ORIGINAL ARTICLE

Journal of Pre-Clinical and Clinical Research, 2008, Vol 2, No 2, 147-152
www.jpccr.eu

Diltiazem enhances the protective activity of oxcarbazepine against maximal electroshock-induced seizures in mice

Anna Zadrożniak[1], Michał K. Trojnar[2], Marcin P. Trojnar[3], Żaneta Kimber-Trojnar[4], Monika Dudra-Jastrzębska[1,5], Marta M. Andres-Mach[1,5], Jarogniew J. Łuszczki[1,5]

[1] *Department of Pathophysiology, Medical University, Lublin, Poland*
[2] *Department of Cardiology, Medical University, Lublin, Poland*
[3] *Department of Internal Medicine, Medical University, Lublin, Poland*
[4] *Department of Obstetrics and Perinatology, Medical University, Lublin, Poland*
[5] *Department of Physiopathology, Institute of Agricultural Medicine, Lublin, Poland*

Abstract: The aim of the study was to assess the effect of diltiazem (a calcium channel antagonist) on the anticonvulsant activity and acute adverse-effect potential of oxcarbazepine (a second-generation antiepileptic drug) in the maximal electroshock seizure (MES) model and chimney test in mice. Total brain concentrations of oxcarbazepine were measured with high pressure liquid chromatography (HPLC) to ascertain any pharmacokinetic contribution to the pharmacodynamic interaction between drugs. Results indicate that diltiazem (at a dose of 5 mg/kg, i.p.) significantly enhanced the anticonvulsant activity of oxcarbazepine in the MES test in mice, by reducing the median effective dose (ED_{50} value) of oxcarbazepine from 14.25 to 9.87 mg/kg ($P<0.01$). In contrast, diltiazem at lower doses of 1.25 and 2.5 mg/kg had no significant impact on the antiseizure action of oxcarbazepine in the MES test in mice. In the chimney test, diltiazem (up to 5 mg/kg, i.p.) did not significantly affect the acute adverse effect potential of oxcarbazepine, and the median toxic dose (TD_{50} value) of the studied antiepileptic drug ranged from 74.16-62.91 mg/kg. Moreover, diltiazem (5 mg/kg) did not significantly alter total brain oxcarbazepine concentrations as measured with HPLC. In conclusion, diltiazem ameliorates the pharmacological profile of oxcarbazepine, when considering both the antiseizure and acute adverse effects of the antiepileptic drug in preclinical study on animals. The observed interaction between oxcarbazepine and diltiazem in the MES test was pharmacodynamic in nature; therefore, this favourable combination deserves more attention from a clinical point of view.

Key words: calcium channel antagonist, diltiazem, oxcarbazepine, maximal electroshock seizure chimney test, protective index, pharmacodynamic interaction

INTRODUCTION

Experimental evidence indicates that calcium ions (Ca^{2+}) play an essential role in the pathophysiology of epilepsy [1]. During seizures, a decrease can be observed in the extracellular calcium concentrations prior to onset of seizure activity, followed by an increase in the intracellular calcium concentrations [1]. Moreover, some calcium channel antagonists (i.e., amlodipine, diltiazem, nimodipine) reduce the incidence of seizures and possess anticonvulsant properties in various experimental seizure models [2-8]. Interestingly, calcium channel antagonists readily penetrating into the brain potentiate the protective efficacy of some antiepileptic drugs in both, preclinical studies on animals [3-8] and clinical settings in humans [9-14]. Several reports have revealed beneficial effects of some calcium channel antagonists (i.e., flunarizine, nimodipine, and diltiazem) as add-on treatment in epileptic patients [10-14]. 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 [1, 15, 16]. It is noteworthy that in epileptology, one of the consequences of calcium channel blockade is the reduced release of neurotransmitters, including glutamate [1, 16, 17]. At present, there are some second-generation antiepileptic drugs that exert their anticonvulsant activity through the blockade of high voltage-activated calcium channels. For instance, one of the antiseizure mechanisms of the action of oxcarbazepine, lamotrigine and topiramate (some second-generation antiepileptic drugs) is related to the reduction of calcium ion fluxes in neurons [18].

