INTRODUCTION

Pregabalin (PGB) is a new antiepileptic drug belonging to the class of derivative of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), usually called gabapentinoids. It has a range of therapeutic activities on the nervous system: besides the antiepileptic effect, it has also anxiolytic and efficient analgesic actions, being used in various types of pain. Its efficacy has been proven especially in neuropathic pain associated with various pathologic conditions: diabetic peripheral neuropathy and postherpetic neuralgia. At present, the current specialists' general opinion is in favor of broadening its spectrum of analgesic activity to other peripheral neuropathic conditions (1, 2). PGB has also been reported to be effective in other types of pain resembling neuropathic pain, like chronic pancreatitis (3).

Lately, various research trials have been conducted in order to evaluate the efficacy and eventual synergistic effect of the gabapentinoids in combination with other analgesic drugs, both opioids and NSAIDS. Many experimental and clinical studies have been conducted for gabapentin. In neuropathic pain, the combinations of gabapentin with opioids (e.g. morphine) were proven to potentiate their antinociceptive effects (4-6). PGB is more efficient than gabapentin as an antiepileptic (7), but many studies suggest that it might be also a stronger analgesic (8); consequently, it could increase the analgesic effect of opioid medication. PGB proved a dose-dependent analgesic effect, with a similar intensity to tramadol in the mouse model of acute thermal pain hot plate test (9, 10).

The data reported by literature reveal that the opioids have low efficacy in neuropathic conditions, especially in single therapy (11). According to certain researchers, the hypothesis claiming that opioidergic pathway could mediate the analgesic effect of pregabalin could be accurate (12). Therefore, we have considered evaluating the effect of the combination between pregabalin and the weak opioid codeine (COD). Although PGB is highly efficient in neuropathic pain management, the combination is justified taking into account the lowering of the needed doses and of side effects of these two substances. Therefore, such a combination is justified because these two substances possess different mechanisms of action, and thus the antinociceptive effect can be increased.

We aimed to prove our hypothesis using the partial nerve ligation model of neuropathic pain in rats, based on our research experience in performing this international accepted standardized model. Ethical reasons have drawn us to choose the partial nerve ligation model: although is more laborious, it has the advantage of being less traumatic for laboratory animals.

MATERIALS AND METHODS

Animals

Experimental protocols were implemented according to recommendations of the ‘Grigore T. Popa’ University Committee for Research and Ethical Issues, in agreement with international ethical normatives (13).
The experiments were carried out on 30 male white Wistar rats (200 – 250 g), distributed into 5 groups of 6 animals each. The animals were treated orally (using an eso-gastric device) with this combination in a single daily dose, 7 days before and 7 consecutive days after the day of surgery:

- Group I (S-O): saline solution (0.5 ml/100 g body weight) - sham-operated;
- Group II (C-1, control with ligation): saline solution (0.5 ml/100 g body weight) - with ligation;
- Group III (PGB): pregabalin (20 mg/kg body weight);
- Group IV (COD): codeine (50 mg/kg body weight);
- Group V (PGB + COD): pregabalin (20 mg/kg body weight) + codeine (50 mg/kg body weight).

The doses were chosen based on our previous experience and reasons to reproduce the manner of administration of these substances in clinical practice. Oral route, involving entero-hepatic circulation, requires higher doses of substance to obtain the same pharmacodynamic effects as the parenteral route.

Laboratory animals were housed in plastic cages, under standard laboratory conditions (relative humidity 55 – 65%, constant room temperature of 23.0 ± 1.0°C and under 12 hours artificial light/dark cycle) and fed with standard diet and water ad libitum, except during the time of the experiments.

Substances

COD and saline solution were purchased from Sigma-Aldrich Co, Germany. The dose of PGB (Mesochem Technology, China) used represents 1/20 of the lethal dose 50. All solutions were prepared extemporaneously in distilled water.

Partial sciatic nerve ligation

Peripheral neuropathic pain after partial sciatic nerve ligation was induced in laboratory animals using a similar procedure to that described by Seltzer et al. for rats (14). Rats were anesthetized with sodium pentobarbital (intraperitoneal, 50 mg/kg body weight, Sigma-Aldrich Co). Unilateral mononeuropathy was induced by a rigid ligation of about one-third to one-half of the left sciatic nerve (ipsilateral) diameter performed with 4 – 0 silk suture. The ligatures were loosely tied until a short flick of the ipsilateral hind limb was observed. In sham-operated rats the sciatic nerve was exposed without ligation.

