Pharmacological Reports
2008, 60, 566–573
ISSN 1734-1140

Short communication

Influence of imperatorin on the anticonvulsant activity and acute adverse-effect profile of lamotrigine in maximal electroshock-induced seizures and chimney test in mice

Jarogniew J. Łuszczki[1,2], Ewa Wojda[1], Grzegorz Raszewski[2], Kazimierz Głowniak[3], Stanisław J. Czuczwar[1,2]

[1]Department of Pathophysiology, Medical University of Lublin, Jaczewskiego 8, PL 20-090 Lublin, Poland

[2]Department of Physiopathology, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950 Lublin, Poland

[3]Department of Pharmacognosy with Medicinal Plant Laboratory, Medical University of Lublin, Chodźki 1, PL 20-093 Lublin, Poland

Correspondence: Jarogniew J. Łuszczki, e-mail: jarogniew.luszczki@gmail.com; jluszczki@yahoo.com

Abstract:
The influence of imperatorin (IMP) on the anticonvulsant activity and acute adverse-effect potential of lamotrigine (LTG, a second-generation antiepileptic drug) was studied in the maximal electroshock-induced seizure (MES) model and chimney test in mice. In order to assess the nature of interaction between IMP and LTG in the MES test, total brain LTG concentrations were evaluated with high-pressure liquid chromatography (HPLC).
Results indicate that IMP administered *ip*, 30 min before the test, at a dose of 50 mg/kg significantly enhanced the anticonvulsant action of LTG in the MES test by reducing the median effective dose (ED_{50}) of LTG from 6.11 to 2.47 mg/kg ($p < 0.05$). In contrast, IMP administered *ip* at doses of 30 and 40 mg/kg did not significantly potentiate the anticonvulsant activity of LTG against MES-induced seizures, although a reduction of the ED_{50} values for LTG from 6.11 to 5.77, and 4.28 mg/kg, respectively, was observed. On the other hand, IMP administered *ip*, at doses of 30, 40 and 50 mg/kg had no impact on the acute adverse effects of LTG, and the median toxic doses for LTG (TD_{50}) were almost unchanged, ranging from 22.13 to 30.04 mg/kg in the chimney test. The protective index (TD_{50} to ED_{50} ratio) for LTG administered alone was 4.90 and increased to 5.21, 6.77, and 8.96 for LTG in combination with IMP at doses of 30, 40 and 50 mg/kg, respectively. Pharmacokinetic evaluation of total brain LTG concentration with HPLC revealed that IMP at the dose of 50 mg/kg did not affect total brain LTG concentration in experimental animals and thus, the observed interaction between IMP and LTG in the MES test was pharmacodynamic in nature.
The present study demonstrates that IMP ameliorates the pharmacological profile of LTG, when considering both, the antiseizure and acute adverse effects of the drug in preclinical study on animals. The combination of LTG with IMP can be of pivotal importance for epileptic patients as a potentially advantageous combination if it is proven that the results of this study can be extrapolated to clinical settings.

Key words:
imperatorin, lamotrigine, maximal electroshock seizure test, chimney test, protective index

Introduction

Imperatorin (IMP; 9-(3-methylbut-2-enyloxy)-7H--furo[3,2-g]chromen-7-one) is a bioactive furanocoumarin isolated from roots of *Angelica dahurica* and fruits of *Angelica archangelica* (*Umbelliferae*) [1]. Experimental evidence indicates that IMP irreversibly inactivates γ-aminobutyric acid (GABA)-transaminase (the enzyme responsible for GABA degradation), thereby increasing the GABA content in the synaptic clefts of neurons and elevating concentration of the inhibitory neurotransmitter GABA in the brain [6]. Moreover, in an autoradiographic study, IMP inhibited the binding of [^{3}H]diazepam to benzodiazepine receptors, suggesting that the compound produced its anticonvulsant effects through the interaction with $GABA_A$-benzodiazepine receptor complex [2].

