DE GRUYTER OPEN

Acta Veterinaria-Beograd 2016, 66 (1), 103-114
UDK: 615.212.015
DOI: 10.1515/acve-2016-0008

Research article

L-ARGININE-NO SYSTEM PARTICIPATES IN THE ANALGESIC EFFECT OF FLUNIXIN MEGLUMINE IN THE RAT

MILOVANOVIĆ Mirjana[1*], VUČKOVIĆ Sonja[2], PROSTRAN Milica[2], TRAILOVIĆ Saša[1], JOVANOVIĆ Milan[1]

[1]Faculty of Veterinary Medicine University of Belgrade, Bul. Oslobodjenja 18, 11000 Belgrade, Serbia.
[2]Medical Faculty University of Belgrade, Dr. Subotica 8, 11000 Belgrade, Serbia

(Received 15 September; Accepted 11 December 2015)

This study investigated whether the L-arginine-NO system participates in the analgesic effect of flunixin meglumine in the rat. Hyperalgesia was induced by intraplantar (i.pl.) administration of carrageenan (500 μg) into the rat's hind paw. Electronic von Frey apparatus was used to determine paw withdrawal threshold induced by pressure as the painful stimulus, measured in grams (g). Flunixin meglumine (FM; 0.09-0.1 mg/kg; s.c.) and N^G-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg; i.p.), given separately as a pre-treatment, i.e. 15 min before i.pl. injection of carrageenan, produced a significant antinociception. When FM (0.09 mg/kg) and a sub-effective dose of L-NAME (5 mg/kg) were co-administered, the antinociceptive effect was significantly increased in comparison with the effect of FM alone. L-arginine (L-ARG;10 mg/kg; i.p.) itself did not produce significant effect on carrageenan-induced hyperalgesia, but significantly reduced the antinociceptive effects of both FM and FM + L-NAME combination. The inhibition of the production of NO might be involved in the mechanism of the analgesic effect of FM.

Key words: Flunixin meglumine, L-NAME, L-arginine, carrageenan, hyperalgesia

INTRODUCTION

Flunixin meglumine is a non-steroidal anti-inflammatory drug (NSAID) that has analgesic and antipyretic properties for use in horses, cattle and swine. It is licensed as a veterinary drug and can be prescribed by a licensed veterinarian. Flunixin meglumine is used in the alleviation of inflammation and pain associated with musculoskeletal disorders and for the alleviation of visceral pain associated with colic in horses; the control of acute inflammation associated with respiratory diseases in cattle and an aid in the treatment of mastitis in dairy cattle, and mastitis metritis agalactia syndrome (MMA) in sows [1,2]. In addition to its analgesic and antipyretic effects, flunixin meglumine has been studied and cited for its antiendotoxic effect in experimental models of septic shock in several species [3-5].

*Corresponding author: e-mail: miram@vet.bg.ac.rs

Flunixin meglumine is a non-selective inhibitor of enzymes cyclooxygenases (COX-1/ COX-2), which control the production of different prostanoids (prostaglandins and thromboxanes) from arachidonic acid being released from cell membrane phospholipids [6,7]. COX-1 enzyme is considered a constitutive enzyme responsible for the production of a variety of cytoprotective prostanoids, which are important for normal gastrointestinal, renal, vascular, and other body system physiologic functions. COX-2 enzymes are induced by inflammatory cytokines and produce prostaglandins involved in inflammation, pain, and fever [8]. COX activity has now been further divided, by some, into COX-3 activity, which appears to act centrally (brain) [9].

In recent times, there is much experimental evidence that the L-arginine-NO system plays an important role in the development of inflammation and inflammatory pain [10,11]. Nitric oxide (NO) is a short-lived signaling molecule that is formed from the amino acid L-arginine in the presence of NO-synthase (NOS). Three NOS isoforms have been characterized: endothelial (eNOS) and neuronal (nNOS), Ca^{2+}/calmodulin dependent or constitutive and iNOS, Ca^{2+}/calmodulin independent, being inducible [12]. NO is potent modulator of pain perception and there is ample evidence to support its role in nociception. Neuronal NO synthase (nNOS) is the predominant form of NOS in the dorsal horn and has a definite role in spinal cord circuits. Besides nNOS, the eNOS is also found in some neuronal populations and blood vessels. Concerning iNOS, it has been proven that this isoform is located beside the astrocytes, in glial cells and macrophages and is expressed in response to inflammatory and pro-inflammatory mediators [13-15].

