Isobolographic Analysis in Mice of the Interaction of Gabapentin and Nortriptyline in Relieving Orofacial Pain

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Aims: *To evaluate the nature of the antinociceptive interaction of systemic administration of a combination of the anticonvulsant gabapentin with the antidepressant nortriptyline, by isobolographic analysis in the formalin orofacial pain test of mice.* ***Methods:*** *The study was carried out in 168 male CF-1 mice weighing 30 g, and the protocol was to test each drug (at dosages of 1, 3, 10, 30, and 100 mg/kg of gabapentin and 0.1, 1, 3, 10, and 30 mg/kg of nortriptyline; ip) alone and in combination. The isobolographic assay has two phases: phase 1 corresponds to the 5-minute period starting immediately after the formalin injection and reflects a tonic acute pain due to peripheral nociceptor sensitization; phase 2 is recorded as the 10-minute period starting 20 minutes after the formalin injection and reflects an inflammatory pain state. Results were analyzed by Student* t *test for independent means.* ***Results:*** *Gabapentin was 1.61 times more potent in phase 2 than in phase 1, and nortriptyline 1.37 times more potent in phase 2 than in phase 1. The combination of both drugs was synergic, with an index of interaction of 0.134 and 0.148 for phase 1 and phase 2, respectively. Differences in the pharmacological profiles of gabapentin and nortriptyline could underlie the synergism of the two drugs.* ***Conclusion:*** *The findings of this study are important, because they are concordant with some clinical studies and also raise the possibility of potential clinical advantages of combining gabapentin and nortriptyline in pain management, since the low doses of the components may potentially have a lower incidence of adverse reactions.* J OROFAC PAIN 2013;27:361–366; doi: 10.11607/jop.1167

Key words: gabapentin, isobolographic analysis, nortriptyline, orofacial pain, synergism

Pain is a complex interaction of different structures in the central and peripheral nervous system, and the management of pain often requires the use of several drugs. Pharmacological treatment of pain typically includes nonsteroidal anti-inflammatory drugs or opioids, but anticonvulsants and antidepressant agents have also been used.[1–3]

Gabapentin is an anticonvulsant drug that has been used for many years in the treatment of epilepsy.[4,5] Gabapentin has also been used to treat neuropathic pain,[6] bipolar depression,[7] anxiety disorders,[8] headache, and neck pain.[9] In animal models, it has been reported that gabapentin reduces nociception in thermal hyperalgesia,[10] spinal nerve ligation,[11] formalin,[12] tail-flick and paw pressure,[13] and cancer-induced bone pain models.[14]

Nortriptyline, an antidepressant drug, has been used as an analgesic in the treatment of chronic low-back pain and postherpetic neuralgia,[15,16] and chronic lumbar root pain.[17] It also has been evaluated as an analgesic in the mouse writhing test,[18] in the rat formalin test,[19]

Table 1 ED_{50} of Gabapentin and Nortriptyline in the Formalin Orofacial Pain Test of Mice

	ED_{50} ± SEM (mg/kg)	
	Phase 1	Phase 2
Gabapentin	5.15 ± 0.59 (n = 30)	3.19 ± 0.45* (n = 30)
Nortriptyline	0.81 ± 0.12 (n = 30)	0.59 ± 0.10* (n = 30)

n = number of animals; *$P < .05$ comparing both phases.

in four nociceptive tests that employ either thermal (hot plate and tail flick tests) or chemical (formalin and acetic acid tests) stimuli,[20] and after its infiltration into the rat tissues.[21]

Given that gabapentin and nortriptyline exert antinociceptive effects in these various experimental pain assays, it is important to evaluate and characterize any interaction between them by an isobolographic analysis[22] in an inflammatory pain assay such as the formalin orofacial pain test.[23,24]

The purpose of this study was to evaluate the nature of the antinociceptive interaction of systemic administration of a combination of gabapentin with nortriptyline by isobolographic analysis in the formalin orofacial pain test.

Materials and Methods

Animals

A total of 168 male CF-1 mice (30 g), housed on a 12-hour light-dark cycle at 22 ± 2°C with ad libitum access to food and water, were used. Experiments were performed according to current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental pain approved by the Animal Care and Use Committee of the University of Chile Medical School. Animals that were acclimatized to the laboratory for at least 2 hours before testing were used only once during the protocol and were sacrificed immediately after the test. The number of animals was kept to a minimum, compatible with consistent effects of the drug treatments. All assays were conducted by an experimented observer who was unaware of the drug treatment of each individual mouse. A study limitation was the use only of male mice; this was done to avoid the estrus cycle of females.

