ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 3, 9 SECTIO DDD 2011

[1]Isobolographic Analysis Laboratory, Institute of Agricultural Medicine, Lublin, Poland
[2]Department of Pathophysiology, Medical University of Lublin, Lublin, Poland

MARTA ANDRES-MACH[1], JAROGNIEW J. ŁUSZCZKI[1,2]

# *Characterization of the anticonvulsant properties of the various p-isopropoxyphenyl-succinimide derivatives in the maximal electroshock-induced seizure test in mice*

Charakterystyka właściwości przeciwdrgawkowych różnych pochodnych p-izoporopoksyfenylo-bursztynimidów w teście maksymalnego wstrząsu elektrycznego u myszy

## INTRODUCTION

Refractory epilepsy is a type of epilepsy defined by an inadequate control of seizures, despite the optimal treatment with conventional drugs. Patients suffering from refractory epilepsy require treatment with more than one antiepileptic drug (AED) [2, 5, 6]. It is widely known that AED therapy with more than one AED may increase a risk of developing of side effects, which may cause alteration in the process of neurogenesis, but also in learning and memory functions as well as may reduce patient's quality of living.

Experimental studies, using different anticonvulsant screening tests in animals, are trying to find new AEDs with more potent anticonvulsant activity, increased neuronal protection and reduced side effects. Various studies on combinations between AEDs with either other AEDs [3, 13, 16], potential antiepileptic agents [15, 12, 2] or herbal substances with anticonvulsant properties [9, 10, 11, 14], have been performed to find out some safe and beneficial anticonvulsant compounds. Results so far obtained from in vivo screening maximal electroshock-induced seizure (MES) test in rodents indicate that N-morpholinemethyl derivative of m-bromophenylsuccinimide, N-piridyl-substituted succinimide and 3-cyclohexylsuccinimide show evident anticonvulsant activity [7, 4, 17].

In the present study we used the mouse MES test to perform the anticonvulsant screening of a series of 15 p-isopropoxyphenylsuccinimide derivatives, whose full names and chemical formulas are presented in Table 1. The MES test is considered as an experimental model of tonic-clonic seizures and partial convulsions with or without secondary generalization in humans [8]. Therefore, this model is very sensible as a first screening test in verification of the possible anticonvulsant properties of the succinimide derivatives.

Table 1. Chemical formulas of the various p-isopropoxyphenylsuccinimide derivatives

| No. | Name | Formula | M.W. |
|---|---|---|---|
| 1 | N-Phenyl-p-isopropoxyphenylsuccinimide | C19H19NO3 | 309.347 |
| 2 | N-(p-Methoxyphenyl)-p-isopropoxyphenylsuccinimide | C20H21NO4 | 339.377 |
| 3 | N-(p-Acetylphenyl)-p-isopropoxyphenyl-succinimide | C21H21NO4 | 351.387 |
| 4 | N-(p-Dimethylaminophenyl)-p-isopropoxyphenylsuccinimide | C21H24N2O3 | 352.420 |
| 5 | N-(m-Bromophenyl)-p-isopropoxyphenyl-succinimide | C19H18BrNO3 | 388.246 |
| 6 | N-(o-Carboxyphenyl)-p-isopropoxyphenylsuccinimide | C20H19NO5 | 353.357 |
| 7 | N-(m-Carboxyphenyl)-p-isopropoxyphenylsuccinimide | C20H19NO5 | 353.357 |
| 8 | N-(p-Carboxyphenyl)-p-isopropoxyphenylsuccinimide | C20H19NO5 | 353.357 |
| 9 | N-(m-Carboxy-p-hydroxyphenyl)-p-isopropoxyphenylsuccinimide | C20H19NO6 | 369.357 |
| 10 | N-(m-Bromoanilinomethyl)-p-isopropoxyphenylsuccinimide | C20H21BrN2O3 | 417.299 |
| 11 | N-(p-Acetylanilinomethyl)-p-isopropoxyphenylsuccinimide | C22H24N2O4 | 380.430 |
| 12 | N-(o-Carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide | C21H22N2O5 | 382.410 |
| 13 | N-(m-Carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide | C21H22N2O5 | 382.410 |
| 14 | N-(p-Carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide | C21H22N2O5 | 382.410 |
| 15 | N-(p-Ethoxycarbonylphenyl)-p-isopropoxyphenylsuccinimide | C23H26N2O5 | 410.460 |

M.W. – molecular weight

## MATERIALS AND METHODS

Animals and experimental conditions. All experiments were performed on adult male albino Swiss mice weighing 22-26 g. The mice were kept in colony cages with free access to food and tap water ad libitum, under standardized housing conditions (natural light-dark cycle, temperature was 21 ± 1ºC). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 mice. Each mouse was used only once. All tests were performed between 9.00 a.m. and 2.00 p.m. Procedures involving animals and their care were conducted in conformity 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 listed below conformed also to the *Guide for the Care and Use of Laboratory Animals* and were approved by the First Local Ethics committee at the Medical University in Lublin and the Second Local Ethics Committee at the University of Life Science in Lublin.

