THE SYNERGISTIC ANTINOCICEPTIVE INTERACTION OF CODEINE AND PREGABALIN IN A SOMATIC PAIN MODEL IN MICE

ELIZA GRATIELA POPA[1], LILIANA MITITELU TARTAU[2]*, RAOUL VASILE LUPUSORU[3], CATALINA ELENA LUPUSORU[2], IULIAN STOLERIU[4], LACRAMIOARA OCHIUZ[1]

[1]University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Pharmacy, Department of Pharmaceutical Technology, Universitatii st. no. 16, code 700115, Iasi, Romania
[2]University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Medicine, Department of Pharmacology-Algesiology, Universitatii st. no. 16, code 700115, Iasi, Romania
[3]University of Medicine and Pharmacy "Grigore T. Popa", Faculty of Medicine, Department of Patho-Physiology, Universitatii st. no. 16, code 700115, Iasi, Romania
[4]University "Al. I. Cuza", Faculty of Mathematics, Carol I Bld, code 700506, Iasi, Romania
** corresponding author: lylytartau@yahoo.com*

Abstract

We aimed to investigate the effects of pregabalin (PGB) and codeine (COD) combination in a somatic pain model (the tail flick test) in mice. In this experiment the substances were administered orally separately and in association; the tail withdrawal reflex latencies, as a response to thermal noxious tail stimulation, were registered at 0, 30, 60 and 90 minutes after substances administration. Differences between the experimental and baseline latencies were interpreted as an index of analgesia. Response latency data from tail flick measurements were also converted to per cent of maximum possible effect. In our experimental conditions oral administration of PGB-COD combination was associated with a stronger increase of latency time response, statistically significant after 15 minutes compared to control group, and to the groups treated with PGB, respectively COD in the tail flick test. We can conclude that oral administration of pregabalin proved to potentiate the antinociceptive effects of codeine in this somatic pain model in mice.

Rezumat

Studiul investighează efectele analgezice ale combinaţiei pregabalin (PGB) - codeină (COD) pe un model de durere somatică la şoareci (testul *tail flick*). Experimentul a urmărit administrarea substanţelor menţionate, separat şi în asociere. Reflexul de retragere, ca răspuns la stimularea termică nocivă a cozii a fost înregistrat la 0, 30, 60 şi 90 minute după administrarea substanţelor. Diferenţele între latenţele experimentale şi cele de la momentul zero, sunt interpretate ca index al analgeziei. Latenţele de răspuns măsurate prin testul *tail flick* au fost de asemenea transformate în procent al efectului maxim posibil. În condiţiile experimentale date, administrarea combinaţiei PGB-COD a relevat o prelungire mai accentuată a timpului de latenţă a răspunsului după 15 minute, în comparaţie cu lotul martor şi cu loturile tratate cu PGB, respectiv COD, prin testul *tail flick* la şoarece. Putem

concluziona că administrarea orală a pregabalinei potenţează efectele antinociceptive ale codeinei pe modelul de durere somatică la şoareci.

Keywords: pregabalin, codeine, combinations, tail flick test.

Introduction

Pregabalin (PGB) is a modern antiepileptic drug, the pharmacologically active *S*-enantiomer of racemic 3-isobutyl GABA [16, 18]. Since 2005 it has been approved in many countries and implemented in therapy as a potent agent in the management of partial-onset seizures, but also in the therapy of neuropathic pain associated with painful diabetic neuropathy and postherpetic neuralgia [16, 18]. At present, much research is being developed for proving its use in pain management in combination with other analgesic drugs [5, 10, 15]; however, few studies have been conducted on animal pain models to prove the synergy of PGB combinations.

Codeine is an alkaloid from opium, with important effects on the central nervous system: cough suppression, analgesia, but also anxiolysis, euphoria, and feelings of relaxation [17]. It is considered a prodrug, since it is metabolized *in vivo* to the primary active compounds morphine and codeine-6-glucuronide [20]. It acts as a selective agonist for the μ opioid receptors localized in the central nervous system to alter processes affecting both perception of pain and the emotional response to pain. It is thought that the analgesic activity of codeine is a result of its conversion to morphine, although the precise mechanism of antinociceptive action remains unknown. Codeine has about one-sixth the analgesic activity of morphine [14].

In the present work we aimed to investigate the effects of pregabalin in combination with low doses of codeine in nociceptive reactivity, using a somatic pain model in mice (the tail flick test).

Materials and Methods

The experiments were carried out on male white Swiss mice (20-25g), distributed into 4 groups of 6 animals each, treated orally (using an esogastric device) with the same volume of solution, during 7 consecutive days as follows: **Group I (Control - C):** saline solution (0.1 mL/10 g of mouse weight); **Group II (PGB):** pregabalin (Mesochem Technology, China; 20 mg/kg body weight - kbw); **Group III (COD,** Sigma Aldrich): codeine (50 mg/kbw); **Group IV (PGB+COD):** pregabalin (20 mg/kbw) + codeine (50 mg/kbw). The mice 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). The animals were fed with standard diet and water *ad libitum*, except during the time of the experiments.

