ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 2, 140 SECTIO D 2004

Department of Pathophysiology, Skubiszewski Medical of Lublin
Isotope Laboratory, Institute of Agricultural Medicine in Lublin
Department of Pharmacology and Toxicology, Skubiszewski Medical University of Lublin

JAROGNIEW J. ŁUSZCZKI, MARTA M. ANDRES, MARIUSZ J. ŚWIĄDER

Effect of lamotrigine combined with felbamate on the horizontal (ambulatory) activity in mice

Although the neurological bases of animal and human behaviours are not yet fully understood, spontaneously emitted behaviours, like motor activity patterns, exploration and stereotypy have been used extensively in pharmacological studies (10). Quite recently, an electronically monitored locomotor activity test has been accepted as an eligible and sophisticated model for evaluating a potential risk of neurotoxic effects produced by drugs affecting central nervous system (CNS) in terms of the impairment of locomotor activity after their administration (6, 12). In animals, this test is able to precisely denote a side-effect profile of AEDs administered alone or in combinations.

One of the novel AEDs, devoid of some deleterious side-effects (at therapeutically relevant doses), seems to be lamotrigine (LTG), clinically used in patients with generalized tonic-clonic convulsions, and partial seizures with or without secondary generalization (1). Lately, the drug has significantly reduced seizure frequency and convulsive attacks in children with Lennox-Gastaut syndrome (1).

In the early 1990s, felbamate (FBM) has been particularly beneficial in children suffering from Lennox-Gastaut syndrome, being the first drug shown to be effective at treating this clinical condition (2). However, the occurrence of rare cases of aplastic anemia and acute liver failure associated with the use of FBM drastically reduced its clinical application. To date, the use of FBM is largely restricted to patients with Lennox-Gastaut syndrome (as third-line treatment) for whom the benefits of treatment prevail over the risks (9, 11).

Most recently, there has appeared a tendency to examine the effects produced by FBM combined with LTG (in preclinical study) in order to create the rationale for polytherapy for the patients with intractable seizures (4). It is understandable that for this critical situation, each treatment regimen offering antiseizure effects is worth recommendation to further clinical practice. Therefore, some emergent questions have been arisen whether or not: 1) FBM combined with LTG is able to improve and/or provide adequate seizure control in patients with Lennox-Gastaut syndrome, 2) the combination therapy is devoid of some life-threatening side-effects, 3) the drugs in combination cause the impairment of patients' normal functioning, or disturb cognitive and locomotor functions.

In the present study, the effect of FBM alone or combined with LTG on the locomotor activity in mice was assessed. To date, there have been no studies on the effects of AEDs on the exploratory and spontaneous behaviors of experimental animals. Therefore, this experiment was designed to investigate the behavioral effects of the combination of FBM with LTG in terms of the exploratory and spontaneous locomotor activities in mice. The alternations in locomotor functioning of animals after acute exposure to FBM alone or combined with LTG were investigated for adequately assessing the adverse-effect profile of these AEDs in combinations, in pre-clinical study.

MATERIAL AND METHODS

Animals. The experiments were performed on Swiss male mice, purchased from a licensed breeder. The animals were kept under standardized laboratory conditions with free access to food (chow pellets) and tap water and a natural light-dark cycle for 7 days prior to the experiments. The experimental groups consisted of 8–10 animals, weighing 20–26 g. All experiments were carried out between 9.00 a.m. and 3.00 p.m. The animals were tested in a strict accordance with the current European Community and Polish legislation on animal experimentation. The experimental procedures listed were approved by the Local Ethics Committee at the Medical University in Lublin (License No. 191/2001/207/01).

Drugs. In this study, LTG (LAMICTAL, Glaxo Wellcome, Kent, UK) and FBM (*TALOXA*, Schering-Plough, Levallois Perret, France) were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA), and injected intraperitoneally (i.p.) in a volume of 10 ml/kg; LTG and FBM – 45 min prior to animals' placement into the activity monitor system. The control animals were injected with a respective amount of vehicle – 45 min before the testing. Drug doses, route of i.p. administration and pre-treatment time before testing were based upon information about their biological activity from our previous study (7).

Locomotor activity monitoring system. Locomotor activity was assessed with 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 placed and tested in the opposite quadrant 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 electronically recorded and analyzed by IBM-PC compatible computer. The activity monitoring system checked for interruptions of each infrared beam at a frequency of 100 Hz. Interruption of any beam was recorded as an activity score. Simultaneous interruptions of two or more consecutive beams separated by at least 1 s were recorded as a movements 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. In the present study, horizontal activity (HA) – measuring the total number of beam interruptions that occurred in the horizontal sensors during a given sample period was analyzed.

