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ORIGINAL ARTICLE

Synergism between gabapentin–tramadol in experimental diabetic neuropathic pain

Hugo F. Miranda[a]*, Fernando Sierralta[b], Nicolas Aranda[b], Paula Poblete[b], Viviana Noriega[c], Juan C. Prieto[b,c]

[a]*Department of Neuroscience, Faculty of Medicine, University of Chile, Indepedencia 1027, Independencia, Santiago, Chile, P.O. 8380453*

[b]*ICBM, Pharmacology Program, Faculty of Medicine, University of Chile, Indepedencia 1027, Independencia, Santiago, Chile, P.O. 8380453*

[c]*Cardiovascular Department, Clinical Hospital, University of Chile, Indepedencia 1027, Independencia, Santiago, Chile, P.O. 8380453*

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*Correspondence and reprints:
hmiranda@med.uchile.cl

ABSTRACT

Neuropathic pain is associated with several conditions such as surgery, cancer, and diabetes and can be induced experimentally. Among the drugs used as monotherapy are gabapentin and tramadol. The purpose of this study was to evaluate the coadministration of gabapentin and tramadol, by isobolographic analysis, in three different algesiometric assays in experimental diabetic neuropathic pain induced by streptozocin in mice. In all the behavioral tests, gabapentin or tramadol produced a dose-dependent antinociception and their coadministration resulted in a positive interaction. This effect can be explained by principles of multimodal analgesia, whereby the different mechanisms of action of each drug contribute to the combined effect in a supra-additive manner. The findings of the present study suggest that the combination of gabapentin and tramadol could be a useful strategy for the treatment of pain induced by diabetic neuropathy.

INTRODUCTION

Neuropathic pain is defined as 'pain caused by a lesion or disease of the somatosensory nervous system' [1] and is induced by post-herpetic neuralgia, HIV, diabetes polyneuropathy, cancer, trigeminal neuralgia, or surgery [2–4]. There are different animal models to evaluate neuropathic pain, such as diabetic neuropathy, streptozocin-induced neuropathy, partial sciatic nerve ligation, spared nerve injury, chemotherapy-induced neuropathy, and orofacial pain.

Diabetic neuropathic pain induced by streptozocin (STZ) is one of the most commonly employed models in many species including rodents and produces more reproducible behavioral changes for a longer period (>3 months). STZ is a model of induction with the ability to synchronize diabetes in a cohort of animal. Mice mimic the hallmarks of diabetes within a short length of time with minimal mouse-to-mouse variation. Also, preclinical data obtained using these animal models have been successively translated to effective pain management in clinical settings [5–7].

Gabapentin and tramadol are among the various drugs used as monotherapy in the pharmacotherapy of neuropathic pain [2]. Gabapentin is an anticonvulsant that has been reported to be effective in the treatment of pain syndromes, including painful diabetic neuropathy. Gabapentin is structurally related to γ-aminobutyric acid (GABA), a neurotransmitter that plays a role in pain transmission and their modulation. Pharmacological actions of gabapentin include interaction with the system L-amino acid transporter, blocking the AMPA-receptor, alteration of synthesis and release of GABA in the brain, high affinity binding to the α2δ subunit of voltage-activated calcium channels, inhibition of voltage-activated sodium channels, alteration of monoamine neurotransmitter release and blood serotonin levels, selective enhancing of NMDA current and neuroprotection in laboratory models of amyotrophic lateral sclerosis [8–10].

Tramadol is a synthetic analgesic drug with opioid and non-opioid properties acting on the nervous system. Antinociception is due to actions on opioid receptors and monoamine systems, blocking norepinephrine, and serotonin reuptake. Tramadol has two chiral centers and is used as 1:1 racemic mixture of R,R-enantiomer ([+]-tramadol) and S,S-enantiomer ([−]-tramadol). The [+]-tramadol is the most potent serotonin reuptake inhibitor, whereas the [−]-enantiomer is the norepinephrine reuptake inhibitor. Tramadol is metabolized into three metabolites and the [+]-M1 metabolite is a high affinity ligand and produces more potent analgesic effect. [11–13].

