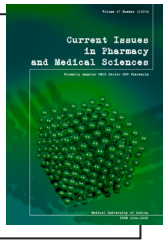
Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/



Effect of N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide on the anticonvulsant action of four classical antiepileptic drugs in the mouse maximal electroshock-induced seizure model

Jarogniew J. Luszczki[1,2*], Ewa Marzeda[1], Maria W. Kondrat-Wrobel[2], Daniel Pyrka[2], Sergey L. Kocharov[3], Magdalena Florek-Luszczki[4]

[1] Isobolographic Analysis Laboratory, Institute of Rural Health, Jaczewskiego 2, 20-950 Lublin, Poland
[2] Department of Pathophysiology, Medical University of Lublin, Ceramiczna 1, 20-150 Lublin, Poland
[3] Mndjoyan's Institute of Fine Organic Chemistry, National Academy of Sciences, Azatutyan Avenue 26, 375014 Yerevan, Republic of Armenia
[4] Department of Public Health, Institute of Rural Health, Jaczewskiego 2, 20-950 Lublin, Poland

ARTICLE INFO

Received 12 March 2014
Accepted 08 April 2014

KEYWORDS:
antiepileptic drugs,
maximal electroshock-induced seizures,
N-(m-bromoanilino-methyl)-p-isopropoxyphenylsuccinimide.

ABSTRACT

The purpose of this study was to determine the effects of N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide (BAM-IPPS – a new succinimide derivative) on the protective action of four classical antiepileptic drugs (AEDs: carbamazepine [CBZ], phenobarbital [PB], phenytoin [PHT] and valproate [VPA]) in the mouse maximal electroshock (MES)-induced tonic seizure model. Tonic hind limb extension (seizure activity) was evoked in adult male albino Swiss mice by a current (sine-wave, 25 mA, 500 V, 50 Hz, 0.2 s stimulus duration) delivered via ear-clip electrodes. BAM-IPPS administered (i.p.) at a dose of 150 mg/kg significantly elevated the threshold for electroconvulsions in mice ($P<0.05$). Lower doses of BAM-IPPS (50 and 100 mg/kg) had no significant impact on the threshold for electroconvulsions in mice. Moreover, BAM-IPPS (100 mg/kg) did not significantly affect the anticonvulsant potency of CBZ, PB, PHT and VPA in the mouse MES model. BAM-IPPS elevated the threshold for electroconvulsions in mice in a dose-dependent manner. However, BAM-IPPS (100 mg/kg) did not affect the anticonvulsant action of various classical AEDs in the mouse MES model, making the combinations of BAM-IPPS with CBZ, PB, PHT and VPA neutral, from a preclinical point of view.

INTRODUCTION

Experimental evidence indicates that several succinimide derivatives possess anticonvulsant properties in animal models of epilepsy [1,2,5-11,14,15].

In our pilot study, we found that N-(m-bromoanilinomethyl)-p-isopropoxyphenyl-succinimide (BAM-IPPS; Fig. 1. – a new succinimide derivative) exerted the anticonvulsant properties by suppressing tonic-clonic seizures in the mouse maximal electroshock-induced seizure (MES) test (unpublished data).

Corresponding author
e-mail: jarogniew.luszczki@gmail.com, jluszczki@yahoo.com
tel: +48 81 718-73-65,
fax: +48 81 718-73-64

The aim of this study was to evaluate the effect of BAM-IPPS on the threshold for electroconvulsions and to assess its influence on the protective activity of four classical antiepileptic drugs (AEDs: carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT), and valproate (VPA)) in the mouse MES-induced seizure model. The MES test in mice is thought to be an experimental model of tonic-clonic seizures and, to a certain extent, of partial convulsions with or without secondary generalization in humans [4,12,13].

