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Short communication

Synergistic interaction of pregabalin with the synthetic cannabinoid WIN 55,212-2 mesylate in the hot-plate test in mice: an isobolographic analysis

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Abstract:
Background: The aim of the study was to determine the type of interaction between pregabalin (a 3rd-generation antiepileptic drug) and WIN 55,212-2 mesylate (WIN – a highly potent non-selective cannabinoid CB1 and CB2 receptor agonist) administered in combination at a fixed ratio of 1:1, in the acute thermal pain model (hot-plate test) in mice.
Methods: Linear regression analysis was used to evaluate the dose-response relationships between logarithms of drug doses and their resultant maximum possible antinociceptive effects in the mouse hot-plate test. From linear equations, doses were calculated that increased the antinociceptive effect by 30% (ED_{30} values) for pregabalin, WIN, and their combination. The type of interaction between pregabalin and WIN was assessed using the isobolographic analysis.
Results: Results indicated that both compounds produced a definite antinociceptive effect, and the experimentally-derived ED_{30} values for pregabalin and WIN, when applied alone, were 29.4 mg/kg and 10.5 mg/kg, respectively. With isobolography, the experimentally derived $ED_{30\ mix}$ value for the fixed ratio combination of 1:1 was 5.7 mg/kg, and differed significantly from the theoretically calculated $ED_{30\ add}$ value of 19.95 mg/kg ($p < 0.01$), indicating synergistic interaction between pregabalin and WIN in the hot-plate test in mice.
Conclusions: Isobolographic analysis demonstrated that the combination of WIN with pregabalin at a fixed ratio of 1:1 exerted synergistic interaction in the mouse model of acute thermal pain. If the results from this study could be adapted to clinical settings, the combination of WIN with pregabalin might be beneficial for pain relief in humans.

Key words:
drug interaction, pregabalin, hot-plate test, isobolographic analysis, maximum possible antinociceptive effect, WIN 55,212-2 mesylate

Introduction

Accumulating evidence indicates that some antiepileptic drugs exert analgesic effects in both preclinical studies on animals [5, 19, 25, 26, 28, 30, 36, 39–41, 51, 54] and clinical settings in humans [1, 17, 46, 58]. At present, several antiepileptic drugs bring pain relief to patients with trigeminal neuralgia (carbamazepine, lamotrigine and oxcarbazepine), diabetic peripheral neuropathy (topiramate, lamotrigine, gabapentin, and pregabalin), post-herpetic neuralgia (topiramate, gabapentin, and pregabalin), phantom limb pain (gabapentin and pregabalin), and other types of chronic pain [1, 16, 46, 58].

Cannabinoids are promising analgesic drugs, and the ability of cannabinoids to inhibit acute nociception is well known [13, 63, 64]. Experimental studies have documented that WIN 55,212-2 mesylate (WIN – a synthetic cannabinoid CB1 and CB2 receptor agonist) reduced the nociceptive behavioral responses in orofacial and temporomandibular joint formalin tests [7], prevented mechanical allodynia induced by chronic administration of the antineoplastic drugs in rats [48, 50, 62], and produced antinociception in the tail-flick test in mice [13]. WIN produced an antiallodynic effect in streptozocin-induced diabetic rats and mice [14, 60]. WIN alleviated hyperalgesia and allodynia in rats subjected to chronic constriction injury of the sciatic nerve [22]. Moreover, the synthetic cannabinoid WIN attenuated allodynia and hyperalgesia in various rat models of neuropathic pain [6, 10, 20, 31, 33]. Additionally, it has been documented with isobolographic analysis that WIN interacted synergistically with bupivacaine (a local anesthetic drug) in the rat formalin test [29], and the combination of WIN with ketorolac (a non-steroidal anti-inflammatory drug) produced additive interaction in the acetic acid-induced writhing and tail-flick tests in mice [61]. Isobolographic analysis also revealed that intrathecal administration of WIN with clonidine (an antihypertensive drug) or neostigmine (a parasympathomimetic drug) produced synergistic interaction during phases 1 and 2 in the formalin test in rats [65].