Investigation of thermal and mechanical hyperalgesia

Nociceptive thermal and mechanical thresholds were evaluated before (baseline) and in the 1st, 3rd, 5th and 7th day after the surgical procedure, 30 minutes after substances administration. Thermal nociceptive reactivity of the hind paw was assessed by using the hot plate test (Ugo Basile). This experimental model consists of rat paws noxious thermal stimulation, followed by counting the response latency period (15). The recommended cut-off time was 15 seconds. To evaluate mechanical withdrawal responses, pressure stimulation analgesimeter test (Ugo Basile) was performed (16). This model consists of mechanical noxious hind paw stimulation by applying increasing pressure and assessing the mechanical threshold (expressed in grams). In order to avoid tissues damage, a cut-off pressure of 450 g was maintained.

Data analysis

Data were analyzed using SPSS 17.0 for Windows software; the one-way ANOVA method was employed to compare the mean values of the groups; the two-way ANOVA and the post-hoc analysis were used for multiple comparisons of the mean values. For the post-hoc analysis the Bonferroni test was used; differences having the P-value less than 0.05 were considered to be significant.

RESULTS

Before surgical intervention, no significant difference was observed between the left and right paws reactivity to heat and pressure stimulation. This procedure induced important decrease in thermal and mechanical nociceptive thresholds observed in the ipsilateral hind paw. No notable modifications were observed in the nociceptive reactivity of the paw controlateral to the sciatic nerve injury. Neither considerable behavioral manifestations, nor major threshold variations were observed between ipsilateral and controlateral hind paw in sham-operated rats.

Partial ligation of sciatic nerve resulted in significant increase of noxious thermal hyperalgesia, proved by a diminution in paw withdrawal threshold (P < 0.05), one day after the surgical intervention as compared to sham-operated group, effect maintained for 7 days in the experiment (Fig. 1). COD administration (50 mg/kg body weight) did not significantly influence the paw withdrawal reaction compared to control group in the hot-plate test. Oral administration of PGB (20 mg/kg body weight) showed an important increase in the latency response compared to control group, during the experiment. The combination PGB + COD significantly attenuated the nerve ligation-induced decreasing nociceptive threshold for thermal hyperalgesia, statistically significant (P < 0.05), compared to control group at each moment of determination in the hot plate test (Fig. 1). The PGB + COD association effects on the paw thermal withdrawal latency were similar to those observed in sham-operated group, 1 day, 3 day and 7 days in the experiment. The combination also increased the latency response to paw noxious stimulation, statistically significant compared to both individual groups PGB and COD at all time moments in the hot plate test (Fig. 1).

Chronic partial ligation of the sciatic nerve was correlated with an important increase of noxious mechanical hyperalgesia (P < 0.05), augmented by reduction in paw withdrawal threshold compared to sham-operated group, effect manifested one day after the surgical procedure and maintained for 7 days in the analgesimeter test (Fig. 2).

PGB (20 mg/kg body weight), but not COD (50 mg/kg body weight) treatment, demonstrated significant (P < 0.05) increase of the latency response compared to control group at every time moments of the determination. The administration of PGB + COD combination resulted in an important attenuation of the nerve ligation-induced reducing nociceptive threshold for mechanical hyperalgesia, statistically significant (P < 0.05) compared to control group during the experiment, in analgesimeter test (Fig. 2). The use of PGB + COD combination was also associated with an increase of latency response, statistically significant compared to both individual groups (PGB, respectively COD), at all times moments in this cutaneous mechanical pain model in rats.

DISCUSSION

Our previous investigations have demonstrated the synergistic analgesic effect of PGB in combination with acetaminophen and tenoxicam in the somatic pain model tail flick test in mice (17). The involvement of prostaglandins system and its interrelation with opioid system was also revealed in different other somatic (hot plate) and visceral (writhing test) experimental pain models in mice (18).
Our results are in agreement with similar studies that have proved the antinociceptive effects of PGB association with different other analgesic drugs in various models of experimental pain. Some experimental researches have revealed an increase of the analgesic effect consecutive to the administration of low analgesic doses of tramadol combined with PGB in two somatic pain models: tail flick and hot plate in mice (10, 19).

**Fig. 1.** Thermal nociceptive thresholds in partial sciatic nerve ligation in ipsilateral paw. Data were expressed as the mean ± S.E.M. of the parameter measured of 6 rats per group and analysed using a two-way ANOVA. In the post-hoc analysis the Bonferroni’s post-hoc test was used. The different symbols indicate a significant difference of the individual groups as: *P < 0.05 vs. pregabalin-codeine combination (PGB + COD); †P < 0.05 vs. sham-operated rats (S-O); ‡P < 0.05 vs. control group (C).