The roles of gabapentin and tramadol as single antinociceptive agents have been examined but the nature of the interaction between them has not been determined. Therefore, the purpose of this study was to evaluate the efficacy of the coadministration of gabapentin and tramadol, by isobolographic analysis, in three different algesiometric assays on the treatment of pain associated with murine diabetic neuropathy.

MATERIAL AND METHODS

CF-1 male mice, weighing 28–30 g, housed in a 12-h light–dark cycle at 22 ± 1 °C with free access to food and water were used. Animals were acclimatized to the laboratory environment for at least 2 h before use. Experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of National Institute of Health and approved by the Institutional Animal Care and Use Committee of University of Chile, Santiago, Chile. Each animal assigned by randomization procedure was used only once, received only one dose of the drugs tested, and testing procedures were conducted on days 3 and 7 after STZ. All drugs were freshly prepared by dissolving them in normal saline and administered intraperitoneally (i.p.) in a constant volume of 10 mg/kg, and the doses of different drugs were selected based on previous pilot study. In this study, mice were allocated at random (by chance alone) to receive one or another drug, and the investigators were blind to the drug protocol used. Control saline animals were run interspersed concurrently with the drug-treated animals (at least two mice per group), which prevented all the controls being run on a single group of mice at one time during the experiment. All experiments were performed by researcher blind to drug treatment.

Streptozocin induction of diabetes

An experimental model of diabetes was induced in CF1 mice by a single administration i.p. of 200 mg/kg of STZ according to the method previously described [14]. Mice were fasted for 3 h before drugs administration. Diabetes was confirmed by determining blood glucose levels taken from tail veins after the STZ treatment, using a hemoglucotest (Accu-check Performa Nano from Roche Diagnostic GmbH, Mannheim, Germany). Mice treated with STZ were considered diabetic when blood glucose levels were $200 \geq$ mg/dL. Mice that failed to reach hyperglycemia were excluded from the study. Control mice were injected with an equal volume of saline.

Algesiometer assays

Tail flick test

The algesiometric test was performed as previously described [14]. A focus of radiant heat (automatic tail flick [TF] algesiometer, U. Basile, Comerio, Italy) was used to measure the response latencies. The light beam was focused on the animal's tail about 4 cm from the tip, and the intensity was adjusted so that baseline readings were between 2 and 3 s. An 8-s cutoff time was imposed to avoid damage to the tail. Control reaction time (latency of the response) was recorded twice, with an interval of 10 min between the readings, the second reading being generally similar to the first. Only animals with baseline reaction times between 2 and 3 s were used in the experiments. TF latencies were converted to % maximum possible effect (MPE). Each animal was used as its own control. Drugs were administered 30 min before the experimental protocol. The dose that produced 50% of the MPE (ED_{50}) was calculated from the linear regression analysis of the curve obtained by plotting the log dose vs. % MPE.

Formalin test (FT)

The method described by Miranda et al. [15] was used. To perform the test, 20 μL of 5% formalin solution was injected subcutaneously (s.c.) into the dorsal surface of the right hind paw of the mice with a 27-gauge needle attached to a 50 μL Hamilton syringe. Each mouse was immediately returned to a Plexiglas observation chamber. The degree of pain intensity was assessed as the total time spent by the animal licking or biting the injected paw, measured by visual observation and a digital time stopwatch. The test shows two clear-cut phases: phase I corresponds to the five-min period starting immediately after the formalin injection and

represents a tonic acute pain response related to peripheral nociceptor sensitization; phase II was recorded as the 10-min period starting 20 min after the formalin injection and represents the activation of central sensitized neurons because of peripheral inflammation stimulus. Drug or saline was administered s.c. 30 min before formalin injection. Control animals ($n = 24$) were injected with saline. For each drug, the analgesic effects were established after the administration of a minimum of four doses in logarithmic increments. The licking times observed were converted to % MPE. The dose that produced 50% of the MPE (ED_{50}) was calculated from the linear regression analysis of the curves obtained by plotting log dose vs. %.