N^G-nitro-L arginine-methyl ester (L-NAME) is a non-selective NOS inhibitor, which administered intraperitoneally produces antinociception in the mouse assessed by the formalin-induced paw licking and acetic acid-induced abdominal constriction models [16]. Also, the same authors have shown that the combined use of L-NAME and non-selective NSAID flurbiprofen or L-NAME and indomethacin combined therapy, resulted in potentiated antinociception in both nociceptive models. In our previous study, we showed that flunixin meglumine, L-NAME and their combination applied in the treatment of carrageenan-induced inflammatory pain exert antinociceptive activity [17]. This study was aimed to investigate whether the L-arginine-NO system participates in the analgesic effect of flunixin meglumine in rats by evaluating the effect of pre-treatment with flunixin meglumine, L-NAME and L-arginine on the carrageenan-induced hyperalgesia in rats.

MATERIALS AND METHODS

Ethical approval

All experimental procedures where performed with the permission of the Ethics Committee for Animal Research and Welfare of Faculty of Medicine, University of Belgrade (permit N°244/10). All of the experiments were approved by the Ethical

Council for Protection of Experimental Animals of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, which operates in accordance with the Animal Welfare Law of the Republic of Serbia and the IASP (International Association for the Study of Pain) Guidelines for the Use of Animals in Research.

Animals

Adult male Wistar rats (200-250g) used in the present study were taken from the Military Academy Breeding Farm, Belgrade, Serbia. The animals were housed in groups of three in home cages (42.5 × 27 × 19 cm) under standard conditions: temperature of 21±1°C, relative humidity of 55-60% and 12/12h light/dark cycle. Food and water were provided *ad libitum*, except during the experimental procedure. The animals were habituated individually in a Plexiglas chamber for 30 min before testing. All behavioral testing was done between 10:00 A.M. and 4:00 P.M.

Drugs and administration

Flunixin meglumine (FM), N^G-nitro-L-arginine methyl ester (L-NAME) and L-arginine (L-ARG) were purchased from Sigma Aldrich, Inc. All drugs were dissolved in saline (vehicle) and administered subcutaneously (FM) and intraperitoneally (L-NAME, L-ARG). Control groups of rats were treated with vehicle alone. Carrageenan (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline (5 mg/ml) and injected i.pl. in a final volume of 0.1 ml/paw, using 1 ml syringe and 26G (0.45 x 12 mm) needle according to Morris [18].

Measurement of hyperalgesia

Hyperalgesia was induced by subcutaneous injection of carrageenan (Ca, 500 μg/paw) into the plantar surface of the hind paw [18]. Hyperalgesia was measured via nociceptive mechanical paw test described by Vivancos and others [19]. Electronic von Frey apparatus (Model 2390, IITC Life Science – USA) was used to determine paw withdrawal threshold in grams. An increasing pressure of maximum 80g was applied to the rat hind paw until the animal presents a paw flexion as the nociceptive threshold. First, the basal (control) reaction was obtained, and then the hyperalgesia was induced by intraplantar (i.pl.) injection of carrageenan. The intensity of hyperalgesia was quantified as the differences in pressures [*d*(g)] applied before and after injection of carrageenan. Measurement was repeated three times at each time point, and the average *d* for each rat was used for further calculations.

Force differences are expressed as a percent antinociceptive activity (%AA) and calculated according to the following formula:

$$\%AA = (\text{control group average } d - \text{test group average } d)/$$
$$(\text{control group average } d) \times 100.$$

If the test group average *d* was greater than control group average *d*, a value of 0% AA was assigned [20].

The percent inhibition (*%I*) of the antinociceptive effect by L-arginine was expressed as follows [20]:

$$\%I = 100 - (\%AA \text{ FM or FM+L-NAME in the presence of L-ARG}) / (\%AA \text{ FM or FM+L-NAME}) \times 100.$$

Experimental protocol

FM and L-NAME were applied before carrageenan (pre-treatment). FM and L-NAME were administered 0.25h (15 min) before carrageenan, subcutaneously and intraperitoneally, respectively. NO-donor, L-arginine was administered intraperitoneally at the time of carageenan injection (0h). Predrug *d* was obtained before i.pl. carrageenan injection. Posdrug *d* was measured at 1, 2, 3, 3.30, 4, 4.30, 5, 5.30 and 6h after drug administration. Control animals received the same volume of vehicle (s.c. or i.p.) instead of the test compound (Fig. 1).

Number of animals used per group was 6 (n=6). Protocols concerning the dose and the time of drug administration were obtained from literature data and pilot experiments.

