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Antinociceptive Interaction of Tramadol with Gabapentin in Experimental Mononeuropathic Pain

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Abstract: Neuropathic pain is the result of injury to the nervous system, and different animal models have been established to meet the manifestations of neuropathy. The pharmacotherapy for neuropathic pain includes gabapentin and tramadol, but these are only partially effective when given alone. The aim of this study was to assess the antinociceptive interaction between both drugs using the isobolographic analysis and changes of the IL-1β concentration in a mouse model of neuropathic pain (partial sciatic nerve ligation or PSNL). The i.p. administration of gabapentin (5–100 mg/kg) or tramadol (12.5–100 mg/kg) displayed a dose-dependent antinociception in the hot plate assay of PSNL mice, and effects induced by gabapentin with tramadol were synergistic. Administration of gabapentin or tramadol reversed significantly the increase in the concentration of IL-1β induced by PSNL after either 7 or 14 days and their combination was significantly more potent in reversing the elevated concentration of IL-1β. The synergism obtained by the co-administration of gabapentin and tramadol is proposed to result from action on different mechanisms in pain pathways. Gabapentin or tramadol or their combination modulates the expression of pro-inflammatory cytokine, IL-1β, in a model of mice PSNL which could be due to an inhibition of glial function.

Neuropathic pain is the result of an injury or malfunction in the peripheral or central nervous system and is characterized by dysaesthesia (an unpleasant abnormal sensation), hyperalgesia (an increased response to painful stimuli) and allodynia (pain in response to a stimulus that does not normally provoke pain) [1]. Different animal models have been developed to model various types of nerve injury, such as spinal cord injury, excitotoxic models, sciatic nerve (neuroma model), sciatic nerve chronic constriction injury, sciatic nerve chronic constriction injury, spinal nerve ligation, polyethylene cuff, partial saphenous nerve injury in mouse, mouse model of trigeminal neuralgia, injection of TNF-α, multiple sclerosis, post-herpetic peripheral neuropathic pain model, HIV-associated sensory neuropathy, diabetic peripheral neuropathic pain model, vincristine-induced peripheral neuropathy model, paclitaxel (taxol)-induced peripheral neuropathy model and cisplatin-induced peripheral neuropathy [2]. The pharmacotherapy of neuropathic pain includes the following: duloxetine, pregabalin, gabapentin, enacarbil, capsaicin, tramadol, botulinum toxin A, lidocaine oxycodone, venlafaxine, amitriptyline, pentadol, valproate, morphine [3]. Amongst drugs that have been tested in neuropathic pain are tramadol and gabapentin. Tramadol, an atypically opioid, is used globally for the treatment of moderate to moderately severe pain, including neuropathic pain [4,5]. On the other hand, gabapentin was developed as an anticonvulsant agent but is helpful for the treatment of chronic neuropathic pain [6]. In addition, gabapentin is recommended as first-line therapy in clinical neuropathy [3]. Furthermore, either tramadol or gabapentin is used in the treatment of neuropathic pain [3,5,7].

Increasing evidence indicates that several inflammatory mediators, such as cytokine IL-1β, play a key role in the development and maintenance of pain [8,9]. There are references for some combinations used in clinical neuropathic pain, such as nortriptyline–morphine [10], imipramine and pregabalin [11], and considering the fact that tramadol and gabapentin, as monotherapy, exert antinociceptive activity in various types of neuropathic pain, the aims of this study were to determine the type of interaction between gabapentin and tramadol (i.e. whether additive or synergistic) and the associated changes of the spinal IL-1β concentration using a murine model of mononeuropathic pain: the partial sciatic nerve ligation (PSNL).

Material and Methods

Animals. In all experiments, CF-1 male mice of 35–40 days of age, weighing 29 ± 1.0 g, housed in a 12-hr light/dark cycle at 22 ± 1°C, with free access to food and water were used. The animals (204 mice) were procured from the Central Laboratory Animal Resource of the Institute and acclimatized to the laboratory environment for at least 2 hr before use. Experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals issued by the National Institute of Health, and experimental procedures were approved by the Institutional Animal Care and Use Committee at the Universidad de Chile, Santiago, Chile (Committee approval N° CBA 0410 FMUCH, 2013). 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 7 and 14 after PSNL. A least-square linear regression analysis of the log

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dose–response curves allows the calculation of the log that produced 50% antinociception (ED_{50}) for each drug, expressed as a maximum possible effect (MPE). 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 on the basis of 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 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 course of the experiment.