D r u g s. The following compounds were used: N-phenyl-p-isopropoxyphenylsuccinimide, N-(p-methoxyphenyl)-p-isopropoxyphenylsuccinimide, N-(p-acetylphenyl)-p-isopropoxyphenylsuccinimide, N-(p-dimethylaminophenyl)-p-isopropoxyphenylsuccinimide, N-(m-bromophenyl)-p-isopropoxyphenylsuccinimide, N-(o-carboxyphenyl)-p-isopropoxyphenylsuccinimide, N-(m-carboxyphenyl)-p-isopropoxyphenylsuccinimide, N-(p-carboxyphenyl)-p-isopropoxyphenylsuccinimide, N-(m-carboxy-p-hydroxyphenyl)-p-isopropoxyphenylsuccinimide, N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide, N-(p-acetylanilinomethyl)-p-isopropoxyphenylsuccinimide, N-(o-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide, N-(m-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide, N-(p-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide, N-(p-ethoxycarbonylphenyl)-p-isopropoxyphenylsuccinimide

(all of them were synthesized by Dr. S.L. Kocharov, from Mndjoyan's Institute of FineOrganic Chemistry of the National Academy of Sciences of the Republic of Armenia, Yerevan, Armenia). All compounds were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally (i.p.) 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 at a constant dose of 300 mg/kg at 4 pretreatment times: 15, 30, 60 and 120 min. before the MES test.

M a x i m a l e l e c t r o s h o c k s e i z u r e t e s t. Electroconvulsions were produced by means of an alternating current (25 mA, 500 V, 50 Hz, 0.2 s stimulus duration) delivered via ear-clip electrodes by a generator (Rodent Shocker, Type 221; Hugo Sachs Electronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hindlimb extension.

## RESULTS

### Time-course of the anticonvulsant effect of the studied p-isopropoxyphenylsuccinimide derivatives in the maximal electroshock-induced seizure test in mice

From all the studied p-isopropoxyphenylsuccinimide derivatives three showed anticonvulsant protection against tonic hindlimb extension in animals subjected to the MES-induced seizures. For N-(o-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide, the percentage of anticonvulsant protection in mice ranged between 62.5-87.5% (Table 2) at four following times. N-(m-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide protected the animals against maximal electroconvulsions in 87.5%; 75%; 75% and 62.5% at four pretreatment times, and N-(p-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide showed anticonvulsant properties in 25%; 37.5%; 37.5%; and 25% after 15, 30, 60 and 120 min, respectively (Table 2). In case of the remaining studied compounds, they did not produce the anticonvulsant action in the mouse MES test (Table 2).

Table 2. Time course of the anticonvulsant effect of the studied p-isopropoxyphenylsuccinimide derivatives in the maximal electroshock-induced seizure test in mice

| Substance No. | Anticonvulsant effect (%) | | | |
|---|---|---|---|---|
| Time (min.) | 15 | 30 | 60 | 120 |
| 1 | 0 | 12.5 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 |
| 8 | 12.5 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 |
| 12 | 62.5 | 75 | 87.5 | 62.5 |
| 13 | 87.5 | 75 | 75 | 62.5 |
| 14 | 25 | 37.5 | 37.5 | 25 |
| 15 | 0 | 0 | 0 | 0 |

Results are presented as percentage of protection against tonic hind limb extension in animals subjected to the MES-induced seizures. The studied p-isopropoxyphenylsuccinimide derivatives were administered i.p. at a constant dose of 300 mg/kg at 4 pretreatment times: 15, 30, 60 and 120 min. before the MES test.

## DISCUSSION

The results indicate that three out of 15 p-isopropoxyphenylsuccinimide derivatives tested in the current study possess strong anticonvulsant properties. N-(o-carboxyanilinomethyl)-p-isopropoxyphenyl-succinimide at 300 mg/kg displayed a 87.5% of the protection against tonic seizure at 60 min after its i.p. administration. N-(m-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide exhibited also 87.5% of the anticonvulsant effect, but at 15 min after its i.p. administration. The lowest, but still significant anticonvulsant properties were shown for N-(p-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide (37% at 30 and 60 min after its i.p. administration).

