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Short communication

Dose-response relationship analysis of vigabatrin doses and their antinociceptive effects in the hot-plate test in mice

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Abstract:
This study was aimed at determining the analgesic effects of vigabatrin (VGB, a newer antiepileptic drug) in the acute thermal pain model (hot-plate test) in mice.
Linear regression analysis was used to evaluate a dose-response relationship between logarithms of VGB doses and their resultant maximum possible antinociceptive effects (MPAE) in the hot-plate test in mice. From the linear equation of dose-response relationship, doses of VGB that increased the antinociceptive effect by 15%, 20% and 25% were calculated and amounted in this study to 144, 383 and 1016 mg/kg, respectively.
In conclusion, VGB in a dose-dependent manner produces the analgesic effects in mice in the hot-plate test. The method allowing for the calculation of doses of VGB increasing the antinociceptive effects by 15%, 20% and 25% can be readily adapted to preclinical studies because these values perfectly characterize the potency of antiepileptic drugs with respect to suppression of acute thermal pain in mice.

Key words:
acute thermal pain, dose-response relationship, hot-plate test, linear regression analysis, maximum possible antinociceptive effect, vigabatrin

Introduction

Accumulating evidence indicates that some antiepileptic drugs (AEDs) exert the analgesic effects in both, preclinical studies on animals [1, 5, 9–11, 17, 19, 25, 27, 28] and clinical settings in humans [2, 3, 7, 8, 12, 21–23, 26]. At present, several AEDs are able to bring relief from pain in patients with trigeminal neuralgia (carbamazepine, lamotrigine and oxcarbazepine), diabetic neuropathy (topiramate, lamotrigine and oxcarbazepine), post-herpetic neuralgia (topiramate, gabapentin, and pregabalin), phantom limb pain (gabapentin, and pregabalin), and other types of chronic pain [2, 3, 7]. Simultaneously, experimental evidence provided information on the efficacy of numerous AEDs (carbamazepine, lamotrigine, oxcarbazepine, gabapentin, pregabalin, topiramate, tiagabine, and vigabatrin [VGB]) in various models of acute and chronic pain, including the hot-plate test and formalin test as well as the constriction sciatic

neuropathy model in rodents [1, 5, 9–11, 17, 19, 25, 27, 28].

Although several reports indicate that AEDs exert analgesic effects, little is known about a dose-response relationship of newer (second-generation) AEDs with respect to the reduction of pain in the acute thermal pain model (the hot-plate test). Therefore, we sought to determine the influence of VGB (a second-generation AED) on the pain response in the hot-plate test in mice.

Previously, it has been found that VGB exerted analgesic effects by prolonging the latency to the first pain reaction in mice subjected to the formalin test [5], step-through passive avoidance task [19], and hot-plate test [17]. Moreover, the direct microinjection of VGB into the rostral agranular insular cortex (RAIC) in rats resulted in a clear and consistent analgesia, which was reversed by co-injection with the $GABA_A$ receptor antagonist, bicuculline [10]. Similarly, the prolonged and enhanced GABA neurotransmission within RAIC produced a long-term (up to 10 days) analgesic effect in freely moving rats [10].

The aim of this study was to determine the dose-response relationship for VGB in the acute thermal pain model (hot-plate test) in mice. Least-squares linear regression analysis was used to establish the dose-response relationship between VGB doses and their resultant antinociceptive effects, expressed as maximum possible antinociceptive effects (MPAE) in the hot-plate test in mice.

Materials and Methods

Animals and experimental conditions

Experiments were performed on adult male Swiss mice weighing 22–26 g. The animals were kept in colony cages with free access to food and tap water, under standardized housing conditions (12 h light-dark cycle, stable temperature of 22 ± 1°C for 24 h). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 mice each. All tests were performed between 9.00 a.m. and 3.00 p.m. Procedures involving animals and their care were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and Polish legislation on animal experimentation. 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 study were approved by the First Local Ethics Committee at the Medical University of Lublin (license no.: 489/2004/525/2004).