Considering the fact that seizure activity depends on calcium ions [17], and some antiepileptic drugs interfere with calcium ion fluxes [1, 10-16, 19], we attempted to study the effects of diltiazem on the protective activity of oxcarbazepine in the mouse maximal electroshock seizure (MES) model. Oxcarbazepine is licensed as an add-on treatment for adults with refractory epilepsy, and as monotherapy in newly diagnosed epilepsy (especially, in patients with generalized tonic-clonic seizures and partial convulsions, with or without secondary generalization) [20]. Oxcarbazepine and its rapidly formed 10-monohydroxy derivative (MHD), at therapeutically relevant concentrations, reduce high-frequency repetitive firing of neurons by an action on sodium channels and enhance potassium currents [18, 21]. Moreover, oxcarbazepine and MHD inhibit high-voltage-activated N-type calcium channels

Corresponding author: Dr. Jarogniew J. Łuszczki, Department of Pathophysiology, Medical University, Jaczewskiego 8, 20-090 Lublin, Poland.
E-mail addresses: jarogniew.luszczki@gmail.com / jluszczki@yahoo.com

Received: 20 October 2008, accepted: 28 November 2008

and reduce glutamatergic transmission at cortical synapses in rat brain slices [22-25].

In preclinical studies, diltiazem had no effect on pentylenetetrazole-induced clonic seizures in mice [8], and the drug did not affect the threshold for maximal electroconvulsions in mice [5, 26]. In contrast, diltiazem reduced the incidence of the tonic phase, but was completely ineffective in preventing clonic and running phases of sound-induced seizures in genetically epilepsy prone rats [7]. Experimental evidence indicates that diltiazem potentiated the antiseizure activity of phenytoin, but not that of phenobarbital and valproate in sound-induced seizures in DBA/2 mice [6]. Diltiazem markedly potentiated the protective action of carbamazepine, phenytoin, phenobarbital, topiramate, and valproate, but not that of lamotrigine in the mouse MES model [5, 26, 27]. The drug enhanced the antiseizure action of ethosuximide, but not that of valproate, phenobarbital and diazepam against pentylenetetrazole-induced clonic seizures in mice [8]. In contrast, the drug did not affect aminophylline-induced convulsions in mice, and had no effect on the protective activity of valproate, phenobarbital, carbamazepine, ethosuximide, and trimethadione against aminophylline-induced seizures in mice [8].

It is widely accepted that the mouse MES test is considered as an experimental animal model, allowing the selection of drugs that are effective in suppressing generalized tonic-clonic seizures and, to a certain extent, of partial seizures, with or without secondary generalization [26]. Thus, it was appropriate to examine the anticonvulsant effects of oxcarbazepine administered alone and in combination with diltiazem in the MES test. Moreover, the acute adverse-effect potential of oxcarbazepine in combination with diltiazem was determined in the chimney test in mice. To confirm or exclude pharmacokinetic characteristics of interactions between oxcarbazepine and the calcium channel antagonist (diltiazem), total brain oxcarbazepine concentrations were measured with high-pressure liquid chromatography (HPLC).