Anticonvulsant properties of succinimide derivatives have been tested for last over 30 years. One of the first results was obtained in 1977 by Lange and coworkers [7]. They have synthesized and tested a series of phenylsuccinimide derivatives. According to their results, N-morpholinemethyl derivative of m-bromophenylsuccinimide was the most interesting drug, mainly because of its long period of activity, very strong anticonvulsant action against pentylenetetrazole (PTZ)-induced clonic seizures and good protection against MES-induced seizures. Amir and coworkers [1] have synthesized and tested a number of N-(5-alkyl-1,3,4-thiadiazol-2-yl)-alpha-aryl/alkyl and N-(cyclohexyl)-alpha-aryl/alkyl succinimides. Some of them were found to be 10 to 50% active against PTZ-induced seizures in mice at a dose of 80 mg/kg. Other studies made by Zejc and colleagues [17] with using N-pyridyl-substituted succinimides also confirmed their anticonvulsant properties in the MES- and the PTZ-

induced seizures in mice. Likewise, results obtained by Kaminski and coworkers [4] clearly showed the anticonvulsant properties of the 1-(2-pyridinyl)-succinimides in the MES and PTZ tests.

## CONCLUSIONS

The screening test used in our study enabled the selection of the potent anticonvulsant substances, which are very important for further more advanced electrophysiological and neurochemical studies. Although the mechanisms of action for these substances are unknown so far, their anticonvulsant properties clearly indicate that additional studies are necessary to better known molecular and neuronal efficacy of these potential anticonvulsants. A better knowledge certainly will make possible the use of these substances as a supplementary compound in further clinical settings.

**Acknowledgements.** This study was supported by grants from the Medical University of Lublin and Institute of Agricultural Medicine (Lublin, Poland). The authors express their thanks to Dr. S.L. Kocharov (Yerevan, Armenia) for synthesis of various p-isopropoxyphenyl-succinimide derivatives. Professor J.J. Luszczki is a Recipient of the Fellowship for Leading Young Researchers from the Ministry of Science and Higher Education (Warszawa, Poland).

## REFERENCES

1. Amir M., Singh E.: Some new N-substituted alpha acryl/alkyl succinimides as possible anticonvulsants. Pharmazie 46, 705, 1991.
2. Czuczwar S.J., Borowicz K.K.: Polytherapy in epilepsy: the experimental evidence. Epilepsy Res. 52, 15, 2002
3. Jonker D.M., Voskuyl R.A., Danhof M.: Synergistic combinations of anticonvulsant agents what its he evidence from animal experiments? Epilepsia 48, 412, 2007.
4. Kaminski K., Obniska J.: Synthesis and properties of new 1-(2-pyridinyl)-3-substituted pyrrolidine-2, 5-dione derivatives. Acta Pol. Pharm. 65, 457, 2008.
5. Kwan P., Brodie M.J.: Early identification of refractory epilepsy. N. Engl. J. Med. 342, 314, 2000.
6. Kwan P., Brodie M.J.: Epilepsy after the first drug fails: substitution or add-on? Seizure 9, 464, 2000.
7. Lange J., Rump S., Gałecka E., et al.: Synthesis and properties of new cyclic derivatives of succinic acid with anticonvulsant activity. Pharmazie 32, 82, 1997.
8. Löscher W., Fassbender C.P., Nolting B.: The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. Epilepsy Res. 8, 79, 1991.
9. Łuszczki J.J., Andres-Mach M., Gleńsk M., et al.: Anticonvulsant effects of four linear furanocoumarins, bergapten, imperatorin, oxypeucedanin, and xanthotoxin, in the mouse maximal electroshock-induced seizure model: a comparative study. Pharmacol. Rep. 62, 1231, 2010.
10. Łuszczki J.J., Czuczwar P., Cioczek-Czuczwar A., et al.: Effect of arachidonyl-2'-chloroethylamide, a selective cannabinoid CB1 receptor agonist, on the protective action of the various antiepileptic drugs in the mouse maximal electroshock-induced seizure model. Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 18, 2010.