On the other hand, in an *in vitro* study IMP induced vasodilatation of rat mesenteric arterial rings *via* inhibiting voltage-dependent (VDCC) and receptor-operated (ROCC) calcium channels [10]. Hence, it is highly likely that this agent possesses properties of a calcium channel antagonist. Accumulating evidence indicates that some calcium channel antagonists reduce the incidence of seizures and exert the anticonvulsant activity in various experimental seizure models in rodents [9, 15, 25, 27, 33, 34]. Generally, it is thought that the blockade of high voltage-activated (L-, N-, P/Q-type) calcium channels is associated with control of partial seizures with or without secondary generalization [11, 15, 26, 32]. Interestingly, calcium channel antagonists readily penetrating into the brain potentiate the protective efficacy of some antiepileptic drugs (AEDs) in both, preclinical studies on animals [3, 7, 12, 13], and clinical trials in humans [8, 28, 29, 31]. To date, several clinical reports revealed beneficial effects of some calcium channel antagonists (i.e., flunarizine, cinnarizine, and nimodipine) as an add-on treatment in epileptic patients [8, 28, 30, 31].

Quite recently, it has been reported that IMP in a dose-dependent manner increased the threshold for electroconvulsions in mice [22]. The time-course and dose-response relationship analyses revealed that the time to peak of the maximum anticonvulsant effect for IMP was established at 30 min after its systemic (*ip*) administration in mice [22]. Moreover, IMP significantly enhanced the antiseizure action of phenytoin, carbamazepine and phenobarbital, but not that of valproate in the mouse maximal electroshock-induced seizure (MES) model [21].

Considering the above-mentioned facts, it was of pivotal importance to evaluate the effects of IMP upon the protective activity of lamotrigine (LTG, a second-generation AED) against MES-induced seizures in mice. It is widely accepted that the MES test is considered to be an experimental model of tonic-clonic seizures and, to a certain extent, of partial seizures with or without secondary generalization [17]. In this model, the anticonvulsant effects produced by conventional AEDs in combination with IMP were determined [21]. Moreover, clinical evidence indicates that LTG is effective in suppression of tonic-clonic seizures, partial simple and complex seizures with or without secondary generalization, the idiopathic generalized epilepsies and Lennox-Gastaut syndrome [5]. Therefore, it was appropriate to use the MES test in order to evaluate the anticonvulsant effects exerted by the combination of IMP with LTG.

Additionally, acute adverse effect profile for LTG administered alone and combined with IMP was determined in the chimney test. The determination of the anticonvulsant activity and acute adverse-effect potential of LTG administered alone and combined with IMP allowed for establishing of the protective index (PI) value for LTG and its combination with IMP. Noteworthy, the PI value in preclinical studies reflects the margin of safety and tolerability between the drug doses exerting acute adverse effects and those providing therapeutic efficacy against seizures [18]. To ascertain whether the observed anticonvulsant effects of the combination of IMP with LTG were consequent to a pharmacodynamic and/or a pharmacokinetic interaction, total brain LTG concentrations were evaluated with high pressure liquid chromatography (HPLC).

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 7 days of adap-

tation to laboratory conditions, the animals were randomly assigned to experimental groups comprising 8 mice. Each mouse was used only once and all tests were performed between 08.00 and 15.00 h.

Procedures involving animals and their care were conducted in accordance with current European Community 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 manuscript were approved by the Local Ethics Committee at the Medical University of Lublin (Licenses no. 14/2006; 37/2006) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

LTG (Lamictal, Glaxo Wellcome, Kent, UK) was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered *ip* as a single injection, in a volume of 5 ml/kg body weight. Fresh drug solutions were prepared on each day of experimentation and administered 60 min before electroconvulsions and motor coordination assessment. IMP [9-(3-methylbut-2-enyloxy)-7H-furo-[3,2-g]chromen-7-one] (Department of Pharmacognosy with Medicinal Plant Laboratory, Lublin, Poland) was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered *ip* at 30 min before electroconvulsions and motor coordination evaluation. The pretreatment times before testing of the drugs were based upon information about their biological activity from the literature and our previous experiments [19–22]. The times to the peak of maximum anticonvulsant effects for LTG and IMP were used as the reference times in the chimney test.

