and chimney test in mice. Log-probit analysis revealed that RTG and TPM, while administered separately, exerted a clear-cut anticonvulsant effects in the MES test in mice (Fig. 1A). The experimentally determined ED_{50} values from the MES test are presented on a graph (Fig. 1A). Linear regression analysis confirmed that the dose-response lines of RTG and TPM in the MES test were non-parallel to one another (Fig. 1A). Similarly, log-probit analysis of RTG and TPM administered alone revealed that the drugs produced a clear-cut acute neurotoxic effect in the chimney test in mice (Fig. 1B). The experimentally derived TD_{50} values from the chimney test are present on a graph (Fig. 1B). With linear regression analysis it was found that the dose-response lines of RTG and TPM from the chimney test were non-parallel to each other (Fig. 1A).

Type I isobolographic analysis of interactions between RTG and TPM in the MES model and chimney test in mice. Type I isobolographic analysis of interaction for non-parallel dose-response effects revealed that the mixture of RTG with TPM at the fixed-ratio of 1:1 exerted an additive interaction in both the tonic-clonic seizure model and chimney test in mice (Tab. 1; Fig. 2A-B). The experimentally derived $ED_{50\,exp}$ and $TD_{50\,exp}$ values for the combination of RTG with TPM at the fixed-ratio of 1:1 did not significantly differ from the theoretically calculated $ED_{50\,add}$ and $TD_{50\,add}$ values from both the lower and upper lines of additivity (Tab. 1; Fig. 2A-B).

Table 1. Isobolographic analysis of interactions between retigabine (RTG) and topiramate (TPM) at the fixed-ratio of 1:1 in the maximal electroshock (MES)-induced seizure model and chimney test in mice

$ED_{50\,add}$	$ED_{50\,exp}$	$TD_{50\,add}$	$TD_{50\,exp}$	PI_{exp}	PI_{add}	BI
^{L}26.36±9.45	34.94±3.85	^{L}168.6±41.23	509.3±35.40	14.58	6.40	2.27
^{U}45.05±9.09	34.94±3.85	^{U}464.4±59.78	509.3±35.40	14.58	10.31	1.41

Median effective dose (ED_{50}) and median toxic dose (TD_{50}) values (in mg/kg ± S.E.M.) were calculated by computerized log-probit analysis. The protective indices (PI – as quotients of the respective TD_{50} and ED_{50} values) for the combination of RTG and TPM were determined experimentally (PI_{exp}) and calculated theoretically (PI_{add}). The benefit indices (BI – as ratios of PI_{exp} and PI_{add}) determine the application of the combination of RTG and TPM in further clinical practice, considering both, the anticonvulsant and neurotoxic effects. ^{L} – indicates $ED_{50\,add}$ and $TD_{50\,add}$ values from the lower line of additivity; ^{U} – indicates $ED_{50\,add}$ or $TD_{50\,add}$ values from the upper line of additivity. The unpaired Student's *t*-test was used to statistically analyze the data and no significant differences were reported.

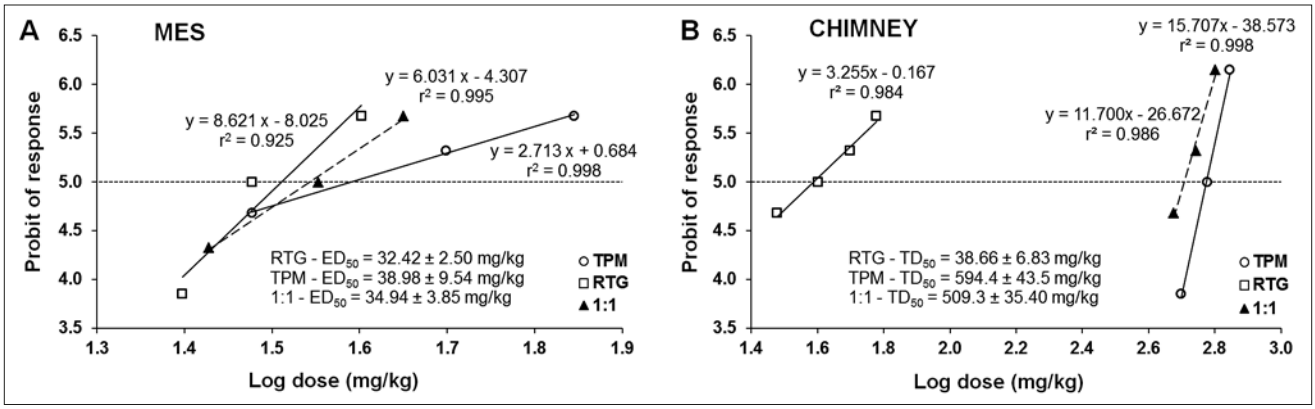

Figure 1 A-B. Log-probit analysis of dose-response effects of retigabine (RTG) and topiramate (TPM) administered alone as well as their combination in the maximal electroshock (MES)-induced seizure model and chimney test in mice.
Legend to Figure 1A-B: Doses of RTG, TPM and their combination at the fixed-ratio of 1:1 were transformed into logarithms to the base 10. The protective effects produced by the drugs against tonic-clonic seizures in mice were transformed into probits. Each point indicates a group of 8 mice. Linear regression equations characterizing dose-response effects for particular drugs administered alone and in combination are presented on the graphs. In the MES and chimney tests, the dose-response effect lines for both, RTG and TPM were not parallel to one another. The experimentally determined median effective doses (ED_{50}) and median toxic doses (TD_{50}) for the drugs and their combination are presented on the graphs.