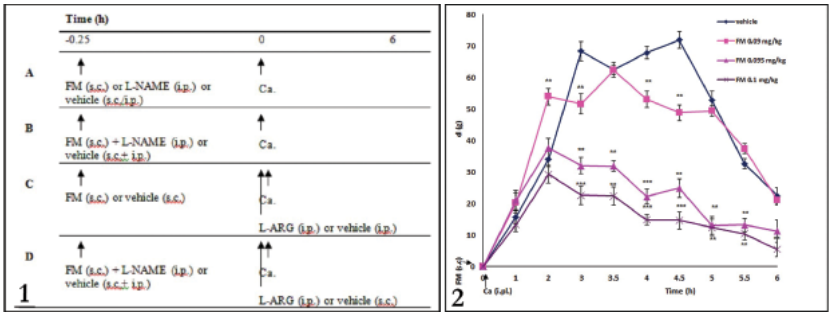

Figure 1. Experimental protocol used in the evaluation of the antinociceptive effects of FM or L-NAME (A), combination of FM+L-NAME (B), FM in the presence of L-ARG (C), and combination of FM+L-NAME in the presence of L-ARG (D) applied in the pre-treatment on the carrageenan-induced hyperalgesia in rats. Control animals received the corresponding subcutaneous (s.c.) or intraperitoneal (i.p.) injections of vehicle.

Figure 2. Antihyperalgesic effect of FM (0.09-0.1mg/kg; s.c.) on carrageenan-induced hyperalgesia. FM was administered 15 min before carrageenan (Ca; 500μg/paw).The symbols denote mean ± SEM of 6 rats *per* group. * $P<0.05$, ** $P<0.01$ vs. vehicle, one-way ANOVA followed by post hoc Tukey`s HSD test.

Statistical analysis

The data were statistically analyzed by One-Way Analysis of Variance (ANOVA) and the post hoc Tukey`s HSD test for multiple comparisons. Probabilities of less than 5% ($P < 0.05$) were considered to be statistically significant.

RESULTS

Effects of flunixin meglumine and L-NAME in the electronic nociceptive mechanical paw test

Flunixin meglumine (FM; 0.09-0.1 mg/kg, s.c.), given 15 minutes before carrageenan injection into the rat hind paw, produced a significant dose-dependent antihyperalgesic effect (Fig. 2).

FM at a dose of 0.09 mg/kg caused a significant reduction of the nociception at time point of 3h (24,5%), with a maximum at 4.5h (32.1%), after which the effect weakened and ceased 5.5h (0%) after carrageenan administration. FM at the higher dose of 0.095 mg/kg achieved the maximum of the antinociceptive activities (*%AA*) at time point of 5h (75.5%), and the antinociceptive effect lasts (67.1%) until the last measurement time point at 6h. FM at the highest dose of 0.1 mg/kg achieved the maximum of the antinociceptive activities (*%AA*) at time point of 4.5h (79.9%), and the antinociceptive effect lasts (75.8%) until the last measurement time point at 6h (not shown).

L-NAME at a dose of 10 mg/kg, (i.p) given 15 minutes before carrageenan, produced antihyperalgesia, while the lower dose of L-NAME (5 mg/kg; i.p.) did not produce any significant effect (Fig. 3).

The influence of L-NAME on the antinociceptive effect of flunixin meglumine in the electronic nociceptive mechanical paw test

Co-administration of FM (the lowest effective dose previously tested =0.09 mg/kg; s.c.) and sub-effective dose of L-NAME (5 mg/kg; i.p.), significantly increased the antihyperalgesic effect in comparison with the effect of FM alone (Fig. 4).

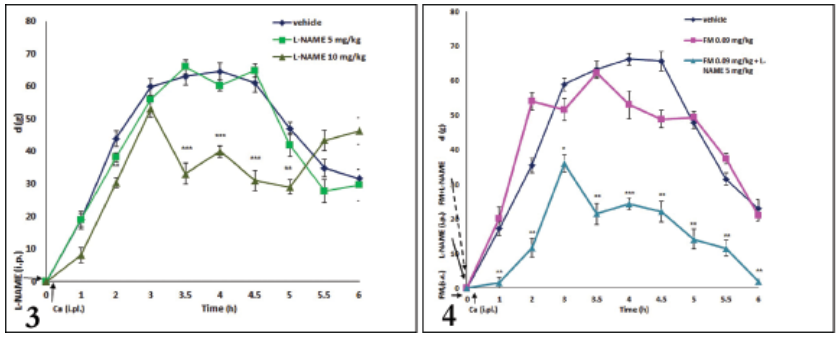

Figure 3. Effect of L-NAME (5, 10 mg/kg; i.p.) on carrageenan-induced hyperalgesia. L-NAME was administered 15 min before carrageenan (Ca; 500μg/paw). The symbols denote mean ± SEM of 6 rats/group. ** *P*<0.01, *** *P*<0.001 vs. vehicle, one-way ANOVA followed by post hoc Tukey`s HSD test.