Maximal electroshock-induced seizures

Electroconvulsions were produced by means of a constant current stimulation (25 mA, 0.2 s stimulus duration) delivered *via* ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension. The protective activity of LTG was determined as its median effective doses (ED_{50} values in mg/kg) against MES-induced seizures. The animals were administered with different doses of LTG so as to obtain a variable percentage of protection against MES-induced seizures, allowing for the construction of a log-probit dose-response curve for LTG administered alone, according to Litchfield and Wilcoxon [16]. To evaluate each ED_{50} value, at least 4 groups of animals (each group consisted of 8 mice) injected with various doses of LTG were subjected to the MES test. The ED_{50} value represents the dose of LTG required to protect half of the animals tested against MES-induced seizures. Similarly, the anticonvulsant activity of a mixture of LTG with IMP was evaluated and expressed as the ED_{50} corresponding to the dose of LTG necessary to protect 50% of mice against tonic hindlimb extension in the MES test. In the present study, LTG was administered at doses ranging between 1–9 mg/kg. This experimental procedure has been described in detail in our earlier studies [20, 21, 23].

Chimney test

The chimney test of Boissier et al. [4] was used to quantify the acute adverse-effect potential of LTG administered alone and in combination with IMP on motor performance in mice. In this test, the animals had to climb backwards up a plastic tube (3 cm inner diameter, 30 cm long), and motor performance impairment was indicated by the inability of the mice to climb backward up the transparent tube within 60 s. The acute adverse effects of LTG administered alone were expressed as its median toxic doses (TD_{50} values), representing the doses at which LTG impaired motor coordination in 50% of the animals tested in the chimney test. To evaluate each TD_{50} value, at least 4 groups of animals (each group consisted of 8 mice) injected with various doses of LTG were challenged with the chimney test. A dose-response relationship curve was calculated on the basis of the percentage of mice showing motor deficits by means of the log-probit method according to Litchfield and Wilcoxon [16]. Additionally, mice were administered combinations of LTG with IMP and were subjected to the chimney test to determine motor performance. In the present study, LTG was administered at doses ranging between 15–40 mg/kg. This experimental procedure has been described in detail in our earlier studies [19, 20, 23].

Protective index

The protective index (PI) for LTG administered alone and in combination with IMP was calculated by dividing a TD_{50} value, as determined in the chimney test, by the respective ED_{50} value, as determined in the MES test. The PI is considered to be an index representing the margin of safety and tolerability between anticonvulsant doses and doses of AEDs exerting acute adverse effects (e.g., sedation, motor coordination impairment, ataxia or other neurotoxic manifestations) [18].

Measurement of total brain lamotrigine concentration

The total brain concentration of LTG was measured after administration of the dose, which corresponded to its ED_{50} value established in the MES test for the combination of LTG with IMP (50 mg/kg). Mice were killed by decapitation at times chosen to coincide with that scheduled for the MES test and the whole brains of mice were removed from skulls, weighed, harvested and homogenized with Abbott buffer (1:2 w/v) in an Ultra-Turrax T8 homogenizer (IKA-Werke, Staufen, Germany). The homogenates were centrifuged at 10,000 × g for 10 min. Next, supernatant samples of 200 μl were added to 200 μl of 3 M trichloroacetic acid, vortexed for 1 min., and centrifuged at 10,000 × g for 10 min. Subsequently, 20 μl of the prepared samples was injected into the HPLC column. The chromatograph (Dionex, Sunnyvale, CA, USA) was equipped with a gradient pump P580 LPG and a UV/VIS detector (UVD 340S) with a sensitivity setting of 0.1 absorbance units full scale (AUFS) and a time constant of 0.1 s. The Rheodyne 3601 injector valve with a 20 μl sample loop was used for sample injection. For HPLC, a stainless steel HP ODS column (200 × 4.6 mm) was used at an ambient temperature of 20°C. The mobile phase was 40 mM triethylammonium phosphate buffer: methanol: acetonitrile (660:80:160 vol/vol/vol; Fluka, HPLC grade). The mobile phase flow rate was 1.2 ml/min, and LTG absorbance was measured at 214 nm. The peak height for LTG was linearly related to its concentrations, which ranged from 0.16 to 5.0 μg/ml. Total brain concentrations of LTG were expressed in μg/ml of supernatant as the means ± SD of 8 separate brain preparations.

