ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXI, N 2, 167 SECTIO D 2006

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Effect of lamotrigine combined with tiagabine on locomotor activity in mice

The combined treatment with two or more antiepileptic drugs (AEDs), as polytherapy, is usually restricted for patients with refractory seizures inadequately treated with current frontline AEDs used in monotherapy (3). However, polytherapy in epilepsy is associated with a high risk of appearance of adverse effects evoked by AEDs alone or in combinations. Generally, AEDs are capable to produce various types of adverse effects, of which: impairment of motor coordination, loss of locomotor activity, memory deficits and/or impairment of neuromuscular tone are the most frequent, disturbing the patients' quality of lives (4). To avoid the adverse effects during the combined therapy, the AEDs are usually applied at low doses, which in turn, owing to interactions between AEDs, offer adequate seizure control (4). The newer AEDs, belonging to the second generation AEDs, are generally characterized by a low propensity to evoke adverse effects in the clinical settings (1).

Recently, the electronically monitored locomotor activity test has been accepted as an eligible and valuable model for evaluating a potential risk of neurotoxic adverse-effects produced by drugs with respect to the impairment of locomotor activity in animals (2, 10). Therefore, it can be considered as an experimental model useful for assessing neurotoxic effects produced by AEDs either alone or in combinations in terms of spontaneous and exploratory activities in animals.

The aim of this study was to evaluate the effects of tiagabine (TGB) and lamotrigine (LTG) – two newer AEDs, used in the clinical settings in patients with partial seizures with or without secondary generalization (1), on the exploratory and spontaneous locomotor activities in mice. The alterations in locomotor functioning of animals after a single administration of TGB alone or in combination with LTG would allow us to determine the adverse-effect profile of these AEDs in preclinical study.

MATERIAL AND METHODS

Animals. The experiments were carried out on male Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food and tap water. The experimental temperature was 21 ± 1°C and mice were on a natural light-dark cycle. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups (consisting of 8 animals). Each mouse was used only once. All procedures listed were approved by the Board for Supervising Ethics in Medical Experiments at the Medical University of Lublin.

Drugs. TGB (GABITRIL; Sanofi Winthrop, Gentilly, France) and LTG (LAMICTAL; Glaxo Wellcome, Kent UK) were suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA) and administered intraperitoneally (i.p.) in a volume of 0.1 ml/g body weigh, TGB – at 15 min and LTG – at 60 min prior to the test.

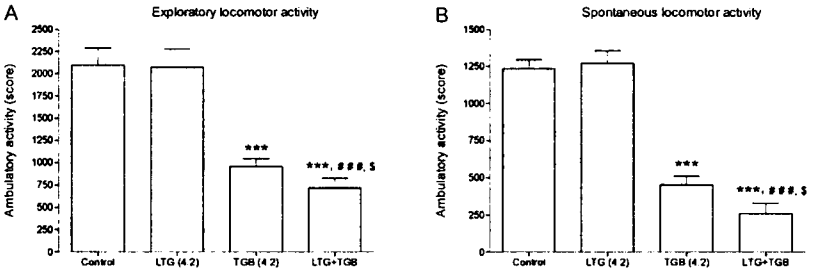
Locomotor activity monitoring. Locomotor activity in animals was assessed by using a Digiscan Animal Activity Monitor System (Omnitech Electronics, Columbus, OH, USA). Each monitor consisted of a 41 x 41 x 32 cm Plexiglas open field box with a grid of infrared beams mounted horizontally every 2.5 cm and vertically every 4.5 cm. Photocells located on the wall directly opposite each photo-beam were activated when the animal interrupted the beam. Each box was partitioned with acrylic cross into four (20 x 20 x 32 cm) quadrants. Mice were tested in the opposite quadrants of each unit (i.e. two mice per box). The photocells of each activity box were connected to the Digiscan analyzer that transmitted the number of beam breaks (activity data) to a computer. During operation, the pattern of beam interruptions was recorded and analyzed by IBM-PC compatible computer. Interruption of any beam was recorded as an activity score. All activity data were collected during two consecutive 15-min periods. Cumulative counts were compiled and downloaded every 15 min into the data collection software, which organized these counts into different motor indices. Two representative motor indices were analyzed as follows: 1) ambulatory activity – measuring the total number of horizontal photo-beam interruptions within the sample period, and 2) rearing activity – measuring the total number of vertical photo-beam interruptions within the sample period.