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, housed under standardized housing conditions (natural light-dark cycle, temperature of 23 ± 1ºC, relative humidity

 DOI: 10.2478/cipms-2014-0017

of 55 ± 5%), were used. After seven days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups each comprised of eight mice. Each mouse was used only once and all tests were performed between 8:00 and 15:00. 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 First Local Ethics Committee at the Medical University of Lublin (License no.: 18/2006) and the Second Local Ethics Committee at the University of Life Sciences in Lublin (License nos.: 79/2009 and 15/2012), and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

The following drugs were used: N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide (BAM-IPPS; Fig.1 – $C_{20}H_{21}BrN_2O_3$ – molecular weight = 417.299; synthesized by Dr. S.L. Kocharov, Mndjoyan's Institute of Fine Organic Chemistry of the National Academy of Sciences of the Republic of Armenia, Yerevan, Armenia), carbamazepine (CBZ - a gift from Polpharma, Starogard Gdański, Poland), phenobarbital (PB - Polfa, Kraków, Poland), phenytoin (PHT - Polfa, Warszawa, Poland) and valproate (VPA - magnesium salt – kindly donated by ICN-Polfa S.A., Rzeszów, Poland). All drugs, except for VPA, were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water, while VPA was directly dissolved in distilled water. All drugs were administered *intraperitoneally* (i.p.), in a volume of 5 ml/kg body weight, as follows: PHT - 120 min, PB and BAM-IPPS - 60 min, CBZ and VPA - 30 min before electroconvulsions. The pretreatment times before testing of the AEDs were based upon information about their biological activity from the literature and our previous experiments [5-10]. The pretreatment time (60 min) before testing BAM-IPPS was established in our pilot study as its peak time of maximum anticonvulsant activity (unpublished data).

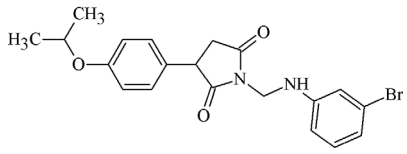


Figure 1. Structural formula of N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide (BAM-IPPS)

Maximal electroshock seizure threshold (MEST) test

The MEST test was first used to assess the anticonvulsant effects of BAM-IPPS administered alone. Electroconvulsions were induced by applying an alternating current (sine-wave, 50 Hz, 500 V) via ear-clip electrodes from a rodent shocker generator (type 221; Hugo Sachs Elektronik, Freiburg, Germany). The stimulus duration was 0.2 s and tonic hind limb extension was used as the endpoint. In this test, at least 4 groups of control mice, each consisting of 8 animals, were challenged with currents of varying intensities ranging between 4 and 8 mA so that 10-30%, 30-50%, 50-70% and 70-90% of animals exhibited the endpoint. After establishing the current intensity-effect curve (i.e., current intensity in mA vs. percentage of mice convulsing) for each dose of BAM-IPPS tested, the electroconvulsive threshold was calculated according to the log-probit method of Litchfield and Wilcoxon [3]. The electroconvulsive threshold was expressed as the median current strength value (CS_{50} in mA) predicted to produce tonic hind limb extension in 50% of the animals tested. This experimental procedure was performed for various increasing doses of BAM-IPPS (50, 100 and 150 mg/kg), until the threshold for electroconvulsions of BAM-IPPS-injected animals was statistically different from that of the control animals. Only doses of BAM-IPPS that did not significantly affect the seizure threshold in the MEST test were selected for testing in combination with four classical AEDs in the MES test (see below). This approach allowed us to rule out any contribution of the intrinsic anticonvulsant efficacy of BAM-IPPS in the effects observed in combination with the AEDs in the MES test.