**Fig. 2.** Mechanical nociceptive thresholds in partial sciatic nerve ligation in ipsilateral paw. Data were expressed as the mean ± S.E.M. of the parameter measured of 6 rats per group and analysed using a two-way ANOVA. In the post-hoc analysis the Bonferroni’s post-hoc test was used. The different symbols indicate a significant difference of the individual groups as: *P < 0.05 vs. pregabalin-codeine combination (PGB + COD); †P < 0.05 vs. sham-operated rats (S-O); ‡P < 0.05 vs. control group (C).
In the present investigation we used a standard model of painful mononeuropathy that induces a slight constriction, swelling, an inflammatory local reaction in lab animals, accompanied by spontaneous pain behavior and increased sensitivity to external noxious stimuli (20). In rats with partial sciatic nerve ligation, the experimental-induced peripheral mononeuropathy was associated with excessive licking and limping of ipsilateral hind paw and intense reactivity to painful heat and pressure stimuli. These results are concordant with other findings which have suggested that PGB may be useful for controlling chronic pain. Several clinical trials have been conducted to investigate PGB-opioid combinations (with morphine, oxycodone, hydromorphone) for various types of neuropathic pain: cancer pain, pre-operative, post-operative pain. The preemptive administration of these drugs is beneficial to control the nociceptive reactivity, especially due to their action on both central components of pain (processing of nociceptive information and psychological interpretation of pain). The use of these substances before the surgical procedure limits the release of algogenic substances, consequently reducing the needed doses of analgesics for pain control (21, 22).

It is known that PGB influences especially the metabolism of inhibitory neurotransmitter GABA in the central nervous system, not acting as direct agonist on GABA-A or GABA-B receptor subtypes. Therefore, its antinociceptive effects are due to the direct action on the neuronal calcium channels, rather than to a miorelaxant activity (23).

Although some papers conclude slightly beneficial effect due to the association of the two types of drugs (24), the results reported in the literature are mostly positive, suggesting a possible PGB-opioid synergic effect (21, 25-28). The benefits of the combination with PGB are represented by the decrease in post-operative opioid consumption and consecutively the diminution of their side effects (25, 29). Literature data suggest the existence of correlations at different levels of GABA and opioid systems, but the intimate mechanism is not yet completely understood. The synergistic effect observed may be explained, partly, by the inhibitory action of these two substances on the voltage-dependent calcium channels. PGB reduces calcium influx into presynaptic terminals and decreases the calcium-dependent release of multiple neurotransmitters (30, 31). It has been postulated that the activation of transient receptor potential ankyrin 1 (TRPA1) calcium-permeable ion channel could mediate the neuropathic hyperalgesia (32), suggesting that the inhibition of spinal TRPA1 channels is a key element involved in the antinociceptive action of pregabalin in peripheral neuropathic pain (33).

Another important protein seems to be the receptors for glucagon-like peptide-1 (GLP-1), their activation stimulating the GABA-A signaling in the central nervous system neurons (34). It was demonstrated that GLP-1 receptors are also involved in the modulation of nociceptive mechanisms, being proved that the analogue of GLP-1 decreased chronic visceral hyperalgesia in the colorectal distention model in rats through serotoninergic pathways (35). The interrelations between GLP-1 receptors, gabaergic and serotoninergic systems may explain the detected visceral antinociceptive effects of pregabalin in colorectal distention model in rats (36).

Pharmacodynamic effects of COD are related to the action of its active metabolite morphine, which binds and stimulates de µ opioid receptors, known to be involved in the modulation of calcium channel current in different central nervous system neurons. Like all opioids, COD closes the N-type voltage-operated calcium channels, inducing the decrease of neuronal excitability (31). It has been suggested that in the modulation of nociceptive hypersensitivity in neuropathic pain several other neurotransmitters, specific receptors and different pathways may be involved, highlighting the interrelations between opioidergic and benzodiazepine, respectively gabaergic pathways, in the mediation of painful reactivity in rats with chronic constriction due to the ligation of sciatic nerve (37).

We proved that the treatment with PGB attenuated thermal and also mechanical hyperalgesia, induced after partial sciatic nerve ligation in rats. Moreover, PGB plus COD association significantly reduced the degree of both thermal and mechanical hyperalgesia in the operated hind paw.

These results suggest that pregabalin-codeine combination exerted amelioriative effect on partial sciatic nerve ligation-induced neuropathic pain in rats, action that can be beneficial in the treatment of different neuropathic chronic pain conditions.

Conflict of interests: None declared.

REFERENCES


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