Figure 4. Antihyperalgesic effect of FM (0.09mg/kg; s.c)+ L-NAME (5mg/kg; i.p.) combination on hyperalgesia induced by carrageenan (Ca; 500μg/paw). FM + L-NAME were administered 15 min before Ca. The symbols denote the mean ± SEM of 6 rats *per* group. ** *P*<0.01 vs. FM, one-way ANOVA followed by post hoc Tukey`s HSD test.

FM and L-NAME administered together caused a significant reduction of the nociception at time point of 3h (47.5%), with a maximum (91.5%) at the last measurement time point at 6h (not shown).

The influence of L-arginine on the antinociceptive effect of flunixin meglumine in the electronic nociceptive mechanical paw test

In the electronic nociceptive mechanical paw test in rats, L-arginine itself (L-ARG; 10 mg/kg, i.p.) did not produce a significant effect on carrageenan-induced hyperalgesia. However, L-ARG (10 mg/kg; i.p.) significantly reduced the antinociceptive effects of FM (0.09 and 0.1 mg/kg; s.c.) (Fig. 5a, Fig. 5b).

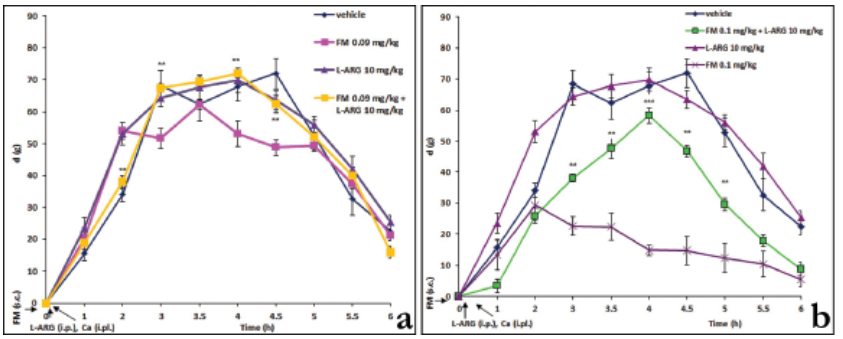

Figure 5a. Influence of L-ARG (10mg/kg; i.p) on the antinociceptive effect of FM (0.09mg/kg; s.c.). FM was administered 15 min before carrageenan and L-ARG was administered at the same time as carrageenan (0h). The symbols denote the mean ± SEM of 6 rats *per* group. ** $P<0.01$ vs. FM, one-way ANOVA followed by post hoc Tukey`s HSD test.
Figure 5b. Influence of L-ARG (10mg/kg; i.p) on the antinociceptive effect of FM (0.1mg/kg; s.c.). FM was administered 15 min before carrageenan and L-ARG was administered at the same time as carrageenan (0h). The symbols denote the mean ± SEM of 6 rats *per* group. ** $P<0.01$, *** $P<0.001$ vs. FM, one-way ANOVA followed by post hoc Tukey`s HSD test.

L-ARG (10 mg/kg, i.p.) abolished the antinociceptive effect of FM administered at the lowest dose of 0.09 mg/kg at the time point of 3h and the effect lasted up to 5.5h after carrageenan administration (Fig. 5a). Also, the inhibitory effect of L-ARG (*%I*) on the antinociceptive effect of the highest dose of FM of 0.1 mg/kg started at time point of 3h (34%) and achieved the maximal reduction of 82% at time point of 4h (82%). The effect was maintained until the last time point measurement, i.e. 6h (20.3%) (not shown).

The influence of L-arginine on the antinociceptive effect of t he combination of flunixin meglumine and L-NAME in the electronic nociceptive mechanical paw test

In the electronic nociceptive mechanical paw test in rats, L-ARG (10 mg/kg, i.p.) decreased significantly the antinociceptive effect of the combination of FM (0.09 mg/kg, s.c.) and L-NAME (5 mg/kg, i.p.) (Fig. 6).

