



## Synergistic interaction of gabapentin with tiagabine in the hot-plate test in mice: an isobolographic analysis

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### Abstract:

This study was aimed at determining the analgesic effect of gabapentin and tiagabine, two antiepileptic drugs that were administered alone and in combination at a fixed ratio of 1:1, in the acute thermal pain model (hot-plate test) in mice.

Linear regression analysis was used to evaluate the dose-response relationships between logarithms of antiepileptic drug doses and their resultant maximum possible antinociceptive effects in the mouse hot-plate test. From linear equations, we calculated doses that increased the antinociceptive effect by 50% (ED<sub>50</sub> values) for gabapentin, tiagabine and their combination. The type of interaction between gabapentin and tiagabine was assessed using the isobolographic analysis.

Results indicated that both antiepileptic drugs produced the definite antinociceptive effect, and the experimentally derived ED<sub>50</sub> values for gabapentin and tiagabine, when applied alone, were 504.4 mg/kg and 5.67 mg/kg, respectively. With isobolography, the experimentally derived ED<sub>50 mix</sub> value for the fixed ratio combination of 1:1 was 139.31 mg/kg and significantly differed from the theoretically calculated ED<sub>50 add</sub> value, which was 255.04 mg/kg ( $p < 0.05$ ), indicating the synergistic interaction between gabapentin and tiagabine in the hot-plate test in mice.

In conclusion, the combination of tiagabine with gabapentin at a fixed ratio of 1:1 exerted a synergistic interaction in the mouse model of nociceptive pain. If the results from this study could be extrapolated to clinical settings, the combination of tiagabine with gabapentin might be beneficial for pain relief in humans.

### Key words:

drug interaction, gabapentin, hot-plate test, isobolographic analysis, maximum possible antinociceptive effect, tiagabine

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### Introduction

Accumulating evidence indicates that some antiepileptic drugs, especially gabapentin and tiagabine, also exert analgesic effects in numerous experimental pain models and in clinical settings in humans. It has been reported that tiagabine is effective in patients with

painful sensory neuropathy [33], painful tonic spasm [39] and chronic pain [45]. Similarly, gabapentin is effective in suppressing and alleviating pain in post-herpetic neuralgia [16, 35], painful diabetic neuropathy [1], migraine [10], trigeminal neuralgia [38] and neuropathic cancer pain [3]. In experimental studies on animals, gabapentin reduced dynorphin-induced

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allodynia [4, 22, 34] and suppressed neuropathic pain [5, 7, 14]. Moreover, the antinociceptive effect of gabapentin has been documented in postoperative pain [6, 14], lumbar adhesive arachnoiditis [21] and cancer-induced bone pain [11] models. Gabapentin also exerted antinociception in the formalin test in rodents [8, 14, 27, 37]. With regard to tiagabine, the drug inhibited both phases of the formalin behaviors in rats [19, 27], produced the antinociceptive effect in dynorphin-induced allodynia in rats and in the hot-plate test in mice [22], increased the latency to the first pain reaction in the mouse grid-shock analgesia test [29, 42] and increased the pain threshold in the paw pressure test [19].

Considering the fact that gabapentin and tiagabine used separately exert antinociceptive activity in both clinical and experimental studies, it was of pivotal importance to determine whether their combination might synergistically interact in terms of the antinociceptive effect in animals. Therefore, we sought to determine the antinociceptive effect for the combination of tiagabine and gabapentin in the hot-plate test in mice. To characterize the type of interaction for the combination of gabapentin with tiagabine, an isobolographic analysis of interaction was used.

