No effect of 3-(N-p-isopropoxyphenyl-succinimidomethylamino)-cinnamic acid on anticonvulsant action of different classical antiepileptic drugs in mouse maximal electroshock-induced seizure model

Jarogniew J. Łuszczki1,2, Tadeusz Marczewski2, Ewa Marzęda1, Dariusz Durmowicz1, Dominika Podgórskia2, Sergey L. Kocharov3, Magdalena Florek-Łuszczki4

1 Isobolographic Analysis Laboratory, Institute of Rural Health, Lublin, Poland
2 Department of Pathophysiology, Medical University, Lublin, Poland
3 Mndjoyan’s Institute of Fine Organic Chemistry, National Academy of Sciences, Yerevan, Republic of Armenia
4 Department of Public Health, Institute of Rural Health, Lublin, Poland


Abstract

Introduction and objective: The aim of the study was to determine the effects of 3-(N-p-isopropoxyphenylsuccinimidomethylamino)-cinnamic acid (IPPSMA-CA – a new succinimide derivative) on the protective action of 4 classical antiepileptic drugs (AEDs): carbamazepine [CBZ], phenobarbital [PB], phenytoin [PHT] and valproate [VPA]), against maximal electroshock (MES)-induced tonic seizures in mice.

Materials and methods: 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 auricular electrodes. Acute adverse-effect profiles of the combination of IPPSMA-CA and 4 classical AEDs (CBZ, PB, PHT and VPA) with respect to motor performance, long-term memory and skeletal muscular strength were measured in the chimney, passive avoidance and grip-strength tests, respectively.

Results: IPPSMA-CA administered at 150 mg/kg (i.p.) significantly elevated the threshold for electroconvulsions in mice (p<0.01). IPPSMA-CA at doses of 50 and 100 mg/kg, however, had no significant impact on the threshold for electroconvulsions in mice. Nor did IPPSMA-CA (100 mg/kg) significantly affect the anticonvulsant activity of CBZ, PB, PHT and VPA in the MES test in mice. None of the examined combinations of IPPSMA-CA (100 mg/kg, i.p.) with CBZ, PB, PHT and VPA (at their ED50 values from the MES-induced seizure test) affected motor coordination in the chimney test, long-term memory in the passive avoidance task, and muscular strength in the grip-strength test in mice. This indicates no possible acute adverse effects in animals.

Conclusions: IPPSMA-CA elevated the threshold for electroconvulsions in mice in a dose-dependent manner. However, IPPSMA-CA at a sub-protective dose of 100 mg/kg did not affect the anticonvulsant action of various classical AEDs in the mouse MES model. Thus, the combinations of IPPSMA-CA with CBZ, PB, PHT and VPA are neutral from a preclinical viewpoint.

Key words

antiepileptic drugs, maximal electroshock-induced seizures, pharmacokinetic/pharmacodynamic interaction, p-isopropoxyphenylsuccinimide derivative

INTRODUCTION

In spite of advances in our knowledge on pathophysiological processes underlying seizure attacks and numbers of antiepileptic drugs (AEDs) licensed and approved for the treatment of epilepsy, there still remain approx. 30% of epilepsy patients inadequately medicated with currently available AEDs. For these patients, some novel AEDs should be created and developed that would protect the patients against seizures, thereby providing them with the state of freedom from seizures. There is undoubtedly a pressing need for developing some novel drugs possessing anticonvulsant properties in both experimental and clinical studies [1].

Experimental in vivo studies indicate that several succinimide derivitives possess anticonvulsant properties in rodents [2, 3, 4, 5, 6, 7, 8]. Previously, it has been reported that 3-cyclohexyl-succinimides [2], N-morpholinemethyl derivative of m-bromophenyl-succinimide [3], N-pyridyl-substituted succinimides [5], N-(anilinomethyl)-p-isopropoxyphenylsuccinimide (AMIPPS) [4], p-isopropoxyphenylsuccinimide monohydrate (IPPS) [6], N-(ortho-carboxyanilinomethyl)
-p-isopropoxyphenylsuccinimide (o-CAMIPPS), N-(meta-carboxyaminomethyl)-p-isopropoxyphenylsuccinimide (m-CAMIPPS), and N-(para-carboxyaminomethyl)-p-isopropoxyphenylsuccinimide (p-CAMIPPS) [7], and N-hydroxymethyl-p-isopropoxyphenylsuccinimide (HMIPPS) [8], exhibited potent anticonvulsant effects in the maximal electroshock-induced seizure (MES) test, recognized as the most widely employed animal seizure model for early identification of candidate anticonvulsant drugs.