MATERIALS AND METHODS

Animals and experimental conditions. Adult male Swiss mice (weighing 22 – 26 g) were kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature of 23 ± 1ºC, relative humidity of 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups, each group comprising 8 mice. Each mouse was used only once, and all tests were performed between 08.00 - 15.00 hours. 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 study were approved by the First Local Ethics Committee in Lublin (License No.: 516/2005/550/2005) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs. The following drugs were used in this study: diltiazem (Polfarmex, Kutno, Poland) and oxcarbazepine (Novartis Pharma AG, Basle, Switzerland). The drugs were suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA) and administered intraperitoneally (i.p.) in a volume of 5 ml/kg body weight. Fresh drug solutions were prepared on each day of experimentation and administered as follows: diltiazem - 60 min, and oxcarbazepine - 30 min, before electroconvulsions and motor coordination evaluation, as well as before brain sampling for the measurement of oxcarbazepine concentrations. The pretreatment times were based on information about their biological activity from the literature and our previous studies [5, 8, 26, 27, 29, 30]. The time to the peak of maximum anticonvulsant effects for oxcarbazepine was used as the reference time in all behavioural tests and pharmacokinetic estimation of total brain oxcarbazepine concentrations.

Maximal electroshock seizure test. Electroconvulsions were produced by current (0.2 s stimulus duration, 50 Hz, fixed current intensity of 25 mA, maximum stimulation voltage of 500 V), delivered via ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The electrical system of the stimulator was self-adjustable so that changes in impedance did not result in alterations of current intensity (i.e. the system provides constant current stimulation). The criterion for the occurrence of seizure activity was the tonic hind limb extension (i.e. the hind limbs of animals outstretched 180° to the plane of the body axis). The protective activity of oxcarbazepine was determined as its median effective dose (ED_{50} value in mg/kg) against MES-induced seizures. The animals received different drug doses in order to obtain a variable percentage of protection against MES-induced seizures, allowing the construction of a log-probit dose-response relationship line for oxcarbazepine administered alone, according to the log-probit method of Litchfield and Wilcoxon [31]. The ED_{50} value represents the dose of a drug required to protect 50% of the animals tested against MES-induced seizures. Similarly, the anticonvulsant activity of mixtures of oxcarbazepine with diltiazem (at doses of 1.25, 2.5 and 5 mg/kg) was evaluated and expressed as ED_{50}, corresponding to the dose of oxcarbazepine necessary to protect 50% of mice against tonic hindlimb extension in the MES test. In this test, oxcarbazepine was administered at doses ranging between 8 - 16 mg/kg. This experimental procedure has been described in detail in our earlier studies [26, 27, 29, 32].

Chimney test. The chimney test of Boissier et al. [33] was used to quantify the acute adverse-effect potential of oxcarbazepine administered alone and in combination with diltiazem (at doses of 1.25, 2.5 and 5 mg/kg) on motor performance in mice. In this test, the animals had to climb backwards up a plastic tube (3 cm inner diameter, 30 cm length), and impairment of motor performance was indicated by the inability of the mice to climb backward up the transparent tube within 60 s. The acute adverse effects of oxcarbazepine administered alone were expressed as its median toxic doses (TD_{50} values in mg/kg), representing the doses at which oxcarbazepine 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 oxcarbazepine were challenged with the chimney test. A log-probit dose-response relationship line was constructed on the basis of

the percentage of mice showing motor deficits by means of the log-probit method according to Litchfield and Wilcoxon [31]. Additionally, mice were administered combinations of oxcarbazepine and diltiazem (at doses of 1.25, 2.5 and 5 mg/kg), and then subjected to the chimney test, which allowed determination of motor performance in mice. In this test, oxcarbazepine was administered at doses ranging between 50- 90 mg/kg. This experimental procedure has been described in detail in our earlier studies [26, 29, 32].

Protective index. The protective index (PI) for oxcarbazepine administered alone and in combination with diltiazem 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 an index of the margin of safety and tolerability between anticonvulsant doses and doses of antiepileptic drugs exerting acute adverse effects (e.g. sedation, motor coordination impairment, ataxia, or other neurotoxic manifestations) [34].

