Journal of Pre-Clinical and Clinical Research, 2009, Vol 3, No 2, 110-113
www.jpccr.eu

ORIGINAL ARTICLE

WIN 55,212-2 mesylate (a highly potent non-selective cannabinoid CB1 and CB2 receptor agonist) elevates the threshold for maximal electroshock-induced seizures in mice

Jarogniew J. Łuszczki[1,2], Marta Misiuta-Krzesińska[1], Aleksandra Wlaź[1], Jakub Zwoliński[1], Janina D. Cioczek[1,2], Dorota Żółkowska[3]

[1] *Department of Pathophysiology, Medical University, Lublin, Poland*
[2] *Department of Physiopathology, Institute of Agricultural Medicine, Lublin, Poland*
[3] *Department of Neurology, UC Davis School of Medicine,Sacramento, USA*

Abstract: The aim of this study was to determine the effect of WIN 55,212-2 mesylate (a highly potent non-selective cannabinoid CB1 and CB2 receptor agonist) on the threshold for maximal electroshock (MEST)-induced seizures in mice. Electroconvulsions were produced in mice by means of a current (sine-wave, 50 Hz, maximum 500 V, strength 4 - 14 mA, *via* ear-clip electrodes, 0.2-s stimulus duration, tonic hind limb extension taken as the endpoint). WIN 55,212-2 mesylate administered systemically (i.p.), 20 min before the MEST test, at doses of 5 and 10 mg/kg, did not alter the threshold for maximal electroconvulsions in mice. In contrast, WIN 55,212-2 mesylate at doses of 15 and 20 mg/kg significantly elevated the threshold for maximal electroconvulsions in mice ($P<0.05$ and $P<0.001$). Linear regression analysis of WIN 55,212-2 mesylate doses and their corresponding threshold increases allowed for the determination of threshold increasing doses by 20% and 50% (TID_{20} and TID_{50} values) that elevate the threshold in drug-treated animals over the threshold in control animals. The experimentally derived TID_{20} and TID_{50} values for WIN 55,212-2 mesylate were 6.3 and 17.2 mg/kg, respectively. Based on this preclinical study, one can ascertain that WIN 55,212-2 mesylate dose-dependently increased the threshold for MEST-induced seizures, suggesting the anticonvulsant action of the compound in this seizure model in mice.

Key words: WIN 55, 212-2 mesylate, threshold for maximal electroshock-induced seizures, TID_{20}, TID_{50}, mice

INTRODUCTION

Anticonvulsant effects produced by drugs or agents are usually evaluated in experimental (preclinical) studies by showing the efficacy of these compounds in animal models of epilepsy [5, 17]. With these models, the maximal electroshock-induced seizure threshold (MEST) test can determine whether or not the examined agents affect the threshold for maximal electroconvulsions in animals. To unequivocally assess the antiseizure potential of agents or drugs in this test, one denotes doses increasing the threshold by 20% and 50% (TID_{20} and TID_{50} values) that elevate the threshold for electroconvulsions in drug-treated animals by 20% and 50% over the threshold in control animals [5, 17]. The TID_{20} and TID_{50} values uniformly describe the antiseizure effects of drugs or agents in preclinical studies. To determine the TID_{20} and TID_{50} values, linear regression analysis of drug doses and their corresponding threshold values is used [5, 17]. The assessment of dose-response relationship with linear regression is a standard and common procedure in pharmacological studies, especially, in those assessing the antielectroshock potential of drugs or agents [6].

WIN 55,212-2 mesylate (a highly potent non-selective cannabinoid type 1 (CB1) and type 2 (CB2) receptor agonist) exerted anticonvulsant effects in the rat pilocarpine-induced seizure model [18-20]. WIN 55,212-2 mesylate suppressed bicuculline-induced convulsions evoked in *in vitro* brainstem-spinal cord preparations [3], and attenuated low-magnesium (Mg^{2+})-induced burst-firing in hippocampal cell cultures [15, 16]. Moreover, WIN 55,212-2 mesylate exerted anticonvulsant action in an experimental *in vivo* model of complex partial seizures (maximal dentate gyrus activation) in rats [14]. WIN 55,212-2 mesylate also attenuated the severity of cocaine-induced convulsive seizures, but not bicuculline- or methyl 6,7-dimethoxy-4-ethyl-beta-carboline-carboxylate (DMCM)-induced convulsive seizures in mice [2]. Additionally, WIN 55,212-2 mesylate antagonized L-glutamic acid and N-methyl-D-aspartate (NMDA)-induced convulsions in mice [2]. Quite recently, it has been documented that WIN 55,212-2 mesylate potentiated the anticonvulsant activity of diazepam (a classical antiepileptic drug [AED]) in the mouse maximal electroshock-induced seizure model [13].