Maximal electroshock seizure (MES) test

Electroconvulsions were induced by applying an alternating current (sine-wave, 50 Hz, 500 V) via ear-clip electrodes from a rodent shocker generator (type 221; Hugo Sachs Elektronik, Freiburg, Germany). The stimulus duration was 0.2 s and tonic hind limb extension was used as the endpoint. In the MES test, mice were challenged with a current of the fixed intensity (25 mA) that was 4-5-fold higher than the CS_{50} value in vehicle-treated control mice [4]. These parameters of stimulation (maximal electroshock) typically result in all mice responding with tonic hind limb extension immediately after stimulation. The AEDs administered alone and their combination with BAM-IPPS were tested for their ability to increase the number of animals not responding with tonus (i.e., protected from tonic hind limb extension) after stimulation. Again, at least 4 groups of mice, each consisting of 8 animals and treated with a different dose of the AEDs alone or in combination with BAM-IPPS, were challenged with a current of 25 mA to yield 10-30%, 30-50%, 50-70% and 70-90% of animals protected from tonic seizures. After constructing a dose-effect curve (i.e., dose in mg/kg vs. percentage of mice protected), the protective median effective dose (ED_{50}) value of the AED tested was calculated according to a log-probit method [3]. Each ED_{50} value represented a dose of the AED (in mg/kg) predicted to protect 50% of mice tested against MES-induced extension of the hind limbs. BAM-IPPS was tested for its ability to affect the anticonvulsive potency of AEDs. As mentioned earlier, BAM-IPPS was administered in a dose of 100 mg/kg that per se had no effect on seizure threshold in the MEST test. In this experimental protocol, an increase in the anticonvulsant potency of the AED tested in combination with BAM-IPPS would be reflected by a lower ED_{50} value of the test AED (i.e., lower dose of the test drug was necessary to protect 50% of mice challenged). In the present study, CBZ and PHT were administered at doses ranging between 8-14 mg/kg, PB at doses ranging

between 15-30 mg/kg and VPA at doses ranging between 225-325 mg/kg.

Statistics

Both CS_{50} and ED_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [3]. Statistical analysis of data from the MEST test was performed with one-way analysis of variance (ANOVA) followed by the post-hoc Tukey-Kramer test for multiple comparisons among four CS_{50} values. Statistical analysis of data from the MES test was performed with log-probit analysis according to Litchfield and Wilcoxon [3] for two ED_{50} values. Differences among values were considered statistically significant if $P<0.05$. All statistical tests were performed using commercially available GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Influence of N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide (BAM-IPPS) upon the threshold for electroconvulsions

BAM-IPPS administered systemically (i.p., 60 min prior to the MEST test) at a dose of 150 mg/kg significantly elevated the threshold for electroconvulsions in mice (by 34%; $P<0.05$; Tab. 1). The experimentally-derived CS_{50} values for animals receiving BAM-IPPS at doses of 50 and 100 mg/kg did not significantly differ from that for control animals subjected to the MEST test (Tab. 1).

Table 1. Effect of N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide (BAM-IPPS) upon the threshold for electroconvulsions in mice

Treatment (mg/kg)	CS_{50} (mA)	n
Vehicle	5.69 ± 0.51	24
BAM-IPPS (50)	5.99 ± 0.40	32
BAM-IPPS (100)	6.71 ± 0.52	16
BAM-IPPS (150)	7.62 ± 0.48*	16
F (3;84) = 2.814; P = 0.0441		

Data are presented as median current strengths (CS_{50} values in mA ± S.E.) required to produce tonic hindlimb extension in 50% of animals tested in the maximal electroshock-induced seizure threshold (MEST) test. BAM-IPPS was administered i.p. 60 min. before the test. Statistical evaluation of the data was performed with log-probit method [3] and one-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons. n – number of animals tested at those current strength intensities, whose seizure effects ranged between 16% and 84%; F – F-statistics from one-way ANOVA; P – probability from one-way ANOVA. *$P<0.05$ vs. the control (vehicle-treated) animals.

Effects of N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide (BAM-IPPS) on the protective action of carbamazepine, phenobarbital, phenytoin and valproate in the mouse maximal electroshock seizure model

All investigated classical AEDs (CBZ, PB, PHT and VPA) administered alone exhibited a definite anticonvulsant activity in the MES test in mice (Tab. 2). When BAM-IPPS (100 mg/kg) was co-administered with CBZ, PB, PHT and VPA, it did not significantly potentiate the anticonvulsant action of the studied AEDs in the MES test. The experimentally-derived ED_{50} values for the AEDs in combination with BAM-IPPS (100 mg/kg) did not considerably differ from those ED_{50} values as documented for the AEDs administered separately (Tab. 2).

