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

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Abstract:
This study was aimed at determining the analgesic effect of gabapentin and tiagabine, two antiepileptic drugs that were administered alone and in combination at a fixed ratio of 1:1, in the acute thermal pain model (hot-plate test) in mice.
Linear regression analysis was used to evaluate the dose-response relationships between logarithms of antiepileptic drug doses and their resultant maximum possible antinociceptive effects in the mouse hot-plate test. From linear equations, we calculated doses that increased the antinociceptive effect by 50% (ED_{50} values) for gabapentin, tiagabine and their combination. The type of interaction between gabapentin and tiagabine was assessed using the isobolographic analysis.
Results indicated that both antiepileptic drugs produced the definite antinociceptive effect, and the experimentally derived ED_{50} values for gabapentin and tiagabine, when applied alone, were 504.4 mg/kg and 5.67 mg/kg, respectively. With isobolography, the experimentally derived $ED_{50\ mix}$ value for the fixed ratio combination of 1:1 was 139.31 mg/kg and significantly differed from the theoretically calculated $ED_{50\ add}$ value, which was 255.04 mg/kg ($p < 0.05$), indicating the synergistic interaction between gabapentin and tiagabine in the hot-plate test in mice.
In conclusion, the combination of tiagabine with gabapentin at a fixed ratio of 1:1 exerted a synergistic interaction in the mouse model of nociceptive pain. If the results from this study could be extrapolated to clinical settings, the combination of tiagabine with gabapentin might be beneficial for pain relief in humans.

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

Introduction

Accumulating evidence indicates that some antiepileptic drugs, especially gabapentin and tiagabine, also exert analgesic effects in numerous experimental pain models and in clinical settings in humans. It has been reported that tiagabine is effective in patients with painful sensory neuropathy [33], painful tonic spasm [39] and chronic pain [45]. Similarly, gabapentin is effective in suppressing and alleviating pain in postherpetic neuralgia [16, 35], painful diabetic neuropathy [1], migraine [10], trigeminal neuralgia [38] and neuropathic cancer pain [3]. In experimental studies on animals, gabapentin reduced dynorphin-induced

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

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

Materials and Methods

Animals and experimental conditions

Adult male Swiss mice (weighing 22–26 g) that were kept in colony cages with free access to food and tap water under standardized housing conditions (natural light-dark cycle, temperature of 23 ± 1°C, relative humidity of 55 ± 5%) were used. After seven days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups containing eight mice each. All tests were performed between eight a.m. and three p.m. Procedures involving animals and their care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this manuscript were approved by the First Local Ethics Committee at the Medical University of Lublin (Licenses no. 7/2007; 59/2007) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

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

Hot-plate test

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