Dose-response curves for intraperitoneal (ip) administration of gabapentin (1, 3, 10, 30, and 100 mg/kg) and nortriptyline (0.1, 1, 3, 10, and 30 mg/kg) were obtained using at least six animals for each of at least five doses, 30 minutes after drug application. A least-square linear regression analysis of the log dose-response curve allowed the calculation of the doses that produced 50% (ED_{50}) antinociception when each drug was administered alone (Table 1).

ED_{50} was used in tests as the equieffective dose for isobolographic analysis because higher doses did not show increased effects without motor impairment.[25] Then, a similar dose-response curve was also obtained and analyzed after the co-administration of gabapentin and nortriptyline, in fixed ratio (1:1) combinations based on the mixture of ½, ¼, ⅛, and 1/16 of their respective ED_{50} values, 30 minutes after the co-administration of the mixture. The following doses expressed in mg/kg were used for the isobolographic study: in phase 1, gabapentin 5.15 ± 0.59 and nortriptyline 0.81 ± 0.12; in phase 2, gabapentin 3.19 ± 0.45 and nortriptyline 0.59 ± 0.10 (Table 1).

Formalin Orofacial Pain Test

The method described by Miranda et al[26] was used. Orofacial formalin-induced responses showed two distinct phases that were separated by a period of relative inactivity with an early short-lasting response (0 to 5 minutes, phase 1) and a continuous prolonged response (20 to 30 minutes, phase 2). To perform the test, mice were randomly assigned to different groups (six per group) and 20 µL of 2% formalin solution was injected into the right upper lip adjacent to the nose, with a 27-gauge needle attached to a 50-µL Hamilton syringe. The applied chemical stimulus (formalin) applied can be considered noxious, since it produces tissue injury, activates Aδ and C nociceptors as well as trigeminal and spinal nociceptive neurons, and produces a painful sensation in humans.[23] Each mouse was immediately returned to the observation chamber. The test shows two clear-cut phases: phase 1 corresponds to the first 5-minute period, starting immediately after the formalin injection, and represents a tonic acute pain due to peripheral nociceptor sensitization, while phase 2, which was recorded as the 10-minute period starting 20 minutes after the formalin injection, represents an inflammatory pain state.[24,25] The nociceptive score was determined for each phase by measuring the total number of seconds that the animals spent grooming the injected area with the ipsilateral fore or hind paw.[27] Drug or saline (n = 6) was administered to animals 30 minutes before formalin injection. Total grooming time in each period was converted to a percentage of maximum possible effect (MPE) as follows:

$$\%\ MPE = 100 - (\text{post–drug grooming time} / \text{post–control grooming time saline}) \times 100$$

The dose that produced 50% of MPE (ED_{50}) was calculated from linear regression analysis of a dose-response curve obtained by plotting log doses vs % MPE.

Isobolographic Analysis

An isobolographic analysis was used to characterize drug interactions. The method of isobolographic analysis has been described previously in detail.[27] The isobologram was built by connecting the ED_{50} of the gabapentin plotted on the abscissa with the ED_{50} of the nortriptyline plotted on the ordinate to obtain the additivity line. For each drug mixture, the ED_{50} and its associated 95% confidence interval were determined by a linear regression analysis of the log dose-response curve (six or eight animals at each dose of at least four doses) and compared by a *t* test to a theoretical additive ED_{50} obtained from the calculation:

$$ED_{50}add = ED_{50}\ gabapentin/(P1 + R \times P2)$$

where R is the potency ratio of the gabapentin alone to nortriptyline alone, and P1 is the proportion of gabapentin and P2 the proportion of nortriptyline in the total mixture. In this study, fixed-ratio proportions were selected by first combining the ED_{50} of each compound and then constructing a dose-response curve in which ED_{50} fractions (½, ¼, ⅛, and 1/16) of gabapentin and nortriptyline combinations were administered. In the equation above, ED_{50}add is the total dose and the variance of ED_{50}add was calculated from the fraction of the ED_{50} values (ie, 0.5) in the combination as:

$$Var\ ED_{50}add = (0.5)^2 Var\ ED_{50}\ gabapentin + (0.5)^2 Var\ ED_{50}\ nortriptyline$$