Hot plate test

The hot plate test (HP) was performed using an automatic analgesiometer (Ugo Basile, Varese, Italy) according to Miranda et al. [14], calibrated at 50 ± 0.5 °C, and the cutoff time was set at 30 s to avoid skin damage. Animal behavior considered as sign of pain is licking of the forelegs or jumping off the hot plate (latency of response in sec). Several measurements were performed with a three-min interval: two without drug administration (baseline latency) and two after i.p. administration of drugs. The baseline latency of control mice injected with saline was 22.10 ± 0.75 s ($n = 24$). Hot plate latencies were converted to % MPE. The animals' behaviors considered as signs of pain included licking of the forelegs. The baseline latency for this behavior was recorded with a stopwatch. The cutoff time was fixed at 30 s to avoid skin damage. Several measurements were performed with three-min interval: two at baseline (without any drug administration) and two after i.p. administration of the test drugs. Hot plate latencies were converted to % MPE. The dose that produced 50% of the MPE (ED_{50}) was calculated from the linear regression analysis of the curve obtained by plotting the log dose vs % MPE.

Interaction of gabapentin with tramadol

Isobolographic analysis was used to characterize the interaction between gabapentin and tramadol on each test. This analysis has been described by Tallarida and adapted by Miranda et al. [16]. The isobologram is a graphical representation of isoeffective doses of gabapentin, or tramadol combined in fixed ratios (1:1) of the corresponding ED_{50}, which was determined in isolation for each drug. The isobologram is constructed by connecting the ED_{50} of tramadol on the abscissa with the ED_{50} of gabapentin on the ordinate, yielding the line of additivity. The experimental ED_{50} of the mixture was obtained by linear regression analysis of the corresponding logarithmic dose–response curve of the mixture and compared with the t-test with theoretical ED_{50}; the theoretical ED_{50} was deduced from: $ED_{50} = ED_{50}$ gabapentin$/(P1 + R \times P2)$, where $P1$ and $P2$ is the ratio of the mixture, and R is the ratio of relative potency of gabapentin or tramadol administered individually. The point representing the experimental ED_{50} will be located in the isobologram, and the site of the graph where the experimental point is located determines the type of interaction. If the experimental point is below the line of additivity and is statistically different from the point of additivity, the effect of the combination of gabapentin with tramadol is synergistic or superadditive. To certify the nature of the mixture of the drugs, the interaction index (I.I.) was also calculated with the following formula I.I. = experimental ED_{50}/theoretical ED_{50}. If the I.I. is less than 1, the interaction is synergistic [16].

Protocols

A dose–response curve for i.p administration of gabapentin (3, 10, 30, 100 mg/kg) and for tramadol (3, 10, 30, 100 mg/kg) and their coadministration was obtained using eight animals with at least four doses expressed on the basis of the salt. A least-square linear regression analysis of the log dose–response curve allows the calculation of the log that produced 50% antinociception (ED_{50}) for gabapentin and tramadol, expressed as % of maximum possible effect (% MPE).

Drugs

All drugs were freshly dissolved in saline solution on a constant volume of 10 mL/kg administered i.p. as mg/kg. Streptozocin, gabapentin, and tramadol were purchased from Sigma Chemical Co, St. Louis, Missouri, USA.

Statistical analysis

All results are presented as means ± standard error of the means (SEM). Analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare the data group. All calculations were performed with the software SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp). P values less than 0.05 ($P < 0.05$) were considered significant.

RESULTS

The different doses of gabapentin and tramadol used did not produce visual-motor dysfunction.

Streptozocin diabetic mouse

Fasting blood glucose measurement was taken at 3 and 7 days following STZ injection. Control mice had an average fasting blood glucose level of 104.80 ± 7.10 mg/dL. Following treatment with STZ (200.00 mg/kg, i.p.), a marked increase in plasma glucose levels, was observed on 3 days (255.60 mg/dL) and on 7 day (386.80 mg/dL). Mice with STZ-induced diabetes also developed neuropathic pain measured by algesiometric tests.

Tail flick antinociception

Gabapentin increased the reaction time of TF test in dose-dependent manner compared to the control mice. The ED_{50} obtained with gabapentin was 17.61 ± 1.84 mg/kg (n = 24). Pretreatment of the mice with 200 mg/kg i.p. of STZ, decreased significantly the control value of ED_{50} on day 3 to 5.97 ± 0.54 mg/kg (n = 24) and on day 7 to 3.30 ± 0.30 mg/kg (n = 24). In STZ mice, gabapentin increased in potency 2.94 times on day 3, and 5.46 times on day 7 (*Figure 1a*).