Statistical analysis

Both, ED_{50} and TD_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [16]. Subsequently, the respective 95% confidence limits were transformed to standard errors (SE) as described previously [23]. Statistical analysis of data from the MES and chimney tests was performed with one-way analysis of variance (ANOVA) followed by the *post-hoc* Tukey-Kramer test for multiple comparisons [20, 21]. Total brain LTG concentrations were statistically analyzed using the unpaired Student's *t*-test. All statistical tests were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). Differences between the means were considered statistically significant if $p < 0.05$.

Results

Effects of imperatorin on the protective action of lamotrigine in the maximal electroshock-induced seizure model in mice

LTG administered alone exhibited a clear-cut anticonvulsant activity in the MES test in mice and its ED_{50} value is presented in Table 1. IMP administered *ip*, 30 min before the test, at a dose of 50 mg/kg significantly enhanced the anticonvulsant action of LTG in the MES test. In this case, the experimentally-derived ED_{50} value for LTG was reduced by 60% from 6.11 to 2.47 mg/kg ($p < 0.05$; Tab. 1). IMP at lower doses of 30 and 40 mg/kg also potentiated the anticonvulsant effect of LTG against MES-induced seizures, however, statistical analysis of data with one-way ANOVA followed by the *post-hoc* Tukey-Kramer test revealed that the reduction of ED_{50} values of LTG (by 6% and 30%, from 6.11 to 5.77, and 4.28 mg/kg after administration of IMP at 30 and 40 mg/kg, respectively) did not attain statistical significance (Tab. 1).

Influence of imperatorin on the acute adverse-effect profile of lamotrigine in the chimney test in mice

LTG administered alone produced a clear-cut motor coordination impairment in the chimney test in mice and its TD_{50} value is presented in Table 1. IMP at

Tab. 1. Effects of imperatorin (IMP) on the anticonvulsant activity and acute adverse-effect (neurotoxic) profile of lamotrigine (LTG) in the maximal electroshock (MES)-induced seizures and chimney test in mice

Treatment (mg/kg)	ED_{50} (mg/kg)	N	SE	TD_{50} (mg/kg)	N	SE	PI
LTG + vehicle	6.11 (4.52–8.25)	24	0.938	29.91 (23.75–37.67)	24	3.519	4.90
LTG + IMP (30)	5.77 (4.28–7.77)	8	0.876	30.04 (23.75–38.00)	24	3.601	5.21
LTG + IMP (40)	4.28 (2.79–6.58)	16	0.939	28.98 (24.14–34.78)	16	2.698	6.77
LTG + IMP (50)	2.47 (1.22–4.99)*	24	0.886	22.13 (15.51–31.59)	24	4.014	8.96
	$F(3, 68) = 3.373$; $p = 0.0233$			$F(3, 84) = 1.159$; $p = 0.3305$			

Data are presented as 1) median effective doses of LTG (ED_{50} values with 95% confidence limits in parentheses), protecting 50% of animals tested against MES-induced seizures and 2) median toxic doses of LTG (TD_{50} values with 95% confidence limits in parentheses), impairing motor coordination in 50% of animals tested subjected to the chimney test. Statistical evaluation of the data was performed with log-probit method [16], followed by one-way analysis of variance (ANOVA) with the *post-hoc* Tukey-Kramer test for multiple comparisons [21]. The drugs were administered systemically (*ip*), as follows: LTG – at 60 min, and IMP – at 30 min prior to the MES and chimney tests. N – total number of animals at those doses whose anticonvulsant and acute adverse effects were between 4 and 6 probits. SE – standard error of the ED_{50} or TD_{50} values. The protective index (PI) was calculated as TD_{50} to ED_{50} ratio based on the chimney test and MES test results, respectively. F – F-statistics from one-way ANOVA accompanied with the respective degrees of freedom in parentheses; p – probability value from one-way ANOVA. * $p < 0.05$ *vs.* the respective control (LTG + vehicle-treated) animals