Confidence limits were calculated from these variances and resolved according to the ratio of the individual drugs in the combination. The ED_{50} for the drug combinations was obtained by linear regression analysis of the dose-response curves. A supra-additive or synergistic effect is defined as the effect of a drug combination that is higher and statistically different (ED_{50} significantly lower) than the theoretical ED_{50} calculated of a drug combination in the same proportion. If the ED_{50} values are not statistically different, the effect of the combination is additive, meaning that each constituent contributes with its own potency to the total effect.

Furthermore, the interaction index (II), or ratio of combination potency to additive potency, indicates the magnitude and nature of the interaction. The II was calculated as:

$$II = experimental\ ED_{50}/theoretical\ ED_{50}$$

If the value is close to 1, the interaction is additive. Values below 1 are an indication of the magnitude of supra-additive or synergistic interactions and values above 1 correspond to sub-additive or antagonistic interactions.[22]

Drugs

All drugs were freshly dissolved in saline solution in a constant volume of 10 mL/kg for ip administration. Gabapentin and nortriptyline hydrochloride were purchased from Sigma Chemical Co, St Louis, MO, USA. Doses were expressed based on the salts.

Statistical Analysis

Results are presented as ED_{50} values ± SEM or with 95% confidence limits (95% CL). The program used to perform statistical procedures was Pharm Tools Pro (version 1.27, The McCary Group Inc) based on Tallarida.[22] Results were analyzed by Student *t* test for independent means; *P* values less than .05 were considered statistically significant.

Results

The different doses of gabapentin and nortriptyline used in this study did not produce significant behavioral or motor dysfunctions in the animals tested.

Antinociception Induced by Gabapentin

Administration of 1, 3, 10, 30, and 100 mg/kg ip of gabapentin produced a dose-related antinociceptive activity with different potencies in phases 1 and 2 of the formalin test. In addition, the dose-response curves obtained were statistically parallel. Gabapentin was 1.61 times more potent in phase 2 than in phase 1 of the formalin test (Fig 1). Table 1 shows the corresponding ED_{50} values of gabapentin in this algesiometric test.

Antinociception Induced by Nortriptyline

Administration of 0.1, 1, 3, 10, and 30 mg/kg ip of nortriptyline produced a dose-related antinociceptive activity with different potencies in phases 1 and 2 of the formalin test. In addition, the dose-response curves obtained were statistically parallel. Nortriptyline was 1.37 times more potent in phase 2 than in phase 1 of the formalin test (see Fig 1). Table 1 shows the corresponding ED_{50} values of nortriptyline in this equieffective assay.

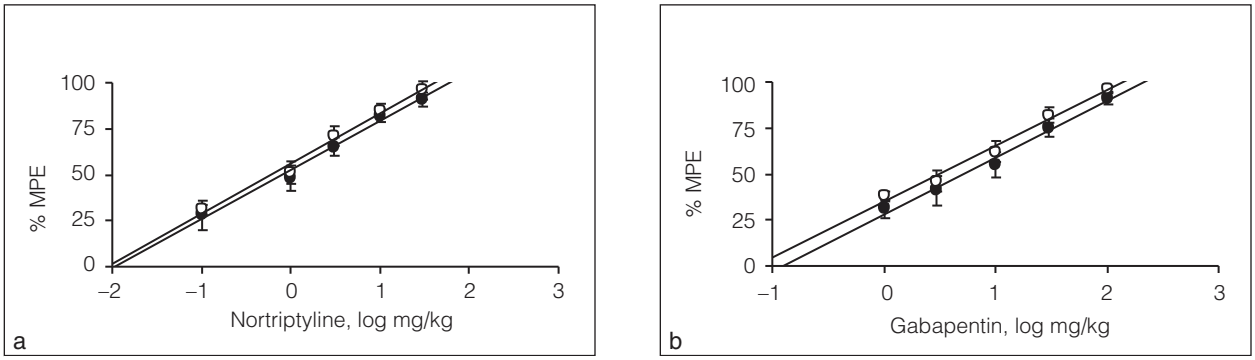

Fig 1 Dose-response curves for the antinociceptive activity in mice induced by *(a)* nortriptyline and *(b)* gabapentin (ip) in phase 1 (•) and phase 2 (○) of the formalin orofacial pain test in mice. Each point is the mean ± SEM of six animals. % MPE = antinociception represented as a percentage of maximum possible effect.