Tramadol increased the reaction time of TF test in dose-dependent manner compared to the control mice. The ED_{50} obtained with tramadol was 9.72 ± 1.34 mg/kg (n = 24). Pretreatment of the mice with 200 mg/kg i.p. of STZ, decreased significantly the control value of ED_{50} on day 3 to 3.30 ± 0.30 mg/kg (n = 24) and on day 7 to 1.77 ± 0.08 mg/kg (n = 24). Tramadol increased in potency on day 3 to 2.94 times and on day 7 to 5.49 times in STZ mice (*Figure 1b*).

The coadministration of gabapentin with tramadol in a 1:1 of ratio of ED_{50} values was subjected to isobolographic analysis. In the TF test, the interaction was synergistic with the following values of their ED_{50} for theoretical control 13.66 ± 1.132 mg/kg; in STZ DN mice, at 3 days the experimental ED_{50} obtained was 9.28 ± 0.85 mg/kg and at 7 days it was 6.00 ± 0.16 mg/kg. The corresponding interaction indices (I.I.) were 0.679 and 0.366, respectively (*Figure 3*). The isobolograms are displayed in *Figure 4*.

Formalin antinociception

Gabapentin induced a dose-related reduction in licking time in the FT in control animals, with an ED_{50} of 9.30 ± 0.80 mg/kg (n = 24) for phase I. Pretreatment of the mice with 200 mg/kg ip. of STZ decreased significantly the control value on day 3 to 2.60 ± 0.95 mg/kg (n = 24) and on day 7 to 2.27 ± 0.87 mg/kg (n = 24).

In this test, gabapentin increased in potency on day 3 to 3.57 and on day 7 to 4.06 times in STZ mice. In phase II responses, gabapentin reduced the licking time of saline control mice, with an ED_{50} of 8.15 ± 0.42 mg/kg (n = 24). Pretreatment of the mice with 200 mg/kg ip. of STZ decreased significantly the control value on day 3, to 2.80 ± 0.23 mg/kg (n = 24) and on day 7, to 1.40 ± 0.28 mg/kg (n = 24). In phase II of the FT,

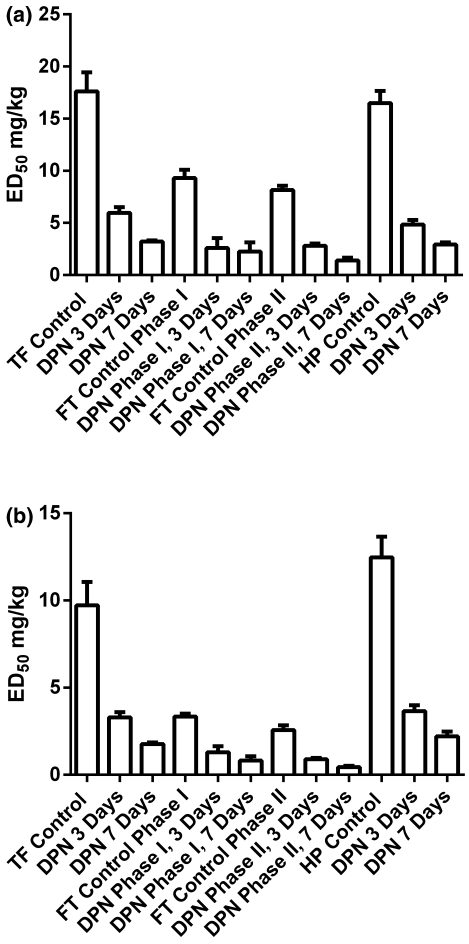

Figure 1 (a) ED_{50} values, in mg/kg ± SEM for the antinociceptive effect of gabapentin in various mice pain models. DPN: diabetic peripheral neuropathy. All result are significant ($P < 0.05$) compared with the respective control. (b) ED_{50} values, in mg/kg ± SEM for the antinociceptive effect of tramadol in various mice pain models. All result are significant ($P < 0.05$) compared with the respective control.

gabapentin reduced the potency on day 3 to 2.91 and on day 7 to 5.82 times in STZ mice (*Figure 1a*).