Recently, we have reported that some second-generation antiepileptic drugs (AEDs, i.e., gabapentin, levetiracetam, stiripentol, tiagabine, and vigabatrin) increased the threshold

Corresponding author: Prof. Jarogniew J. Łuszczki, Department of Pathophysiology, Medical University, Jaczewskiego 8, 20-090 Lublin, Poland.
E-mail: jarogniew.luszczki@gmail.com/jluszczki@yahoo.com
Received: 23 November 2009; accepted: 15 December 2009

for electroconvulsions in mice that allowed the calculation of TID_{20} and TID_{50} values in mice [8-12].

Considering the above-mentioned facts, it was of pivotal importance to determine the TID_{20} and TID_{50} values for WIN 55,212-2 mesylate in the MEST test in mice.

MATERIALS AND METHODS

Animals and experimental conditions. All experiments were performed on adult male Swiss mice weighing 22-26 g. The animals were purchased from a licensed breeder (Dr. T. Górzkowska, Warsaw, Poland). The mice were kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature 21 ± 1ºC). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8 mice per group. Each mouse was used only once. All tests were performed between 09:00-14:00. 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 to the *Guide for the Care and Use of Laboratory Animals* and approved by the Local Ethics Committee in Lublin. The total number of animals used in this study was 160.

Drugs. WIN 55,212-2 mesylate [(R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate] (Tocris Bioscience, Bristol, UK) was dissolved in distilled water and administered intraperitoneally (i.p.) in a volume of 0.005 ml/g body weight 20 min before MEST-induced seizures. The pretreatment time was based on the biological activity of the compound in the literature [13, 18-20].

Maximal electroshock seizure threshold (MEST) test. Electroconvulsions were produced by means of an alternating current (sine-wave, 0.2 s stimulus duration, 50 Hz, maximum stimulation voltage of 500 V, current strength ranging from 4-14 mA) 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 in order 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). To evaluate the threshold for maximal electroconvulsions, at least 4 groups of mice, consisting of 8 animals per group, were challenged with electroshocks of various intensities to yield 10-30%, 30-50%, 50-70%, and 70-90% of animals with seizures. A current intensity-response curve was then constructed, according to the log-probit method by Litchfield and Wilcoxon [4], from which a median current strength (CS_{50} in mA) was calculated. Each CS_{50} value represented the current intensity required to induce tonic hindlimb extension in 50% of the mice challenged. The CS_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [4]. Subsequently, the 95% confidence limits were transformed to their SEM according to the procedure described in our earlier study [7, 11]. After administration of a single dose of WIN 55,212-2 mesylate to 4 groups of animals, the mice were again subjected to electroconvulsions (each group with a constant current intensity). The threshold for maximal electroconvulsions was recorded for 4 different doses of WIN 55,212-2 mesylate: 5, 10, 15 and 20 mg/kg. Statistical analysis of CS_{50} values was performed with one-way ANOVA followed by the *post-hoc* Tukey-Kramer test for multiple comparisons. Subsequently, the percentage of increase in CS_{50} values for animals injected with increasing doses of WIN 55,212-2 mesylate over the control (vehicle-treated animals) was calculated. The doses of WIN 55,212-2 mesylate and the resultant percentage of threshold increase over the control (vehicle-treated animals) were graphically plotted in rectangular coordinates of the Cartesian plot system and examined with least-squares linear regression analysis [1]. From the linear regression equation the TID_{20} and TID_{50} values were determined as recommended by Löscher et al. [5] and Swinyard et al. [17]. The experimental procedure has been described in more detail in our earlier studies [8-12]. Statistical evaluation of data was performed using commercially available GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA, USA). Differences between the respective values were statistically significant at $P<0.05$.

