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Short communication

Synergistic interaction of pregabalin with the synthetic cannabinoid WIN 55,212-2 mesylate in the hot-plate test in mice: an isobolographic analysis

Jarogniew J. Łuszczki[1,2], Magdalena Florek-Łuszczki[3]

[1]Isobolographic Analysis Laboratory, Institute of Rural Health, Jaczewskiego 2, PL 20-950 Lublin, Poland

[2]Department of Pathophysiology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, Poland

[3]Department of Public Health, Institute of Rural Health, Jaczewskiego 2, PL 20-950 Lublin, Poland

Correspondence: Jarogniew J. Łuszczki, e-mail: jarogniew.luszczki@gmail.com, jluszczki@yahoo.com

Abstract:
Background: The aim of the study was to determine the type of interaction between pregabalin (a 3rd-generation antiepileptic drug) and WIN 55,212-2 mesylate (WIN – a highly potent non-selective cannabinoid CB1 and CB2 receptor agonist) administered in combination at a fixed ratio of 1:1, in the acute thermal pain model (hot-plate test) in mice.
Methods: Linear regression analysis was used to evaluate the dose-response relationships between logarithms of drug doses and their resultant maximum possible antinociceptive effects in the mouse hot-plate test. From linear equations, doses were calculated that increased the antinociceptive effect by 30% (ED_{30} values) for pregabalin, WIN, and their combination. The type of interaction between pregabalin and WIN was assessed using the isobolographic analysis.
Results: Results indicated that both compounds produced a definite antinociceptive effect, and the experimentally-derived ED_{30} values for pregabalin and WIN, when applied alone, were 29.4 mg/kg and 10.5 mg/kg, respectively. With isobolography, the experimentally derived $ED_{30\ mix}$ value for the fixed ratio combination of 1:1 was 5.7 mg/kg, and differed significantly from the theoretically calculated $ED_{30\ add}$ value of 19.95 mg/kg ($p < 0.01$), indicating synergistic interaction between pregabalin and WIN in the hot-plate test in mice.
Conclusions: Isobolographic analysis demonstrated that the combination of WIN with pregabalin at a fixed ratio of 1:1 exerted synergistic interaction in the mouse model of acute thermal pain. If the results from this study could be adapted to clinical settings, the combination of WIN with pregabalin might be beneficial for pain relief in humans.

Key words:
drug interaction, pregabalin, hot-plate test, isobolographic analysis, maximum possible antinociceptive effect, WIN 55,212-2 mesylate

Introduction

Accumulating evidence indicates that some antiepileptic drugs exert analgesic effects in both preclinical studies on animals [5, 19, 25, 26, 28, 30, 36, 39–41, 51, 54] and clinical settings in humans [1, 17, 46, 58]. At present, several antiepileptic drugs bring pain relief to patients with trigeminal neuralgia (carbamazepine, lamotrigine and oxcarbazepine), diabetic peripheral neuropathy (topiramate, lamotrigine, gabapentin, and pregabalin), post-herpetic neuralgia (topiramate, gabapentin, and pregabalin), phantom limb pain (gabapentin and pregabalin), and other types of chronic pain [1, 16, 46, 58].

Cannabinoids are promising analgesic drugs, and the ability of cannabinoids to inhibit acute nociception is well known [13, 63, 64]. Experimental studies have documented that WIN 55,212-2 mesylate (WIN – a synthetic cannabinoid CB1 and CB2 receptor agonist) reduced the nociceptive behavioral responses in orofacial and temporomandibular joint formalin tests [7], prevented mechanical allodynia induced by chronic administration of the antineoplastic drugs in rats [48, 50, 62], and produced antinociception in the tail-flick test in mice [13]. WIN produced an antiallodynic effect in streptozocin-induced diabetic rats and mice [14, 60]. WIN alleviated hyperalgesia and allodynia in rats subjected to chronic constriction injury of the sciatic nerve [22]. Moreover, the synthetic cannabinoid WIN attenuated allodynia and hyperalgesia in various rat models of neuropathic pain [6, 10, 20, 31, 33]. Additionally, it has been documented with isobolographic analysis that WIN interacted synergistically with bupivacaine (a local anesthetic drug) in the rat formalin test [29], and the combination of WIN with ketorolac (a non-steroidal anti-inflammatory drug) produced additive interaction in the acetic acid-induced writhing and tail-flick tests in mice [61]. Isobolographic analysis also revealed that intrathecal administration of WIN with clonidine (an antihypertensive drug) or neostigmine (a parasympathomimetic drug) produced synergistic interaction during phases 1 and 2 in the formalin test in rats [65].

Considering the facts that pregabalin and WIN used separately exert antinociceptive effects in various experimental models of acute and chronic pain, it was important to determine the interaction between these agents using the hot-plate test in mice (a standard model used to determine the antinociceptive efficacy of compounds with respect to acute thermal nociception). To characterize the type of interaction for the combination of pregabalin with WIN, an isobolographic analysis of interaction was used.

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 23 ± 1°C, relative humidity 55 ± 5%) were used. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups containing 8 mice each. All tests were performed between 8:00 – 15:00. Procedures involving animals and their care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. 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 article were approved by the Second Local Ethics Committee at the University of Life Sciences in Lublin (License Nos. 58/2009; 60/2009; 11/2011) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

The following drugs were used in the present study: pregabalin (Lyrica®, Pfizer Ltd., Sandwich, Kent, UK) and WIN 55,212-2 mesylate (WIN – ((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). Pregabalin was suspended in a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA), while WIN was dissolved in distilled water, and the drugs were administered *via* intraperitoneal (*ip*) injection in a volume of 0.005 ml/g of body weight. The drugs were administered as follows: WIN at 20 min and pregabalin at 60 min before the hot-plate test. These pretreatment times were chosen based upon information about their biological activity from the literature and authors' previous studies [36, 42].