Tramadol 1–100 mg/kg i.p. induced a dose–response reduction of the licking time in phase I of the FT in saline control animals, with an ED_{50} of 3.35 ± 0.16 mg/kg (n = 24). Pretreatment of the mice with 200 mg/kg ip. of STZ decreased significantly the control value on day 3, to 1.30 ± 0.34 mg/kg (n = 24) and on day 7, to 0.82 ± 0.25 mg/kg (n = 24). In phase I of the test, tramadol increased in potency on day 3 to 2.57 and on day 7 to 4.08 times in STZ mice. In phase II, tramadol reduced the licking time of saline control mice, with an ED_{50} of 2.57 ± 0.27 mg/kg (n = 24). Pretreatment of the mice with 200 mg/kg ip. of STZ decreased significantly the control value, on day 3 to 0.90 ± 0.07 mg/kg (n = 24) and on day 7 to 0.44 ± 0.08 mg/kg (n = 24). In phase II, tramadol potency was increased on day 3 to 2.85 and on day 7 to 5.84 times in STZ mice, as shown in *Figure 1b*.

ED_{50} values were subjected to isobolographic analysis. The interaction of both drugs was synergistic: in phase I, the theoretical control was 6.32 ± 0.40 mg/kg; in STZ DN mice at 3 days it was 3.90 ± 1.30 mg/kg and at 7 days it was 3.10 ± 0.96 mg/kg. In phase II, the values were 5.36 ± 0.25 mg/kg for theoretical control; in STZ DN mice, they were 3.80 ± 0.30 mg/kg at 3 days and 1.85 ± 0.10 mg/kg at 7 days (see *Figure 2*). The respective isobolograms are displayed in *Figure 4*. Also, the II values for phase I were 0.617 and 0.490 for animals pretreated with STZ at 3 days and 7 days, respectively. The I.I. values for phase II were 0.709 and 0.345 for animals with STZ at 3 days and 7 days, respectively (see *Figure 3*).

Hot plate antinociception

Gabapentin administered i.p. at doses of 3–100 mg/kg increased the control latency time, in dose-dependent manner compared to the control mice with an ED_{50} of 16.49 ± 1.17 mg/kg (n = 24). Pretreatment of the mice with 200 mg/kg i.p. of STZ reduced significantly the control value on day 3 to 4.84 ± 0.44 mg/kg and on day 7 to 2.92 ± 0.22 mg/kg (n = 24). Gabapentin increased in potency on day 3 to 3.40 and on day 7 to 5.66 in STZ mice, as shown in *Figure 1a*.

Tramadol administered i.p. at the doses of 1–100 mg/kg i.p. increased the control latency time, compared to the control mice with an ED_{50} of 12.47 ± 1.18 mg/kg (n = 24). Pretreatment of the mice with