Drug

VGB (Sabril, Marion Merrell S.A., Puteaux, France) was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in saline and administered intraperitoneally (*ip*) in a volume of 5 ml/kg of body weight. VGB was administered 240 min before the hot-plate test. This pretreatment time was chosen based upon information about the biological activity of VGB from the literature and our previous studies [14, 15, 17–19].

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 that described by Eddy and Leimbach [6]. 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 56.0 ± 0.1°C. Mice were placed on the hot plate, and the time to either licking of the fore or hind-paws, shaking the hind-paws or jumping off was recorded by a stopwatch. First, animals were tested before drug treatment (baseline value) 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 4 and 8 s. Subsequently, the animals were administered VGB alone at increasing doses, and were subjected to the hot-plate test at the time of peak of anticonvulsant activity of VGB (240 min after treatment). A maximum cut-off time of 30 s was chosen to avoid tissue damage. Mice not responding within 30 s were removed and assigned a score of 30 s. The maximum possible antinociceptive effect (MPAE) was defined as the lack of a nociceptive response in mice during the exposure to the heat stimulus, and the percentage of MPAE was calculated according to the for-

mula presented by Schmauss and Yaksh [24], as follows: $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where T_0 and T_1 were the latencies obtained before and after drug administration, and T_2 was the cutoff time of 30 s.

Statistical analysis

Linear regression analysis of VGB doses (transformed into logarithms to the base 10) *vs*. their corresponding antinociceptive effects (expressed as MPAE) was performed according to Motulsky and Christopoulos [20]. The doses of VGB (as logarithms) were plotted on the X-axis of the Cartesian system of coordinates, whereas MPAE was plotted on the Y-axis. Subsequently, from the equation of linear dose-response relationship, the doses increasing antinociceptive effect by 15%, 20%, and 25% ($AEID_{15}$, $AEID_{20}$, and $AEID_{25}$) were calculated in this study. Least-squares linear regression analysis was performed using commercially available GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA, USA).

Results

VGB administered systemically (*ip*), 240 min before the hot-plate test, prolonged in a dose-dependent manner the latency to the first pain reaction in the acute thermal pain model in mice. The experimentally-derived MPAE values for VGB administered at doses ranging between 100–1200 mg/kg were between 13.92–26.76% (Tab. 1). Subsequently, the MPAE values for animals injected with increasing doses of VGB (expressed as logarithms to the base 10) were graphically plotted in rectangular coordinates of the Cartesian system of coordinates and examined with least-squares linear regression analysis. This method allowed for the determination of equation for dose-response relationship for VGB, as follows: $y = 11.80 x - 10.48$ ($r^2 = 0.9703$); where y – is the MPAE in %, x – is the logarithm of a drug dose, and r^2 – coefficient of determination (Fig. 1). From this equation, one can readily calculate the VGB doses increasing antinociceptive effect by 15%, 20%, and 25% ($AEID_{15}$, $AEID_{20}$, and $AEID_{25}$), which were estimated at 144 mg/kg, 383 mg/kg, and 1016 mg/kg in the hot-plate test in mice, respectively (based on logarithms of VGB dose of 2.156, 2.583, and 3.007, respectively) (Fig. 1).

Tab. 1. Antinociceptive effect of vigabatrin (VGB) in the hot-plate test in mice

VGB dose (mg/kg)	Log dose	MPAE (%)
100	2.000	13.92 ± 2.88
200	2.301	16.49 ± 2.56
400	2.602	19.08 ± 3.22
600	2.778	21.84 ± 2.93
800	2.903	23.09 ± 3.71
1000	3.000	25.61 ± 3.86
1200	3.079	26.76 ± 3.79