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## Materials and Methods

### Animals and experimental conditions

Adult male Swiss mice (weighing 22–26 g) that were kept in colony cages with free access to food and tap water under standardized housing conditions (natural light-dark cycle, temperature of  $23 \pm 1^\circ\text{C}$ , relative humidity of  $55 \pm 5\%$ ) were used. After seven days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups containing eight mice each. All tests were performed between eight a.m. and three p.m. Procedures involving animals and their care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. 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 manuscript were approved by the First Local Ethics Committee at the Medical Uni-

versity of Lublin (Licenses no. 7/2007; 59/2007) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

### Drugs

In the present study, gabapentin (Neurontin, Parke-Davis, Berlin, Germany) and tiagabine (Gabitril, Sanofi Winthrop, Gentilly, France) were suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA) and administered *via* intraperitoneal (*ip*) injection in a volume of 0.005 ml/g of body weight. The antiepileptic drugs were administered as follows: tiagabine at 15 min and gabapentin at 60 min before the hot-plate test. These pretreatment times were chosen based upon information about their biological activity from the literature and our previous studies [26, 27, 29].

### Hot-plate test

The hot-plate test, a standard model used to determine the antinociceptive efficacy of compounds with respect to acute thermal nociception, was conducted according to the procedure described by Eddy and Leimbach [12], with minor modifications. The device consisted of an electrically heated surface and an open Plexiglas tube (17 cm high  $\times$  22 cm diameter) to confine the animals to the heated surface (Ugo Basile, Varese, Italy). The temperature was set at  $55.0 \pm 0.1^\circ\text{C}$ . Mice were placed separately on a heated surface, and the time interval (in s) between placement and a shaking, licking, or tucking of the fore- or hind-paws was recorded by a stopwatch as the predrug latency response. Animals were tested once before baselines were taken, and this trial served as the control reaction time for the animals. Mice showing a reaction time greater than 10 s were excluded from the subsequent test. The predrug latencies were between 5 and 8 s. Subsequently, the animals were administered tiagabine and gabapentin alone at increasing doses and at times to the peak of their anticonvulsant activity (i.e., 15 and 60 min, respectively). The same procedure was repeated, and the animals were placed again on the heated surface. In other words, each animal was subjected to the hot-plate test twice. To perform the first evaluation of time to the first pain reaction in animals in the hot-plate test, the naive mice were randomly assigned to experimental groups (consisting of eight mice per group) and consecutively numbered on their tails with multi-colored markers. Then, the ani-

mals were challenged with the hot-plate test to determine the latency to the first pain reaction for each mouse separately. Next, the marked animals received the antiepileptic drugs, either alone or in combination at a fixed ratio of 1:1. Then, after reaching the peak of the maximum anticonvulsant effects, the mice were subjected to the second evaluation of time to the first pain reaction in the same animals. In other words, both pre- and post-treatment reaction times were recorded in the same animals. In the present study, tiagabine was administered *ip* at doses ranging between 0.5 and 10 mg/kg, whereas gabapentin was administered at doses ranging from 100 to 1000 mg/kg. A maximum cut-off time of 30 s was chosen to prevent injury to animals. Mice not responding within 30 s were removed from the heated surface and assigned a score of 30 s. The maximum possible antinociceptive effect was defined as the lack of a nociceptive response in mice during the exposure to the heat stimulus, and the percentage of maximum possible antinociceptive effect was calculated according to the formula presented by Schmauss and Yaksh [36], as follows:  $[(T_1 - T_0)/(T_2 - T_0)] \times 100$ ; where  $T_0$  and  $T_1$  are the latencies obtained before and after drug administration, and  $T_2$  is the cut-off time of 30 s. Next, gabapentin and tiagabine doses were transformed to logarithms to the base 10 and plotted on the x-axis of the Cartesian system of coordinates. Simultaneously, the maximum possible antinociceptive effect, corresponding to the antiepileptic drug doses, was plotted on the y-axis, and both values were analyzed with least-squares linear regression analysis according to Motulsky and Christopoulos [31]. Subsequently, from the equation of the linear dose-response relationship, the dose of an antiepileptic drug that increased the antinociceptive effect by 50% ( $ED_{50}$  value) was calculated. This experimental procedure was described in more detail in our earlier study [25].