All drugs were diluted in saline solution and prepared extemporaneously. Pregabalin was administered orally (using an esogastric device) 7 consecutive days. The dose of pregabalin used represents 1/20 of the lethal dose 50 (LD50) [10]. In the 7th day of the experiment codeine was administered *via* the same route.

Somatic nociceptive reactivity evaluation

Somatic antinociception was assessed using the tail-flick test (on a Panlab Hardvard Apparatus). Mice were placed on a flat surface and held gently by the operator. Tail withdrawal latencies were recorded in response to thermal stimulus from a light beam focused on the dorsal surface of the tail [3, 13, 22]. When the animal flicks its tail, the light beam activates the photocell, closing a switch which turns off the heat source. The amount of time taken for the animal to move its tail away from the heat representing the response latency period, was recorded [6, 9, 22]. The tail-withdrawal latency (seconds) was measured before administration of any drug or vehicle. The baseline latency (before drug injection) in the tail flick test was 4.2 ± 0.2 seconds (mean ± standard error of mean -SEM). The recommended cut-off time of 12 seconds was used to prevent tissue damage. The tail withdrawal reflex periods were registered at 0, 30, 60 and 90 minutes after the administration of the substances. Differences between the experimental and baseline latencies are interpreted as an index of analgesia. An increase of the latency for the mouse to flick its tail is indicative of analgesia, while a decrease of the tail-flick latency is indicative of hyperalgesia [4].

Response latency data from the tail flick measurements were converted to per cent of maximum possible effect (% MPE) according to the formula:

$$\%\ MPE = [(observed\ latency - baseline\ latency) / (cut\ off\ time - baseline\ latency)] \times 100$$ [21].

Results of the tail flick response from each group were calculated as mean with its corresponding confidence limits (95%) and were presented in graphs as means ± SEM (standard error of mean) of latency time (seconds) for six mice.

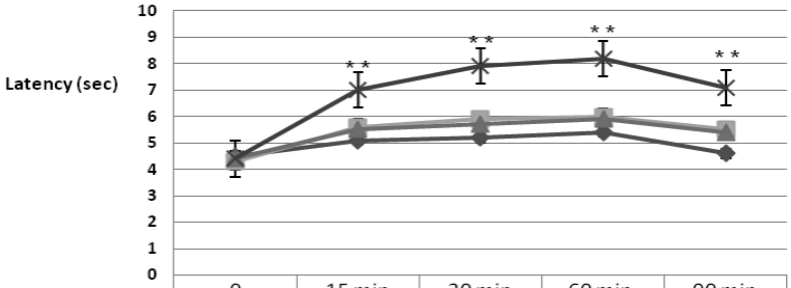
The obtained data were processed using the ANOVA test implemented in *SPSS 17 for Windows* software; p-values less than 0.05

were considered statistically significant compared to those of the control groups.

The experimental protocol was implemented according to the recommendations of the "Grigore T. Popa" University Committee for Research and Ethical Issues and in concordance with the guidelines of the IASP Committee for Research and Ethical Issues and with the Directive 86/609/EEC/24.11.1986, regarding the protection of animals used for experimental and other scientific purposes [23]. In particular, the duration of the experiment was kept as short as possible as well as and the number of mice. For ethical reasons, all the animals were sacrificed at the end of the experiment.

Results and Discussion

Statistical analysis of the results obtained in the tail-flick test showed that the administration of low doses of codeine determined a slight increase of the latency period of the response, but statistically non-significant compared to C group in the tail flick test. Codeine exhibited a maximum effect after 60 minutes in the present experiment (19.7 ± 1.2), (Figure 1).



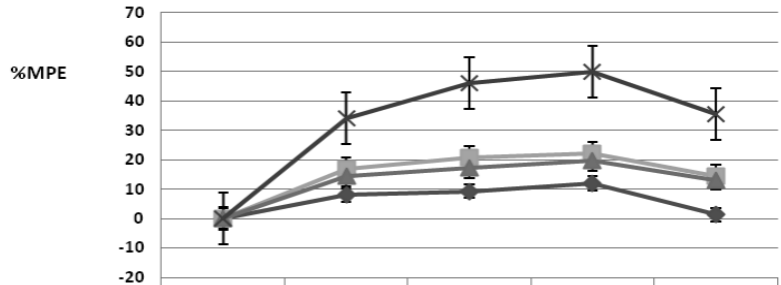
	0	15 min	30 min	60 min	90 min
C	4,5	5,1	5,2	5,4	4,6
PGB	4,3	5,6	5,9	6	5,5
COD	4,4	5,5	5,7	5,9	5,4
PGB+COD	4,4	7	7,9	8,2	7,1

Figure 1.
The latency response of PGB, COD, PGB-COD in the tail flick test. (Each point is the mean ± SEM of latency time (s.) for six mice. **p<0.01; single substances vs. control group, combination vs. single substances.)