11. Łuszczki J.J., Andres-Mach M., Barcicka-Klosowska B., et al.: Effects of WIN 55,212-2 mesylate (a synthetic cannabinoid) on the protective action of clonazepam, ethosuximide, phenobarbital and valproate against pentylenetetrazole-induced clonic seizures in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry DOI:10.1016/j.pnpbp.2011.07.001, 2011.
12. Łuszczki J.J., Antkiewicz-Michaluk L., Czuczwar S.J.: Isobolographic analysis of interactions between 1-methyl-1,2,3,4-tetrahydroisoquinoline and four conventional antiepileptic drugs in the mouse maximal electroshock-induced seizure model. Eur. J. Pharmacol. 602, 298, 2009.
13. Łuszczki J.J., Andres M.M., Czuczwar P., et al.: Levetiracetam selectively potentiates the acute neurotoxic effects of topiramate and carbamazepine in the rotarod test in mice. Eur. Neuropsychopharmacol. 15, 609, 2005.
14. Łuszczki J.J., Andres-Mach M., Cisowski W., et al.: Osthole suppresses seizures in the mouse maximal electroshock seizure model. Eur. J. Pharmacol. 607, 107, 2009.
15. Łuszczki J.J., Sacharuk A., Wojciechowska A., et al.: 7-Nitroindazole enhances dose-dependently the anticonvulsant activities of conventional antiepileptic drugs in the mouse maximal electroshock-induced seizure model. Pharmacol. Rep. 58, 660, 2006.
16. Łuszczki J.J., Andres M.M., Czuczwar P., et al.: Pharmacodynamic and pharmacokinetic characterization of interactions between levetiracetam and numerous antiepileptic drugs in the mouse maximal electroshock seizure model: an isobolographic analysis. Epilepsia 47, 10, 2006.
17. Zejc A., Obniska J., Wilimowski M., et al.: Synthesis and properties of some arylsuccinate methylpyridylimides. Pol. J. Pharmacol. Pharm. 42, 69, 1990.

## SUMMARY

The aim of the study was to examine whether some p-isopropoxyphenylsuccinimide derivatives display anticonvulsant properties in the mouse maximal electroshock seizure (MES) model. Fifteen p-isopropoxyphenylsuccinimide derivatives used in this study were administered i.p. at a constant dose of 300 mg/kg at four pretreatment times: 15, 30, 60 and 120 min and mice were subjected to electroconvulsions by applying an alternating current (25 mA, 50 Hz, 500 V, 0.2 s of stimulus duration) via ear-clip electrodes. Three out of 15 p-isopropoxyphenylsuccinimide derivatives showed strong anticonvulsant properties against MES-induced seizures. The protection against maximal electroconvulsions was noticed for: N-(o-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide (o-CAMIPPS), N-(m-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide (m-CAMIPPS) and N-(p-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide (p-CAMIPPS). All compounds, except for m-CAMIPPS, showed the highest percentage of the anticonvulsant activity at 60 min after i.p. administration. Only m-CAMIPPS displayed the highest anticonvulsant activity at 15 min after its i.p. administration.

Anticonvulsant properties of the selected p-isopropoxyphenylsuccinimide derivatives indicate that these substances certainly are worthy of further and more detailed studies in other animal seizure models to determine their anticonvulsant profile in preclinical studies.

*Keywords:* epilepsy, p-isopropoxyphenylsuccinimide derivatives, maximal electroshock-induced seizure test,

STRESZCZENIE

Celem przeprowadzonych badań było określenie, czy niektóre pochodne p-izoporopoksyfenylobursztynimidów wykazują właściwości przeciwdrgawkowe w teście maksymalnego wstrząsu elektrycznego myszy. Piętnaście pochodnych p-izoporopoksyfenylobursztynimidów zastosowanych w doświadczeniu podawane było dootrzewnowo w stałej dawce 300 mg/kg w czterech czasach: 15, 30, 60 i 120 min a następnie myszy poddane zostały wstrząsom elektrycznym przy użyciu prądu zmiennego (25 mA, 50 Hz, 500 V, 0,2 s-czas trwania stymulacji) dostarczanym poprzez elektrody uszne. Trzy z piętnastu pochodnych izopropoksyfenylobursztynimidów wykazało silne właściwości przeciwdrgawkowe w teście drgawek MES. Ochronę przeciw drgawkom wstrząsu elektrycznego wykazały: N-(o-karboksyanilinometylo)-p-izopropoksyfenylobursztynimid (o-CAMIPPS), N-(m-karboksyanilinometylo)- p-izopropoksyfenylobursztynimid (m-CAMIPPS) oraz N-(p- karboksyanilinometylo)- p-izopropoksyfenylobursztynimid (p-CAMIPPS). Wszystkie substancje za wyjątkiem m-CAMIPPS wykazały najwyższy procent aktywności przeciwdrgawkowej po 60 min od podania dootrzewnowego. Tylko m-CAMIPPS wykazał najwyższą ochronę przeciwdrgawkową 15 min od jego podania dootrzewnowego. Właściwości przeciwdrgawkowe wybranych pochodnych p-izoporopoksyfenylobursztynimidów wskazują, że te substancje z pewnością są warte dalszych i bardziej zaawansowanych badań w innych modelach padaczkowych w celu określenia ich profilu przeciwkdrgawkowego w badaniach przedklinicznych.

*Słowa kluczowe:* padaczka, pochodne -izoporopoksyfenylobursztynimidów, test drgawek indukowanych maksymalnym wstrząsem elektrycznym.