In our pilot study, it was found that 3-(N-p-isopropoxyphenylsuccinimidomethylamino)-cinnamic acid (IPPSMA-CA) exerted anticonvulsant properties by suppressing tonic-clonic seizures in the mouse MES test (unpublished data). We sought, therefore, to evaluate the effect of IPPSMA-CA on the threshold for electroconvulsions, and to assess its influence on the protective activity of 4 classical AEDs (carbamazepine (CBZ), phenobarbital (PB), phenytoin (PB), and valproate (VPA)) in the mouse MES-induced seizure model. The test evaluating the threshold for electroconvulsions and the MES test in mice are both thought to be experimental models of tonic-clonic seizures and, to a certain extent, of partial convulsions with or without secondary generalization in humans [9]. In these experimental tests, one can readily determine the anticonvulsant potential of agents and compounds having anticonvulsant properties and evaluate their effects on various classical AEDs, which are effective in suppression of tonic-clonic seizures in humans [9]. Additionally, acute side effects of IPPSMA-CA administered alone and in combination with the 4 classical AEDs were investigated regarding impairment of motor coordination, long-term memory and muscular strength utilizing the chimney test, step-through passive avoidance task, and grip-strength test, respectively.

**MATERIALS AND METHODS**

**Animals and experimental conditions.** Adult male Swiss mice (weighing 22-26 g) were used, kept in colony cages with free access to food and tap water, housed under standardized housing conditions (natural light-dark cycle, temperature of 23 ± 1°C, and relative humidity of 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups each comprised of 8 mice. Each mouse was used only once and all tests were performed between 08:00-15:00. 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 study were approved by the First Local Ethics Committee at the Medical University in Lublin (License Nos.: 18/2006) and the Second Local Ethics Committee at the University of Life Sciences in Lublin (License Nos.: 79/2009, 15/2012), and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

**Drugs.** The following drugs were used:

- 3-(N-p-isopropoxyphenylsuccinimidomethylamino)-cinnamic acid (IPPSMA-CA – C_{23}H_{24}N_{2}O_{5} – molecular weight = 408.440, synthesized by Dr. S.L. Kocharov, Mndjoyan’s Institute of Fine Organic Chemistry of the National Academy of Sciences in Yerevan, Republic of Armenia);
- carbamazepine (CBZ – a gift from Polpharma, Starogard Gdański, Poland);
- phenobarbital (PB – Polfa, Kraków, Poland);
- phenytoin (PHT – Polfa, Warsaw, Poland);
- valproate (VPA – magnesium salt (donated by ICN-Polfa SA, 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 IPPSMA-CA – 60 min, CBZ and VPA – 30 min before electroconvulsions, motor coordination, grip-strength and long-term memory tests. The pretreatment times before testing the AEDs were based on information concerning their biological activity from the literature, and from own previous experiments [4, 6, 7, 8]. The times to the peak of maximum anticonvulsant effects for all AEDs were used as the reference times in all behavioural tests. The pretreatment time (60 min.) before testing IPPSMA-CA was established in a pilot study as its peak time of maximum anticonvulsant activity (unpublished data).

**Maximal electroshock seizure threshold (MEST) test.** The MEST test was used first to assess the anticonvulsant effects of IPPSMA-CA administered alone. Electroconvulsions were induced by applying an alternating current (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 5-8 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 IPPSMA-CA tested, the electroconvulsive threshold was calculated according to the log-probit method of Litchfield and Wilcoxon [10]. 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 IPPSMA-CA (50, 100 and 150 mg/kg), until the threshold for electroconvulsions of IPPSMA-CA-injected animals was statistically different from that of the control animals. Only doses of IPPSMA-CA that did not significantly affect the seizure threshold in the MEST test were selected for testing in combination with the 4 classical AEDs in the MES test (see below). This approach allowed the ruling out any contribution of the intrinsic anticonvulsant efficacy of IPPSMA-CA in the effects observed in combination with the AEDs in the MES test.