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 AED doses, the mice were placed in the centre of individual cages and recording of their locomotor activity started throughout two consecutive 15 min-intervals. In order to diminish the response of the animals to the mild stress produced by handling and injection, the mice were placed in the respective monitor quadrants, at least 45 min (not immediately) after the i.p. injection of AEDs or vehicle. The first measure of animals' activity is considered as a rate of habituation to a novel environment. 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 a rate of spontaneous activity of mice. The test procedure was conducted automatically without the presence of the experimenter in the test room.

Statistics. Baseline values were means for vehicle injection (1% solution of Tween 80) for each examined parameter. Statistical analysis was performed with the analysis of variance (ANOVA). *Post-hoc* test was conducted to determine which drug effects differed significantly from control values. All differences tested *post-hoc* were considered significant at $p < 0.001$ following Bonferroni's correction for multiple tests.

RESULTS

LTG injected separately at the dose of 2.3 mg/kg did not affect horizontal activity of animals in terms of the exploratory and spontaneous locomotor activities. Moreover, LTG at the dose of 2.3 mg/kg combined with FBM (25.7 mg/kg) drastically reduced HA scores by 29%, evaluated during the first ($F_{(3, 28)} = 16.543$; $p < 0.001$), and by 52% in the second ($F_{(3, 28)} = 9.256$; $p < 0.001$) period of time measured, respectively (Table 1, Fig. 1). Similar effects were observed for FBM (25.7 mg/kg) administered alone, which considerably reduced HA scores by 29% and 32%, either in the first ($F_{(3, 28)} = 16.543$; $p < 0.001$) or the second 15-min interval ($F_{(3, 28)} = 9.256$; $p < 0.001$) of locomotor activity (Table 1, Fig. 1).

Table 1. Influence of felbamate administered alone or combined with lamotrigine on the horizontal (ambulatory) activity scores of animals tested

	Treatment (mg/kg)	N	HA scores	ANOVA test
ELA				
	Control	8	2672.0 ± 84.8	
	LTG (2.3)	8	2598.3 ± 93.0	$F_{(3, 28)} = 16.543$; $p < 0.001$
	FBM (25.7)	8	1895.6 ± 89.9 ***	
	LTG (2.3) + FBM (25.7)	8	1903.3 ± 90.2 ***	
SLA				
	Control	8	1481.7 ± 128.8	
	LTG (2.3)	8	1317.0 ± 118.8	$F_{(3, 28)} = 9.256$; $p < 0.001$
	FBM (25.7)	8	918.7 ± 97.1 ***	
	LTG (2.3) + FBM (25.7)	8	705.8 ± 90.2 ***	

Table data are presented as means of horizontal activity scores ± S.E.M. of 8 determinations. Statistical analysis of data was performed by the use of ANOVA test followed by *post-hoc* Bonferroni multiple comparisons test. The p value is < 0.001 (considered extremely significant), hence, variation among column means is significantly greater than expected by chance. ELA – exploratory locomotor activity; SLA – spontaneous locomotor activity; N – number of animals tested; HA – horizontal activity; F – Fischer's test for 3 (between columns) and 28 (within columns) degrees of freedom, respectively; LTG – lamotrigine; FBM – felbamate. ***$p < 0.001$ vs the respective control value

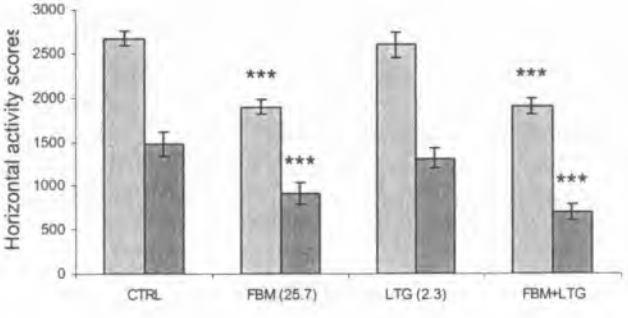

Fig. 1. Influence of LTG alone or combined with FBM upon the horizontal activity of animals in the locomotor activity test

The light-grey columns represent on the graph the means of horizontal activity scores of the animals tested (with S.E.M. as the error bars) evaluated during the first period of time (0–15 min) – habituation to a novel

environment. The dark-grey columns represent the means of horizontal activity scores of the animals tested (with S.E.M. as the error bars) evaluated during the second period of time (15–30 min). Statistical analysis performed with the use of the analysis of variance (ANOVA) followed by *post-hoc* Bonferroni multiple comparisons test, revealed significant reduction of horizontal activity scores as compared to the control (CTRL) values at ***$p < 0.001$.