RESULTS

Effect of WIN 55,212-2 mesylate on the threshold for maximal electroshock-induced seizures. WIN 55,212-2 mesylate administered systemically (i.e. 20 min prior to the test), at doses of 5 and 10 mg/kg did not affect the threshold for maximal electroconvulsions in mice (Table 1). In this case, the experimentally derived CS_{50} values for animals receiving WIN 55,212-2 mesylate did not differ significantly from the CS_{50} value determined for control animals in the MEST test in mice (Table 1). In contrast, WIN 55,212-2 mesylate

Table 1 Effect of WIN 55,212-2 mesylate on the threshold for electroconvulsions in mice

Treatment (mg/kg)	CS_{50} (mA)	n	TI (%)
Vehicle	6.31 ± 0.43	16	-
WIN 55,212-2 mesylate (5)	7.52 ± 0.56	24	19.2
WIN 55,212-2 mesylate (10)	7.93 ± 0.72	32	25.7
WIN 55,212-2 mesylate (15)	9.12 ± 0.43 *	24	44.5
WIN 55,212-2 mesylate (20)	10.72 ± 0.76 ***	16	58.6
F (4, 107) = 5.283; P = 0.0006			

Data are presented as median current strengths (CS_{50} values in mA ± S.E.M.) required to evoke seizure activity (tonic hindlimb extension) in 50% of animals tested. The CS_{50} values were calculated according to the log-probit method by Litchfield and Wilcoxon (1949). WIN 55,212-2 mesylate was administered systemically (i.p.) 20 min before the initiation of electroconvulsions in mice. The threshold for control (vehicle-treated) animals was considered as a baseline (reference) value for calculations of percentage in the threshold increase (TI) following WIN 55,212-2 mesylate administration. Statistical analysis of data was performed with one-way ANOVA followed by the *post-hoc* Tukey-Kramer test for multiple comparisons. TI – threshold increase in % (the control value of 6.31 mA was considered as 100%).
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 value from one-way ANOVA
*P<0.05 and ***P<0.001 *versus* the control CS_{50} value for vehicle-treated animals.

administered at doses of 15 and 20 mg/kg significantly elevated the threshold for maximal electroconvulsions in mice ($P<0.05$ and $P<0.001$, respectively; Table 1). It was found that WIN 55,212-2 mesylate at doses of 5, 10, 15 and 20 mg/kg elevated the threshold for MEST-induced seizures by 19.2%, 25.7%, 44.5% and 58.7%, respectively (Table 1, Figure 1). The experimentally derived TID_{20} and TID_{50} values for WIN 55,212-2 mesylate were 6.3 and 17.2 mg/kg, respectively, in the MEST test in mice (Figure 1).

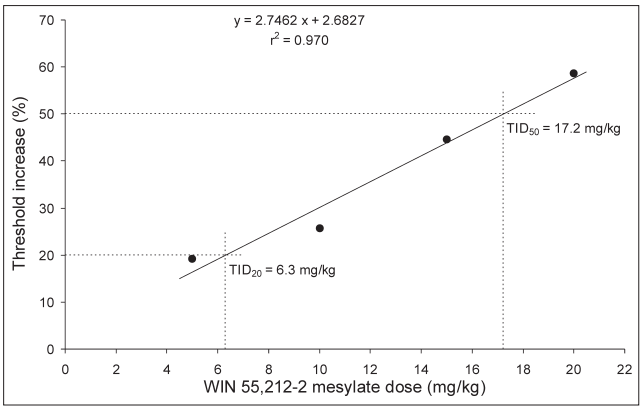

Figure 1 Dose-threshold increase relation for WIN 55,212-2 mesylate in maximal electroshock seizure threshold (MEST) test in mice.
Points on the graph represent threshold increasing doses of WIN 55,212-2 mesylate, experimentally denoted in the MEST test in mice. Linear regression analysis allowed for the determination of equation for dose-threshold increase relation for WIN 55,212-2 mesylate, as follows: $y = 2.746 x + 2.683$ ($r^2 = 0.970$); where y – threshold increase in %, x – the dose of WIN 55,212-2 mesylate, and r^2 – coefficient of determination [1]. From this equation one denotes the TID_{20} and TID_{50} (threshold increasing doses by 20% and 50%) for the MEST test. In this study, these values were 6.3 and 17.2 mg/kg, respectively. WIN 55,212-2 mesylate was administered i.p., 20 min before the threshold evaluation.