Considering the facts that pregabalin and WIN used separately exert antinociceptive effects in various experimental models of acute and chronic pain, it was important to determine the interaction between these agents using the hot-plate test in mice (a standard model used to determine the antinociceptive efficacy of compounds with respect to acute thermal nociception). To characterize the type of interaction for the combination of pregabalin with WIN, an isobolographic analysis of interaction was used.

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 23 ± 1°C, relative humidity 55 ± 5%) were used. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups containing 8 mice each. All tests were performed between 8:00 – 15:00. 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 article were approved by the Second Local Ethics Committee at the University of Life Sciences in Lublin (License Nos. 58/2009; 60/2009; 11/2011) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

The following drugs were used in the present study: pregabalin (Lyrica®, Pfizer Ltd., Sandwich, Kent, UK) and WIN 55,212-2 mesylate (WIN – ((R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate), Tocris Bioscience, Bristol, UK). Pregabalin was suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA), while WIN was dissolved in distilled water, and the drugs were administered *via* intraperitoneal (*ip*) injection in a volume of 0.005 ml/g of body weight. The drugs were administered as follows: WIN at 20 min and pregabalin at 60 min before the hot-plate test. These pretreatment times were chosen based upon information about their biological activity from the literature and authors' previous studies [36, 42].

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 [15], with minor modifications. The device consisted of an electrically-heated surface and an open Plexiglas tube (17 cm high × 22 cm diameter) to confine the animals to the heated surface (Ugo Basile, Varese, Italy). The temperature was set at 55.0 ± 0.1°C. Mice were placed separately on the heated surface, and the time interval (in s) between placement and the 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 5–8 s. Subsequently, the animals were administered pregabalin and WIN alone at increasing doses and at times to the peak of their antinociceptive activity (i.e., 60 and 20 min, respectively). The same procedure was repeated, and the animals were placed again on the heated surface. Thus, 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 8 mice per group) and consecutively numbered on their tails with multi-colored markers. The animals were then challenged with the hot-plate test to determine the latency to the first pain reaction for each mouse separately. Next, the marked animals received WIN and pregabalin, either alone or in combination at a fixed ratio of 1:1. After reaching the peak of the maximum antinociceptive effects, the mice were subjected to the second evaluation of time to the first pain reaction in the same animals. Therefore, both pre- and post-treatment reaction times were recorded in the same animals. The behavioral measures were scored by trained observers blind to the experimental conditions. In the presented study, WIN was administered *ip* at doses ranging between 1.25–15 mg/kg, whereas pregabalin was administered at doses ranging from 6.25–75 mg/kg. A maximum cut-off time of 30 s was chosen to prevent injury to the 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 [52], 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, pregabalin and WIN 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 drug doses, was plotted on the y-axis, and both values were analyzed with least-squares linear regression analysis according to Motulsky and Christopoulos [45]. Subsequently, from the equation of the linear dose-response relationship, the dose of a drug that increased the antinociceptive effect by 30% (ED_{30} value) was calculated. This experimental procedure has been described in more detail in our earlier studies [36, 39, 40].

Isobolographic analysis of interactions

The interaction of pregabalin with WIN, 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 were presented [38]. Notably, the $ED_{30\ add}$ represents the total additive dose of pregabalin and WIN in the mixture that theoretically increases the antinociceptive effect by 30% in the hot-plate test in mice. The $ED_{30\ mix}$ is an experimentally determined total dose of a mixture of 2 component drugs at a fixed ratio combination of 1:1, which is sufficient for a 30% increase in the antinociceptive effect in mice challenged with the hot-plate test. The additive dose of pregabalin and WIN in combination that increased the antinociceptive effect by 30% in the hot-plate test ($ED_{30\ add}$ value) was calculated from the 'equation of additivity' presented by Loewe [34], as follows: $x/X + y/Y = 1$; where x and y are, respectively, the doses of pregabalin and WIN co-administered in the mixture and exert a 30% maximum possible antinociceptive effect in the hot-plate test in mice. X and Y, respectively, are the doses of the antiepileptic drugs administered separately in order to obtain the same effect (30% maxi-