Values are presented as the means of maximum possible antinociceptive effect (MPAE) ± SE of 8 mice. VGB was administered *ip*, at 240 min before evaluation of the antinociceptive effect. The MPAE was defined as the lack of a nociceptive response in mice during the exposure to the heat stimulus (56.0 ± 0.1°C), and the percentage of MPAE was calculated according to the formula proposed by Schmauss and Yaksh [24]: $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where: T_0 and T_1 – latencies obtained before and after drug administration, and T_2 – cutoff time of 30 s

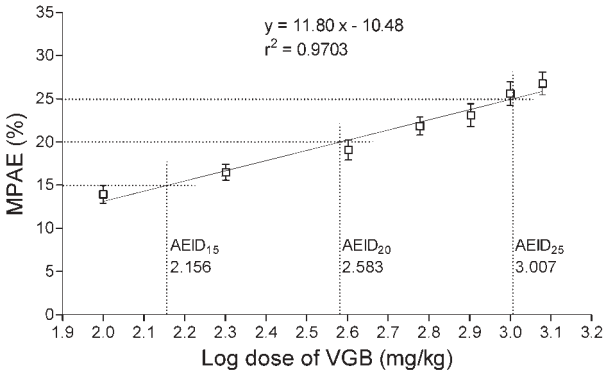

Fig. 1. Dose-response relationship between vigabatrin (VGB) doses and respective maximum possible antinociceptive effect (MPAE) values in the hot-plate test in mice. Doses of VGB were transformed in logarithms to the base 10 and the antinociceptive effects produced by VGB were transformed into maximum possible antinociceptive effect (MPAE ± SE as the error bars, n = 8). Log doses of VGB and respective MPAE values were plotted in the Cartesian system of coordinates and analyzed with linear regression to determine the dose-response relationship between doses of VGB and respective antinociceptive effects in the acute thermal pain model (hot-plate test) in mice. Linear regression analysis allowed for the determination of equation of dose-response relationship for VGB, as follows: $y = 11.80 x - 10.48$ ($r^2 = 0.9703$); where: y – MPAE in %, x – logarithm of the drug dose to the base 10, and r^2 – coefficient of determination [20]. From this equation one can calculate the $AEID_{15}$, $AEID_{20}$ and $AEID_{25}$ values (VGB doses that increase the antinociceptive effects by 15%, 20% and 25%) in the hot-plate test. In this study, the experimentally derived logarithms of $AEID_{15}$, $AEID_{20}$ and $AEID_{25}$ values were 2.156, 2.583, and 3.007, that corresponded to VGB doses of 144, 383 and 1016 mg/kg, respectively. For more detailed information see the legend to Table 1

Discussion

Results presented in this study indicate that VGB produced a dose-dependent antinociceptive effect in the acute thermal pain model (hot-plate test) in mice. Linear regression analysis of this dose-response relationship allowed for the determination of $AEID_{15}$, $AEID_{20}$ and $AEID_{25}$, i.e., doses of VGB that increased the antinociceptive effects by 15%, 20% and 25%, respectively, in the hot-plate test in mice. The procedure for the determination of MPEA for VGB doses and the calculation of $AEID_{15}$, $AEID_{20}$ and $AEID_{25}$ values can be readily adapted to perform similar experiments in preclinical studies for other AEDs in order to characterize the analgesic potential of conventional and newer AEDs. Thus, one can classify AEDs based on their antinociceptive properties by evaluating their potency in reduction of acute thermal pain. It should be stressed that the method described in this study allows to unequivocally determine doses of drugs that fulfill identical criteria, i.e., producing the increase in the antinociceptive effects by 15%, 20% and 25%. There is no doubt that the AEID values are very helpful during the assessment of analgesic potency of drugs because they can select the most effective drugs, offering strong antinociception at low doses.