Measurement of total brain oxcarbazepine concentration. The measurement of total brain concentration of oxcarbazepine was undertaken at a dose of oxcarbazepine which corresponded to its ED_{50} value from the MES test for the combination of oxcarbazepine with diltiazem (at a dose of 5 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 using distilled water (1:2 w/vol) in an Ultra-Turrax T8 homogenizer (IKA-Werke, Staufen, Germany). The homogenates were centrifuged at 10,000×*g* for 10 min. The supernatant samples (400 µl) were analyzed by HPLC for oxcarbazepine content. The chromatograph (Laboratorij Pristroje, Prague, Czech Republic) was equipped with a 305 micropump (LCP 3001) and an ultraviolet (UV) detector (HP 1050) with a sensitivity setting of 0.1 AUFS (absorbance units full scale) and a time constant of 0.1 s. The Rheodyne 7125 injector valve with a 100 µl sample loop was used for sample injection. For HPLC, a stainless-steel Hypersil ODS column (200 × 4.6 mm) was used at an ambient temperature of 22°C. The mobile phase was methanol: acetonitrile: acetate buffer (5 mM acetic acid/ 50 mM sodium acetate); 15:15:70 vol/vol/vol (Baker HPLC grade). The mobile phase flow rate was 1 ml/min. Brain supernatants of 400 µl were added to 400 µl of distilled water and shaken. Subsequently, the external standard of 0.15 µg of carbamazepine in 150 µl of methanol: water solution (1:1) was added. The samples were shaken again, and to each sample a volume of 4 ml of tertbuthyl-methyl ether was added and centrifuged for 4 min at 2,000×*g*. The samples were evaporated to dryness under a vacuum system, redissolved in 1 ml of naphthyl ether (HPLC, Aldrich), and again evaporated to dryness under a vacuum system. The remains were redissolved in 100 µl of the mobile phase; samples of 50 µl were then injected into the chromatograph. Oxcarbazepine concentrations were calculated according to the external standard method using the original Gilson 715 software. The amount of oxcarbazepine was determined by comparing their peak area with the peak area of the external standard (carbamazepine). The wave excitation and emission parameters for detection of oxcarbazepine were 220 and 310 nm, respectively. The limit of detection of the method was 0.01 µg/ml and the within-batch and between-batch precisions were <5% and <6%, respectively. Total brain concentrations of oxcarbazepine were expressed in µg/ml of brain supernatants as means ± S.D. of at least 8 separate brain preparations.

Statistical analysis. The ED_{50} and TD_{50} values, with their 95% confidence limits, were calculated by computer-assisted log-probit analysis according to Litchfield and Wilcoxon [31]. Subsequently, the respective 95% confidence limits were transformed to their corresponding standard errors (SE), as described previously [29]. 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 [26, 27, 35]. Total brain oxcarbazepine concentrations were statistically compared using the unpaired Student's *t*-test. Differences among values were considered statistically significant if $P<0.05$. All statistical tests were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Effects of diltiazem on the protective action of oxcarbazepine in the mouse maximal electroshock-induced seizure model. Oxcarbazepine administered alone (i.p.) produced a clear-cut anticonvulsant effect against MES-induced seizures in mice, and its ED_{50} value is presented in Table 1. Diltiazem co-administered with oxcarbazepine enhanced dose-dependently the antielectroshock action of the latter drug by reducing its ED_{50} value in the MES test. One-way ANOVA, followed by the *post-hoc* Tukey-Kramer test for multiple comparisons, revealed that diltiazem at a dose of 5 mg/kg significantly decreased the ED_{50} value of oxcarbazepine from 14.25 to 9.87 mg/kg ($P<0.01$; Table 1). Diltiazem at lower doses of 1.25 and 2.5 mg/kg also reduced the ED_{50} value of oxcarbazepine from 14.25 to 11.54, and 11.32 mg/kg, respectively (Table 1). Statistical analysis of data, however, did not attain significance with one-way ANOVA.