mum possible antinociceptive effect in the hot-plate test in mice). Further details regarding these concepts have been published elsewhere [9, 37, 55]. Of note, the isobolographic notation of the fixed-ratio of 1:1 for the combination of WIN with pregabalin is based on fractions of both drugs used separately. According to the equation presented by Loewe [34], drugs in mixture are usually combined in fixed fractions of their effective doses. In other words, a 2-drug mixture at the fixed-ratio of 1:1 is composed of 2 combined in equal proportions (1:1) of their median effective doses. Hence, the proportions for the fixed-ratio of 1:1 are based on fractions of doses that produce a defined effect in animals, but not on milligram doses of the drugs used. For more details see our earlier studies [36–41]. In this study, we determined the ED_{30} values for pregabalin, WIN and the mixture of both drugs that corresponded to doses of drugs and their mixture, which produced a 30% antinociceptive effect in the hot-plate test in mice. Of note, a 100% antinociceptive effect can be observed only in fully anesthetized animals. In the hot-plate test (a model of thermal pain), the antinociceptive effect observed in mice could not reach a 100% effect because the animals would be unable to fulfill and respond to the thermal stimulus, which would be destructive and harmful for the animals. On the other hand, a 30% antinociceptive effect for WIN and pregabalin was strong enough to detect the antinociceptive properties of drugs and their mixture in animals, without any acute adverse effects produced by the drugs at doses corresponding to their ED_{30} values. Previously, we have reported that WIN at doses higher than 15 mg/kg produced acute adverse effects in mice manifesting various symptoms including ataxia, impairment of motor coordination and skeletal muscular strength, as well as long-term memory deficits [42]. This is the reason we did not evaluate the ED_{50} or ED_{90} values in this study.

Statistical analysis

The maximum possible antinociceptive effect values with their SE were calculated by using the formula presented by Schmauss and Yaksh [52]. The ED_{30} values with their SE were calculated from least-squares linear regression analysis according to Motulsky and Christopoulos [45]. Statistical evaluation of the isobolographic interaction between pregabalin and WIN was performed by the use of Student's *t*-test with Welch's correction in order to detect the differences between the experimentally-derived ($ED_{30\ mix}$) and theoretical additive ($ED_{30\ add}$) values, according to Tallarida [55]. All statistical tests were performed using commercially available GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). Differences between values were considered statistically significant if $p < 0.05$.

Results

Effects of WIN, pregabalin and their combination on the antinociception in the hot-plate test in mice

WIN administered *ip* 20 min before the acute thermal pain test prolonged the latency to the first pain reaction in mice in a dose-dependent manner. The experimentally-derived values of the maximum possible antinociceptive effect for WIN (administered at increasing doses of 1.25–15 mg/kg) were between 11.99% – 34.62% (Fig. 1). The equation of the dose-response relationship, as denoted from a least-squares linear regression, for WIN was: $y = 20.096\ x + 9.478$ ($r^2 = 0.976$); where y is the maximum possible antinociceptive effect in %, x is the logarithm of the WIN dose, and r^2 is the coefficient of determination (Fig. 1). The experimentally denoted logarithm of the ED_{30} value for WIN in the hot-plate test in mice was 1.021, which corresponded to a drug dose of 10.50 ± 2.04 mg/kg (Fig. 1).

Similarly, pregabalin 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 pregabalin (administered at increasing doses of 6.25–75 mg/kg) ranged between 15.95% – 42.22% (Fig. 1). The equation for the pregabalin dose-response relationship was: $y = 23.476\ x - 4.480$ ($r^2 = 0.960$; Fig. 1). Thus, the experimentally calculated logarithm of the dose of pregabalin that increased the antinociceptive effect by 30% (ED_{30} value) in the hot-plate test in mice was 1.469, corresponding to the drug dose of 29.43 ± 2.85 mg/kg (Fig. 1).

The mixture of pregabalin with WIN 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 maximum possible

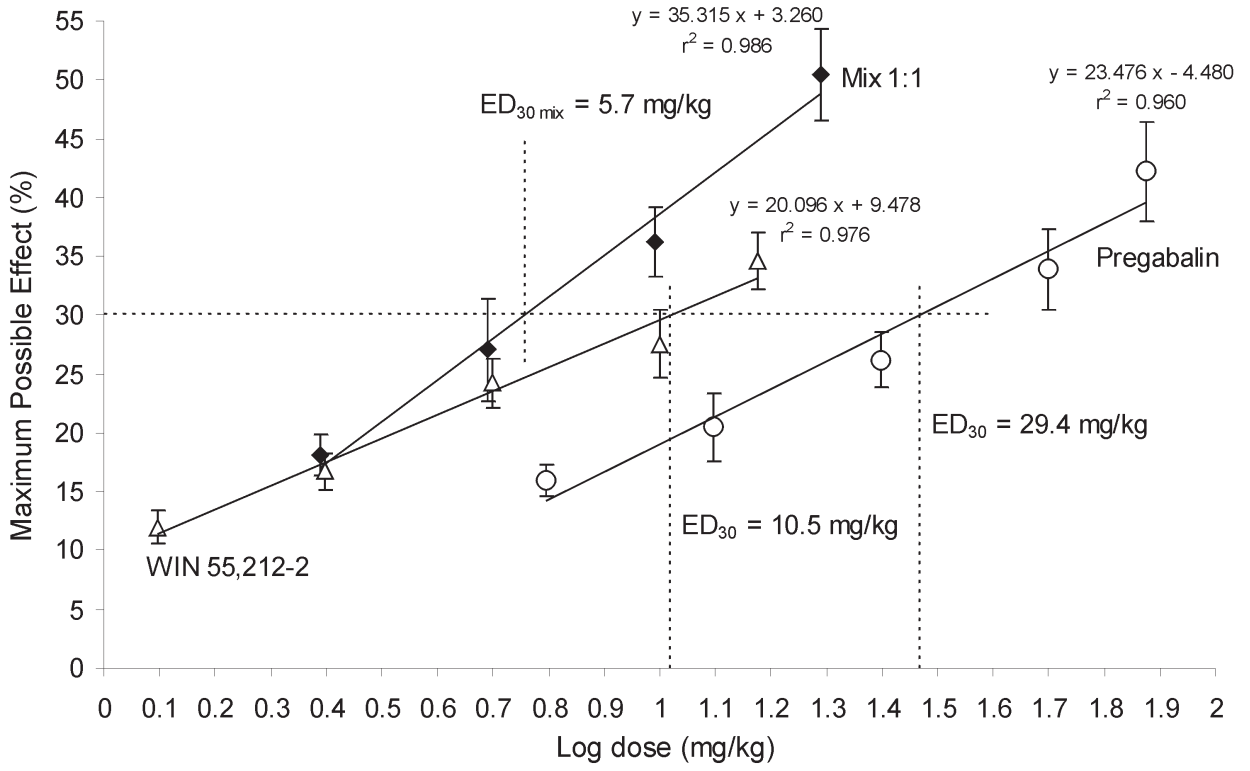

Fig. 1. Dose-response effects of pregabalin, WIN and the combination of the 2 drugs at a fixed ratio of 1:1 in the hot-plate test in mice. Doses of pregabalin, WIN, and the mixture of their combination at a fixed ratio of 1:1 (in mg/kg) were transformed to logarithms to the base 10 (log), whereas the antinociceptive effects produced by pregabalin, WIN, and the mixture of both drugs at the ratio of 1:1 were transformed to the maximum possible antinociceptive effect (maximum possible effect in % ± SE as the error bars, n = 8). Pregabalin and WIN were administered *ip* at 60 and 20 min, respectively, before the antinociceptive effect evaluation. Log doses of pregabalin, WIN and their combination at the fixed ratio of 1:1, together with their resultant maximum possible 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 the tested drugs and their respective antinociceptive effect in the hot-plate test in mice. The linear equations for WIN, pregabalin, and the combination of the 2 drugs are presented in Figure 1; where y is the maximum possible effect value (in %), x is the log dose (in mg/kg) of pregabalin or WIN administered alone, or the mixture of WIN and pregabalin in combination, at a fixed ratio of 1:1; and r^2 is the coefficient of determination. The log of ED_{30} value for WIN was 1.021 and corresponded to a WIN dose of 10.5 mg/kg. The experimentally calculated log of ED_{30} value for pregabalin was 1.469, which corresponded to a pregabalin dose of 29.4 mg/kg. The log of $ED_{30\ mix}$ value for the combination of WIN with pregabalin at a fixed ratio of 1:1 was 0.757 and corresponded to a dose of 5.7 mg/kg of the mixture. The test for parallelism of 2 dose-response lines (for pregabalin and WIN) compared their slopes with Student's *t*-test [56]. In our study, the computed *t* value was 0.186, whereas the tabular value for 6 degrees of freedom was 2.447. Since the computed *t* value is lower than the tabular value, the 2 regression lines (for pregabalin and WIN) are parallel to one another

antinociceptive effect values for the mixture, administered at doses ranging between 2.45 – 19.55 mg/kg, ranged from 18.06% – 50.39% (Fig. 1). Least-squares linear regression revealed that the experimentally-derived equation for the mixture of pregabalin with WIN at a fixed ratio of 1:1 was: y = 35.315 x + 3.260 (r^2 = 0.986; Fig. 1). Thus, the logarithm of the experimentally determined $ED_{30\ mix}$ value in the hot-plate test in mice was 0.757, which corresponded to the dose of the mixture of 5.72 ± 3.24 mg/kg (Fig. 1).

The test for parallelism of 2 dose-response lines (for pregabalin and WIN) was performed according to the procedure described by Tallarida and Murray [54]. This procedure compares the slopes of 2 regression lines with Student's *t*-test. If the computed *t* value exceeds the tabular value, the slopes differ significantly and the hypothesis of parallelism is rejected [54]. In the presented study, the computed *t* value was 0.186, and the tabular value for 6 degrees of freedom was 2.447 (Fig. 1). Since the computed *t* value is lower than the tabular value, the slopes for WIN and pregabalin do not differ significantly, therefore, the hypothesis of parallelism is accepted, indicating that the 2 dose-response regression lines for pregabalin and WIN are parallel to one another. This is why we used the type I isobolographic analysis for parallel dose-response curves to characterize the interaction between WIN and pregabalin.

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

$Pregabalin_{add}$	WIN_{add}	$ED_{30\ add}$	n_{add}	$ED_{30\ mix}$	$Pregabalin_{mix}$	WIN_{mix}	n_{mix}
14.70	5.25	19.95 ± 2.45	76	5.72 ± 3.24**	4.22	1.50	32

Data are presented as doses of the mixture of pregabalin and WIN at a fixed ratio of 1:1 that increased the antinociceptive effect by 30% (ED_{30} ± SE) from the hot-plate test in mice. The ED_{30} values were either experimentally determined from the mixture of 2 tested drugs ($ED_{30\ mix}$), or theoretically calculated from the equation of additivity ($ED_{30\ add}$). Additionally, the actual doses of pregabalin and WIN that comprised the mixture at a fixed ratio combination of 1:1, for both $ED_{30\ mix}$ and $ED_{30\ add}$ values, are presented in separate columns as $Pregabalin_{add}$, WIN_{add}, $Pregabalin_{mix}$ and WIN_{mix} 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 the doses at which the expected antinociceptive effect was greater than 16%, denoted for the experimental mixture of drugs (n_{mix}) and theoretically calculated (n_{add}) from the equation of additivity; ** $p < 0.01$ *vs.* the $ED_{30\ add}$