Experimental design. In this experiment, the mice were not habituated to the test apparatus; therefore, the test procedure consisted of two independent, consecutive measures. After injection of vehicle or the respective TGB or LTG doses, the mice were placed in the centre of individual cages and recording of their locomotor activities started throughout two consecutive 15-min intervals. The first measure of animals' activity is the rate of exploration and habituation to a novel environment (exploratory activity). Thus, during prolonged exposure to a new environment, animals typically spend progressively less time in movements and exploration. So, the second measure is considered as the rate of spontaneous activity of mice.

Statistics. Statistical evaluation of data was performed with one-way analysis of variance (ANOVA) followed by the *post-hoc* Bonferroni's test for multiple comparisons.

RESULTS

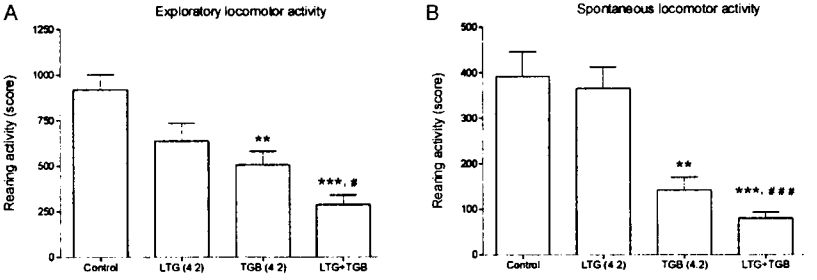
Exploratory locomotor activity testing. TGB administered i.p., at a dose of 4.2 mg/kg markedly reduced ambulatory activity score in mice from 2096 ± 193 to 956 ± 90 ($p<0.001$; Fig. 1A). Likewise, the combination of LTG (4.2 mg/kg) with TGB (4.2 mg/kg) produced a significant decrease in ambulatory activity scores from 2096 ± 193 to 719 ± 69 ($p<0.001$; Fig. 1A). In contrast, LTG (4.2 mg/kg) administered alone did not affect ambulatory activity score in animals tested (Fig. 1A). Moreover, statistical analysis of data with the *post-hoc* Bonferroni's test revealed that TGB (4.2 mg/kg) injected singly or in combination with LTG (4.2 mg/kg) significantly decreased rearing activity scores in mice from 920 ± 81 to 507 ± 54 ($p<0.01$), and 288 ± 32 ($p<0.001$), respectively (Fig. 2A). In contrast, LTG (4.2 mg/kg) administered alone had no impact on rearing activity scores in mice subjected to the electronically monitored locomotor activities (Fig. 2A).



Columns represent ambulatory (horizontal) locomotor activities scores of the animals tested (as means ± SEM, as the error bars) evaluated during two consecutive periods of time, as follows: (0–15 min) – reflecting exploratory locomotor activity (Fig. A), and (15–30 min) – corresponding to spontaneous locomotor activity (Fig. B). Statistical analysis of data was performed with one-way ANOVA followed by the *post-hoc* Bonferroni's test.
***$p<0.001$ vs. control (vehicle-treated animals); ###$p<0.001$ vs. LTG-treated animals; and $^{\$}p<0.05$ vs. TGB-treated animals

Fig. 1A–B. Effects of lamotrigine (LTG), tiagabine (TGB), and their combination (LTG+TGB) on the exploratory and spontaneous ambulatory (horizontal) activities in mice

Spontaneous locomotor activity testing. TGB at 4.2 mg/kg significantly reduced ambulatory activity score in mice from 1233 ± 113 to 452 ± 56 ($p<0.001$; Fig. 2A). Similarly, the combination of LTG (4.2 mg/kg) with TGB (4.2 mg/kg) produced a significant reduction in ambulatory activity scores from 1233 ± 113 to 260 ± 36 ($p<0.001$; Fig. 2A). In contrast, LTG (4.2 mg/kg) administered alone did not affect ambulatory activity score in animals tested (Fig. 2A). Furthermore, it was found that TGB (4.2 mg/kg) injected singly or in combination with LTG (4.2 mg/kg) significantly decreased rearing activity scores in mice from 392 ± 41 to 142 ± 28 ($p<0.01$), and 79 ± 13 ($p<0.001$), respectively (Fig. 2B). Only, LTG (4.2 mg/kg) administered separately did not significantly alter rearing activity score in mice (Fig. 2B).