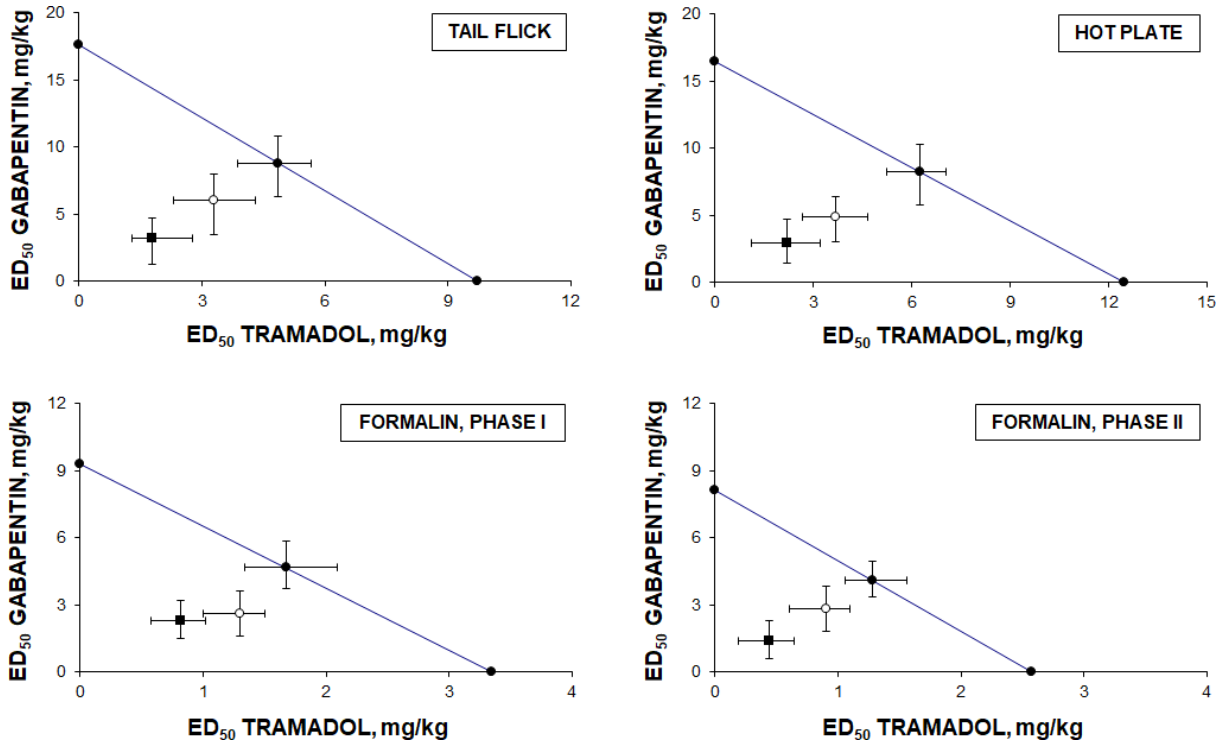

Figure 2 Theoretical and experimental ED_{50} values, in mg/kg ± SEM for the antinociceptive effect of the combination gabapentin with tramadol in various mice pain models. All experimental results are significant ($P < 0.05$) compared with the theoretical values.

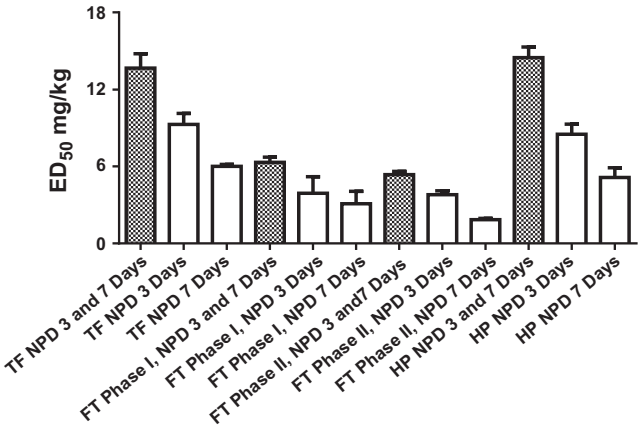

Figure 3 Interaction index (I.I) of the antinociceptive effect of the combination gabapentin with tramadol in various mice pain models.

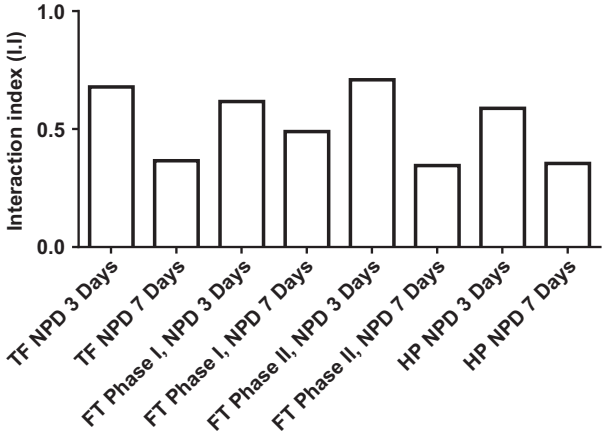

Figure 4 Isobolograms for the administration of the combination of gabapentin and tramadol, i.p., in the tail flick, hot plate, formalin, phase I and formalin, phase II of mice. Theoretical ED_{50} value with SEM (●) Experimental and ED_{50} value with SEM (○).