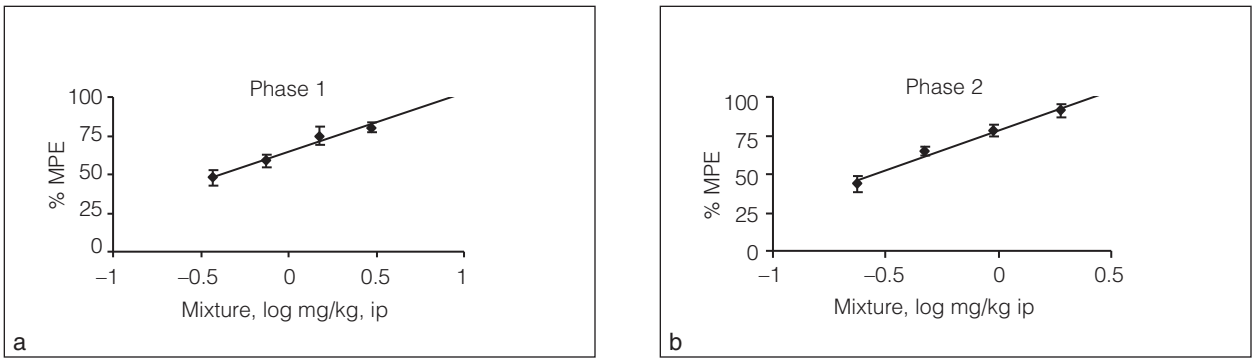

Fig 2 Dose-response curves for the antinociceptive activity in mice induced by the combination of gabapentin and nortriptyline (ip) in *(a)* phase 1 and *(b)* phase 2 of the formalin orofacial pain test in mice. Each point is the mean ± SEM of six animals. % MPE = antinociception represented as a percentage of maximum possible effect.

Table 2 ED_{50} ± SEM (mg/kg) and Interaction Index (II) for the Isobolographic Analysis of Gabapentin with Nortriptyline in the Formalin Orofacial Pain Test in Mice

	ED_{50} ± SEM		
Test	Theoretical	Experimental	II
Orofacial formalin phase 1, ip	2.98 ± 0.30	0.40 ± 0.08*	0.134
Orofacial formalin phase 2, ip	1.89 ± 0.05	0.28 ± 0.08*	0.148

*$P < .05$ comparing ED_{50} theoretical with ED_{50} experimental, Student *t* test.

Interaction Between Gabapentin and Nortriptyline

The interactions between gabapentin and nortriptyline administered on the basis of the fixed ratio (1:1) of their ED_{50} values alone were calculated by isobolographic analysis. Fixed ratios (1:3 and 3:1) of their ED_{50} were analyzed, but the data are not shown, since these mixtures were also synergic. The dose-response curves of experimental combination of gabapentin with nortriptyline are shown in Fig 2. Furthermore, the theoretical additive ED_{50} values and the experimental ED_{50} values for the fixed ratio (1:1) combination are shown in Table 2. Statistical analysis using the data from the isobolographic analysis indicated that synergistic interaction occurred between gabapentin and nortriptyline in the formalin test. These results are shown in Fig 3.

Furthermore, the II, indicating the magnitude and nature of the interaction when two drugs are combined, demonstrated the following rank of potencies for the combination of gabapentin and nortriptyline: 0.134 and 0.148 for phase 1 and phase 2 of the formalin test, respectively. All these results are shown in Table 2.