Tab. 2. Effect of imperatorin (IMP) on total brain concentrations of lamotrigine (LTG) in mice

Treatment (mg/kg)	Brain concentration (μg/ml)
LTG (2.5) + vehicle	0.789 ± 0.111
LTG (2.5) + IMP (50)	0.799 ± 0.144

Data are presented as mean concentrations (in μg/ml ± SD of at least 8 separate brain samples). The drugs were administered *ip* at doses corresponding to the ED_{50} value from the MES test. Total brain concentrations of LTG were measured with HPLC technique. Data were statistically verified by using the unpaired Student's *t*-test

doses of 30, 40 and 50 mg/kg did not significantly affect the acute adverse effects of LTG in the chimney test (Tab. 1). According to one-way ANOVA followed by the *post-hoc* Tukey-Kramer test for multiple comparisons, the TD_{50} values for LTG combined with IMP (30, 40 and 50 mg/kg) did not differ significantly from the respective value for LTG administered alone (Tab. 1).

Protective index

The PI (as a ratio of TD_{50} and ED_{50} values) for LTG administered alone was 4.90 (Tab. 1). The PI for the combination of LTG with IMP at 30 mg/kg was 5.21, and that for the combination of LTG with IMP at 40 mg/kg increased to 6.77 (Tab. 1). The highest PI value was observed for the combination of LTG with IMP at the dose of 50 mg/kg, which amounted to 8.96 (Tab. 1).

Total brain LTG concentrations

Total brain concentration of LTG for the mixture of LTG (2.5 mg/kg) with IMP (50 mg/kg) was 0.799 ± 0.144 μg/ml and did not significantly differ from that evaluated for LTG administered alone at 2.5 mg/kg, which was 0.789 ± 0.111 μg/ml (Tab. 2).

Discussion

The results indicate that IMP at the dose of 50 mg/kg significantly enhanced the anticonvulsant action of LTG in the MES test and had no impact on the acute adverse effects of LTG in the chimney test. However, as reported earlier, IMP at the dose of 50 mg/kg significantly increased (by 38%) the threshold for electroconvulsions in mice [22]. Thus, the protective action of LTG against MES-induced seizures was enhanced by 60% after co-administration of IMP (at the dose of 50 mg/kg), despite that IMP by itself increased the threshold for electroconvulsions in mice. Pharmacokinetic evaluation of interaction between LTG and IMP revealed that IMP had no impact on the total brain LTG concentrations in experimental animals and thus, the observed interaction between IMP and LTG in the MES test was pharmacodynamic in nature. The lack of the pharmacokinetic interaction between drugs is in agreement with the fact reported

earlier that IMP had no impact on the total brain concentrations of phenytoin and phenobarbital in experimental animals [21].