Table 2. Effect of N-(m-bromoanilinomethyl)-p-isopropoxyphenylsuccinimide (BAM-IPPS) on the protective activity of four classical antiepileptic drugs against maximal electroshock-induced seizures in mice

Treatment (mg/kg)	ED_{50} (mg/kg)
CBZ + vehicle	10.6 (8.8-12.8)
CBZ + BAM-IPPS (100)	9.5 (8.5-10.7)
PB + vehicle	22.1 (17.7-27.5)
PB + BAM-IPPS (100)	16.2 (11.8-22.4)
PHT + vehicle	9.9 (8.3-11.7)
PHT + BAM-IPPS (100)	9.4 (8.5-10.4)
VPA + vehicle	262.7 (246.2-280.4)
VPA + BAM-IPPS (100)	236.1 (215.1-259.1)

Results are presented as median effective doses (ED_{50} in mg/kg, with 95% confidence limits in parentheses) of AEDs, protecting 50% of animals tested against maximal electroshock (MES)-induced seizures. All AEDs were administered i.p.: PHT – 120 min., PB – 60 min., CBZ and VPA – 30 min. prior to the MES test. BAM-IPPS was administered i.p. at 60 min. before the MES test. Statistical analysis of data was performed with log-probit method according to Litchfield and Wilcoxon [3]. CBZ – carbamazepine, PB – phenobarbital, PHT – phenytoin, and VPA – valproate.

DISCUSSION

Results presented herein indicate that BAM-IPPS in a dose dependent manner increased the threshold for electroconvulsions in mice. However, BAM-IPPS at the subprotective dose of 100 mg/kg (i.e., the dose that by itself did not significantly affect the threshold for electroconvulsions) had no impact on the protective action of CBZ, PB, PHT and VPA against MES-induced tonic seizures in mice, thus indicating neutral interactions between these drugs in the mouse MES model.

Previously, we have reported that p-isopropoxyphenylsuccinimide monohydrate enhanced the anticonvulsant action of PHT and VPA, but not that of CBZ and PB [9]. Moreover, N-(anilino-methyl)-p-isopropoxyphenylsuccinimide, N-(p-acetylphenyl)-p-isopropoxyphenylsuccinimide, N-hydroxymethyl-p-isopropoxyphenyl-succinimide and N-morpholinomethyl-p-isopropoxyphenyl-succinimide significantly potentiated the anticonvulsant action of PB and VPA, but not that of CBZ and PHT in the mouse MES model [6,7,10,15]. On the other hand, N-(ortho-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide reduced the anticonvulsant action of CBZ and had no significant impact on the protective action of PHT, PB and VPA against MES-induced seizures in mice [5]. As regards, N-(meta-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide, N-(para-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide and 3-(N-p-isopropoxyphenylsuccinimido-methylamino)-cinnamic acid, all these succinimide derivatives had no effect on the protective action of the classical AEDs in the mouse MES model [5,8]. It is important to note that the anticonvulsant profile of BAM-IPPS when combined with four classical AEDs is similar to that reported earlier for N-(meta-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide, N-(para-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide and 3-(N-p-isopropoxyph

enylsuccinimidomethylamino)-cinnamic acid in the mouse MES model.

Although BAM-IPPS significantly raised the threshold for maximal electroconvulsions in mice, it did not affect the protective action of four different classical AEDs in the mouse MES model. Thus, one can ascertain that BAM-IPPS possesses the anticonvulsant action against electrically-evoked tonic seizures in experimental animals, but this action seems to be too weak in order to enhance the protective activity of different classical AEDs in the mouse MES-induced tonic seizure model. Perhaps, BAM-IPPS will suppress clonic or limbic seizures in other experimental models of epilepsy. To confirm this hypothesis, more advanced studies are required.

CONCLUSIONS

The co-administration of BAM-IPPS with CBZ, PB, PHT and VPA was neutral in the mouse MES model.

ACKNOWLEDGEMENTS

This study was supported by grants from the Medical University of Lublin and Institute of Rural Health (Lublin, Poland). Professor J.J. Luszczki is a Member of the Academy of Young Scientists (Polish Academy of Sciences, Warszawa, Poland). The authors are grateful for the generous gifts of carbamazepine from Polpharma S.A. (Starogard Gdański, Poland) and valproate from ICN-Polfa S.A. (Rzeszów, Poland).