### Isobolographic analysis of interactions

The interaction of gabapentin with tiagabine with respect to the antinociceptive effect produced by both drugs in the hot-plate test was analyzed according to the methodology previously detailed in our earlier studies, where the precise descriptions of theoretical background with the respective equations showing how to undertake isobolographic calculations have been presented [24, 26–29]. Notably, the  $ED_{50 \text{ add}}$  represents the total additive dose of gabapentin and tiaga-

bine in the mixture that theoretically increases the antinociceptive effect by 50% in the hot-plate test in mice. The  $ED_{50 \text{ mix}}$  is an experimentally determined total dose of a mixture of two component drugs, at a fixed ratio combination of 1:1, that is sufficient for a 50% increase in the antinociceptive effect in mice challenged with the hot-plate test.

The additive dose of gabapentin and tiagabine in combination that increased the antinociceptive effect by 50% in the hot-plate test ( $ED_{50 \text{ add}}$  value) was calculated from the “equation of additivity” presented by Loewe [23], as follows:  $x/X + y/Y = 1$ ; where  $x$  and  $y$  are, respectively, the doses of gabapentin and tiagabine that are co-administered in the mixture and exert a 50% maximum possible antinociceptive effect in the hot-plate test in mice.  $X$  and  $Y$  are, respectively, the doses of the antiepileptic drugs administered separately in order to obtain the same effect (50% maximum possible antinociceptive effect in the hot-plate test in mice). Further details regarding these concepts have been published elsewhere [7, 24, 26–29, 43].

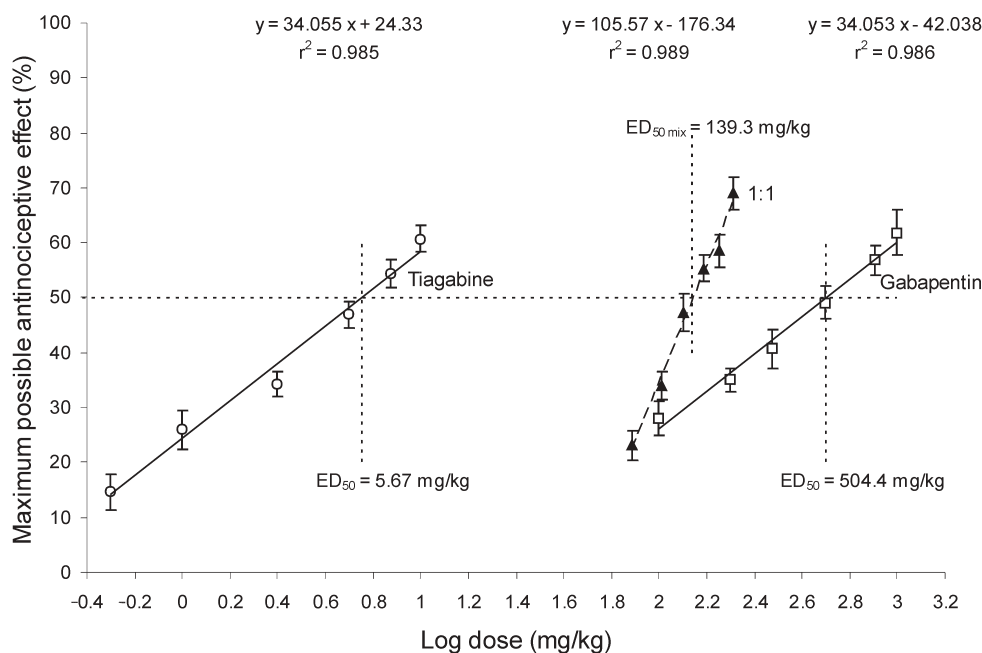
### Statistical analysis

The maximum possible antinociceptive effect values with their SE were calculated by using the formula presented by Schmauss and Yaksh [36]. The  $ED_{50}$  values with their SE were calculated from least-squares linear regression analysis according to Motulsky and Christopoulos [31]. Statistical evaluation of the isobolographic interaction between gabapentin and tiagabine was performed by the use of Student's *t*-test with Welch's correction in order to detect the differences between the experimentally-derived ( $ED_{50 \text{ mix}}$ ) and theoretical additive ( $ED_{50 \text{ add}}$ ) values, according to Tallarida [43]. All statistical tests were performed using commercially available GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

## Results

### Effects of tiagabine, gabapentin and their combination on the antinociception in the hot-plate test in mice

Tiagabine administered *ip* 15 min before the acute thermal pain test prolonged the latency to the first pain reaction in mice in a dose-dependent manner.



