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Influence of WIN 55,212-2 on the anticonvulsant and acute neurotoxic potential of clobazam and lacosamide in the maximal electroshock-induced seizure model and chimney test in mice

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Summary The influence of WIN 55,212-2 mesylate (WIN) on the anticonvulsant activity and acute neurotoxic potential of clobazam (CLB) and lacosamide (LCM) was studied in the maximal electroshock-induced seizure (MES) model and chimney test in mice.
Results: indicate that WIN administered intraperitoneally, at doses of 2.5 and 5 mg/kg significantly enhanced the anticonvulsant action of CLB in the MES test by reducing its median effective dose (ED_{50}) from 20.80 mg/kg to 12.05 mg/kg ($P < 0.05$), and 8.22 mg/kg ($P < 0.001$), respectively. In contrast, WIN (1.25 mg/kg) did not significantly potentiate the anticonvulsant activity of CLB against MES-induced seizures. Similarly, WIN at doses of 1.25, 2.5 and 5 mg/kg had no significant impact on the anticonvulsant action of LCM in the MES test. On the other hand, WIN (5 mg/kg) had no impact on the acute neurotoxic effects of CLB and LCM in the chimney test and the median toxic doses (TD_{50}) for CLB and LCM were almost unchanged. Thus, WIN (5 mg/kg) elevated the protective index values for CLB (from 1.41 to 3.07) and LCM (from 3.60 to 4.91).

Abbreviations: AED, antiepileptic drug; CLB, clobazam; LCM, lacosamide; MES, maximal electroshock-induced seizures; PI, protective index; WIN, WIN 55,212-2.

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In conclusion, WIN potentiates suppression of tonic—clonic seizures produced by CLB in the mouse MES model, without affecting acute neurotoxic adverse effects of CLB in the chimney test in mice, which is favorable from a preclinical point of view.

Introduction

In spite of advanced knowledge about pathophysiological processes underlying seizure initiation and propagation in the brain (Bender and Baram, 2007; Stafstrom and Sutula, 2005), as well as, numbers of antiepileptic drugs (AEDs) approved for the treatment of epilepsy, there is still approx. 30% of patients, who fail seizure control with the AEDs (Hao et al., 2013; Kwan et al., 2011). For these patients, there is an urgent need to discover some novel treatment options, based on rational polytherapy with AEDs (French and Faught, 2009; St Louis, 2009; Wasterlain et al., 2011) or AEDs combined with naturally occurring substances or agents that could offer the epileptic patients a state of seizure-freedom (Stephen and Brodie, 2012; Stephen et al., 2012).

Previously, we have documented that WIN 55,212-2 mesylate (WIN—a synthetic non-selective cannabinoid CB_1 and CB_2 receptor agonist) enhanced the anticonvulsant action of some classical and second-generation AEDs in the mouse maximal electroshock-induced seizure (MES) model (Luszczki et al., 2011; Luszczki et al., 2013).

Considering the above-mentioned facts, it was of importance to continue our experiments and evaluate the effects of WIN on the anticonvulsant activity of two novel AEDs (i.e., clobazam (CLB) and lacosamide (LCM)) against MES-induced seizures in mice. Of note, the MES test in rodents is considered as an experimental model of tonic—clonic seizures and, to a certain extent, of partial seizures with or without secondary generalization in humans (Loscher et al., 1991; Loscher and Nolting, 1991).

Clobazam (CLB—7-chloro-1-methyl-5-phenyl-1,5-benzodiazepine-2,4(3*H*)-dione)—a second-generation AED is used as an add-on (adjunctive) medication for primary generalized and partial epilepsies (Giarratano et al., 2012; Ng and Collins, 2007). The drug is approved for adjunctive use in tonic—clonic, complex partial, and myoclonic seizures as well as for the treatment of seizures associated with Lennox—Gastaut syndrome (Owen, 2012; Yang and Scott, 2012).

Lacosamide (LCM—N^2-acetyl-*N*-benzyl-D-homoserinamide)—a third-generation AED is approved as adjunctive therapy for partial-onset seizures in certain adult patients with epilepsy (Doty et al., 2013; Luk et al., 2012). The drug is also used for treating epilepsy in children with Lennox—Gastaut syndrome (Grosso et al., 2014), and as adjunctive treatment for uncontrolled primary generalized tonic—clonic seizures in patients with idiopathic generalized epilepsy (Doty et al., 2013; Hofler and Trinka, 2013).

Because both AEDs are licensed for adjunctive treatment of tonic—clonic seizures, partial simple and complex seizures with or without secondary generalization, it was appropriate to use the MES test so as to evaluate the anticonvulsant effects exerted by the combinations of CLB and LCM with WIN.

Additionally, acute adverse (neurotoxic) effect profiles for CLB and LCM administered alone and combined with WIN were determined in the chimney test. The evaluation of the anticonvulsant activity and acute adverse-effect (neurotoxic) potentials of both AEDs administered alone and in combination with WIN allowed to establish protective index (PI) values for CLB and LCM and their combinations with WIN. Noteworthy, the PI value in preclinical studies reflects the margin of safety and tolerability between the AED doses exerting acute adverse (neurotoxic) effects and those providing therapeutic (protective) efficacy against seizures (Loscher and Nolting, 1991).

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, under standardized housing conditions (natural light-dark cycle, temperature of $23 \pm 1\,^{\circ}C$, relative humidity of $55 \pm 5\%$), were used. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups comprising of 8 mice. Each mouse was used only once and all tests were performed between 08.00 and 15.00 h. 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 Second Local Ethics Committee at the University of Life Sciences in Lublin (License no. 46/2013) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

The following drugs were used in this study: CLB (Frisium®, Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany), LCM (Vimpat®, UCB Pharma, Brussels, Belgium) and WIN 55,212-2 ((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). All drugs, except for WIN, were suspended in a 1% solution of Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) in distilled water, while WIN was dissolved in distilled water only. All drugs were administered intraperitoneally (i.p.) as a single injection in a volume of 5 ml/kg body