Influence of diltiazem on the acute adverse-effect profile of oxcarbazepine in the chimney test in mice. Oxcarbazepine administered alone produced a clear-cut motor coordination impairment in the chimney test in mice; its TD_{50} value is presented in Table 1. When diltiazem at 1.25, 2.5 and 5 mg/kg was co-administered with oxcarbazepine, it did not significantly affect the acute adverse effects of oxcarbazepine in the chimney test, and the experimentally derived TD_{50} values of oxcarbazepine in combination with diltiazem were almost similar to the TD_{50} value denoted for oxcarbazepine alone (Table 1). With one-way ANOVA, followed by the *post-hoc* Tukey-Kramer test for multiple comparisons, the TD_{50} values for oxcarbazepine combined with diltiazem at 1.25, 2.5 and 5 mg/kg did not significantly differ from that denoted for oxcarbazepine alone (Table 1).

Effect of diltiazem on the protective index of oxcarbazepine. The PI (as a ratio of TD_{50} and ED_{50} values) for oxcarbazepine administered alone was 5.20 (Table 1). The PI values for the combination of oxcarbazepine with diltiazem at doses of 1.25, 2.5 and 5 mg/kg increased to 6.02, 6.40 and 6.37, respectively (Table 1).

Table 1 Anticonvulsant and acute adverse effects of oxcarbazepine administered alone and in combination with diltiazem in the maximal electroshock seizure model and chimney test in mice.

Treatment (mg/kg)	ED_{50}	n	SE	TD_{50}	n	SE	PI
Oxcarbazepine + vehicle	14.25 (12.79-15.89)	16	0.790	74.16 (65.48-83.98)	16	4.705	5.20
Oxcarbazepine + Diltiazem (1.25)	11.54 (10.15-13.13)	16	0.759	69.52 (61.61-78.45)	24	4.282	6.02
Oxcarbazepine + Diltiazem (2.5)	11.32 (9.96-12.86)	16	0.737	72.50 (64.97-80.89)	16	4.052	6.40
Oxcarbazepine + Diltiazem (5)	9.87 (8.31-11.71)**	24	0.862	62.91 (54.85-72.14)	16	4.394	6.37
One-way ANOVA	F (3;68) = 5.067; P = 0.0032			F (3;68) = 1.116; P = 0.3488			

Results are presented as: 1) median effective doses (ED_{50} in mg/kg; with 95% confidence limits in parentheses) required to protect 50% of animals tested against MES-induced seizures, and 2) median toxic doses (TD_{50} in mg/kg; with 95% confidence limits in parentheses) required to impair motor coordination in 50% of animals challenged with the chimney test. Both ED_{50} and TD_{50} values were calculated by use of log-probit method [31], followed by the method transforming 95% confidence limits to their corresponding SE [29]. Diltiazem and oxcarbazepine were suspended in 1% aqueous solution of Tween 80 and administered systemically (i.p.) as follows: diltiazem at 60 min. and oxcarbazepine at 30 min. before the MES and chimney tests. Statistical analysis of data was performed with one-way ANOVA, followed by the *post-hoc* Tukey-Kramer test for multiple comparisons.
n – number of animals at those doses, whose anticonvulsant and acute adverse effects ranged between 16% and 84%;
SE – standard error of ED_{50} or TD_{50}.
Protective index (PI) values were calculated by dividing the TD_{50} by the respective ED_{50} values.
F – F-statistics from one-way ANOVA;
P – probability.
** P<0.01 vs. respective control group (oxcarbazepine + vehicle-treated animals).

Influence of diltiazem on total brain concentration of oxcarbazepine. With HPLC technique it was found that the total brain concentration of oxcarbazepine administered alone (at a dose of 9.9 mg/kg) was 0.52 ± 0.11 μg/ml, and did not differ significantly from that for the combination of oxcarbazepine (9.9 mg/kg) with diltiazem (5 mg/kg), which amounted to 0.54 ± 0.10 μg/ml (results not shown).