The PSNL developed by Malmberg and Basbaum [12] was used. In this assay, the mice were anaesthetized with 7% of chloral hydrate, the left thigh was shaved, and the sciatic nerve was exposed. Then, the dorsal one-third to one-half of the nerve was loosely ligated with a 7.0 silk suture, and the wound closed. In the control mice, the nerve was exposed without ligation.

Algesiometric assay. The hot plate test was performed with an automatic device (Ugo Basile, Italy) according to the method described by Miranda *et al.* [13]. The animals were free to move, and the assay temperature was 50°C ± 1°C. The animal behaviours considered as signs of pain included licking of the forelegs or jumping off the hot plate (latency in sec.). The cut-off time was fixed at 30 sec. to avoid skin damage. Several measurements were performed with a 3-min. interval: two at baseline (without drug administration) and two after i.p. administration of the test drug of either gabapentin or tramadol. Hot plate latencies were converted to a MPE % as follows: $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where T_0 and T_1 are the hot plate latencies for control and after treatment, respectively, and T_2 is the cut-off time. The latency period, in sec., for saline sham control group animals was 21.09 ± 0.79 (n = 12).

Isobolographic analysis. Isobolographic analysis was used to characterize interaction between gabapentin and nortriptyline in the hot plate test. This analysis has been described by Tallarida and adapted by Miranda *et al.* [14]. 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 gabapentin on the abscissa with the ED_{50} of tramadol on the ordinate, yielding the line of additivity. The experimental ED_{50} of the mixture was obtained with a 95% confidence level (95% CL) by a 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 the following: $ED_{50} = ED_{50}$ gabapentin$/(P1 + R \times P2)$, where $P1$ and $P2$ are the ratios 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 opioids is synergistic or superadditive. To certify the nature of the mixture of the drugs, the interaction index (I.I.) was also calculated with the formula I.I. = experimental ED_{50}/theoretical ED_{50}. If the I.I. value is close to 1, the interaction is additive; if the I.I. is <1, the interaction is synergistic; and if the resulting value is >1, the interaction is subadditive [14].

Determinations of IL-1β. IL-1β concentrations were determined by commercially available ELISA kits [14]. Spinal cord samples from mice were cut into small pieces in tissue lysis buffer containing 20 mM Tris, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 1 mM EDTA and 0.1% SDS (pH 7.5) with protease inhibitors cocktail (Sigma, ST.LOUIS, MO, USA). Tissue was then homogenized and centrifuged for 20 min. at 20820 g (rpm 13000; radius of rotor 110 mm). Ultracentrífuge, Sorvall model OTD-65B at 4°C. Supernatants were collected, and the amount of protein was quantified using protein microassay (Bio-Rad, Hercules, CA, USA). The protein concentrations in all samples were diluted to 5 mg/ml. IL-1β was determined by commercially available ELISA kits (eBioscience, San Diego, CA, USA) according to manufacturer's instructions (500 µg proteins) of each diluted sample that was used in the assay. The results were expressed as IL-β concentration (pg/mg protein).

Protocol. Dose–response curves for the antinociceptive effect of gabapentin or tramadol and determinations of spinal IL-1β were obtained using at least six to eight animals for each of the four or five doses administered (i.p.). Testing procedures were conducted on days 7 and 14 after PSNL. A least-squares linear regression analysis of the log dose–response curves allows the calculation of the log that produced 50% antinociception (ED_{50}) for each drug, expressed as a MPE. A dose–response curve was also obtained by the i.p. co-administration of fractions of their respective ED_{50} values: 1/2, 1/4, 1/8 and 1/16. An isobolographic analysis was used to determine the drug interactions. The method has been described in detail by Miranda *et al.* [14]. Supra-additivity, or synergistic effect, is defined as the effect of a drug combination that is higher and statistically different than the theoretically calculated effects of the drug combination with the same proportions in addition. The interaction index (I.I) was calculated as the experimental ED_{50}/the theoretical ED_{50}.

Drugs. The antinociception of the drugs, individually and in combination, was evaluated after 30 min. of administration of the drugs, time of peak efficacy of the drugs, determined previously [13,14]. The drugs were freshly dissolved in a saline solution in a constant volume of 10 ml/kg. Gabapentin and tramadol hydrochloride were purchased from Sigma Chemical Co., ST.LOUIS, MO, USA.