It is important to note that IMP has been found to induce vasodilatation *via* inhibiting voltage-dependent calcium channels and receptor-mediated calcium influx [10], therefore, one can ascertain that IMP possesses, at least in part, properties of a calcium channel antagonist. In order to explain the observed interaction between IMP and LTG in the MES test, one should analyze the effect produced by the combination of LTG with three calcium channel antagonists: amlodipine, diltiazem and verapamil. Quite recently, it has been found that amlodipine, but not diltiazem or verapamil significantly potentiated the antiseizure action of LTG in the MES test in mice [24]. The ED_{50} value of LTG against MES-induced seizures after co-administration of amlodipine (at a dose of 20 mg/kg) was reduced by 55% from 6.33 to 2.87 mg/kg [24]. In the present study, the reduction of ED_{50} value of LTG after co-administration of IMP (at the dose of 50 mg/kg) attained 60% from 6.11 to 2.47 mg/kg. Moreover, the experimental studies indicated that IMP at the dose of 50 mg/kg increased the threshold for electroconvulsions in mice by 38% [21], whereas amlodipine at the dose of 20 mg/kg elevated the threshold for electroconvulsions in mice by 20% [24]. Thus, based on comparison of the effects of IMP and amlodipine on the anticonvulsant action of LTG in the MES test in mice, one can hypothesize that IMP is quite similar to amlodipine, especially, if one relates the pharmacological activity of both drugs in combination with LTG. There are distinct similarities between IMP and amlodipine confirming the hypothesis about the functional resemblance of both drugs. For instance, amlodipine enhanced the anticonvulsant action of carbamazepine, phenobarbital and valproate, but not that of phenytoin in the MES test in mice [13]. The pharmacokinetic estimation of free plasma AED concentrations revealed that amlodipine significantly elevated free plasma concentrations of carbamazepine, remaining without any effect on free plasma concentrations of phenobarbital or valproate in mice [13]. With respect to IMP, the drug enhanced the antiseizure action of carbamazepine, phenobarbital and phenytoin, but not that of valproate in the MES test in mice [21]. Similarly, IMP significantly increased total brain concentrations of carbamazepine, remaining without any effect on total brain concentrations of phenobarbital or phenytoin [21]. The quite identical profile of interaction of IMP and amlodipine may suggest the functional resemblance of these drugs in preclinical studies. Although this hypothesis is highly speculative, the blockade of calcium channels by IMP can readily explain the observed interaction between LTG and IMP in the MES test in mice.

Moreover, this study extended our previous findings by investigating the effect of IMP on the acute adverse-effect profile of LTG, specifically on the motor coordination impairment in mice. Noteworthy, the chimney test is considered to be an experimental animal model allowing to assess the effects of drugs on motor performance, including the influence of drugs on synchronic movements of fore- and hind-limbs, coordination of movements and normal muscular strength in rodents [18]. The evaluation of acute adverse-effect potential of LTG administered alone and in combination with IMP in the chimney test revealed that IMP did not significantly alter motor coordination in mice as compared to the animals receiving LTG alone. Therefore, the investigated combination of LTG with IMP seems to be safe and well tolerated by experimental animals. Our findings are in agreement with those reported earlier by Kleiner et al. [14] and Okamoto et al. [29], who have found that IMP administered chronically at up to 70 mg/kg/day and acutely at up to 200 mg/kg produced no toxicity in rodents. Since a close correlation exists between the acute adverse effects observed in animals in preclinical studies and the acute neurotoxic effects documented in clinical settings in humans, one can ascertain that the combination of LTG with IMP should be safe and well tolerated by patients.

The results presented herein also indicate that IMP ameliorated the pharmacological profile of LTG, by increasing its PI value. Noteworthy, the PI values calculated for the combination of LTG with IMP were higher than that for LTG administered alone, indicating a favorable profile for the combination of LTG with IMP. Generally, the PI reflects both, the antiseizure activity of a drug and its ability to produce acute adverse effects in preclinical studies.

Conclusions

IMP ameliorated pharmacological profile of LTG, especially, by enhancing its antiseizure activity with unaltered acute adverse effect liability in preclinical studies on animals. If the experimental data from this

study can be extrapolated to clinical settings, IMP should enhance the antiseizure activity of LTG in epileptic patients that would create a new therapeutic option for epileptic patients. However, the hypothesis about the beneficial effect of combined treatment with LTG and IMP in epileptic patients needs an additional confirmation in further clinical trials.

Acknowledgments:
This study was supported by a grant from Medical University of Lublin, Poland. The authors express their thanks to Miss Katarzyna Drozd for her technical assistance.