DISCUSSION

It was found that diltiazem (at 5 mg/kg) significantly enhanced the antiseizure action of oxcarbazepine, whereas, the drug at lower doses of 1.25 and 2.5 mg/kg had no significant effect on the anticonvulsant activity of oxcarbazepine in the mouse MES test. Thus, our findings are in agreement with those reported earlier showing that diltiazem enhanced the antiseizure action of carbamazepine, phenytoin, phenobarbital, topiramate and valproate, but not that of lamotrigine in the MES test in mice [5, 8, 26, 27]. It should be stressed that the doses of diltiazem used in this study (up to 5 mg/kg) did not affect the threshold for electroconvulsions, because we have recently reported that diltiazem at doses up to 10 mg/kg had no impact on the electroconvulsive threshold in mice [26]. Moreover, in the present study, the ED_{50} and TD_{50} values of oxcarbazepine administered alone and in combination with diltiazem were statistically analyzed with one-way ANOVA, followed by the *post-hoc* Tukey-Kramer test for multiple comparisons. In contrast, the results presented earlier were compared separately with their control ED_{50} values using the log-probit method only [5, 8]. Quite recently, it has been recommended to statistically analyze data obtained from log-probit method with one-way ANOVA, followed by the *post-hoc* test for multiple comparisons [36]. Thus, one-way ANOVA has gained priority during the statistical comparisons of data among several groups. The crucial differences between these two statistical methods have been presented and discussed in more detail elsewhere [36]. This is why in this study, diltiazem was administered at doses up to 5 mg/kg, whereas in those by Czuczwar et al. [5, 8], diltiazem was administered at doses up to 2.5 mg/kg.

To scientifically explain the appearance of the favourable interaction between oxcarbazepine and diltiazem against MES-induced seizures, one should consider the molecular mechanisms of the action of both drugs. Regarding the anticonvulsant effect of oxcarbazepine, it has been documented that the drug acts at voltage-dependent sodium channels to decrease the presynaptic release of the excitatory neurotransmitter glutamate [18, 21]. Oxcarbazepine blocks high voltage activated N-type calcium channels [37]. In contrast, diltiazem is considered to be the L-type calcium channel antagonist [38]. Thus, it seems that diltiazem enhanced the antiseizure action of oxcarbazepine due to the complementary mechanisms of action, because both L- and N-type calcium channel antagonists (oxcarbazepine and diltiazem) might cooperate in terms of reduction of seizure activity in the MES test in mice. Although this hypothesis can readily explain the observed effects between oxcarbazepine and diltiazem in the MES test in mice, more advanced studies are required to elucidate this phenomenon.

Furthermore, diltiazem did not affect the acute adverse-effect profile of oxcarbazepine in the chimney test in mice and the TD_{50} values of oxcarbazepine, as determined in the chimney test in mice, were unchanged after co-administration of diltiazem. The experimental determination of TD_{50} and ED_{50} values allowed calculation of the PI values for oxcarbazepine administered alone and in combination with diltiazem. In preclinical studies, the PI values describe the margin of safety and tolerability of drug doses, providing information about the range of drug doses producing neurotoxic effects - i.e. the doses that impair motor coordination in the chimney test in mice - and drug doses, offering the antiseizure protection against MES-induced seizures [34]. The comparison of PI values for oxcarbazepine revealed that the PI values for the combination of oxcarbazepine with diltiazem were greater than the PI value for oxcarbazepine administered alone. On the other hand, it should be stressed that the combined administration of diltiazem with oxcarbazepine did not enhance the acute adverse effects produced by oxcarbazepine in the chimney test in mice. Therefore, one can indirectly ascertain that the blockade of L- and N-type calcium channels does not seem to be responsible for the induction and potentiation of acute

adverse effects in the chimney test in mice. It is highly likely that other molecular mechanisms of action of both drugs are responsible for their acute adverse effects in mice.