**Fig. 1.** Dose-response effects of gabapentin, tiagabine and the combination of the two drugs at a fixed ratio of 1:1 in the hot-plate test in mice. Doses of gabapentin, tiagabine and the mixture of their combination at a fixed ratio of 1:1 (in mg/kg) were transformed to logarithms to the base 10 (log), whereas the antinociceptive effects produced by gabapentin, tiagabine and the mixture of both drugs at a ratio of 1:1 were transformed to the maximum possible antinociceptive effect (in %  $\pm$  SE as the error bars,  $n = 8$ ). Gabapentin and tiagabine were administered *ip* at 15 and 60 min, respectively, before the antinociceptive effect evaluation. The maximum possible antinociceptive effect was defined as the lack of a nociceptive response in mice during exposure to the heat stimulus ( $55.0 \pm 0.1^\circ\text{C}$ ), and the percentage of maximum possible antinociceptive effect was calculated according to the formula by Schmauss and Yaksh [36], as follows:  $[(T_1 - T_0)/(T_2 - T_0)] \times 100$ , where  $T_0$  and  $T_1$  are the latencies obtained before and after drug administration and  $T_2$  is the cut-off time of 30 s. Log doses of gabapentin, tiagabine and their combination at a fixed ratio of 1:1 along with their resultant maximum possible antinociceptive effects were plotted into the Cartesian system of coordinates and analyzed with least-squares linear regression to determine the dose-response relationship between the doses of antiepileptic drugs and their respective antinociceptive effect in the hot-plate test in mice. The linear equations for tiagabine, gabapentin and the combination of the two drugs are presented in Fig. 1; where  $y$  is the maximum possible antinociceptive effect value (in %),  $x$  is the log dose (in mg/kg) of an antiepileptic drug administered alone or the mixture of tiagabine and gabapentin in combination at a fixed ratio of 1:1; and  $r^2$  is the coefficient of determination. The log of  $ED_{50}$  value for tiagabine was 0.754 and corresponded to a tiagabine dose of 5.67 mg/kg. The experimentally calculated log of  $ED_{50}$  value for gabapentin was 2.703, which corresponded to a gabapentin dose of 504.4 mg/kg. The log of  $ED_{50\text{mix}}$  value for the combination of tiagabine with gabapentin at a fixed ratio of 1:1 was 2.144 and corresponded to a dose of 139.3 mg/kg of the mixture

The experimentally-derived values of the maximum possible antinociceptive effect for tiagabine (administered at increasing doses of 0.5 to 10 mg/kg) were between 14.71% and 60.65% (Fig. 1). The equation of the dose-response relationship, as denoted from a least-squares linear regression, for tiagabine was:  $y = 34.055x + 24.33$  ( $r^2 = 0.985$ ); where  $y$  is the maximum possible antinociceptive effect in %,  $x$  is the logarithm of the tiagabine dose and  $r^2$  is the coefficient of determination (Fig. 1). The experimentally denoted logarithm of the  $ED_{50}$  value for tiagabine in the hot-plate test in mice was 0.754, which corresponded to a drug dose of  $5.67 \pm 0.30$  mg/kg (Fig. 1).

Similarly, gabapentin administered *ip* 60 min before the hot-plate test prolonged the latency to the first pain reaction in the mouse hot-plate test in a dose-

dependent manner. The experimentally-derived values of the maximum possible antinociceptive effect for gabapentin (administered at increasing doses of 100 to 1000 mg/kg) ranged between 28.06% and 61.81% (Fig. 1). The equation for the gabapentin dose-response relationship was:  $y = 34.053x - 42.038$  ( $r^2 = 0.986$ ; Fig. 1). Thus, the experimentally calculated logarithm of the dose of gabapentin that increased the antinociceptive effect by 50% ( $ED_{50}$  value) in the hot-plate test in mice was 2.703, corresponding to the drug dose of  $504.4 \pm 96.15$  mg/kg (Fig. 1).