References:

1. Baek NI, Ahn EM, Kim HY, Park YD: Furanocoumarins from the root of *Angelica dahurica*. Arch Pharm Res, 2000, 23, 467–470.
2. Bergendorff O, Dekermendjian K, Nielsen M, Shan R, Witt R, Ai J, Sterner O: Furanocoumarins with affinity to brain benzodiazepine receptors in vitro. Phytochemistry, 1997, 44, 1121–1124.
3. Binnie CD, de Beukelaar F, Meijer JW, Meinardi H, Overweg J, Wauquier A, van Wieringen A: Open dose-ranging trial of flunarizine as add-on therapy in epilepsy. Epilepsia, 1985, 26, 424–428.
4. Boissier JR, Tardy J, Diverres JC: A new simple method to explore the tranquilizing action: a chimney test (in French). Med Exp (Basel), 1960, 3, 81–84.
5. Brodie MJ, Schachter SC: Fast Facts. Epilepsy, 2nd edn., Health Press, Oxford, 2001.
6. Choi SY, Ahn EM, Song MC, Kim DW, Kang JH, Kwon OS, Kang TC, Baek NI: In vitro GABA-transaminase inhibitory compounds from the root of *Angelica dahurica*. Phytother Res, 2005, 19, 839–845.
7. Czuczwar SJ, Chodkowska A, Kleinrok Z, Małek U, Jagiełło-Wójtowicz E: Effects of calcium channel inhibitors upon the efficacy of common antiepileptic drugs. Eur J Pharmacol, 1990, 176, 75–83.
8. De Falco FA, Bartiromo U, Majello L, Di Geronimo G, Mundo P: Calcium antagonist nimodipine in intractable epilepsy. Epilepsia, 1992, 33, 343–345.
9. De Sarro GB, Meldrum BS, Nistico G: Anticonvulsant effects of some calcium entry blockers in DBA/2 mice. Br J Pharmacol, 1988, 93, 247–256.
10. He JY, Zhang W, He LC, Cao YX: Imperatorin induces vasodilatation possibly via inhibiting voltage dependent calcium channel and receptor-mediated Ca^{2+} influx and release. Eur J Pharmacol, 2007, 573, 170–175.
11. Heinemann U, Hamon B: Calcium and epileptogenesis. Exp Brain Res, 1986, 65, 1–10.
12. Jagiełło-Wójtowicz E, Czuczwar SJ, Chodkowska A, Szponar J, Kleinrok Z: Influence of calcium channel blockers on pentylenetetrazol and electroshock-induced convulsions in mice. Pol J Pharmacol Pharm, 1991, 43, 95–101.
13. Kamiński R, Jasiński M, Jagiełło-Wójtowicz E, Kleinrok Z, Czuczwar SJ: Effect of amlodipine upon the protective activity of antiepileptic drugs against maximal electroshock-induced seizures in mice. Pharmacol Res, 1999, 40, 319–325.
14. Kleiner HE, Vulimiri SV, Miller L, Johnson WH Jr, Whitman CP, DiGiovanni J: Oral administration of naturally occurring coumarins leads to altered phase I and II enzyme activities and reduced DNA adduct formation by polycyclic aromatic hydrocarbons in various tissues of SENCAR mice. Carcinogenesis, 2001, 22, 73–82.
15. Kułak W, Sobaniec W, Wojtal K, Czuczwar SJ: Calcium modulation in epilepsy. Pol J Pharmacol, 2004, 56, 29–41.
16. Litchfield JT, Wilcoxon F: A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther, 1949, 96, 99–113.
17. Löscher W, Fassbender CP, Nolting B: The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. Epilepsy Res, 1991, 8, 79–94.
18. Löscher W, Nolting B: The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. IV. Protective indices. Epilepsy Res, 1991, 9, 1–10.
19. Łuszczki JJ, Czuczwar SJ: Interaction between lamotrigine and felbamate in the maximal electroshock-induced seizures in mice: an isobolographic analysis. Eur Neuropsychopharmacol, 2005, 15, 133–142.
20. Łuszczki JJ, Czuczwar M, Gawlik P, Sawiniec-Późniak G, Czuczwar K, Sawicka KM, Dudra-Jastrzębska M, Czuczwar SJ: Influence of N^G-nitro-L-arginine on the anticonvulsant and acute adverse effects of some newer antiepileptic drugs in the maximal electroshock-induced seizures and chimney test in mice. Pharmacol Rep, 2006, 58, 955–960.
21. Łuszczki JJ, Głowniak K, Czuczwar SJ: Imperatorin enhances the protective activity of conventional antiepileptic drugs against maximal electroshock-induced seizures in mice. Eur J Pharmacol, 2007, 574, 133–139.
22. Łuszczki JJ, Głowniak K, Czuczwar SJ: Time-course and dose-response relationships of imperatorin in the mouse maximal electroshock seizure threshold model. Neurosci Res, 2007, 59, 18–22.
23. Łuszczki JJ, Ratnaraj N, Patsalos PN, Czuczwar SJ: Isobolographic analysis of interactions between loreclezole and conventional antiepileptic drugs in the mouse maximal electroshock-induced seizure model. Naunyn Schmiedebergs Arch Pharmacol, 2006, 373, 169–181.
24. Łuszczki JJ, Trojnar MK, Trojnar MP, Kimber-Trojnar Z, Szostakiewicz B, Zadrożniak A, Borowicz KK, Czuczwar SJ: Effects of three calcium channel antagonists (amlodipine, diltiazem and verapamil) on the protective action of lamotrigine in the mouse maximal electroshock-induced seizure model. Pharmacol Rep, 2007, 59, 672–682.
25. Marinho MM, de Bruin VM, de Sousa FC, Aguiar LM, de Pinho RS, Viana GS: Inhibitory action of a calcium