DISCUSSION

The objective of this study was to determine the TID_{20} and TID_{50} values for WIN 55,212-2 mesylate in the MEST test. Linear regression analysis of WIN 55,212-2 mesylate doses and their corresponding threshold increasing values over the threshold in control animals in the MEST test revealed that there was a close relation between the doses of WIN 55,212-2 mesylate and their biological effects in terms of seizure suppression in mice (Table 1, Figure 1).

It is important to note that linear regression analysis was used in this study to determine the doses of WIN 55,212-2 mesylate increasing the threshold for electroconvulsions in animals by a fixed and previously established percentages (20% and 50%), as mentioned earlier [8, 10]. With the log-probit method, followed by one-way ANOVA alone (without linear regression analysis), one can determine the effects exerted by the drugs tested at various doses. Therefore, to determine other effects, more experimental groups were required. In contrast, the linear regression analysis allows prediction of the doses increasing the threshold for electroconvulsions by the respective percentage, without testing additional groups of experimental animals [8, 10]. Moreover, with the linear regression analysis one can readily assess the same effect for various agents and drugs, contributing to the evaluation of their antiseizure potency by comparing their TID_{20} and TID_{50} values.

Results presented in this study are comparable with those reported earlier for some classical and second-generation AEDs (Table 2). It has been documented that carbamazepine, clonazepam, diazepam, gabapentin, levetiracetam, phenobarbital, phenytoin, stiripentol, tiagabine, valproate and vigabatrin increased the threshold for electroconvulsions in mice [5, 8-12]. The direct comparison of TID_{50} values of the classical and second-generation AEDs with those calculated for WIN 55,212-2 mesylate revealed that carbamazepine, clonazepam, diazepam, phenobarbital, phenytoin and tiagabine had TID_{50} values lower than those of WIN 55,212-2 mesylate (Table 2). In contrast, the TID_{50} values denoted for gabapentin, levetiracetam, stiripentol, valproate and vigabatrin were considerably higher than those denoted for WIN 55,212-2 mesylate in this study (Table 2). Thus, one can ascertain that WIN 55,212-2 mesylate possesses a strong anticonvulsant action in the MEST model in mice.

Table 2 Characteristics of the various antiepileptic drugs in the MEST test in mice.

Drug	TID_{20}	TID_{50}	References
Carbamazepine	ND	1.5	[5]
Clonazepam	ND	0.65	[5]
Diazepam	ND	2.7	[5]
Gabapentin	70.0	112.7	[12]
Levetiracetam	44.0	150.0	[8]
Phenobarbital	ND	4.0	[5]
Phenytoin	ND	5.4	[5]
Stiripentol	103.2	195.8	[10]
Tiagabine	4.4	8.0	[9]
Valproate	ND	69	[5]
Vigabatrin	226.2	ND	[9,12]
WIN 55,212-2	6.3	17.2	[present study]

Results are presented as doses of the drugs increasing the threshold for electroconvulsions by 20% and 50% (TID_{20} and TID_{50} in mg/kg), experimentally denoted in the MEST test in mice. ND – not determined.

In our opinion, the procedure of TID_{20} and TID_{50} evaluation in preclinical studies should be introduced as a paradigm allowing the precise characteristics of drugs possessing anticonvulsant properties in rodents.

Based on this preclinical study, one can ascertain that WIN 55,212-2 mesylate suppressed MEST-induced tonic seizures in mice. The linear regression analysis accompanied with calculation of TID_{20} and TID_{50} values should be added to other methods evaluating the threshold for electroconvulsions in mice, because it facilitates the comparison of the anticonvulsant potency of various drugs tested in preclinical studies.

ACKNOWLEDGEMENTS

This study was supported by grants from the Medical University and the Institute of Agricultural Medicine, both in Lublin, Poland. Professor J. J. Luszczki is a Recipient of the Fellowship for Leading Young Researchers from the Ministry of Science and Higher Education in Warsaw, Poland.