The mixture of gabapentin with tiagabine at a fixed ratio of 1:1 prolonged the latency to the first pain reaction in the hot-plate test in mice in a dose-dependent manner. The experimentally-derived maxi-

**Tab. 1.** Isobolographic characterization of the interaction between gabapentin and tiagabine at a fixed ratio of 1:1 in the hot-plate test in mice

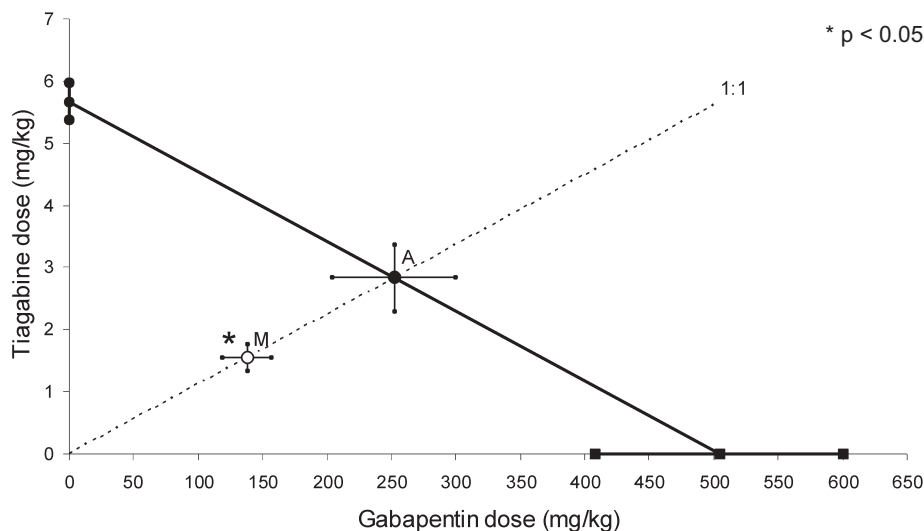
Gabapentin <sub>add</sub>	Tiagabine <sub>add</sub>	ED <sub>50 add</sub>	n <sub>add</sub>	ED <sub>50 mix</sub>	Gabapentin <sub>mix</sub>	Tiagabine <sub>mix</sub>	n <sub>mix</sub>
252.20	2.84	255.04 ± 48.22	84	139.30 ± 18.97 *	137.75	1.55	48

Data are presented as doses of the mixture of gabapentin and tiagabine at a fixed ratio of 1:1 that increased the antinociceptive effect by 50% (ED<sub>50</sub> ± SE) from the hot-plate test in mice. The ED<sub>50</sub> values were either experimentally determined from the mixture of two antiepileptic drugs (ED<sub>50 mix</sub>) or theoretically calculated from the equation of additivity (ED<sub>50 add</sub>). Additionally, the actual doses of gabapentin and tiagabine that comprised the mixture at a fixed ratio combination of 1:1, for both ED<sub>50 mix</sub> and ED<sub>50 add</sub> values, are presented in separate columns as Gabapentin<sub>add</sub>, Tiagabine<sub>add</sub>, Gabapentin<sub>mix</sub> and Tiagabine<sub>mix</sub> values. Statistical evaluation of the data was performed using the unpaired Student's *t*-test with Welch's correction. n = total number of animals used at those doses whose expected antinociceptive effect was greater than 16%, denoted for the experimental mixture of drugs (n<sub>mix</sub>) and theoretically calculated (n<sub>add</sub>) from the equation of additivity. \* *p* < 0.05 vs. the ED<sub>50 add</sub>

imum possible antinociceptive effect values for the mixture, administered at doses ranging between 75.66 and 201.77 mg/kg, ranged from 23.19% to 69.01% (Fig. 1). Least-squares linear regression revealed that the experimentally-derived equation for the mixture of gabapentin with tiagabine at a fixed ratio of 1:1 was:  $y = 105.57x - 176.34$  ( $r^2 = 0.989$ ; Fig. 1). Thus, the logarithm of the experimentally determined ED<sub>50 mix</sub> value in the hot-plate test in mice was 2.144, which corresponded to the dose of the mixture of 139.3 ± 18.97 mg/kg (Fig. 1).