Statistical analysis. Results are presented as mean values ± S.E.M., or ED_{50} value with a 95% CL. Statistical analysis of the isobolograms, related to the difference between the theoretical and experimental values, was assessed by *t*-tests for independent means. All calculations were performed with the program Pharm Tools Pro (version 1.27; The McCary Group Inc., Allentown, PA, USA), based on Tallarida [15]. *p*-Values < 0.05 ($p < 0.05$) were considered significant.

Results

Effect of gabapentin and tramadol.

The doses of gabapentin and tramadol did not induce visuo-motor dysfunction. The latency period, in sec., in the hot plate assay, for the saline control group of animals was 21.09 ± 0.79 (n = 12); PSNL significantly decreased this value, on day 7, to 17.25 ± 0.85 (n = 12) and on day 14 to 15.23 ± 0.57 (n = 12). The latency period in the assay for sham-operated mice was 20.85 ± 0.45 (n = 12). These data are shown in fig. 1A. The i.p. administration of gabapentin (5–100 mg/kg) or tramadol (12.5–100 mg/kg) displayed a dose-dependent curve in the hot plate assay of PSNL mice with different efficacy, because the ED_{50} for gabapentin was 12.51 ± 0.71 (n = 12) and for tramadol 28.72 ± 0.20 (n = 12). The corresponding dose–response curves are displayed in fig. 1A,B. Based on the equi-effective doses, gabapentin was 2.29 times more potent than tramadol.

Isobolographic analysis of the interaction between gabapentin and tramadol.

In the hot plate test performed with control or sham mice, the nocifensive responses induced by gabapentin with tramadol combined in a 1:1 ratio based on their analgesic potency (ED_{50}) were synergistic, as can be seen in fig. 2A for control mice and fig. 2B for sham mice. The combination of gabapentin with tramadol at 1:1 ratio of their ED_{50} likewise generated isobolograms at mice PSNL of 7 and 14 days, as can be seen in fig. 2C for 7 days and 2D for 14 days. The data generated by the isobolograms corresponding to the ED_{50}'s theoretical and the experimental and the interaction index are shown in table 1.

Spinal cord levels of IL-1β.

The mice spinal cord levels of IL-1β at 7 and 14 days after PSNL procedure were measured to determine whether they are involved in the process after PSNL. Concentrations of IL-1β were significantly elevated at 7 and 14 days after PSNL (fig. 3A, table 2). The administration of gabapentin or tramadol reversed significantly the increase in concentration of IL-1β induced by PSNL either at 7 or 14 days, as can be seen in fig. 3B,C and table 2. The combination of gabapentin and tramadol was significantly more potent in reversing the elevated concentration of IL-1β induced by PSNL, indicating a synergistic effect of the combination, as shown in fig. 3A,B and table 2.

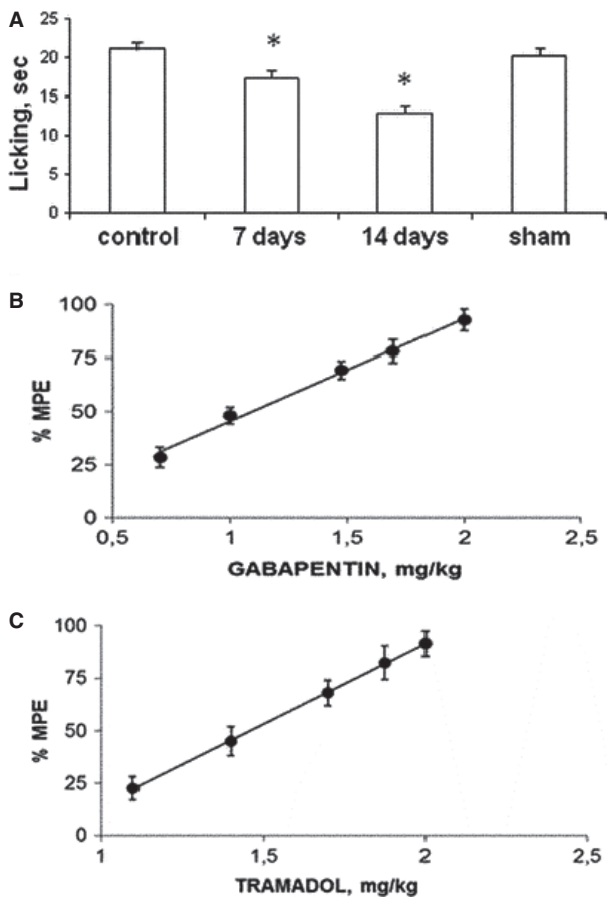

Fig. 1. (A) Licking time in sec., on latency period of hot plate in control, partial sciatic nerve ligation and sham mice. Data are mean ± S.E.M. *$p < 0.05$ *versus* control B and C. Dose–response curves for the antinociceptive activity induced by gabapentin and tramadol via i.p. in the hot plate assay. Each point is the mean ± S.E.M. of eight animals. % MPE = antinociception as a percentage of the maximum possible effect.