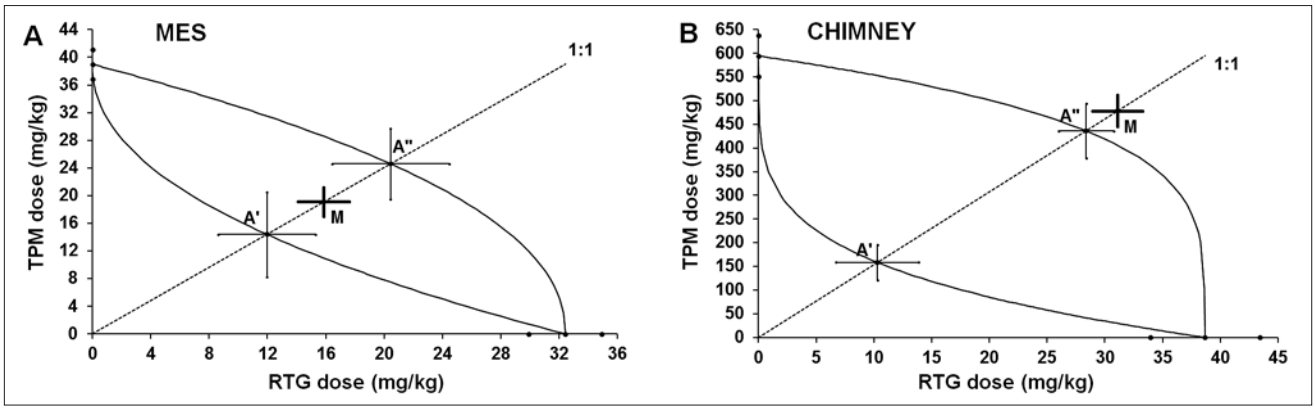

Figure 2 A-B. Isobolograms with additive interactions between retigabine (RTG) and topiramate (TPM) in the maximal electroshock (MES)-induced seizure model and chimney test in mice.
Legend to Figure 2A-B: Median effective doses (ED_{50} ± S.E.M.) and median toxic doses (TD_{50} ± S.E.M.) for RTG and TPM are plotted graphically on the X- and Y-axes, respectively. Lower and upper isoboles of additivity represent the curves connecting the ED_{50} and TD_{50} values for RTG and TPM administered separately. The points A' and A" depict the theoretically calculated $ED_{50\,add}$ and $TD_{50\,add}$ values for both, lower and upper isoboles of additivity. The point M illustrates the experimentally determined $ED_{50\,exp}$ and $TD_{50\,exp}$ values for total dose of the mixture that produced 50% anticonvulsant effects in the MES test and 50% acute adverse (neurotoxic) effects in the chimney test in mice, respectively.

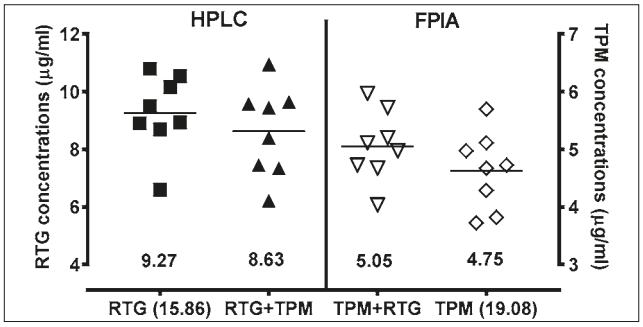

Figure 3. Effect of topiramate (TPM) on total brain concentrations of retigabine (RTG) and inversely, RTG on total brain concentrations of TPM in mice.
Legend to Figure 3: Total brain concentrations (means in μg/ml) of RTG and TPM were assayed with high-pressure liquid chromatography (HPLC) and fluorescence polarization immunoassay (FPIA), respectively. Doses of RTG and TPM correspond to the $ED_{50\ exp}$ value from the MES test (for more details see Table 1). The unpaired Student's t-test was used to statistically analyze the data and no significant differences were reported.