REFERENCES

1. Glantz SA, Slinker BK: Primer of applied regression and analysis of variance 2nd ed), McGraw-Hill Inc., New York 2001.
2. Hayase T, Yamamoto Y, Yamamoto K: Protective effects of cannabinoid receptor agonists against cocaine and other convulsant-induced toxic behavioural symptoms. *J Pharm Pharmacol* 2001, **53,** 1525-1532.

3. Koda S, Takeda S, Onimaru H, Akada S, Sakamoto A: Cannabinoid suppressed bicuculline-induced convulsion without respiratory depression in the brainstem-spinal cord preparation from newborn rats. *Biomed Res* 2005, **26,** 241-247.
4. Litchfield JT, Wilcoxon F: A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* 1949, **96,** 99-113.
5. 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.
6. Löscher W, Wauquier A: Use of animal models in developing guiding principles for polypharmacy in epilepsy. *Epilepsy Res* 1996 (Suppl.), **11,** 61-65.
7. Łuszczki JJ, Antkiewicz-Michaluk L, Czuczwar SJ: 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* 2009, **602,** 298-305.
8. Łuszczki JJ, Czuczwar SJ: How significant is the difference between drug doses influencing the threshold for electroconvulsions? *Pharmacol Rep* 2005, **57,** 782-786.
9. Łuszczki JJ, Czuczwar SJ: Isobolographic characterization of interactions between vigabatrin and tiagabine in two experimental models of epilepsy. *Prog Neuropsychopharmacol Biol Psychiatry* 2007, **31,** 529-538.
10. Łuszczki JJ, Dudra-Jastrzębska M, Andres-Mach M, Czernecki R, Barcicka-Kłosowska B, Misiuta-Krzesińska M, Zwoliński J, Filip D: Stiripentol in a dose-dependent manner elevates the threshold for maximal electroshock-induced seizures in mice. *J Pre-Clin Clin Res* 2007, **1,** 155-157.
11. Łuszczki JJ, Ratnaraj N, Patsalos PN, Czuczwar SJ: Isobolographic analysis of interactions between loreclezole and conventional antiepileptic drugs in the mouse maximal electroshock-induced seizure model. *Naunyn Schmiedebergs Arch Pharmacol* 2006, **373,** 169-181.
12. Łuszczki JJ, Ratnaraj N, Patsalos PN, Czuczwar SJ: Isobolographic and behavioral characterizations of interactions between vigabatrin and gabapentin in two experimental models of epilepsy. *Eur J Pharmacol* 2008, **595,** 13-21.
13. Naderi N, Aziz Ahari F, Shafaghi B, Najarkolaei AH, Motamedi F: Evaluation of interactions between cannabinoid compounds and diazepam in electroshock-induced seizure model in mice. *J Neural Transm* 2008, **115,** 1501-1511.
14. Rizzo V, Ferraro G, Carletti F, Lonobile G, Cannizzaro C, Sardo P: Evidences of cannabinoids-induced modulation of paroxysmal events in an experimental model of partial epilepsy in the rat. *Neurosci Lett* 2009, **462,** 135-139.
15. Shen M, Thayer SA: Delta9-tetrahydrocannabinol acts as a partial agonist to modulate glutamatergic synaptic transmission between rat hippocampal neurons in culture. *Mol Pharmacol* 1999, **55,** 8-13.
16. Shen M, Thayer SA: The cannabinoid agonist Win55,212-2 inhibits calcium channels by receptor-mediated and direct pathways in cultured rat hippocampal neurons. *Brain Res* 1998, **783,** 77-84.
17. Swinyard EA, Brown WC, Goodman LS: Comparative assays of antiepileptic drugs in mice and rats. *J Pharmacol Exp Ther* 1952, **106,** 319-330.
18. Wallace MJ, Blair RE, Faleński KW, Martin BR, DeLorenzo RJ: The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. *J Pharmacol Exp Ther* 2003, **307,** 129-137.
19. Wallace MJ, Martin BR, DeLorenzo RJ: Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity. *Eur J Pharmacol* 2002, **452**, 295-301.
20. Wallace MJ, Wiley JL, Martin BR, DeLorenzo RJ: Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. *Eur J Pharmacol* 2001, **428**, 51-57.