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Grip-strength test. The effects of combinations of IPPSMA-CA (100 mg/kg) with classical AEDs at doses corresponding to their ED$_{50}$ values from the MES test on skeletal muscular strength in mice were quantified by the grip-strength test of Meyer et al. [12]. The time before the commencement of the grip-strength test (after drug administration) was identical to that for the MES test. The grip-strength apparatus (BioSeb, Chaville, France) was equipped with a wire grid ($8 \times 8$ cm) connected to an isometric force transducer (dynamometer). The mice were lifted by the tails so that their forepaws could grasp the grid. The mice were then gently pulled backward by the tail until the grid was released. The maximal force exerted by the mouse before losing grip was recorded. The mean of three measurements for each animal was calculated and subsequently, the mean maximal force of 8 animals per group was determined. The muscular strength in mice is expressed in N (Newtons) as means ± S.E. of at least 8 determinations. This experimental procedure has also been described in detail in our earlier studies [4, 6, 7, 8].

Chimney test. Potential adverse effects of combinations of classical AEDs administered at doses corresponding to their ED$_{50}$ values from the MES test with IPPSMA-CA (100 mg/kg) were evaluated using the chimney test of Boissier et al. [13]. In this test, the mice were placed at the bottom of a transparent plastic tube (30 cm long, 3 cm inner diameter,) and had to climb backwards up to the top of the tube. Impairment of motor performance was indicated by the inability of the mice to climb out of the tube within 60 s., and the results were expressed as the percentages of animals that failed to perform the task. This experimental procedure, too, has been described in detail in our earlier studies [4, 6, 7, 8].

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 [10]. Statistical analysis of data from the MES test was performed with one-way analysis of variance (ANOVA) followed by the post-hoc Tukey-Kramer test for multiple comparisons among 4 CS$_{50}$ values. Statistical analysis of data from the MES test was performed with log-probit analysis according to Litchfield and Wilcoxon [10] for 2 ED$_{50}$ values.

The results obtained in the step-through passive avoidance task were statistically evaluated using Kruskal-Wallis nonparametric ANOVA. The results from the grip-strength test were verified with one-way ANOVA. Qualitative variables from the chimney test were compared by use of the Fisher's exact probability test. 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 3-(N-p-isopropoxyphenylsuccinimido-methylamino)-cinnamic acid (IPPSMA-CA) on the threshold for electroconvulsions. IPPSMA-CA 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 from 5.27 ± 0.45 mA – 7.60 ± 0.49 mA (p<0.01) (Fig. 1). The experimentally-derived CS$_{50}$

Step-through passive avoidance task. The effects of IPPSMA-CA (100 mg/kg) applied alone or in combination with classical AEDs (at doses equal to their ED$_{50}$ values against MES-induced tonic seizures) on long-term memory were evaluated in the step-through, passive avoidance task. Each animal was administered an AED, either alone or in combination with IPPSMA-CA, on the first day before training. The pretreatment time of drugs in the passive avoidance task was identical to that of the MES test. Subsequently, the animals were placed in an illuminated box ($10 \times 13 \times 15$ cm) connected to a larger dark box ($25 \times 20 \times 15$ cm) equipped with an electric grid floor. Entrance of pretreated animals to the dark box was punished by an adequate electric shock to the foot (0.6 mA for 2 s). The animals that did not enter the dark compartment were excluded from subsequent experimentation.

On the following day (24 h later), the pre-trained animals were again placed into the illuminated box and observed for up to 180 s. Mice that avoided the dark compartment for 180 s were considered to remember the task. The time that the mice took to enter the dark box was noted and the median latencies (retention times) with 25th and 75th percentiles were calculated. The step-through passive avoidance task gives information about ability to acquire a task (learning) and to recall a task (retrieval). Therefore, it may be regarded as a measure of long-term memory [11]. This experimental procedure has been described in detail in our earlier studies [4, 6, 7, 8].