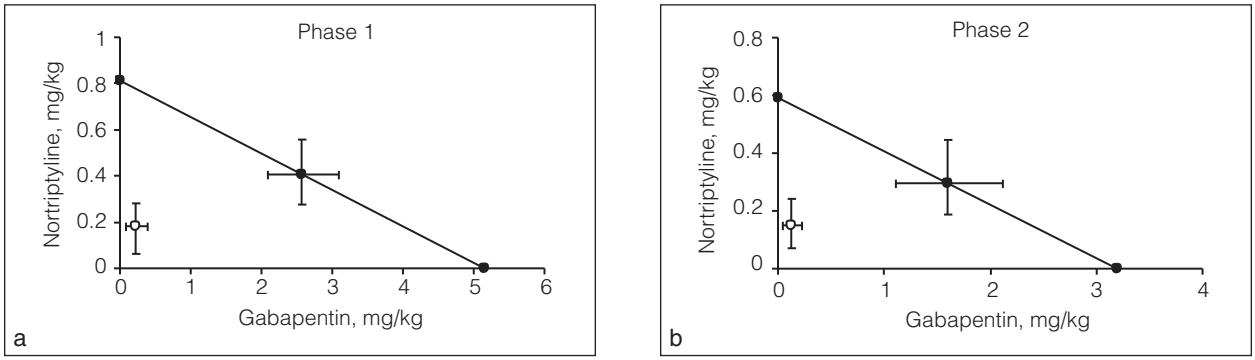

Fig 3 Isobolograms for the administration of the combination of gabapentin and nortriptyline (ip) in *(a)* phase 1 and *(b)* phase 2 of the formalin orofacial pain test in mice. Theoretical ED_{50} value with 95% CL (•). Experimental ED_{50} value with 95% CL (○).

Discussion

The findings of this study have demonstrated that gabapentin and nortriptyline possess a marked antinociceptive dose-dependent activity, in both phases of the formalin orofacial pain test. In addition, a different relative potency of gabapentin and nortriptyline was found in the algesiometric assay. The antinociceptive action of gabapentin and nortriptyline was reflected in parallel dose-response curves, for phase 1 and phase 2 of the formalin test. The parallelism obtained for each drug is indicative of activation of a common mechanism of action for phase 1 and for phase 2.[28]

Several studies with different algesiometric models of inflammatory and tissue injury are concordant with antinociceptive effects induced by gabapentin and nortriptyline in this model.[29] On the other hand, recent findings have demonstrated that pregabalin, an anticonvulsant closely related to gabapentin, can also reduce nociception in an acute inflammatory orofacial pain model as well as in an orofacial neuropathic pain model.[30,31] However, gabapentin has been reported to be ineffective in phase 1 of the formalin orofacial pain test.[29] Furthermore, it has been reported that the noradrenergic tricyclic nortriptyline did not elicit analgesia and inconsistently affected morphine analgesia.[32] Nonetheless, nortriptyline has been shown to be effective for the management of neuropathic pain, independent of its antidepressant property.[33] These findings are concordant with clinical implications, since some studies have shown that the combination of gabapentin and nortriptyline is useful for the relief of neuropathic pain.[34,35]

The synergism of the combination of gabapentin with nortriptyline found in the present study could possibly be related to the activation of different groups of receptors and their subtypes; nevertheless, the mechanism of the synergism is complex and may be influenced by other antinociceptive systems. Additionally, the differences in the pharmacological profiles of gabapentin and nortriptyline could help explain the synergism obtained in this study. Thus, for gabapentin, several hypotheses have been proposed for its mechanisms of action. They include selective activation or modulation of gamma-aminobutyric acid type B ($GABA_B$), N-methyl-D-aspartate (NMDA), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors; the adenosine triphosphate (ATP)-sensitive K^+ channels; and $\alpha_2\delta$ Ca^{+2} channels.[29] The antinociceptive mechanism of nortriptyline is due to its preferential action as a noradrenaline reuptake inhibitor and also a 5-hydroxytryptamine (5-HT2) receptor antagonist.[36] Also, the effect of nortriptyline implicates the endogenous opioid system, in particular delta- and kappa-opioid receptors.[37] However, Bohren et al[38] has recently demonstrated that μ-opioid receptors are not critical for nortriptyline action. Although the mechanisms underlying synergistic interactions are not well understood, the synergy shown in the present study could be the result of the simultaneous action of the two agents at two different sites. In addition, the combination of gabapentin and nortriptyline also could be used in other orofacial pain models with a neuropathic component, eg, infraorbital nerve ligation.

In conclusion, the findings of this study are important because they are concordant with some clinical studies and also raise the possibility of potential clinical advantages in combining gabapentin and nortriptyline in pain management, since the low doses of the components may be a potential index of lower incidence of adverse reactions. The combination of the drugs may prove useful clinically in inflammatory as well as neuropathic pain states.

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