Pharmacokinetic estimation of total brain oxcarbazepine concentrations revealed that diltiazem had no significant impact on total brain oxcarbazepine concentrations in experimental animals, and thus, the observed interaction between oxcarbazepine and diltiazem was pharmacodynamic in nature. This finding is in agreement with previous reports documenting no pharmacokinetic interactions between diltiazem and conventional antiepileptic drugs or topiramate in mice [5, 8, 27].

In conclusion, diltiazem enhanced the anticonvulsant action of oxcarbazepine against MES-induced seizures, produced no acute adverse effects when combined with oxcarbazepine in the chimney test, and had no impact on total brain concentrations of the studied antiepileptic drug in experimental animals. If the results from this study can be extrapolated to the clinical setting, a novel therapeutic option in the management of epilepsy may be created for epileptic patients. Thus, diltiazem deserves more attention from a preclinical point of view, as a potentially favourable drug that could be applied in patients treated with oxcarbazepine, who additionally need a calcium channel antagonist treatment for reasons other than epilepsy.

ACKNOWLEDGEMENTS

This study was supported by Grant KBN 2P05D 096 29 from the State Committee for Scientific Research, Warsaw, Poland. The authors express their thanks to Mr. W. Zgrajka (Institute of Agricultural Medicine, Lublin, Poland) for skilful determination of the brain concentrations of oxcarbazepine.