### Isobolographic analysis of interaction between gabapentin and tiagabine at a fixed ratio of 1:1 in the hot-plate test in mice

Statistical evaluation of data with unpaired Student's *t*-test followed by Welch's correction revealed that the combination of gabapentin with tiagabine at a fixed ratio of 1:1 was supra-additive (synergistic) in the hot-plate test in mice (Tab. 1; Fig. 2). The experimentally-derived ED<sub>50 mix</sub> for the fixed ratio of 1:1 was 139.3 mg/kg, which significantly differed from the ED<sub>50 add</sub> of 255.04 mg/kg (*p* < 0.05; Tab. 1; Fig. 2).



**Fig. 2.** Isobologram illustrating the supra-additive (synergistic) interaction for the combination of tiagabine with gabapentin in the hot-plate test in mice. Doses increasing the antinociceptive effect by 50% (ED<sub>50</sub>) in the hot-plate test in mice for gabapentin and tiagabine are plotted graphically on the x- and y-axes of the Cartesian system of coordinates. The solid lines on the axes represent SE for the antiepileptic drugs administered alone. The straight line connecting these two ED<sub>50</sub> values represents the theoretical line of additivity for a continuum of different fixed dose ratios (ED<sub>50 add</sub> values). Point (A) represents the theoretical additive ED<sub>50 add</sub> (± SE as the error bars) for the total dose expressed as the proportion of gabapentin and tiagabine that produced a 50% antinociceptive effect. Point (M) depicts on the graph the experimentally-derived ED<sub>50 mix</sub> (± SE as the error bars) for the total dose expressed as the proportion of gabapentin and tiagabine that produced a 50% antinociceptive effect. The ED<sub>50 mix</sub> for the fixed ratio of 1:1 is placed significantly below the line of additivity, indicating the supra-additive (synergistic) interaction between tiagabine and gabapentin in the hot-plate test in mice (\* *p* < 0.05)

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The separate doses of gabapentin and tiagabine in the mixture at the fixed-ratio of 1:1, calculated from the  $ED_{50\text{ add}}$  and  $ED_{50\text{ mix}}$  values, are presented in Table 1.

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## Discussion

Results in this study indicate that both antiepileptic drugs produce the antinociceptive effect in the hot-plate test in mice in a dose-dependent manner. Thus, our findings are in agreement with results presented by other authors who have tested tiagabine and gabapentin in the hot-plate test in rodents [19, 22]. Previously, it has been documented that tiagabine administered *ip* at doses up to 10 mg/kg produced a significant antinociception in the hot-plate test in mice [19, 22]. Moreover, tiagabine at doses up to 4 mg/kg significantly increased the latency to the first pain reaction in mice, showing a potent antinociception in the mouse grid-shock analgesia test [29]. Additionally, tiagabine has been found to produce the antinociceptive effect in the mouse grid-shock analgesia test in a dose-dependent manner with an  $ED_{50}$  of 4.01 (1.17–13.71) mg/kg [42]. In the present study, tiagabine was examined at doses up to 10 mg/kg, and the drug produced a clear-cut antinociceptive effect with the  $ED_{50}$  value of 5.67 mg/kg, which confirmed the antinociceptive effect of tiagabine in the hot-plate test. With respect to gabapentin, it has been shown that the drug administered *ip* at doses up to 100 mg/kg had no impact on antinociception in the hot-plate test in mice [19]. Moreover, gabapentin, at doses up to 75 mg/kg, did not prolong the latency to the first pain reaction in the mouse grid-shock analgesia test [29]. In contrast, in the present study, gabapentin administered *ip* at doses up to 1000 mg/kg exerted the antinociceptive effect in the hot-plate test in mice, and the experimentally-derived  $ED_{50}$  value for gabapentin was 504.4 mg/kg. The apparent difference between the results for gabapentin presented earlier and those shown in the present study can be readily explained by comparing the doses of gabapentin used in both experiments. It is highly likely that gabapentin administered *ip* at doses up to 100 mg/kg was virtually ineffective with respect to antinociception in the acute thermal pain model in mice. In contrast, gabapentin at higher doses (up to 1000 mg/kg) produced antinociception in the acute thermal pain model in mice with