Noteworthy, the doses of VGB were transformed into logarithms to the base 10, as recommended by Motulsky and Christopoulos [20], whereas, the antinociceptive effects produced by VGB were expressed as percentage of maximum possible antinociceptive effects (MPAE), according to the method described by Schmauss and Yaksh [24]. In such a case, linear regression analysis revealed a strong linear correlation between VGB doses and their antinociceptive effects. The calculation of MPAE takes into account the threshold for the first pain reaction in each mouse individually. Therefore, the procedure used to determine MPAE requires two experimental evaluations of times to the first pain reaction in mice challenged with the hot-plate test, i.e., before VGB administration and at the peak VGB activity, which was established as 240 min after the *ip* administration of VGB [14, 15]. Thus, the procedure of MPAE calculation eliminates any differences in individual reactions of mice subjected to the hot-plate test.

Previously, it has been documented that VGB dose-dependently prolonged the latency to the first pain reaction in the step-through passive avoidance task [19]. The drug administered at doses of 185 and 332 mg/kg exerted antinociception corresponding to 14.3% MPAE, and 17.2% MPAE in the hot-plate test in mice [17]. Similarly, VGB applied at doses ranging between 50–260 mg/kg produced the antinociceptive activity in the formalin test in mice by prolonging phases I and II to the pain reaction [5]. The doses of VGB required to inhibit 50% of the control response in the formalin test in mice were 148 and 91 mg/kg for the phase I and II, respectively [5]. Considering the results from the formalin test, step-through passive avoidance task and hot-plate test, one can ascertain that VGB possesses analgesic properties in preclinical studies on animals.

It is important to note that the antinociception exerted by VGB was observed at doses that did not produce the acute neurotoxic effects in animals, because as reported earlier VGB at doses up to 3.0 g/kg did not significantly affect motor coordination in mice in the chimney test [19]. Thus, the maximal tested dose of VGB in this study (1200 mg/kg) had no impact on the acute adverse effects in animals and, therefore, one can ascertain that the inhibition of the nociceptive reflex in animals was sensory. Moreover, it should be noted that doses of VGB used in this study (ranging between 100 and 1200 mg/kg) were ~2–28 times higher than those used clinically in epileptic patients, who usually receive VGB at doses up to 3.0 g a day [4]. On the other hand, there exists no direct extrapolation of results from preclinical studies on animals to clinical conditions, therefore, doses of AEDs used in preclinical studies in mice are higher than those used in epileptic patients [13]. For instance, the median effective dose of valproate (ED_{50}) against maximal electroshock-induced seizures in mice was 262.7 mg/kg [16], that should theoretically correspond to the dose of valproate of 18.4 g for a patient weighing 70 kg. However, in clinical practice valproate is administered at doses ranging between 500 mg – 2.0 g a day [4].

Noteworthy, the antinociceptive effects produced by VGB may be of great importance for patients with epilepsy, who similarly to healthy people suffer from incidental pain (headaches, migraine, odontalgias, algomenorrhoeas, etc.) [12, 21, 22]. Thus, VGB by offering the antiseizure protection and simultaneously producing strong analgesic effects, may eliminate the incidental intake of analgesic drugs that may interact with the applied AEDs, changing their pharmacological and therapeutic profiles. It seems that VGB, by ex-

erting the antiseizure and analgesic effects, could substantially reduce the incidental polytherapy associated with treatment of pain in patients with epilepsy and thus, VGB would be able to ameliorate the quality of living of epileptic patients. On the other hand, some AEDs are used in patients with chronic neuropathic pain resistant to conventional analgesic drugs, providing them with pain relief or alleviating the pain sensation [2, 22]. Experimental studies indicate that VGB belongs to the AEDs that produce antinociceptive effects in rodents. However, to confirm or exclude the hypothesis that VGB would be effective in patients with chronic neuropathic pain, more advanced studies and further clinical trials should be conducted.

Conclusions

VGB dose-dependently increased the antinociceptive effects in the hot-plate test. The procedure describing the calculation of doses increasing the antinociceptive effects by 15%, 20% and 25% ($AEID_{15}$, $AEID_{20}$ and $AEID_{25}$ values) can become a paradigm in preclinical studies allowing for characterization of the antinociceptive properties of AEDs and for comparison of the potency of AEDs in relieving pain in acute thermal pain model in mice.

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