REFERENCES

1. Heinemann U, Hamon B: Calcium and epileptogenesis. *Exp Brain Res* 1986, **65**, 1-10.
2. Czuczwar SJ, Turski WA, Kleinrok Z: Interactions of excitatory amino acid antagonists with conventional antiepileptic drugs. *Metab Brain Dis* 1996, **11**, 143-152.
3. 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.
4. 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.
5. 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.
6. De Sarro GB, De Sarro A, Trimarchi GR, Nistico G: Effects of some calcium antagonists upon the activity of common antiepileptic compounds on sound-induced seizures in DBA/2 mice. *Gen Pharmacol* 1992, **23**, 75-82.
7. De Sarro G, De Sarro A, Federico F, Meldrum BS: Anticonvulsant properties of some calcium antagonists on sound-induced seizures in genetically epilepsy prone rats. *Gen Pharmacol* 1990, **21**, 769-778.
8. Czuczwar SJ, Małek U, Kleinrok Z: Influence of calcium channel inhibitors upon the anticonvulsant efficacy of common antiepileptics against pentylenetetrazol-induced convulsions in mice. *Neuropharmacology* 1990, **29**, 943-948.
9. Wajima Z, Yoshikawa T, Ogura A, Imanaga K, Shiga T, Inoue T, Ogawa R: The effects of diltiazem on hemodynamics and seizure duration during electroconvulsive therapy. *Anesth Analg* 2001, **92**, 1327-1330.
10. 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.
11. De Falco FA, Bartiromo U, Majello L, Di Geronimo G, Mundo P: Calcium antagonist nimodipine in intractable epilepsy. *Epilepsia* 1992, **33**, 343-345.
12. Nakane Y, Seino M, Yagi A, Kaji S, Yamauchi T: Effects of flunarizine therapy on intractable epilepsy. *Arzneimittelforschung* 1989, **39**, 793-798.
13. 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.
14. 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.
15. Kułak W, Sobaniec W, Wojtal K, Czuczwar SJ: Calcium modulation in epilepsy. *Pol J Pharmacol* 2004, **56**, 29-41.
16. 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.
17. Hotson JR, Prince DA: A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. *J Neurophysiol* 1980, **43**, 409-419.
18. Macdonald RL, Greenfield LJ: Mechanisms of action of new antiepileptic drugs. *Curr Opin Neurol* 1997, 10, 121-128.
19. Trojnar MP, Kimber-Trojnar Z, Trojnar MK, Łuszczki JJ, Czuczwar SJ: Clinical considerations on the relationship between epilepsy and cardiovascular diseases. *JPCCR* 2007**, 1,** 6-8.
20. Brodie MJ, Schachter SC: Fast Facts. Epilepsy (2nd ed.). *Health Press*, Oxford 2001.
21. McLean MJ, Schmutz M, Wamil AW, Olpe HR, Portet C, Feldmann KF: Oxcarbazepine: mechanism of action. *Epilepsia* 1994, **35**, 5-9.
22. Calabresi P, De Murtas M, Stefani A, Pisani A, Sancesario G, Mercuri NB, Bernardi G: Action of GP 47779, the active metabolite of oxcarbazepine, on the corticostriatal system. I. Modulation of corticostriatal synaptic transmission. *Epilepsia* 1995, **36**, 990-996.
23. Stefani A, Pisani A, DeMurtas M, Mercuri NB, Marciani MG, Calabresi P. Action of GP 47779, the active metabolite of oxcarbazepine on the corticostriatal system. II. Modulation of High-Voltage-Activated Calcium Currents. *Epilepsia* 1995, **36**, 997-1002.
24. Stefani A, Spadoni F, Bernardi G: Voltage-activated calcium channels: targets of antiepileptic drug therapy? *Epilepsia* 1997**, 38,** 959-965.
25. Waldmeier PC, Martin P, Stöcklin K, Portet C, Schmutz M: Effect of carbamazepine, oxcarbazepine and lamotrigine on the increase in extracellular glutamate elicited by veratridine in rat cortex and striatum. *Naunyn-Schmiedeberg's Arch Pharmacol* 1996, **354**, 164-172.
26. Łuszczki JJ, Trojnar MK, Trojnar MP, Kimber-Trojnar Ż, 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.
27. Łuszczki JJ, Trojnar MK, Trojnar MP, Kimber-Trojnar Ż, Szostakiewicz B, Zadrożniak A, Borowicz KK, Czuczwar SJ: Effects of amlodipine, diltiazem, and verapamil on the anticonvulsant action of topiramate against maximal electroshock-induced seizures in mice. *Can J Physiol Pharmacol* 2008, **86**, 113-121.
28. 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.
29. Łuszczki JJ, Borowicz KK, Świąder M, Czuczwar SJ: Interactions between oxcarbazepine and conventional antiepileptic drugs in the maximal electroshock test in mice: an isobolographic analysis. *Epilepsia* 2003, **44**, 489-499.
30. Łuszczki JJ: Isobolographic analysis of interaction between oxcarbazepine and valproate in pentylenetetrazole-induced seizures in mice. *JPCCR* 2008**, 2,** 40-45.
31. Litchfield JT, Wilcoxon F: A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* 1949, **96,** 99-113.
32. Łuszczki JJ, Zadrożniak A, Barcicka-Kłosowska B, Bednarski J, Misiuta-Krzesińska M, Filip D, Zwoliński J, Czernecki R: Influence of 7-nitroindazole and NG-nitro-L-arginine on the anticonvulsant activity of loreclezole in maximal electroshock-induced seizures in mice. *JPCCR* 2007, **1**, 146-149.

33. Boissier JR, Tardy J, Diverres JC: Une nouvelle méthod e simple pour explorer l'action tranquilisante: le test de la cheminée. *Med Exp (Basel)* 1960, **3**, 81-84.
34. 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.
35. Hinkle DE, Wiersma W, Jurs SG: Applied statistics for the behavioral science, 3rd ed.). *Houghton Mifflin Company*, Boston 1994.
36. Łuszczki JJ, Czuczwar SJ: How significant is the difference between drug doses influencing the threshold for electroconvulsions? *Pharmacol Rep* 2005, **57**, 782-786.
37. Stefani A, Spadoni F, Bernardi G: Voltage-activated calcium channels: targets of antiepileptic drug therapy? *Epilepsia* 1997, **38**, 959-965.
38. Miljanich GP, Ramachandran J: Antagonists of neuronal calcium channels: structure, function, and therapeutic implications. *Annu Rev Pharmacol Toxicol* 1995, **35**, 707-734.