Oral administration of PGB was associated with a rapid increase of the latency time response to noxious tail stimulation, statistically significant ($p < 0.01$) compared to C group, effect maintained at all moments of time in this somatic experimental pain model in mice. The effects of PGB were

more intense than those of COD in the experiment (Figure 1). Its maximum possible effect was observed after 60 minutes (% MPE_{60} = 23.7 ± 2.1%), statistically significant compared to the % MPE_{60} (15.8 ± 0.9%) of C group in the tail flick test (Figure 2).

The treatment with the combination PGB-COD resulted in a prolongation of the latency time response to thermal noxious tail stimulation, statistically significant ($p < 0.01$) compared to C group, and also to single PGB, respectively COD administration at all-time intervals during the tail flick test (Figure 1). Its maximal antinociceptive effect achieved after 60 minutes from the administration (% MPE_{60} = 50.0 ± 0.7%) was biologically significant compared to C group, and also PGB, respectively COD groups in the experiment (Figure 2).



	0	15 min	30 min	60 min	90 min
C	0	8	9,3	12	1,3
PGB	0	16,9	20,8	22,1	14,3
COD	0	14,5	17,1	19,7	13,2
PGB+COD	0	34,2	46,1	50	35,5

Figure 2.
Time course of the % MPE of PGB, COD, PGB-COD in the tail flick test. (Each point is the mean ± SEM of percentage of maximum possible effect for six mice.)

The starting point of our study was the idea of the possibility to investigate and further exploit the synergistic effects of PGB-COD combination, in order to improve the quality of analgesia and lower the doses of the individual drugs, consequently reducing the adverse effects. As expected, the low doses of COD tested did not influence significantly the latency time reaction to thermal noxious tail stimulation. PGB administration resulted in a significant analgesic activity in the tail flick test. The association of PGB with COD determined a synergic analgesic effect in this experimental pain model in mice.

In one of our previous experimental studies we have demonstrated that PGB potentiated the antinociceptive effects of two non-opioid analgesic

drugs: acetaminophen and tenoxicam in the tail flick test, but also in a visceral pain model in mice (the writhing test) [11]. Our results are in agreement with other findings which have suggested that PGB may be useful for controlling different types of pain. Literature data showed that PGB proves antinociceptive effects in various acute and chronic, somatic and visceral, inflammatory and neuropathic experimental pain models. Many research studies have revealed that acute treatment with PGB prevents hyperalgesia in the hot plate test and also thermal hyperalgesia (in the tail flick test), in streptozotocin induced diabetic peripheral neuropathy [8, 10] and in various models of experimental-induced peripheral inflammation with carrageenan or formalin [1]. It has also been demonstrated that systemic or intrathecal PGB reduced mechanical allodynia in tail compression test or chronic hyperalgesia in experimental neuropathic pain after peripheral or central nerve injury in rats [7, 16, 19]. Other studies demonstrated a synergic effect of pregabalin-tramadol combination in the tail flick and in the hot plate test [2, 5] and of pregabalin-morphine on mechanical allodynia in tibial neuroma transposition model in rats [12].

Conclusions

We used a standard somatic pain model, the tail flick test, to evaluate the effect of pregabalin-codeine association on the nociceptive reactivity in mice. The 7 days treatment with an unique daily sub-analgesic dose of pregabalin (20 mg/kgb.w.), respectively of codeine (50 mg/kgb.w.) did not significant modify the somatic nociceptive sensitivity in mice.

The administration of pregabalin-codeine combination, using sub-analgesic doses of these drugs, resulted in a significant antinociceptive effect in this experimental cutaneous pain model in mice.

We can conclude that the synergistic analgesic action determined by oral administration, for 7 days, of pregabalin associated with low doses of codeine is an advantage compared to single administration of pregabalin, respectively codeine, due to higher intensity of effect and on the other hand to lower side effects.

References

1. Bardin L., Gregoire S., Aliaga M., Malfetes N., Vitton O., Ladure P., Newman-Tancredi A., Comparison of milnacipran, duloxetine and pregabalin in the formalin paintest and in a model of stress-induced ultrasonic vocalizations in rats. *Neurosci. Res*., 2010; 66: 135-140.
2. Cannon C.Z., Kissling G.E., Hoenerhoff M.J., King-Herbert A.P., Blankenship-Paris T., Evaluation of dosages and routes of administration of tramadol analgesia in rats using hot-plate and tail-flick tests. *Lab. Anim*., 2010; 39: 342-351.