Effects of RTG on total brain concentrations of TPM and inversely, TPM on total brain concentrations of RTG. Estimation of total brain concentrations of RTG with HPLC revealed that the co-administration of RTG with TPM was associated with no significant changes in total brain RTG concentrations in mice (Fig. 3). Moreover, with FPIA it was found that total brain concentrations of TPM did not significantly differ after concurrent administration of RTG (Fig. 3).

Isobolographic parameters for combinations of RTG with TPM. The PI for RTG and TPM administered alone were 1.19 and 15.25, respectively (results not shown). The isobolographically calculated BI for the combination of RTG with TPM at the fixed ratio of 1:1 ranged from 1.41–2.27 (Tab. 1).

DISCUSSION

Results presented in this study indicate clearly that the combination of RTG with TPM produced additive interaction in both, the MES model and chimney test in mice. These findings are similar to those published earlier for the combinations of RTG with OXC, LTG, CBZ and PHT at the fixed-ratio of 1:1, for which additive interactions were reported in the mouse MES model [10–12]. In contrast, the combination of RTG with LEV and VPA produced supra-additive (synergistic) interaction in the mouse MES model [10, 13]. However, in the case of the combination of RTG with LEV, the type II isobolographic analysis was used to characterize the synergistic interaction between AEDs in the mouse MES model [13]. Generally, type II isobolographic analysis is used in preclinical studies on animals if one of the tested drugs in mixture does not have any activity in the studied experimental seizure model. In the case of the combination of RTG with LEV, the latter drug did not produce anticonvulsant activity in the mouse MES model; therefore, it is considered to be virtually inactive in the mouse MES model [25]. This is the reason for testing the combination of RTG and LEV with type II isobolographic analysis. In contrast, with type I isobolographic analysis for parallel dose-response lines, the combination of RTG with VPA produced supra-additive (synergistic) interaction in the mouse MES model [10].

It should be mentioned that this study additionally characterized the types of interaction between drugs administered in high toxic doses that evoked acute side-neurotoxic effects, manifesting in form of ataxia and impairment of motor coordination in mice. Results analyzed isobolographically revealed that the combination of RTG with TPM produced additivity with a slight tendency to antagonism in the chimney test – an experimental model, in which the neurotoxic- effects for the AED combination in mice were assessed. Previously, the authors also found that the combinations of RTG with PHT and OXC produced an additive interaction with a tendency towards antagonism in the chimney test [11, 12].

A pharmacokinetic study revealed that the combination of RTG with TPM, at doses corresponding to the $ED_{50\ exp}$ value, was devoid of any significant changes in total brain concentrations of both drugs, since it was found that neither RTG significantly affected total brain concentrations of TPM, nor TPM considerably affected total brain concentrations of RTG in mice. There is no doubt that this bidirectional measurement of total brain concentrations of both drugs provided the authors with detailed information about pharmacokinetic properties of drugs combined together. Lack of any pharmacokinetic interactions between RTG and TPM is consistent with previously published results. More specifically, the authors have confirmed the lack of any pharmacokinetic interactions between RTG and OXC, PHT, LTG, CBZ and VPA [10–12]. Thus, when translating the results from the current study to clinical settings, no pharmacokinetic interactions between RTG and TPM are expected in humans.

On the other hand, the molecular mechanisms of action of both AEDs, i.e., RTG and TPM, contributing to the observed additive interaction in the mouse MES model should be discussed briefly. As regards RTG, the drug is a positive modulator of M-current of potassium channels and, additionally, it potentiates $GABA_A$ receptor-mediated responses [26]. In the case of TPM, molecular studies revealed that the drug blocks voltage-dependent sodium channels, modulates AMPA/kainate receptors, potentiates $GABA_A$ receptor-mediated responses, inhibits R-type, but activates L-type calcium channels, inhibits carbonic anhydrase II and IV isoenzymes and changes intra- and/or extracellular pH [27]. Accumulating experimental evidence indicates that the combinations of AEDs with diverse and different mechanisms of action are favourable and result in synergistic interactions [5]. Unfortunately, despite various and complementary molecular mechanisms of the anticonvulsant activities of RTG and TPM, neither drug synergistically interacted together. Instead of the expected synergy, the additive interaction was observed in the mouse MES model.

It should be stressed that this study evaluated both the anticonvulsant and acute neurotoxic (adverse) effects produced by the combination of RTG with TPM at the fixed-ratio of 1:1. Owing to the isobolographic analysis, the BI could be calculated for the combination of RTG with TPM. Generally, the BI allows researchers to classify the interactions with respect to their potential application in further clinical settings [6, 28]. As recommended earlier, a BI higher than 1.3 indicate clinically favourable combinations of AEDs. Since the BI for the combination of RTG with TPM ranged from 1.41 – 2.27, it can be ascertained that the beneficial two-drug combination is worth recommending for patients with pharmacoresistant epilepsy.

CONCLUSIONS

If the results from this study could be translated into clinical trials, the combination of RTG with TPM would be beneficial for epilepsy patients remaining refractory to currently available AEDs.

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