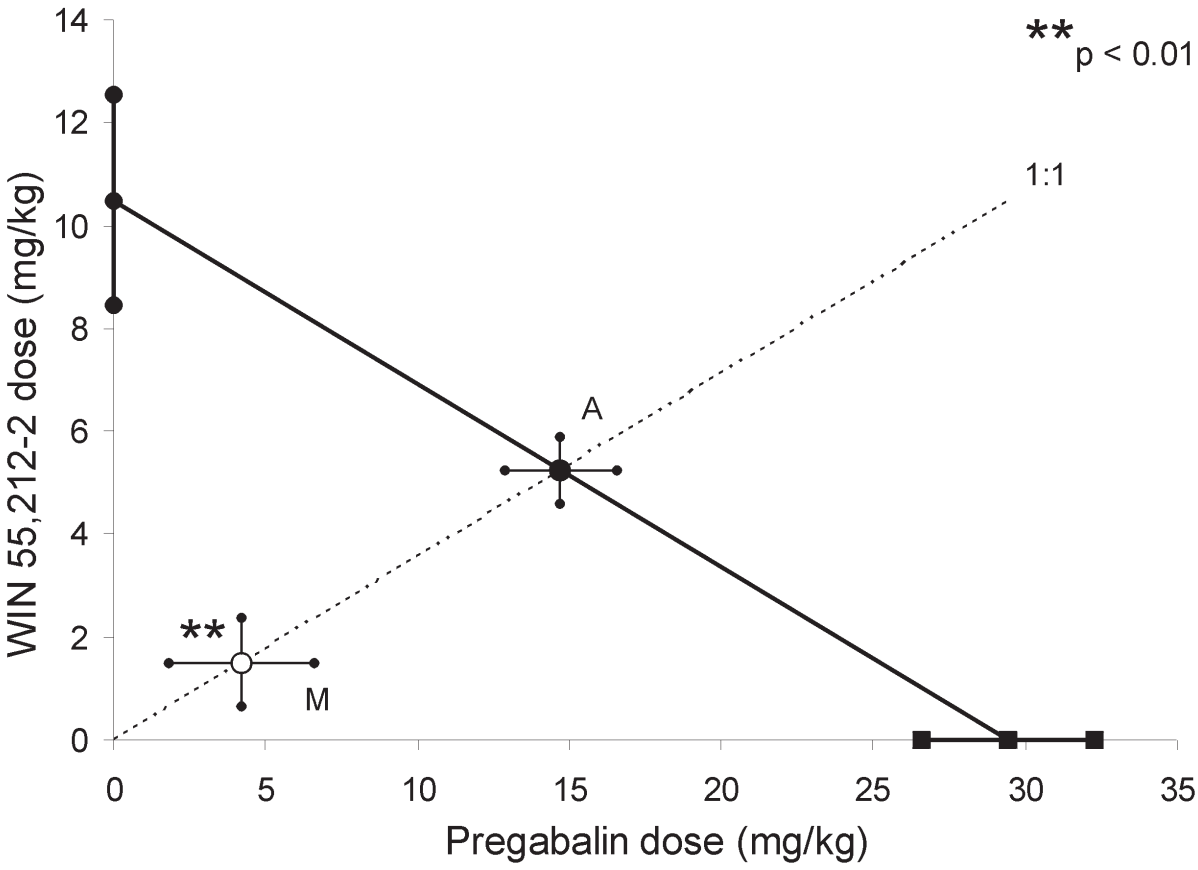

Fig. 2. Isobologram illustrating the supra-additive (synergistic) interaction for the combination of WIN with pregabalin in the hot-plate test in mice. Doses increasing the antinociceptive effect by 30% (ED_{30}) in the hot-plate test in mice for pregabalin and WIN are plotted graphically on the x- and y-axes of the Cartesian system of coordinates. The solid lines on the axes represent SE for the drugs administered alone. The straight line connecting these two ED_{30} values represents the theoretical line of additivity for a continuum of different fixed dose ratios ($ED_{30\ add}$ values). **Point A** represents the theoretical additive $ED_{30\ add}$ (± SE as the error bars) for the total dose expressed as the proportion of pregabalin and WIN that produced a 30% antinociceptive effect. **Point M** on the graph depicts the experimentally-derived $ED_{30\ mix}$ (± SE as the error bars) for the total dose expressed as the proportion of pregabalin and WIN that produced a 30% antinociceptive effect. The $ED_{30\ mix}$ for the fixed ratio of 1:1 is placed significantly below the line of additivity, indicating the supra-additive (synergistic) interaction between WIN and pregabalin in the hot-plate test in mice

Isobolographic analysis of interaction between pregabalin and WIN 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 pregabalin with WIN 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 $ED_{30\ mix}$ for the fixed ratio of 1:1 was 5.72 mg/kg, which significantly differed from the $ED_{30\ add}$ of 19.95 mg/kg ($p < 0.01$; Tab. 1; Fig. 2). The separate doses of pregabalin and WIN in the mixture at the fixed-ratio of 1:1, calculated from the $ED_{30\ add}$ and $ED_{30\ mix}$ values, are presented in Table 1.

Discussion

The presented results indicate that pregabalin and WIN produced antinociceptive effects in a dose-dependent manner using the acute thermal pain model (hot-plate test) in mice. WIN was examined at doses up to 15 mg/kg, and the drug produced a clear-cut antinociceptive effect with the ED_{30} value of 10.5 mg/kg, which confirmed the antinociceptive effect of WIN in the hot-plate test. Similarly, pregabalin was examined at doses up to 75 mg/kg, and the antiepileptic drug produced a clear-cut antinociceptive effect with the ED_{30} value of 29.4 mg/kg in the hot-plate test. Notably, the ED_{30} values for WIN and pregabalin, 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 [36, 42].