3. Firulescu S., Negres S., Mihele D., Experimental pharmacological researches valuating the analgesic activity for novel hybrid prodrugs obtained by esterification of NSAIDS compounds with prostaglandinic compounds. *Farmacia*, 2010; 58(6): 695-702.
4. Keefe F.J., Fillingim R.B., Williams D.A., Behavioral Assessment of Pain: Nonverbal Measures in Animals and Humans. *ILAR Journal*, 1991; 33(1-2): 3-13.
5. Keyhanfar F., Meymandi M.S., Sepehri G., Rastegaryanzadeh R., Heravi G., Evaluation of antinociceptive effect of pregabalin in mice and its combination with tramadol using tail-flick test. *Iranian Journal of Pharmaceutical Research*, 2013; 12(3): 483-493.
6. Le Bars D., Gozariu M., Cadden S.W., Animal models of nociception. *Pharmacological Reviews*, 2001; 53(4): 597-652.
7. Lim H.S., Kim J.M., Choi J.G., Ko Y.K., Shin Y.S., Jeon B.H., Park J.B., Lee J.H., Kim H.W., Intrathecal ketamine and pregabalin at sub-effective doses synergistically reduces neuropathic pain without motor dysfunction in mice. *Biol. Pharm. Bull*., 2013; 36(1): 125-130.
8. Luszczki J.J., Dose-response relationship analysis of pregabalin doses and their antinociceptive effects in hot-plate test in mice. *Pharmacol. Reports*, 2010; 62(5): 942-948.
9. Ma C., Animal models of pain. *Int. Anesthesiol. Clin*., 2007; 45 (2): 121-131.
10. Meymand M.S., Keyhandfar F., Pregabalin antinociception and its interaction with tramadol in acute model of pain. *Pharmacol. Rep*., 2012; 64(3): 576-585.
11. Mititelu-Tartau L., Popa E.G., Lupusoru R.V., Lupusoru C.E., Stoleriu I., Ochiuz L., Synergic effects of pregabalin-acetaminophen combination in somatic and visceral nociceptive reactivity. *Pharmacology*, 2014; 93(5-6): 253-259.
12. Miyazaki R., Yamamoto T., The efficacy of morphine, pregabalin, gabapentin, and duloxetine on mechanical allodynia is different from that on neuroma pain in the rat neuropathic pain model. *Anesth. Analg*,, 2012; 115(1): 182-188.
13. Mogil J.S., Animal models of pain: progress and challenges. *Nat. Rev. Neurosci*., 2009, 10: 283-294.
14. Moore A., Collins S., Carroll D., Paracetamol with and without codeine in acute pain: a quantitative systematic review. *Pain*, 1997; 70: 193-201.
15. Nishihara M., Arai Y.C.P., Yamamoto Y., Nishida K., Arawawa M., Ushida T., Ikeuchi M., Combinations of Low-Dose Antidepressants and Low-Dose Pregabalin as Useful Adjuvants to Opioids for Intractable, Painful Bone Metastases. *Pain Physician*, 2013; 16: E547-E552, at http://www.painphysicianjournal.com.
16. Sikandar S., Dickenson A.H., Pregabalin modulation of spinal and brainstem visceral nociceptive processing. *Pain*, 2011; 152(10): 2312–2322.
17. Srinivasan V., Wielbo D., Tebbett I.R., Analgesic effects of codeine-6-glucuronide after intravenous administration. *Eur. J. Pain*, 1997; 1(3): 185-190.
18. Stahl S.M., Porreca F., Taylor C.P., Cheung R., Thorpe A.J., Clair A., The diverse therapeutic actions of pregabalin: is a single mechanism responsible for several pharmacological activities? *Trends in Pharmacological Sciences*, 2013, 34(6): 332-339.
19. Takenchi Y., Takasu K., Ono H., Tanabe M., Pregabalin S-(+)-3-isobutylgaba activates the descending noradrenergic system to alleviate neuropathic pain in mouse partial sciatic nerve ligation model. *Neuropharmacology*, 2007; 53: 842-853.
20. Vree T.B., van Dongen R.T., Koopman-Kimenai P.M., Codeine analgesia is due to codeine-6-glucuronide, not morphine. *Int. J. Clin. Pract*., 2000; 54 (6): 395–398.
21. Walker E.A., Butelman E.R., DeCosta B.R., Opioid thermal antinociception in rhesus monkeys: receptor mechanisms and temperature dependency. *J. Pharmacol. Exp. Ther*., 1993; 267: 280–286.
22. Zbarcea N.E., Negres S., Cristea A.N., Chirita C., The effect of dextromethorphan, gabapentin, amitriptyline and tramadol on a mouse model of vincristine - induced peripheral neuropathy. *Farmacia*, 2011; 50(6): 809-811.
23. Zimmerman M., Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*, 1983, 16: 109-110.

Manuscript received: January 2014