200 mg/kg i.p. of STZ reduced significantly the control value on day 3 to 3.66 ± 0.33 mg/kg an on day 7 to 2.20 ± 0.28 mg/kg (n = 24). In the HP test, tramadol increased in potency on day 3 to 3.40 and on day 7 to 5.66 in STZ mice (data in *Figure 1b*).

ED_{50} values were subjected to isobolographic analysis. The interaction of both drugs was synergistic. The theoretical control was 14.48 ± 0.83 mg/kg; in STZ DN mice, at 3 days it was 8.51 ± 0.78 mg/kg and at 7 days it was 5.13 ± 0.78 mg/kg (*Figure 2*). The corresponding isobolograms are displayed in *Figure 4*. In addition, the I.I. values were 0.588 and 0.354 for animals pretreated with STZ at 3 days and 7 days, respectively.

DISCUSSION

The present study demonstrated that antinociception induced by gabapentin combined to tramadol was superior to their monotherapy effect. These results are in agreement with multiple animal studies that used various experimental pain models in which dose-dependent antinociception has been demonstrated with gabapentin or tramadol in different murine pain models, such as tail flick, formalin hind paw, hot plate, acetone test, and von Frey test [17–25].

The exact mechanism of action of gabapentin at cellular level and after neuropathy is unknown, despite the fact that gabapentin is similar to GABA but it has no effect on GABA binding, uptake, or degradation. Molecular and transgenic studies strongly support α2δ-1 as the molecular target for the analgesic actions of gabapentin and to inhibit transmitter release [8]. Moreover, the precise mechanism of action of tramadol remains to be elucidated. It has been proposed that G protein-coupled receptor (GPCR) and ion channels are targets for tramadol. Besides, it has been reported that spinal and peripheral adenosine A(1) receptors contribute to antinociception by tramadol [25–29].

In neuropathic pain, insufficient pain relief occurs with the monotherapy currently used. Combination therapy in the pain treatment with synergistic effect, using two drugs with different mechanisms of action, is of great interest. In addition, a reduction in the adverse effects of drugs is added. Examples of this multimodal analgesia are the preclinical studies in a neuropathic pain model, the combination of morphine and cannabinoid [30], nortriptyline, and morphine in neuropathic

rats [31] , morphine with clonidine [32], morphine with tramadol [33]. It has also been reported that amitriptyline, duloxetine, sitagliptin, and pregabalin, and their combinations on STZ-induced diabetic neuropathy [34], proglumide with celecoxib in neuropathy [35], of proglumide with NSAIDs in neuropathy [33], proglumide with ibuprofen with hydrocodone in neuropathy [36]. Clinically, combinations of morphine and pregabalin [37], morphine with nortriptyline [38], tricyclic antidepressants, gabapentin, and pregabalin in the treatment of neuropathic pain [39] have been reported. Also, the effect of imipramine, pregabalin, and their combination in painful polyneuropathy [40], as well the action of tramadol/dexketoprofen [41], combination of dexketoprofen and tramadol [42], and the mixture of tramadol and diclofenac [43] are favorable.

To determine whether gabapentin and tramadol interact synergistically, an isobolographic analysis was necessary because it is the most rigorous method available to assess whether the interaction is additive, synergistic, or subadditive [44]. Coadministration of gabapentin with tramadol resulted in a nociceptive synergistic interaction in a dose-dependent manner. This synergistic effect could be explained according to a pharmacodynamic interaction based on the different mechanisms of action of each drug of the association. As gabapentin and tramadol affect different targets, all involved in modulating pain, coadministration could result in an enhancement of each other's activity. Pharmacokinetic and pharmacodynamic mechanisms mediated by either intercellular or intracellular mechanisms, depending on the activation of the receptors involved, could contribute to explain synergistic interactions between gabapentin and tramadol.

CONCLUSION

It is recognized that neuropathic pain is a common and refractory chronic pain; therefore, the findings of the present study suggest that synergistic combination of gabapentin and tramadol could be a new strategy for use in the treatment of pain induced by diabetic neuropathy.

CONFLICT OF INTEREST

The authors declare that they have no competing interest related to the work in this study.

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