L-arginine (L-ARG; 10 mg/kg, i.p.) exhibited a pronounced inhibitory effect on the combination induced antinociception of 86.6% and 100% at time points of 3.5h and 5h, respectively (not shown).

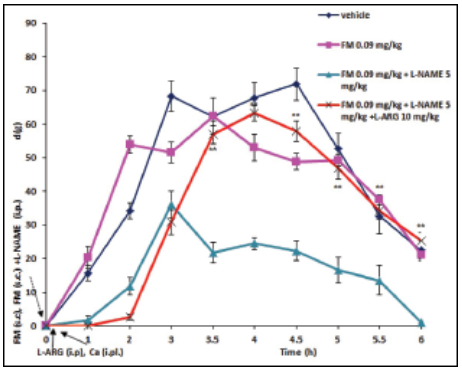

Figure 6. Influence of L-ARG (10mg/kg; i.p.) on the antinociceptive effect of FM (0.09mg/kg; s.c.) + L-NAME (5mg/kg; i.p.) combination. FM and L-NAME were administered 15 min before and L-ARG was administered at the same time as carrageenan (Ca; 500μg/paw). The symbols denote the mean ± SEM of 6 rats *per* group. ***P*<0.01 vs. FM + L-NAME combination, one-way ANOVA followed by post hoc Tukey`s HSD test.

DISCUSSION

The present study revealed that non-selective COX inhibitor flunixin meglumine and non-selective NOS inhibitor L-NAME caused a significant reduction of the nociception induced by carrageenan in an electronic nociceptive mechanical paw test in rats.

The effects of flunixin meglumine and L-NAME on the inflammatory hyperalgesia had already been studied in rats and other animals [21-23]. Antinociceptive activity of flunixin meglumine presented in our study is in agreement with those reported by Ciofalo et al. [6]. They showed that flunixin meglumine administered subcutaneously (ED_{50}=2.4 mg/kg) and intraperitoneally (ED_{50}=4.8 mg/kg) significantly decreased hyperalgesia in the rat paw induced by brewer`s yeast suspension. Also, flunixin meglumine administered intramuscularly (2.2 mg/kg) to sows with chemically induced lameness, enhance the nociceptive threshold to noxious mechanical stimulation [24]. Schulz et al. found that flunixin meglumine was effective in providing analgesia for dairy steers with transient lameness due to amphotericin B-induced transient arthritis [25]. Finally, in our previous study, we have demonstrated that flunixin meglumine administered subcutaneously in the treatment of carrageenan–induced hyperalgesia in rats, caused a dose-dependent antinociceptive effect [17].

The antinociceptive effect of L-NAME has been previously described by Morgan et al. [16]. They showed that L-NAME administered i.p. (10-100 mg/kg) as a pre-treatment decreased formalin-induced hyperalgesia in the mouse paw in a dose-dependent manner. Also, when L-NAME was co-administered with formalin intraplantarly [26] or given intrathecally (i.t.) before induction of inflammation [27], dose-dependent antinociceptive activity was observed. Furthermore, Duarte and Ferreira demonstrated that L-NAME administered i.pl. 30 min. before PGE_2-induced nociception in the mouse caused dose-dependent antinociceptive effect [22]. In the same study, L-NAME administered i.p. in pre-treatment reduced the number of acetic acid-induced abdominal constrictions in the mouse in a dose-dependent manner. Finally, in our previous study, we have demonstrated that L-NAME (2.5 and 5 mg/kg) administered i.p. in the treatment of carrageenan–induced hyperalgesia in rats, caused a dose-dependent antinociceptive effect [17].

Particular interest in our present study was to examine the influence of the inhibitor of NO-synthase on the analgesic effect of flunixin meglumine. Thus, it was demonstrated that the combined use of the lowest effective dose of flunixin meglumine and sub-effective dose of L-NAME in pre-treatment of carrageenan–induced hyperalgesia, provided significantly more potent antinociceptive effect, compared to the effect of flunixin meglumine alone. The potentiation of the analgesic action of NSAIDs in the presence of L-NAME has been previously presented by other authors. Using somatic (injection of formalin into the mouse paw) and visceral inflammatory pain (i.p. administration of acetic acid), Morgan et al. demonstrated synergistic interaction between L-NAME and flurbiprofen/indomethacin [16]. Ketoprofen and L-NAME co-infused *in vitro*, significantly inhibited the production of a highly-sensitive excitatory postsynaptic potential, which is obtained by electrical stimulation of the isolated spinal cord of neonatal rats [28]. However, there are literature data that show the opposite effect, *i.e.* that L-NAME reduces the analgesic effect of NSAIDs. Thus, in the model of formalin-induced inflammatory pain in rats, L-NAME administered i.pl. in pre-treatment reversed antinociceptive effect of diclofenac administered i.pl., while not acting on antinociceptive effect of indomethacine (i.pl.) [29]. In rats with experimentally induced ankle arthritis the analgesic effects of indomethacine [30] and rofecoxibe [31] were significantly reduced with pre-treatment with intra-articular L-NAME.

Based on literature data and analysis of the results of this study it appears that there are differences in the activity of L-NAME, non-selective inhibitor of NO-synthase, on the analgesic activity of NSAIDs. What is common in all these studies is that inhibition of antinociceptive effect of NSAIDs is obtained when L-NAME was administered locally, and contrary to this, the potentiating of the antinociceptive effect of NSAIDs when L-NAME was administered systemically. As we know that FM inhibits the activity of cyclooxygenase, and that L-NAME inhibits the activity of NO-synthase, it is obvious that both prostaglandins (PG) and nitric oxide (NO) are involved in the development of inflammatory hyperalgesia induced by carrageenan. Prostaglandins (PGE_2, PGI_2)

are well studied pro-inflammatory and pain-producing mediators [32, 33]. For this reason, the antinociceptive effect is achieved by the inhibition of the production of these mediators, both at the site of inflammation (primary hyperalgesia), and centrally, in the spinal cord level (secondary hyperalgesia) [34]. On the other hand, the role of NO in the development of nociception is not sufficiently understood. One thing is for sure that NO has dual nociceptive effect, which depends on the location of its production (local - at the site of inflammation or at the level of the spinal cord), and the produced amounts of NO [35].

In the continuation of our study we have shown that the NO-precursor L-arginine (L-ARG) administered i.p. in a pre-treatment did not affect the carrageenan-induced hyperalgesia in the rat. However, when L-ARG was combined with FM in pre-treatment of carrageenan-induced hyperalgesia, the antinociceptive effect of FM was significantly decreased in a dose-dependent manner. This indicated that L-ARG directly affected the analgesic activity of FM. The NO-precursor L-ARG in our study increased the production of NO, which then reduced or reversed the analgesic effect of FM in the rats. A similar result announced Bjorkman et al. where they showed that the antinociceptive effects of acetaminophen in NMDA and substance P-induced nociception were antagonized by L-arginine [36].

At the end of this study we have shown that L-ARG applied in the pre-treatment also significantly inhibits the antinociceptive effect of FM + L-NAME combination. At the same time, the simultaneous pre-treatment with sub-effective doses of L-ARG and L-NAME did not affect the intensity of the carrageenan-induced hyperalgesia in the rats.

CONCLUSION

The results of the present study indicate that flunixin meglumine, a non-steroidal anti-inflammatory drug, and L-NAME, a non-selective NOS inhibitor, applied in the pre-treatment reduced the carrageenan-induced hyperalgesia in rats. Also, L-NAME increased and NO-precursor L-arginine (L-ARG) reduced the antinociceptive effect of flunixin meglumine suggesting that L-arginine-NO system probably plays a role in the mechanism of analgesic activity of flunixin meglumine in rats. Whether this combined therapy has the potential clinical application in achieving analgesic effects warrants further investigation.

Authors' contributions

MM, SV and MP were administered drugs and measured of nociception by vonFrey apparatus. ST and MJ performed statistical analysis of data. MM wrote the text of the article.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Boothe DM: The analgesic, antipyretic, anti-inflammatory drugs. In: Veterinary Pharmacology and Therapeutics, 8th edn. The Iowa State University Press, Ames; 2001, 442-443.
2. EMEA/CVMP/MRL/Summary Report (2) of Flunixin 2000. [http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014325.pdf].
3. Margolis JH, Bottoms GD, Fessler JF: The efficacy of dexamethasone and flunixin meglumine in treating endotoxin-induced changes in calves. Vet Res Commun 1987, 11(5):479-491.
4. Anderson KL, Hunt E, Davis BJ: The influence of anti-inflammatory therapy on bacterial clearance following intramammary Escherichia coli challenge in goats. Vet Res Commun 1991, 15(2):147-161.
5. Daels PF, Stabenfeldt GH, Hughes JP, Odensvik K, Kindahl H: Effects of flunixin meglumine on endotoxin-induced prostaglandin F2 alpha secretion during early pregnancy in mares. Am J Vet Res 1991, 52(2):276-281.
6. Ciofalo VB, Latranyi MB, Patel JB, Taber RI: Flunixin meglumine: A non-narcotic analgesic. J Pharmacol Exp Ther 1977, 200(3):501-507.
7. Beretta C, Garavaglia G, Cavalli M: COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: an in vitro analysis. Pharmacol Res 2005, 52(4):302-306.
8. Giuliano F, Warner TD: Ex vivo assay to determine the cyclooxygenase selectivity of non-steroidal anti-inflammatory drugs. Br J Pharmacol 1999, 126(8):1824-1830.
9. Botting R, Ayoub SS: COX-3 and the mechanism of action of paracetamol/acetaminophen. Prostaglandins Leukot Essent Fatty Acids 2005, 72(2) 85-87.
10. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, Currie MG: Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paws inflammation. Br J Pharmacol 1996, 118(4):829-838.
11. Bhat AS, Tandan SK, Kumar D, Krishna V, Prakash VR: The Interaction between Inhibitors of Nitric Oxide Synthase and Cyclooxigenase in Formaline-Induced Pain in Mice: An Isobolographic Study. Anesth Analg 2008, 106(3):978-984.
12. Moncada S, Palmer RM.J, Higgs EA: Nitric oxide physiology, pathophysiology and pharmacology. Pharmacol Rev 1991, 43(2):109–142.
13. Freire MA, Guimaraes JS, Leal WG, Pereira A: Pain modulation by nitric oxide in the spinal cord. Front Neurosci 2009, 3(2):175–181.
14. Vojvodic D, Miljanovic O, Djurdjevic D, Gataric S, Stanojevic I, Obradovic D, Surbatovic M, Francuski J: Effects of different anesthetic agents on GM-CSF, MCP1, IL1 and TNF levels in rat sepsis model. Acta Vet (Beograd) 2013, 63(2-3):125-136.

15. Miclescu A, Gordh T: Nitric oxide and pain: 'Something old, something new'. Acta Anaesthesiol Scand 2009, 53(9):1107–1120.
16. Morgan CV, Babbedge RC, Gaffen Z, Wallace P, Hart SL & Moore PK: Synergistic anti-nociceptive effect of L-NG-nitro arginine methyl ester (L-NAME) and flurbiprofen in the mouse. Br J Pharmacol 1992, 106(2):493-497.
17. Milovanovic M, Vuckovic S, Prostran M, Jezdimirovic M, Cupic V: The effects of flunixin meglumine and L-NAME on the carrageenan-induced hyperalgesia in rats. 9th Congress of the European Association for Clinical Pharmacology and Therapeutics-EACPT, 12-15 July, Edinburgh, UK. International Proceedings 2009, 163-167.
18. Morris CJ: Carrageenan-Induced Paw Edema in the Rat and Mouse. Methods Mol Biol 2003, 225:115-121.
19. Vivancos GG, Verri Jr WA, Cunha TM, Schivo IRS, Parada CA, Cunha FQ, Ferreira SH: An electronic pressure-meter nociception paw test for rats. Braz J Med Biol Res 2004, 37(3):391-399.
20. Srebro DP, Vuckovic S, Vujovic KS, Prostran M: Anti-hyperalgesic effect of systemic magnesium sulfate in carrageenan-induced inflammatory pain in rats: influence of the nitric oxide pathway. Magnes Res 2014, 27(2):77-85.
21. Welsh EM, Nolan AM: Effect of flunixin meglumine on the thresholds to mechanical stimulation in healthy and lame sheep. Res Vet Sci 1995, 58(1):61-66.
22. Duarte ID, Ferreira SH: L-NAME causes antinociceptive by stimulation of the arginine –NO-cGMP pathway. Mediators Inflamm 2000, 9(1):25-30.
23. Coble DJ, Taylor DK, Mook DM: Analgesic effects of meloxicam, morphine sulfate, flunixin meglumine, and xylazine hydrochloride in African-clawed frogs (Xenopus laevis). J Am Assoc Lab Anim Sci 2011, 50(3):355-360.
24. Pairis-Garcia MD, Johnson AK, Stalder KJ, Karriker LA, Coetzee JF, Millman ST: Measuring the efficacy of flunixin meglumine and meloxicam for lame sows using nociceptive threshold tests. Animal Welfare 2014, 23(2):219-229.
25. Schulz KL, Anderson DE, Coetzee JF, White BJ, Miesner MD: Effect of flunixin meglumine on the amelioration of lameness in dairy steers with amphotericin B-induced transient synovitis-arthritis. Am J Vet Res 2011, 72(11):1431-1438.
26. Kawabata A, Manabe S, Manabe Y, Takagi H: Effect of topical administration of L-argentine on formalin-induced nociception in the mouse: a dual role of peripherally formed NO in pain modulation. Br J Pharmacol 1994, 112(2):547-550.
27. Sakurada C, Sugiyama A, Nakayama M, Yonezawa A, Sakurada S, Tan-No K, Kisara K, Sakurada T: Antinociceptive effect of spinally injected L-NAME on the acute nociceptive response induced by low concentrations of formalin. Neurochem Int 2001, 38(5):417-423.
28. Lizarraga I, Chambers JP, Johnson CB: Synergistic depression of NMDA receptor-mediated transmission by ketamine, ketoprofen and L-NAME combinations in neonatal rat spinal cord in vitro. Br J Pharmacol 2008, 153(5):1030-1042.
29. Ortiz MI, Granados-Soto V, Castaneda-Hernandez G: The NO-cGMP-K+ channel pathway participates in the antinociceptive effect of diclofenac, but not of indomethacin. Pharmacol Biochem Behav 2003, 76(1):187-195.
30. Ventura-Martinez R, Deciga-Campos M, Diaz-Reval I, Gonzalez-Trujano E, Lopez-Munoz FJ: Peripheral involvement of the nitric oxide-cGMP pathway in the indomethacine-induced antinociception in rat. Eur J Pharmacol 2004, 503(1-3):43-48.

31. Deciga-Campos M, Lopez-Munoz FJ: Participation of the L-arginine-nitric oxide-cyclic GMP-ATP-sensitive K+ channel cascade in the antinociceptive effect of rofecoxib. Eur J Pharmacol 2004, 484(2-3):193-199.
32. Nau C, Kress M:Modulation of TRPV1by protein Kinasa A. In: Hyperalgesia: Molecular Mechanisms and Clinical Implication, IASP Press, Seattle; 2004, 49-56.
33. Kassuya CA, Ferreira J, Claudino RF, Calixto JB: Intraplantar PGE2 causes nociceptive behaviour and mechanical allodynia: the role of prostanoid E receptors and protein kinases. Br J Pharmacol 2007, 150(6):727-737.
34. Zeilhofer HU: Prostanoids in nociception and pain. Biochem Pharmacol 2007, 73(2) 165-174.
35. Cury Y, Picolo G, Pacciari Gutierrez V, Ferreira SH: Pain and analgesia: The dual of nitric oxide in the nociceptive system. Nitric Oxide 2011, 25:243-254.
36. Bjorkman R, Hallman KM, Hedner J, Hedner T, Henning M: Acetaminophen blocks spinal hyperalgesia induced by NMDA and substance P. Pain 1994, 57(3):259–264.

UČEŠĆE L-ARGININ-NO SISTEMA U ANALGETIČKOM EFEKTU FLUNIKSIN MEGLUMINA KOD PACOVA

MILOVANOVIĆ Mirjana, VUČKOVIĆ Sonja, PROSTRAN Milica, TRAILOVIĆ Saša, JOVANOVIĆ Milan

Ova studija ispituje da li L-arginin-NO sistem učestvuju u analgetskom dejstvu fluniksin meglumina kod pacova. Hiperalgezija je izazvana intraplantarnom (i.pl.) aplikacijom karagenina (500 μg) u zadnju šapu pacova. Elektronski von Frey aparat se koristi za određivanje praga osetljivosti šape izazvane pritiskom kao bolnim stimulusom, i meri se u gramima (g). Fluniksin meglumin (FM; 0,09-0,1 mg/kg, s.c.) i N^G-nitro-L-arginin metil estar (L-NAME; 10 mg/kg, i.p.), dati odvojeno u pretretmanu, tj. 15 min pre i.pl. aplikacije karagenina, prouzrokuju značajnu antinocicepciju. Kada se FM (0,09 mg/kg) i L-NAME u sub-efektivnoj dozi (5 mg/kg) primene zajedno, antinociceptivni efekat se značajno povećava u odnosu na efekat primene samog FM. L-arginin (10 mg/kg; i.p.) sam ne deluje na hiperalgeziju izazvanu karageninom, ali značajno smanjuje antinociceptivno dejstvo FM i kombinacije FM + L-NAME. Inhibicija produkcije NO-a može biti uključena u mehanizam analgetičkog efekta FM.