European Journal of Pharmacology 628 (2010) 75–82

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

Analysis of the antinociceptive interactions in two-drug combinations of gabapentin, oxcarbazepine and amitriptyline in streptozotocin-induced diabetic mice

Maja A. Tomić [a,*], Sonja M. Vučković [b], Radica M. Stepanović-Petrović [a], Ana M. Micov [a], Nenad D. Ugrešić [a], Milica Š. Prostran [b], Bogdan Bošković [c]

[a] Department of Pharmacology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P.O. Box 146, 11221 Belgrade, Serbia
[b] Department of Pharmacology, Clinical Pharmacology & Toxicology, School of Medicine, University of Belgrade, Dr Subotića Starijeg 1, P.O. Box 38, 11000 Belgrade, Serbia
[c] Medical Military Academy, Crnotravska 17, 11 000 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 9 June 2009
Received in revised form 26 October 2009
Accepted 10 November 2009
Available online 14 November 2009

Keywords:
Gabapentin
Oxcarbazepine
Amitriptyline
Streptozotocin
Diabetic neuropathy
Synergistic interaction

ABSTRACT

Antiepileptic and antidepressant drugs are the primary treatments for pain relief in diabetic neuropathy. Combination therapy is a valid approach in pain treatment, where a reduction of doses could reduce side effects and still achieve optimal analgesia. We examined the effects of two-drug combinations of gabapentin, oxcarbazepine, and amitriptyline on nociception in diabetic mice and aimed to determine the type of interaction between components. The nociceptive responses in normal and diabetic mice were assessed by the tail-flick test. The testing was performed before and three weeks after the diabetes induction with streptozotocin (150 mg/kg; i.p.), when the antinociceptive effects of gabapentin, oxcarbazepine, amitriptyline and their two-drug combinations were examined. Gabapentin (10–40 mg/kg; p.o.) and oxcarbazepine (20–80 mg/kg; p.o.) produced a significant, dose-dependent antinociception in diabetic mice while amitriptyline (5–60 mg/kg; p.o.) produced weak antinociceptive effect. In normal mice, neither of the drugs produced antinociception. Gabapentin and oxcarbazepine, co-administered in fixed-dose fractions of the ED_{50} to diabetic mice, induced significant, dose-dependent antinociception. Isobolographic analysis revealed synergistic interaction. Oxcarbazepine (10–60 mg/kg; p.o.) + amitriptyline (5 mg/kg; p.o.) and gabapentin (10–30 mg/kg; p.o.) + amitriptyline (5 mg/kg; p.o.) combinations significantly and dose-dependently reduced nociception in diabetic mice. Analysis of the log dose–response curves for oxcarbazepine or gabapentin in a presence of amitriptyline and oxcarbazepine or gabapentin applied alone, revealed a synergism in oxcarbazepine–amitriptyline and additivity in gabapentin–amitriptyline combination. These findings provide new information about the combination therapy of painful diabetic neuropathy and should be explored further in patients with diabetic neuropathy.

1. Introduction

Diabetic neuropathy is one of the most common causes of neuropathic pain. Sensory disorders, ranging from loss of sensation in the extremities to pain, are often the earliest manifestations of diabetic neuropathy. Pain is estimated to occur in 5–15% of patients (Calcutt and Backonja, 2007). The management of diabetic neuropathy consists of maximizing diabetic control and administering medications to alleviate pain. Evidence from placebo-controlled studies has shown that systemic opioids, antiepileptic and antidepressant drugs are effective in symptomatic pain-relief treatment of diabetic patients with neuropathy (Chong and Hester, 2007; Zin et al., 2008). Gabapentin, an antiepileptic drug, has been shown to be effective in treating painful diabetic neuropathy in a number of placebo-controlled trials (Backonja et al., 1998; Backonja, 1999; other studies are summarized in Mellegers et al., 2001) and is extensively used. Recently, two placebo-controlled studies demonstrated that oxcarbazepine also provides clinically meaningful pain relief in this condition (Beydoun et al., 2006; Dogra et al., 2005). Oxcarbazepine has better tolerability because it is metabolized by a non-CYP450 pathway, thereby avoiding numerous metabolic drug–drug interactions described for carbamazepine (Beydoun and Kutluay, 2002). Of the antidepressants, the tricyclic drugs have been shown to be effective in symptomatic pain relief in diabetic neuropathy and have been considered for many years as the first-line treatment (Chong and Hester, 2007; Zin et al., 2008). In the pivotal study of Max et al. (1987), amitriptyline was more effective than placebo in alleviating painful diabetic neuropathy and its efficacy has also been confirmed in comparative studies (Morello et al., 1999; Vrethem et al., 1997).