Isobolographic analysis revealed that the combination of both drugs produced a supra-additive (synergistic) interaction in the hot-plate test in mice. To explain the observed synergistic interaction between pregabalin and WIN in this study, one should consider their molecular mechanisms of action. With respect to pregabalin, this drug binds with high affinity to the $\alpha_2\delta$ type 1 and 2 subunits of calcium channels [57] and inhibits calcium influx through presynaptic P/Q-type voltage-gated calcium channels [32]. The inhibition of calcium influx reduces potassium-evoked excitatory transmitter release, thereby decreasing postsynaptic excitability [47]. Experimental studies have revealed that the binding of pregabalin to the $\alpha_2\delta$ auxiliary subunits of the calcium channels is necessary and sufficient for analgesic effects [57]. For instance, transgenic mice expressing the mutant gene for the $\alpha_2\delta$ auxiliary subunit of the calcium channels (R217A mutant mice) have much smaller quantities of drug binding in the forebrain and spinal cord. They also completely lack analgesic-like actions of pregabalin with unaltered analgesia from morphine and amitriptyline [18]. It has been reported recently that pregabalin activates the descending noradrenergic system to facilitate spinal noradrenaline turnover, resulting in analgesic effects mediated by spinal α_2-adrenoceptors after peripheral nerve injury in the mouse partial sciatic nerve ligation model [54]. Moreover, the antiallodynic effect of pregabalin is correlated with the up-regulation of $\alpha_2\delta$ subunits of voltage-dependent calcium channels in the spinal cord and/or dorsal root ganglia [35]. Pregabalin impairs anterograde trafficking of the $\alpha_2\delta$-1 subunit, resulting in its decrease in presynaptic terminals, which reduces neurotransmitter release and spinal sensitization in rats with unilateral lumbar spinal nerve ligation [2].

In the case of WIN, by activating cannabinoid CB1 receptors the compound inhibits adenylyl cyclase [8, 23], blocks N-type and P/Q-type calcium channels [43, 59], stimulates A-type and inwardly rectifying potassium (K_{ir}) channels [11, 21, 44], and activates mitogen-activated protein kinase (MAPK) signaling pathway [4]. Experimental studies have documented that the inhibition of presynaptically located N-type and P/Q-type calcium channels reduces presynaptic entry of calcium, thereby inhibiting neurotransmitter release from CB1-presynaptic terminals in cultured rat hippocampal neurons [24, 59]. Moreover, WIN produced antinociception *via* cannabinoid CB1 receptor- mediated mechanisms through activation of descending serotonergic pathways to the spinal cord by acting on serotonine $5HT_7$ and $5HT_{2A}$ receptors [53].

Bearing in mind the molecular mechanisms of action of pregabalin and WIN, it can be ascertained that the inhibition of N-type and P/Q-type calcium channels are likely to be responsible for the observed synergistic interaction in the hot-plate test in mice. One can suppose that the similar mechanisms of action of the studied compounds would result in the inhibition of nociceptive transmission to the central and peripheral nervous system.

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 [12, 49]. In contrast, the drugs with diverse mechanisms of action may complete their own activities, thereby producing a synergistic interaction [3, 12, 49]. In the presented study, both pregabalin and WIN block P/Q-type calcium channels. However, it is possible that the different sites of action of these drugs may be responsible for the observed synergistic interaction in the hot-plate test. Such interaction may occur when both drugs affect different critical points along a common pathway [3]. Hence, the action of WIN and pregabalin may independently alter P/Q-type calcium channels and mediate a synergistic interaction. Moreover, functional interaction may result from distinct drug effects at separate anatomic sites that may act independently and together to inhibit spinal nociceptive processing. It is possible that the drugs (pregabalin and WIN) possess both presynaptic and postsynaptic actions. Therefore, simultaneous engagement of pre- and post-synaptic mechanisms may augment the antinociceptive action produced by either drug acting at one site independently.

In addition, comparing the doses of pregabalin and WIN in the mixture at the fixed ratio of 1:1 that exerted a 30% increase in the antinociceptive effect (4.22 mg/kg for pregabalin and 1.50 mg/kg for WIN) with the doses of pregabalin and WIN producing the same 30% effect when administered alone (29.4 mg/kg for pregabalin and 10.5 mg/kg for WIN), one can observe a considerable reduction of drug doses when both drugs were used in combination. Thus, the reduction of drug doses during the treatment with these drugs may contribute to the limitation of the acute adverse effects exerted by these drugs when applied alone at high effective doses [49]. There is no doubt that the decreased doses of both drugs in combination will be better tolerated than higher doses of the drugs used separately, especially if the antinociceptive effect is unchanged. In other words, the combination of pregabalin with WIN fulfills all the criteria of multimodal analgesia [16, 27], therefore, it can be recommended as an advantageous combination in further clinical trials. Moreover, the presented results confirm that pregabalin in combination with WIN exerts synergistic interaction in the acute thermal pain model. Nevertheless, our results describing the synergistic interaction of pregabalin with WIN should be confirmed in additional models of acute and/or chronic pain.

Conclusion

Pregabalin and WIN produced an antinociceptive effect, and the combination of both compounds at a fixed ratio of 1:1 exerted a supra-additive (synergistic) interaction in the hot-plate model of nociceptive pain in mice. If the results from this study can be adapted to clinical settings and additionally confirmed in different experimental models of pain, the combination of WIN with pregabalin might be useful in the management of pain in patients.

Disclosure of conflicts of interest:
The authors have no disclosures to declare.

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