Columns represent rearing (vertical) locomotor activities scores of the animals tested (as means ± SEM, as the error bars) evaluated during exploratory (Fig. A), and spontaneous locomotor activities (Fig. B). Statistical analysis of data was performed with one-way ANOVA followed by the *post-hoc* Bonferroni's test **$p<0.01$ vs. control (vehicle-treated animals); ***$p<0.001$ vs. control; #$p<0.05$ vs. LTG-treated animals; and ###$p<0.001$ vs. LTG-treated animals

Fig. 2A–B. Effects of lamotrigine (LTG), tiagabine (TGB), and their combination (LTG+TGB) on the exploratory and spontaneous rearing (vertical) activities in mice

DISCUSSION

Results presented in this study are consistent with our previous findings reporting that LTG administered alone had no effect on both ambulatory and rearing activities in animals during the exploratory and spontaneous locomotor activity testing (5). In case of TGB, the drug administered alone and combined with LTG produced a considerable reduction in locomotor activities in mice. It is highly likely that TGB might reduce the activity in epileptic patients receiving TGB, since a high positive correlation exists between the adverse-effect profiles of AEDs evaluated in rodents and humans (9).

Noteworthy, in this study LTG was administered at a dose corresponding to its median effective dose (ED_{50}) – protecting 50% of animals tested against maximal electroshock-induced seizures in mice (7). In case of TGB, the drug at a dose of 4.2 mg/kg elevated the threshold for electroconvulsions by ~20% (6).

Considering molecular mechanisms of action of both AEDs, one can ascertain that the selective activation of GABA-ergic neurotransmitter system in the brain through the drug blocking re-uptake of GABA from synaptic clefts (TGB, 8) produced more pronounced reduction in locomotor activity of animals than the AED inhibiting Na^+ and Ca^{2+} currents in neurons (LTG, 8).

It should be highlighted that the electronically monitored locomotor activity test in rodents seems to be a good screening allowing preselection of drugs affecting central nervous system and changing locomotor functioning in experimental animals.

CONCLUSIONS

1. The utmost caution is advised when administering TGB to epileptic patients since the drug reduced both exploratory and spontaneous locomotor activities in animals.

2. Lack of any significant changes in locomotor activity of animals injected with LTG, during the exploratory and spontaneous activities testing, is of some clinical importance, worthy of further consideration.

Acknowledgement. This study was supported by a grant (KBN 6P05D 098 21) from the State Committee for Scientific Research, Warszawa, Poland.

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SUMMARY

The objective of this study was to determine the effects of tiagabine, administered alone or in combination with lamotrigine, on the exploratory and spontaneous locomotor activities in mice. In the electronically monitored locomotor activity test, the ambulatory (horizontal) and rearing (vertical) activities were evaluated within two 15-min periods. Results indicate that tiagabine (4.2 mg/kg) administered separately or combined with lamotrigine (4.2 mg/kg) considerably reduced all the investigated parameters of the exploratory and spontaneous locomotor activities in mice. In contrast, lamotrigine (4.2 mg/kg) administered alone had no impact on the ambulatory and rearing activities in animals with respect to the exploratory and spontaneous locomotor activities. In conclusion, since tiagabine reduced locomotor activities in experimental animals, the utmost caution is advised when combining the drug with lamotrigine in clinical practice.

Wpływ lamotryginy w kombinacji z tiagabiną na aktywność ruchową myszy

Celem pracy było wyznaczenie wpływu tiagabiny, podawanej osobno lub w kombinacji z lamotryginą, na poznawczą i spontaniczną aktywność ruchową u myszy. W teście elektronicznie monitorowanej aktywności ruchowej oceniano ruchliwość poziomą (horyzontalną) i pionową (wertykalną) zwierząt w ciągu 2 okresów 15-min. Wyniki wykazują, że tiagabina (4,2 mg/kg) podawana osobno lub w kombinacji z lamotryginą (4,2 mg/kg) znacząco zmniejszała wszystkie oceniane parametry aktywności poznawczej i spontanicznej u myszy. Przeciwnie, lamotrygina (4,2 mg/kg) podawana osobno nie miała żadnego wpływu na ruchliwość poziomą i pionową zwierząt w odniesieniu do aktywności poznawczej i spontanicznej. Wnioskując, skoro tiagabina zmniejszała aktywność motoryczną u zwierząt doświadczalnych, zaleca się znaczną ostrożność podczas łącznego podawania leku z lamotryginą w warunkach klinicznych.