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

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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% (TID20 and TID50 values) that elevate the threshold in drug-treated animals over the threshold in control animals. The experimentally derived TID20 and TID50 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, TID20, TID50, 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% (TID20 and TID50 values) that elevate the threshold for electroconvulsions in drug-treated animals by 20% and 50% over the threshold in control animals [5, 17]. The TID20 and TID50 values uniformly describe the antiseizure effects of drugs or agents in preclinical studies. To determine the TID20 and TID50 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 (Mg2+)-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
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 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 (CS50) in mA was calculated. Each CS50 value represented the current intensity required to induce tonic hindlimb extension in 50% of the mice challenged. The CS50 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 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 TID20 and TID50 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 CS50 values for animals receiving WIN 55,212-2 mesylate did not differ significantly from the CS50 value determined for control animals in the MEST test in mice (Table 1). In contrast, WIN 55,212-2 mesylate
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\textsubscript{20} and TID\textsubscript{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).

**DISCUSSION**

The objective of this study was to determine the TID\textsubscript{20} and TID\textsubscript{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\textsubscript{20} and TID\textsubscript{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\textsubscript{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\textsubscript{50} values lower than those of WIN 55,212-2 mesylate (Table 2). In contrast, the TID\textsubscript{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.

In our opinion, the procedure of TID\textsubscript{50} and TID\textsubscript{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\textsubscript{50} and TID\textsubscript{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.

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