Discussion

In this study, a murine neuropathic model induced by the unilateral loose ligation sciatic nerve of mice was used. In this assay, gabapentin, tramadol and the combination of them were able to induce a dose-dependent antinociception, in which gabapentin was 2.29 times more potent than tramadol; results are concordant with previous reports [16–18].

The interaction between the mixture of gabapentin and tramadol induced a dose-dependent antinociceptive effect, and the experimental ED_{50} was shifted to the left with respect to the theoretical ED_{50} which is a clear demonstration that these drugs displayed a synergistic effect, accompanied by a significant lower interaction index than 1. The changes obtained in the experimental ED_{50} of the combination in the PSNL mice represent a significant degree of animal plasticity in the antinociceptive activity. In addition, a new finding is the dose-dependent antinociception of the mixture of gabapentin with tramadol.

The synergism obtained by the co-administration of gabapentin and tramadol is based on different pathways of antinociception induced by each drug of the combination. Thus, the mechanism of action of gabapentin is related to the activation of several receptors such as AMPA, GABA, NMDA, ACh-muscarinic and adenosine, and it has been proposed to be the result of impaired trafficking of α2δ1 subunit with a consequent diminished expression of functional calcium channels. Recent research also suggests that gabapentin acts by blocking new synapse formation [20]. Tramadol antinociception derives from relatively weak μ-opioid receptor (MOR) agonism, plus norepinephrine and serotonin reuptake inhibition, muscarinic and nicotinic receptor inhibition and modulation of the dopaminergic system [4].

On the other hand, it has been reported that IL-1β plays a key role in the development and maintenance of neuropathic pain by activating neurons directly and modulating nociception indirectly via the activation of non-neuronal nervous system cells (e.g. glial cells) and infiltration of immune cells [21–23].

Another interesting finding of the present study is the demonstration that gabapentin or tramadol or their combination modulates the expression of pro-inflammatory cytokine, IL-1β, in a model of mice PSNL. This finding suggests that the antinociceptive effect of gabapentin and tramadol may be partially due to inhibition of glial function which will be demonstrated by the diminution in the release of a glial-derived agent: IL-1β, as it has been proposed that an increase in inflammatory response may lead to inflammation, which in turn can stimulate microglia that are associated with an

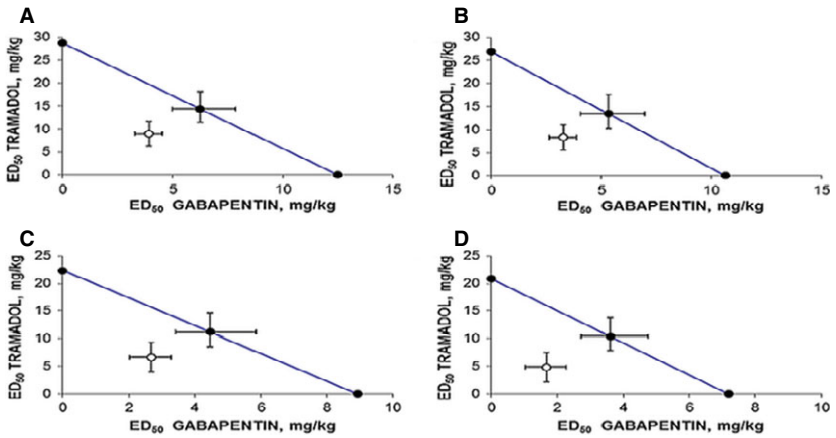

Fig. 2. Isobolographic representation of the antinociceptive activity of the combination of gabapentin with tramadol, via i.p., in the hot plate assay in control mice (A), sham mice (B), partial sciatic nerve ligation (PSNL) mice of 7 days (C) and PSNL mice of 14 days (D). Filled circle indicates the theoretical ED_{50} with 95% confidence limits; open circles are the experimental ED_{50} with 95% confidence limits.