channel blocker (nimodipine) on seizures and brain damage induced by pilocarpine and lithium-pilocarpine in rats. Neurosci Lett, 1997, 235, 13–16.
26. Meyer FB, Anderson RE, Sundt TM Jr, Sharbrough FW: Selective central nervous system calcium channel blockers – a new class of anticonvulsant agents. Mayo Clin Proc, 1986, 61, 239–247.
27. Meyer FB, Anderson RE, Sundt TM Jr, Yaksh TL, Sharbrough FW: Suppression of pentylenetetrazole seizures by oral administration of a dihydropyridine Ca^{2+} antagonist. Epilepsia, 1987, 28, 409–414.
28. Nakane Y, Seino M, Yagi A, Kaji S, Yamauchi T: Effects of flunarizine therapy on intractable epilepsy. Arzneimittelforschung, 1989, 39, 793–798.
29. Okamoto T, Yoshida S, Kobayashi T, Okabe S: Inhibition of concanavalin A-induced mice hepatitis by coumarin derivatives. Jpn J Pharmacol, 2001, 85, 95–97.
30. Overweg J, Binnie CD, Meijer JW, Meinardi H, Nuijten ST, Schmaltz S, Wauquier A: Double-blind placebo-controlled trial of flunarizine as add-on therapy in epilepsy. Epilepsia, 1984, 25, 217–222.
31. Starreveld E, de Beukelaar F, Wilson AF, McLean DR, Findlay HP: Double-blind cross-over placebo controlled study of flunarizine in patients with therapy resistant epilepsy. Can J Neurol Sci, 1989, 16, 187–190.
32. Stefani A, Spadoni F, Bernardi G: Voltage-activated calcium channels: targets of antiepileptic drug therapy? Epilepsia, 1997, 38, 959–965.
33. Thomas J: The effect of nimodipine on picrotoxin-induced seizures. Brain Res Bull, 1990, 24, 11–15.
34. Wurpel JN, Iyer SN: Calcium channel blockers verapamil and nimodipine inhibit kindling in adult and immature rats. Epilepsia, 1994, 35, 443–449.

Received:
December 18, 2007; in revised form: May 5, 2008.