Although various drug treatments are available for symptomatic relief of painful diabetic neuropathy, these agents do not provide satisfactory relief to all patients. Moreover, some patients do not

* Corresponding author. Tel.: +381 11 39 51 374; fax: +381 11 39 70 840.
E-mail address: majat@pharmacy.bg.ac.rs (M.A. Tomić).

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.11.016

tolerate well long-term treatment due to dose-related adverse effects (Zin et al., 2008). Combinations of analgesics having different mechanisms of action offer the possibility of efficient analgesia with less side effects as a result of lower doses of one or both drugs. Evidence for combined drug therapy of pain in diabetic neuropathy is sparse. It has been demonstrated that opioids enhance the analgesic effects of gabapentin in diabetic patients (Gilron et al., 2005; Hanna et al., 2008). However, the effects of combined use and the interactions between gabapentin, oxcarbazepine and amitriptyline have not been examined in either experimental models or in patients with diabetic neuropathy.

The aim of the present study was to examine the effects of three different two-drug combinations of gabapentin, oxcarbazepine and amitriptyline administered by the clinically preferred oral route to diabetic mice and to determine the type of interaction between the respective components, i.e. synergism, additivity or antagonism.

2. Materials and methods

2.1. Animals

Experiments were performed on 22–28 g male Swiss Webster mice (Military Academy Breeding Farm, Belgrade, Serbia). The animals were housed in groups of 15 in home cages (42.5× 27×19 cm) and maintained on a 12/12 h light/dark cycle at 22± 1 °C. Food and water were freely available, except during the experimental procedure. On the day of experiment, the animals were taken to the laboratory at 8:00 o'clock. Pre-treatment latencies in a tail-flick test were measured in all experimental groups at 11:30. The drugs/drug combinations were administered at 12:00. The post-treatment latencies were subsequently measured 30, 60, 90, 120, 240, 180 and 240 min after the treatment. All experimental groups consisted of 6 to 10 mice. All experiments were approved by the Institutional Animal Ethics Committee which is in accordance with Revised Guide for the Care and Use of Laboratory Animals (NIH Guide, Volume 25, Number 28, 1996).

2.2. Drugs administration

Streptozotocin (Sigma-Aldrich Chemie, Germany) was dissolved in 0.03 mol/l citrate buffer at pH 4.5 immediately before i.p. injection. Gabapentin (Neurontin, Pfizer H.C.P. Corporation, New York, USA), oxcarbazepine (Trileptal, Novartis Pharma AD, Basel, Switzerland), and amitriptyline (Amizol, Lek, Ljubljana, Slovenia) were suspended in distilled water. Drugs/drug combinations were administered to mice orally in a volume of 10 ml/kg.

2.3. Induction and assessment of diabetes

To induce diabetes, mice received a single i.p. injection of streptozotocin (150 mg/kg body weight) following an overnight fast. One group of ten age-matched control mice received an equivalent volume of citrate buffer. The diabetes was confirmed 2 weeks after the streptozotocin injection by measuring the blood glucose level with an automatic analyzer (GlucoSure Plus, Apex Biotechnology Corp, Hsinchu, Taiwan) using glucose oxidase peroxidase enzyme reagent stripes. Blood was withdrawn from the retro-orbital sinus of mice with a heparinized capillary tube. Animals with a non-fasting serum glucose levels above 250 mg/dl were considered diabetic and used for the experiments.