DISCUSSION

Results presented herein indicate clearly that FBM injected alone or combined with LTG drastically reduced both exploratory and spontaneous activities of the animals tested in the electronically monitored locomotor activity test. It is noteworthy that FBM at the dose of 25.7 mg/kg combined with LTG (2.3 mg/kg) considerably reduced HA of the mice. The doses of applied AEDs were based upon the results from our previous study, concerning the isobolographic analysis of interactions in the maximal electroshock-induced seizure test in mice, and were given to animals at the fixed-ratio combination of 1:1 (7). This is why the drug doses were injected at the fixed proportion. Moreover, it was reported that FBM (25.7 mg/kg) significantly reduced the exploratory and spontaneous locomotor activities of the mice. It has to be stressed that the above-mentioned combination has exerted a pure additive interaction against maximal electroshock-induced seizures in mice (8), providing the possibility of its clinical utility in patients with drug-resistant epilepsy. The observed effects produced by the combination of LTG (2.3 mg/kg) with FBM (25.7 mg/kg) possessed a pharmacodynamic characteristics, since FBM did not affect the free plasma and brain concentrations of LTG (results not shown). Similarly, in clinical practice, it was observed that FBM had no significant impact on LTG's concentration (5) and *vice versa* – LTG on FBM (3). So, whatever pharmacokinetic events, which might disturb the observed interaction, are not probable.

Additionally, a significant decrease of exploratory and spontaneous locomotor activities was observed at $p < 0.001$ in both 15-min intervals of monitoring for HA. The combination of FBM with LTG drastically reduced all examined locomotor activity parameters to the same extent than FBM injected alone at $p < 0.001$. Hence, one can ascertain that FBM may *per se* impair the exploratory and spontaneous locomotor activities. In contrast, LTG injected separately at the dose of 2.3 mg/kg affected no parameters of locomotor activity of the animals examined in our study.

On the other hand, the observed substantial impairment of locomotor activity (after FBM administration) may be explained by the fact that this AED inhibits excitatory neurotransmitter system within the brain, and through this, may decrease the exploratory and spontaneous behaviours of the animals tested. Thus, the observed reduction in ambulatory activity may result from the inhibition of central nervous system (CNS)-related activity of the animals receiving AEDs.

Locomotor activity measure seems to be the most optimal method for determining the exploratory and spontaneous activities of animals, after being treated with AEDs, in order to elaborate some algorithms for the rational and the safest treatment for patients with intractable epilepsy, who need 2 or more AEDs in combinations for suppressing the epileptic attacks. Moreover, clinicians should pay close attention to the fact on how combining AEDs in polytherapy, in order to provide absolute safety during the therapy of drug-resistant epilepsy, considering AEDs' individual antiseizure effects and side-effect profile offered by AED-combinations.

CONCLUSIONS

1. FBM administered alone or combined with LTG produces some adverse effects in terms of hypoactivity. Its clinical application should be restricted for the patients, whose seizures are refractory to all available AEDs, or as the "*ultima ratio*" treatment

regimen in some catastrophic and devastating epileptic attacks (Lennox-Gastaut syndrome).

2. LTG injected alone seems to be devoid of any adverse locomotor effects, worth further clinical consideration for the patients with intractable seizures.

3. The locomotor activity test should be included as a very good paradigm for testing AEDs' propensity to evoke some adverse (neurotoxic) effects as regards the detection of any deficits in exploratory and spontaneous activities of animals receiving some AEDs alone or in combinations.

This study was supported by a grant (6P05F 026 20) from the State Committee for Scientific Research, Warsaw, Poland.

REFERENCES

1. Brodie M. J., Schachter S. C.: Epilepsy. Health Press, Oxford, UK, 2001.
2. Brown W. M., Aiken S. P.: Felbamate: clinical and molecular aspects of a unique antiepileptic drug. Crit. Rev. Neurobiol., 12, 205, 1998.
3. Colucci R. et al.: Effect of felbamate on the pharmacokinetics of lamotrigine. J. Clin. Pharmacol., 36, 634, 1996.
4. Cuadrado A. et al.: Synergistic interaction between felbamate and lamotrigine against seizures induced by 4-aminopyridine and pentylenetetrazole in mice. Eur. J. Pharmacol., 465, 43, 2003.
5. Gidal B. E. et al.: Lamotrigine pharmacokinetics in patients receiving felbamate. Epilepsy Res., 27, 1, 1997.
6. Kolasiewicz W., Maj J.: Locomotor hypoactivity and motor disturbances – behavioral effects induced by intracerebellar microinjections of dopaminergic DA – D2/D3 receptor agonists. Pol. J. Pharmacol., 53, 509, 2001.
7. Łuszczki J. et al.: Interactions of lamotrigine with topiramate and first-generation antiepileptic drugs in the maximal electroshock test in mice: an isobolographic analysis. Epilepsia, 44, 1003, 2003.
8. Łuszczki J. et al.: Interactions of lamotrigine with some antiepileptic drugs: an isobolographic analysis. Pol. J. Pharmacol., 54, 82, 2002.
9. Morton L. D., Pellock J. M.: Overview of childhood epilepsy and epileptic syndromes and advances in therapy. Curr. Pharm. Des., 6, 879, 2000.
10. Robbins T. W., Everitt B. J.: Functional studies of the central catecholamines. Int. Rev. Neurobiol., 23, 303, 1982.
11. Schmidt D., Bourgeois B. F.: A risk-benefit assessment of therapies for Lennox-Gastaut syndrome. Drug Saf., 22, 467, 2000.
12. Stanford J. A. et al.: Acute locomotor effects of fluoxetine, sertraline, and nomifensine in young versus aged Fischer 344 rats. Pharmacol. Biochem. Behav., 71, 333, 2002.

SUMMARY

Exploratory and spontaneous locomotor activities, after administration of some novel antiepileptic drugs (felbamate and lamotrigine alone or in combination), were evaluated in mice by means of the electronically monitored locomotor activity system (Digiscan analyzer). Felbamate (injected intraperitoneally, at the single dose of 25.7 mg/kg) significantly reduced behavioral activity in terms

of horizontal (ambulatory) activity in mice. The combination of felbamate (25.7 mg/kg) with lamotrigine (2.3 mg/kg) drastically reduced this parameter during the first 15-min period (habituation to a novel environment – being the rate of exploratory locomotor activity) and the second 15-min interval (spontaneous locomotor activity). Moreover, neither the exploratory, nor spontaneous locomotor activity of the mice, were affected by lamotrigine (at the separate dose of 2.3 mg/kg) with respect to the ambulatory activity of the animals tested. Felbamate administered alone or combined with lamotrigine considerably impaired exploratory and spontaneous locomotor activities of the animals tested in terms of horizontal activity.

Wpływ lamotryginy w kombinacji z felbamatem na poziomą aktywność ruchową u myszy

Poznawcza i spontaniczna aktywność ruchowa zwierząt po podaniu niektórych nowych leków przeciwpadaczkowych (felbamatu i lamotryginy – aplikowanych oddzielnie jak również w kombinacji) była oceniana z wykorzystaniem elektronicznego systemu monitorującego aktywność ruchową u myszy (analizator Digiscan). Felbamat (podawany dootrzewnowo, w pojedynczej dawce 25,7 mg/kg) istotnie zmniejszał aktywność motoryczną zwierząt w odniesieniu do ruchliwości poziomej (horyzontalnej). Kombinacja felbamatu (25,7 mg/kg) z lamotryginą (2,3 mg/kg) drastycznie zmniejszała oceniany parametr podczas pierwszych 15 minut (przyzwyczajenia się do nowego otaczającego środowiska – wskaźnika aktywności poznawczej u zwierząt) oraz podczas następnych 15 minut okresu spontanicznej ruchliwości zwierząt. Ani poznawcza, ani spontaniczna aktywność ruchowa zwierząt nie była zmieniona po podaniu lamotryginy (w pojedynczej dawce 2,3 mg/kg) w odniesieniu do badanej ruchliwości poziomej zwierząt badanych. Felbamat podawany osobno lub w połączeniu z lamotryginą istotnie upośledzał aktywność poznawczą i spontaniczną badanych zwierząt w zakresie aktywności poziomej.