the  $ED_{50}$  value of 504.4 mg/kg. Quite recently, a similar difference in the antinociceptive activity of gabapentin has been observed in mice subjected to the formalin test. It has been documented that gabapentin administered subcutaneously (*sc*) exerted antinociception by inhibiting only phase II of the formalin behaviors in rats, being virtually ineffective in phase I of the formalin test in rats [14]. In contrast, our earlier studies indicated that gabapentin administered *ip* produced the antinociceptive effect in both phases of the formalin test in mice [8, 27]. However, the experimentally-derived median inhibitory dose ( $ID_{50}$  value – the dose of gabapentin required to reduce the nociceptive response to formalin by 50%, as compared to control animals) for gabapentin in phase I of the formalin test was 34.5 mg/kg, whereas that for phase II was 11.3 mg/kg [8]. Similarly, the experimentally-derived  $ED_{50}$  value for gabapentin in phase I of the formalin test was 29.6 mg/kg, whereas that for phase II was 8.2 mg/kg, evidently indicating that gabapentin suppressed nociception in mice challenged with the formalin test [27]. Similarly, in the study by Shannon et al. [37], gabapentin administered *ip* at doses up to 300 mg/kg inhibited both phases of the formalin test in rats. Hence, a virtual inactivity of gabapentin in phase I of the formalin test in rodents, as presented by Field et al. [14], was probably evoked by an inappropriate dose of gabapentin and/or the route of administration of the drug.

Notably, the  $ED_{50}$  values for tiagabine and gabapentin, as determined in the hot-plate test, were considerably lower than those producing acute adverse effects in the chimney test, which is used as an experimental model in preclinical studies to determine potential adverse effects of drugs on motor coordination in mice. As documented in our earlier studies, the median toxic doses ( $TD_{50}$  values) for tiagabine and gabapentin in the chimney test were 13.6 mg/kg and 957.8 mg/kg, respectively [25, 26]. Since the experimentally derived  $ED_{50}$  values for tiagabine and gabapentin in the hot-plate test were 5.67 mg/kg and 504.4 mg/kg (~2-fold lower than their  $TD_{50}$  values), one can assume that the antinociceptive effect afforded by tiagabine and gabapentin did not result from the acute adverse effects produced by these drugs in animals.

To explain the observed synergistic interaction between gabapentin and tiagabine in this study, one should consider their molecular mechanisms of action. With regard to tiagabine, the drug is a selective

GABA reuptake inhibitor [41]. Tiagabine inhibits the GABA transporter GAT-1 on presynaptic neurons and glial cells, and, thus, the drug prolongs the duration, but not the magnitude, of the peak inhibitory postsynaptic current associated with endogenously released GABA in synapses [30]. With respect to gabapentin, at therapeutically relevant concentrations, this drug binds with high affinity to the  $\alpha_2\delta$  type 1 and 2 subunits of calcium channels [17] and inhibits calcium influx through presynaptic P/Q-type voltage-gated calcium channels [15]. The inhibition of calcium influx reduces potassium-evoked excitatory transmitter release and, thus, decreases postsynaptic excitability [32]. Gabapentin is considered to be an agonist of GABA<sub>B</sub> (gb1a-gb2) heterodimers coupled to inward rectifying potassium channels [32]. The activation of GABA<sub>B</sub> receptors leads to a suppression of excitatory amino acid release from neuronal terminals, and this postsynaptic mechanism seems to be independent of the presynaptic inhibition of P/Q-type voltage-gated calcium channels. Moreover, gabapentin increases the hyperpolarization-activated cation current ( $I_h$ ) in dendrites, and, thereby, the drug additionally reduces neuronal excitability [40]. Gabapentin competes with transport of branched chain amino acids (L-leucine, L-valine, L-phenylalanine); therefore, some pharmacological properties of the drug may arise from changes in cytosolic concentrations of endogenous amino acids in neurons [44].