DISCLOSURE OF CONFLICTS OF INTEREST

The authors have no disclosures to declare.

REFERENCES

1. Kamiński K., Obniska J.: Synthesis and anticonvulsant properties of new 1-(2-pyridinyl)- 3-substituted pyrrolidine-2,5-dione derivatives. *Acta Pol. Pharm*., 65, 457, 2008.
2. Lange J. et al.: Synthesis and properties of new cyclic derivatives of succinic acid with anticonvulsant activity. Pharmazie, 32, 82, 1977.
3. Litchfield J.T., Wilcoxon F.: A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther*., 96, 99, 1949.
4. 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.
5. Łuszczki J.J. et al.: Effects of three N-(carboxyanilinomethyl) derivatives of p-isopropoxyphenylsuccinimide on the anticonvulsant action of carbamazepine, phenobarbital, phenytoin and valproate in the mouse maximal electroshock-induced seizure model. *Eur.* J. *Pharmacol*., 648, 74, 2010.
6. Łuszczki J.J. et al.: Influence of N-(p-acetylphenyl)-p-isopropoxyphenylsuccinimide on the protective action of classical antiepileptic drugs against maximal electroshock-induced seizures in mice. *Curr. Issues Pharm. Med. Sci*., 26, 76, 2013.
7. Łuszczki J.J. et al.: Influence of N-hydroxymethyl-p-isopropoxyphenylsuccinimide on the anticonvulsant action of different classical antiepileptic drugs in the mouse maximal electroshock-induced seizure model. *Epilepsy Res*., 100, 27, 2012.
8. Łuszczki J.J. et al.: No effect of 3-(N-p-isopropoxyphenylsuccinimid omethylamino)-cinnamic acid on anticonvulsant action of different classical antiepileptic drugs in mouse maximal electroshock-induced seizure model. *J. Pre-Clin. Clin. Res*., 6, 20, 2012.
9. Łuszczki J.J., Kocharov S.L., Czuczwar S.J.: Effect of p-isopropoxyphenylsuccinimide monohydrate on the anticonvulsant action of carbamazepine, phenobarbital, phenytoin and valproate in the mouse maximal electroshock-induced seizure model. *Pharmacol. Rep*., 62, 194, 2010.
10. Łuszczki J.J., Kocharov S.L., Czuczwar S.J.: N-(anilinomethyl)-p-isopropoxyphenyl-succinimide potentiates the anticonvulsant action of phenobarbital and valproate in the mouse maximal electroshock-induced seizure model. *Neurosci. Res*., 64, 267, 2009.
11. Rogawski M.A., Bazil C.W.: New molecular targets for antiepileptic drugs: alpha(2)delta, SV2A, and K(v)7/KCNQ/M potassium channels. *Curr. Neurol. Neurosci. Rep*., 8, 345, 2008.
12. Stables J.P., Kupferberg H.J. (1997). The NIH Anticonvulsant Drug Development (ADD) Program: preclinical anticonvulsant screening project. In: Molecular and cellular targets for anti-epileptic drugs. Avanzini G., Regesta G., Tanganelli P., Avoli M. (editors). London: John Libbey; p. 191.
13. White H.S. et al. (2002). Discovery and preclinical development of antiepileptic drugs. In: Antiepileptic drugs. 5th ed. Levy R.H., Mattson R.H., Meldrum B.S., Perucca E. (editors). Philadelphia: Lippincott Williams & Wilkins; p. 36.
14. Zejc A. et al.: Synthesis and anticonvulsant properties of some arylsuccinate methylpyridylimides. *Pol. J. Pharmacol. Pharm*., 42, 69, 1990.
15. Żółkowska D. et al.: Effects of N-(morpholinomethyl)-p-isopropoxyphenylsuccinimide on the protective action of different classical antiepileptic drugs against maximal electroshock-induced tonic seizures in mice. *Pharmacol. Rep*., 65, 389, 2013.