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Values for animals receiving IPPSMA-CA at doses of 50 and 100 mg/kg did not significantly differ from that for control animals subjected to the MEST test (Fig. 1).

**Figure 1.** Effect of 3- (N-p-isopropoxyphenylsuccinimidomethylamino) -cinnamic acid (IPPSMA-CA) on the threshold for electroconvulsions in mice

**Table 1.** Effect of 3- (N-p-isopropoxyphenylsuccinimidomethylamino) -cinnamic acid (IPPSMA-CA) on the protective activity of 4 classical AEDs against MES-induced seizures in mice

**Table 2.** Effects of 3- (N-p-isopropoxyphenylsuccinimidomethylamino) -cinnamic acid (IPPSMA-CA) and its combinations with 4 classical AEDs on long-term memory, muscular strength and motor performance in mice

**DISCUSSION**

The results of the presented study indicate that IPPSMA-CA elevated in a dose-dependent manner the threshold for electroconvulsions in mice. IPPSMA-CA at the sub-protective 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.

Comparing the effects produced by IPPSMA-CA with those reported earlier for AMIPPS, IPPS, o-CAMIPPS, m-CAMIPPS, p-CAMIPPS, and HMIPPS, it can be ascertained that IPPSMA-CA had no impact on the anticonvulsant properties of the 4 classical AEDs. Previously, we have documented that p-isopropoxyphenylsuccinimide monohydrate (IPPS) potentiated the anticonvulsant action of PHT and VPA, but not that of CBZ and PB [6]. Moreover, AMIPPS and HMIPPS significantly enhanced the
anticonvulsant action of PB and VPA, but not that of CBZ and PHT in the mouse MES model [4, 8].

On the other hand, o-CAMIPPS attenuated 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 [7]. With regards to m-CAMIPPS and p-CAMIPPS, both compounds had no impact on the protective action of CBZ, PHT, PB and VPA in the mouse MES model [7]. Of note, the anticonvulsant profile of IPPSMA-CA when combined with classical AEDs is similar to that reported earlier for m-CAMIPPS and p-CAMIPPS in the mouse MES model.

There is another fact worth mentioning while explaining the results obtained in the presented study. It was found that IPPSMA-CA-administered either alone or in combination with classical AEDs, at doses corresponding to their ED₅₀ values from the MES test, did not affect the motor coordination of mice in the chimney test. Additionally, IPPSMA-CA had no impact on long-term memory and muscular strength in mice subjected to the step-through passive avoidance task and grip-strength test, respectively. Since IPPSMA-CA had no impact on the acute adverse-effect profile of classical AEDs, the tested succinimide derivative seems to be neutral in preclinical studies.

Although IPPSMA-CA significantly raised the threshold for maximal electroconvulsions in mice, it did not affect the protective action of different classical AEDs in the mouse MES model. Thus, one can ascertain that IPPSMA-CA has an anticonvulsant action against electrically-evoked tonic seizures in experimental animals. This action, however, is too weak to enhance the protective activity of different classical AEDs in the mouse MES-induced tonic seizure model. Perhaps IPPSMA-CA will be effective in suppression of clonic or limbic seizures in other experimental models of epilepsy. To confirm or reject this hypothesis, more advanced studies are required.

CONCLUSION

Finally, based on the results from this study, one can ascertain that the co-administration of IPPSMA-CA with various classical AEDs (CBZ, PB, PHT and VPA) was neutral in the mouse MES model. Additionally, IPPSMA-CA had no impact on acute adverse-effect profiles of classical AEDs, as determined in the passive avoidance, grip-strength and chimney tests in mice.

Acknowledgments

The study was supported by grants from the Medical University and Institute of Rural Health in Lublin, Poland. The authors express their thanks to the firms of Polpharma SA, Starogard Gdański, Poland, and ICN-Połża SA, Rzeszów, Poland, for the generous gifts of carbamazepine and valproate, respectively.

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