Bearing in mind the molecular mechanisms of action of gabapentin and tiagabine, one can ascertain that their different and complementary mechanisms of action are likely responsible for the observed synergistic interaction in the hot-plate test in mice. Generally, it is accepted that drugs with similar mechanisms of action produce an additive interaction as a result of summation of the partial effects produced by each component drug in the mixture [9]. In contrast, the drugs with diverse mechanisms of action may complete their own activities and, thus, produce a synergistic interaction [9]. Considering the possibility of the application of multimodal analgesia with gabapentin and tiagabine, it seems plausible that the different mechanisms of action of these drugs may be favorable in clinical settings. In addition, comparing the doses of gabapentin and tiagabine in the mixture at the fixed ratio of 1:1 that exerted a 50% increase in the antinociceptive effect (137.75 mg/kg for gabapentin and 1.55 mg/kg for tiagabine) with the doses of gabapentin and tiagabine producing the same 50% effect

when administered alone (504.4 mg/kg for gabapentin and 5.67 mg/kg for tiagabine), one can observe a considerable reduction of drug doses without the loss of the antinociceptive effect in the combination. Thus, the reduction of drug doses during the treatment with these antiepileptic drugs may contribute to the limitation of the acute adverse effects exerted by these antiepileptic drugs when applied alone at high effective doses. There is no doubt that the decreased doses of both antiepileptic drugs will be better tolerated than higher doses of the antiepileptic drugs used separately, especially if the antinociceptive effect is unchanged. In other words, the combination of gabapentin with tiagabine fulfills all the criteria of multimodal analgesia [13, 20]; therefore, it can be recommended as an advantageous combination in further clinical trials. Moreover, in this study, we confirmed that some antiepileptic drug combinations exert synergistic interactions in the acute thermal pain model. Additionally, it has recently been documented that the combination of gabapentin with tiagabine at a fixed ratio of 1:1 exerted a supra-additive (synergistic) interaction in phase II of the formalin test in mice; thus, our findings presented here are consistent with those documented earlier [27]. Nevertheless, our hypothesis concerning the synergistic interaction of gabapentin with tiagabine should be confirmed in additional models of acute and/or chronic pain.

It is notable that the first pain reaction in rodents subjected to the hot-plate test differs individually and may manifest in the form of shaking, tucking or licking of the fore- and hind-paws and/or jumping. With regard to the licking of the forepaws, there is a strong difference between the licking of the forepaws observed in the hot-plate test and that reported during grooming behavior. Generally, apart from the licking of the forepaws in grooming rodents, one can distinguish the activities associated with cleaning the fur, which are not observed in animals subjected to the hot-plate test. In contrast, the first pain reaction in animals challenged with the hot-plate test is accompanied not only with licking the fore- and hind-paws but also with other behavioral responses, such as shaking or tucking of the hindpaws that characterize the first pain reaction to the thermal nociceptive stimulation.

In conclusion, gabapentin and tiagabine produced an antinociceptive effect, and the combination of both antiepileptic drugs at a fixed ratio of 1:1 exerted a synergistic interaction in the hot-plate model of nociceptive pain in mice. If the results from this study

can be extrapolated to clinical settings and additionally confirmed in different experimental models of pain, the combination of tiagabine with gabapentin might be beneficial for pain relief in patients.

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