Table 1.
Isobolographic parameters of the antinociception from gabapentin with tramadol administered i.p. to control, sham and partial sciatic nerve ligation (PSNL), in a mice model of the hot plate assay.

Mice	Theoretical $ED_{50} \pm$ S.E.M., mg/kg	Experimental $ED_{50} \pm$ S.E.M., mg/kg	Interaction index
Control	20.61 ± 0.37	12.88 ± 0.58	0.625
Sham	18.75 ± 0.40	11.58 ± 0.67	0.618
PSNL 7 days	15.65 ± 0.33	9.38 ± 0.42	0.599
PSNL 14 days	14.02 ± 0.31	6.54 ± 0.19	0.466

increase in IL-1β [24]. Furthermore, reduction in interleukin-1β contributed to the synergistic effects of gabapentin and tramadol on PSNL. This indicates that gabapentin and tramadol, drugs reported to be associated with the process of pain, also take part in the modulation of the IL-1β an important mediator of inflammation [21,22]. An additional advantage is that IL-1β could be a potential target in the management of neuropathic pain after injury, in agreement with a previous report that suggests that IL-1β contributes to neuropathic pain by acting locally at the site of peripheral nerve injury [23].

Conclusion

The present results indicate that both gabapentin and tramadol administered alone relieve peripheral nerve injury-induced neuropathic pain effectively. Furthermore, the co-administration of gabapentin and tramadol exerts a synergistic effect on neuropathic pain reduction by inhibiting pro-inflammatory factor IL-1β activity. Therefore, the present study supports a novel strategy for treating peripheral nerve injury-induced neuropathic pain.

Acknowledgement

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Conflict of interests

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

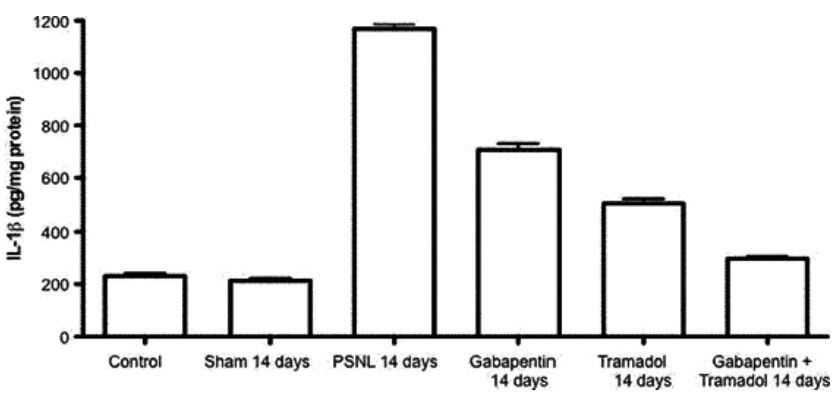

Fig. 3. Effect of gabapentin (ED_{50}) and tramadol (ED_{50}), via i.p. on the mice spinal cord levels of IL-1β, expressed as pg/ml protein, in control mice, sham mice and 7 and 14 days after partial sciatic nerve ligation mice in the hot plate assay. Each point is the mean ± S.E.M. of eight animals.

Table 2.
Levels of IL-1β as pg/mg protein in control, sham and partial sciatic nerve ligation (PSNL) mice in a model of the hot plate assay.

Mice	IL-1β 7 days	ED_{50} ± S.E.M. (pg/ml protein) 14 days
Control	208.51 ± 19.5	231–85 ± 39.8
Sham	221.58 ± 27.7	213.68 ± 25.7
PSNL	1033.13 ± 131.4*	1169.48 ± 49.6*
Gabapentin	902.21 ± 57.8*·**	707.26 ± 68.3*·**
Tramadol	636.03 ± 61.4*·**	507.76 ± 47.8*·**
Gabapentin + tramadol	251.10 ± 28.1*·**·***	296.60 ± 24.1*·**·***

All groups n = 6.
*$p < 0.05$ with respect to sham 7 and 14 days; **$p < 0.05$ with respect to PSNL at 7 days and 14 days; ***$p < 0.05$ with respect to gabapentin and tramadol alone.

Authors' contributions

All authors participated in planning research, performing experiments and data interpretation and collaborated in writing of the manuscript. Also, all authors read and approved the final manuscript.

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