2.4. Assessment of thermal nociceptive responses

The nociceptive responses in both normal and diabetic mice were evaluated by the radiant heat tail-flick test. This test is commonly used for assessing sensory functions in diabetic animals since it is similar to certain quantitative sensory tests that are used in diabetic patients (Calcutt, 2002). The tail-flick test displays the activity of a simple spinal reflex arc. It provides information concerning peripheral nerve and spinal functions in isolation from higher nociceptive processing and cognitive systems (Calcutt, 2002; Le Bars et al., 2001). Thus, it is very unlikely that motor impairment or sedation of animals caused by either neuropathic changes in diabetic mice or by the drug treatments in both normal and diabetic mice could interfere with the ability of an animal to react to nociceptive stimuli.

Three measurements of baseline tail-flick latencies were performed before the streptozotocin injection, and the average latency for each mouse was used for further calculations. Three weeks after the induction of diabetes, tail-flick latencies were retested and the effects of the drugs and drug combinations were examined. That time point was chosen in order to avoid substantial loss of animals due to death, and to provide conditions for performing the experiments with sufficiently healthy mice.

To measure the latency of the tail-flick response, the radiant heat was focused on a spot in the mid-region at the dorsal surface of the animal tail (about 4 cm from the tip of the tail). The latency between the application of the stimulation heat and the flicking of the animal's tail was recorded. When the animal flicks its tail, its exposes a photocell in the apparatus (Hugo Sachs Elektronik, Cat. No. 7360, March-Hugstetten, Germany) and the time is recorded automatically. The heat lamp was set to provide a pre-treatment (baseline) latency time of the tail-flick response of 4.5–5.5 s. The intensity of the heat lamp and the laboratory temperature was constant throughout all measurements. A cut-off time of 10 s was used to prevent blistering.

The percentage of the maximal possible effect of each treatment (MPE) was calculated for each animal using the following formula:

$$MPE = (\text{post-treatment latency} - \text{pre-treatment latency}) / (10 - \text{pre-treatment latency}) \times 100.$$

When testing the signs of diabetic neuropathy development in streptozotocin-treated mice, the shorter post-streptozotocin latencies than the pre-streptozotocin latencies would result in negative values of MPE, indicating hyperalgesia.

When examining the antinociceptive effects of drug/drug combinations in diabetic and normal mice, for the shorter post-drug/drug combinations latencies than the pre-drug/drug combinations latencies, a value of 0% MPE was assigned, indicating the absence of antinociception. The ED_{50} (the dose that was expected to result in 50% MPE) with S.E.M. and 95% confidence limits were estimated from regression analysis of the linear portion of the corresponding log dose–response curves at the time of peak effect (Tallarida and Murray, 1986).

2.5. Analysis of type of interaction between co-administered drugs

2.5.1. Analysis of interaction between two drugs with high antinociceptive efficacy

The interaction between gabapentin and oxcarbazepine (drugs that exerted high antinociceptive efficacy and dose-dependent effect in diabetic mice) was evaluated by co-administration of fixed proportions of each drug, and performing an isobolographic analysis (Tallarida et al., 1997). At first, an ED_{50} value of each drug has to be obtained from the corresponding log dose–response curves. In the next step, gabapentin and oxcarbazepine were co-administered at fixed-dose fractions of the ED_{50} ($0.125\ ED_{50\ GABAPENTIN} + 0.125\ ED_{50\ OXCARBAZEPINE}$, $0.25\ ED_{50\ GABAPENTIN} + 0.25\ ED_{50\ OXCARBAZEPINE}$, $0.33\ ED_{50\ GABAPENTIN} + 0.33\ ED_{50\ OXCARBAZEPINE}$ and $0.5\ ED_{50\ GABAPENTIN} + 0.5\ ED_{50\ OXCARBAZEPINE}$). For drug mixture, experimental ED_{50} ($ED_{50\ mix}$) and its associated 95% confidence intervals were determined by linear regression analysis of the log dose–response curve and compared to a theoretical additive ED_{50} ($ED_{50\ add}$) obtained from the calculation: