International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:9, No:5, 2015

(Anti)Depressant Effects of Non-Steroidal Antiinflammatory Drugs in Mice

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Abstract**—**Purpose: The study aimed to assess the depressant or antidepressant effects of several Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in mice: the selective cyclooxygenase-2 (COX-2) inhibitor meloxicam, and the non-selective COX-1 and COX-2 inhibitors lornoxicam, sodium metamizole, and ketorolac. The current literature data regarding such effects of these agents are scarce.

Materials and methods: The study was carried out on NMRI mice weighing 20-35 g, kept in a standard laboratory environment. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy „Carol Davila", Bucharest. The study agents were injected intraperitoneally, 10 mL/kg body weight (bw) 1 hour before the assessment of the locomotor activity by cage testing (n=10 mice/ group) and 2 hours before the forced swimming tests (n=15). The study agents were dissolved in normal saline (meloxicam, sodium metamizole), ethanol 11.8% v/v in normal saline (ketorolac), or water (lornoxicam), respectively. Negative and positive control agents were also given (amitryptilline in the forced swimming test). The cage floor used in the locomotor activity assessment was divided into 20 equal 10 cm squares. The forced swimming test involved partial immersion of the mice in cylinders (15/9cm height/diameter) filled with water (10 cm depth at 28°C), where they were left for 6 minutes. The cage endpoint used in the locomotor activity assessment was the number of treaded squares. Four endpoints were used in the forced swimming test (immobility latency for the entire 6 minutes, and immobility, swimming, and climbing scores for the final 4 minutes of the swimming session), recorded by an observer that was „blinded" to the experimental design. The statistical analysis used the Levene test for variance homogeneity, ANOVA and post-hoc analysis as appropriate, Tukey or Tamhane tests.

Results: No statistically significant increase or decrease in the number of treaded squares was seen in the locomotor activity assessment of any mice group. In the forced swimming test, amitryptilline showed an antidepressant effect in each experiment, at the 10 mg/kg bw dosage. Sodium metamizole was depressant at 100 mg/kg bw (increased the immobility score, p=0.049, Tamhane test), but not in lower dosages as well (25 and 50 mg/kg bw). Ketorolac showed an antidepressant effect at the intermediate dosage of 5 mg/kg bw, but not so in the dosages of 2.5 and 10 mg/kg bw, respectively (increased the swimming score, p=0.012, Tamhane test). Meloxicam and lornoxicam did not alter the forced swimming endpoints at any dosage level.

Discussion: 1) Certain NSAIDs caused changes in the forced swimming patterns without interfering with locomotion. 2) Sodium metamizole showed a depressant effect, whereas ketorolac proved antidepressant. Conclusion: NSAID-induced mood changes are not class effects of these agents and apparently are independent of the type of inhibited cyclooxygenase (COX-1 or COX-2).

Disclosure: This paper was co-financed from the European Social Fund, through the Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU /159 /1.5 /S /138907 "Excellence in scientific interdisciplinary research, doctoral and postdoctoral, in the economic, social and medical fields -EXCELIS", coordinator The Bucharest University of Economic Studies.

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Keywords**—**Antidepressant, depressant, forced swim, NSAIDs.

I. Introduction

LITERATURE data on possible per se (anti)depressant effects of nonsteroidal anti-inflammatory drugs (NSAIDs) in non-clinical tests, namely forced swimming test (FST), tail suspension test (TST), and on the interactions between NSAIDs and antidepressants are scarce. In TST and FST performed in rats, aspirin, piroxicam, celecoxib, and ibuprofen showed per se antidepressant effects [1]-[3]. In TST and FST performed in mice with "sickness behaviour" (after bacillus Calmette-Guérin or endotoxin inoculation), ibuprofen, indomethacin, nimesulide, naproxen, and rofecoxib showed antidepressant effects [4]-[6]. On the other hand, ketoprofen showed depressant per se effects, while ibuprofen, naproxen, aspirin and acetaminophen antagonized the antidepressant effects of selective serotonin reuptake inhibitors [7], [8]. Our study aimed to assess the depressant or antidepressant effects of several NSAIDs in mice: the selective cyclooxygenase-2 (COX-2) inhibitor meloxicam, and the non-selective COX-1 and COX-2 inhibitors lornoxicam, sodium metamizole, and ketorolac.

II. Materials and Methods

A. Laboratory Animals

Rodents were used – NMRI mice, Swiss albino strain, bred in the biological hatchery of the University of Medicine and Pharmacy "Carol Davila", Bucharest. The animals were brought in from the hatchery 3 days before the experiments for adaptation to the new environment. They were kept under standard laboratory conditions, accommodated in acrylic plastic cages with the floor covered by wood shaving, 12 mice per cage, with *ad libitum* granulated food and water, at an environmental temperature of 21-22°C and relative humidity of 45-60%, under normal lighting conditions (between 07:00 – 19:00 hrs). The experiments were carried out in accordance with the current Romanian legislation and were approved by the Ethics Committee of the University of Medicine and Pharmacy „Carol Davila", Bucharest.

B. Test Agents

The following agents were used: meloxicam, lornoxicam, sodium metamizole, ketorolac. The following pharmaceutical

forms were used: injectable preparations (Movalis, Boehringer Ingelheim; Xefo, Nycomed Austria GmbH; Algocalmin, Zentiva; Ketorol, Dr Reddy's Laboratories) that were administered intraperitoneally in a dosage of 10 mL/kg body weight (bw).

- The control group: the injectable preparations were dissolved either in normal saline (meloxicam, lornoxicam, sodium metamizole) or a solvent consisting of distilled water, ethanol 11.5% vol/vol, and normal saline (for ketorolac).
- The reference group: amitryptilline (98% purity, from Sigma Aldrich) was used for its antidepressant effect in forced swimming test, and was dissolved in normal saline administered intraperitoneally at 10 mL/kg, 30 min before the experiments.

C. Test Equipment and Conditions

In order to assess the locomotor activity acrylic plastic cages illuminated with light from the ceiling of the room were used. The cage floor was divided into 20 equal 10 cm squares. The cage arena was cleaned following each trial.

The forced swimming test or Porsolt test was also used. The test equipment consisted of Berzelius glasses 18 cm high, 10 cm diameter, water height 12 cm, water temperature 28°C, and video recording systems.

The experiments were carried out in daylight conditions, between 08:30 and 16:30 hours.

D. Protocol

In the test for locomotor activity the number of animals in each group was 10-12. The time between the test agent administration and testing was 1 hour.

The locomotor activity was assessed in 3 experiments: In the first experiment meloxicam 5 mg/kg and 10 mg/kg, lornoxicam 2.5 mg/kg and 5 mg/kg and amitryptilline 10 mg/kg, in the second experiment sodium metamizole 25 mg/kg, 50 mg/kg, and 100 mg/kg, and in the third experiment ketorolac 2.5 mg/kg, 5 mg/kg, and 10 mg/kg.

The endpoint used in the locomotor activity assessment was the number of treaded squares for 5 minutes.

In forced swimming test the number of animals in each group was set at 15. The larger number of animals in the forced swimming groups was prompted by earlier research, that have set the real mean difference between the control and the reference (treated with antidepressants) groups at 50%. Using a sample size calculation software – Piface from Russel Lenth, version 1.76, with sigma 1 (for the control group) of 0.4 (40%), sigma 2 (for the test groups) of 0.5 (50%), the mean difference was found to be 0.5 (50%), which for alpha = 0.05 and test power of 0.81 (81%) results in a number of 14 animals per group in both test and control groups.

The time between the test agent administration and testing was 2 hours in forced swimming test, with the exception of amitryptilline, which was given 30 minutes before the experiments.

The dosages of active substances were increased in geometric progression:

- Meloxicam 5 mg/kg and 10 mg/kg, lornoxicam 2.5 mg/kg and 5 mg/kg
- Meloxicam 1.25 mg/kg, 2.5 mg/kg, and 5 mg/kg (a second experiment)
- Sodium metamizole 25 mg/kg, 50 mg/kg, and 100 mg/kg
- Ketorolac 2.5 mg/kg, 5 mg/kg, and 10 mg/kg
- All experiments: amitryptilline 10 mg/kg

The animals were left to swim for 6 minutes. Endpoints used included immobility latency across 6 minutes, and 5-second intervals' scoring of the last 4 minutes of immobility, swimming, and climbing respectively (thus resulting in 48 scoring intervals for each animal). This method was used before, as in [9]. The scoring was performed by staff trained for this purpose, 1-2 persons per experiment. The results were read in a blinded manner (the mice were assigned in three Berzelius glasses simultaneously, varying the immersion order into the glasses 1, 2, and 3 of each animal group), in accordance with the test protocol.

Immobility was defined as lack of movement, with the exceptions of respiratory movements and movements required for keeping the head above the water, with no significant active movements, either horizontally or vertically. Swimming was defined as active horizontal movements at the water surface. Climbing was defined as active vertical movements at the water surface.

Immobility latency increases with the intensity of the antidepressant effect. The immobility score decreases with the intensity of the antidepressant effect, and a high score is associated with a possible depressant effect. The swimming score increases with the intensity of the antidepressant effect. The climbing score increases with the intensity of the psychomotor stimulating effect.

E. Statistical Analysis

Microsoft Excel and SPSS version 15 were used for the purposes of statistical analysis. Also used were normality tests of the results' distribution, homogeneity dispersion tests, ANOVA, and parametric post hoc tests – the Tukey test (based on homogenous dispersion), and the Tamhane test (not involving homogenous dispersion). $P<0.05$ was considered to indicate a statistically significant difference.

III. Results

Results will be presented in a table for locomotor activity assessment (see Table I) and in graph form for forced swimming test (only a part of statistically significant results will be shown in this form, see Figs. 1-4). For forced swimming test, all recorded results will be shown in table form, for an overall outlook on the performed tests (see Table II).

TABLE I
RESULTS OF THE LOCOMOTOR ASSESSMENT IN THE CAGE OF LOCOMOTION

Experiment	Drugs	Mean number of squares traded ± standard error
1	Saline	89.43±10.92
1	Meloxicam 5 mg/kg	88.67±12.99
1	Meloxicam 10 mg/kg	91.00±10.62
1	Lornoxicam 2.5 mg/kg bw	108.17±12.66
1	Lornoxicam 5 mg/kg bw	97.00±12.14
1	Amitryptilline 10 mg/kg bw	103.08±5.11
2	Saline	129.2±10.96
2	Sodium metamizole 25 mg/kg bw	107.7±17.65
2	Sodium metamizole 50 mg/kg bw	134.2±14.89
2	Sodium metamizole 100 mg/kg bw	128.9±7.86
3	Saline + distilled water + ethanol 11.5% v/v	115.3±13.77
3	Ketorolac 2.5 mg/kg bw	99.5±10.45
3	Ketorolac 5 mg/kg bw	81.4±8.27
3	Ketorolac 10 mg/kg bw	103.5±9.83

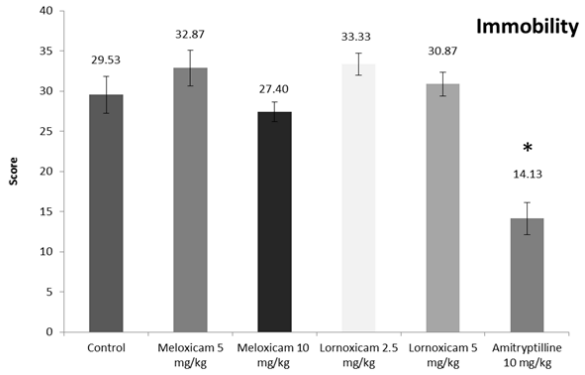

Fig. 1 Immobility score in forced swimming test after administration of meloxicam (2 doses) and lornoxicam (2 doses), and after administration of amitryptilline 10 mg/kg. Each column shows the score recorded in the final 4 minutes of the test, for each agent administered.*p<0.05 vs. the control group

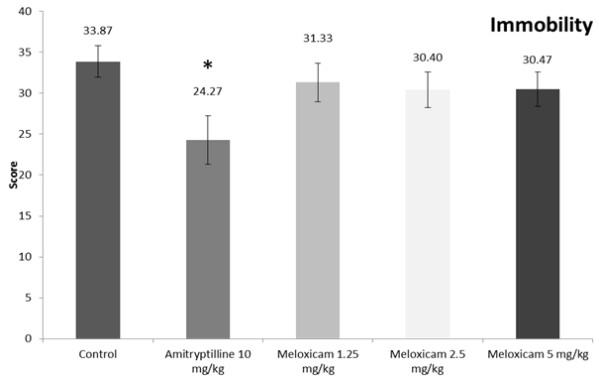

Fig. 2 Immobility score in forced swimming test after administration of meloxicam (3 doses) and after administration of amitryptilline 10 mg/kg. Each column shows the score recorded in the final 4 minutes of the test, for each agent administered. *p<0.05 vs. the control group

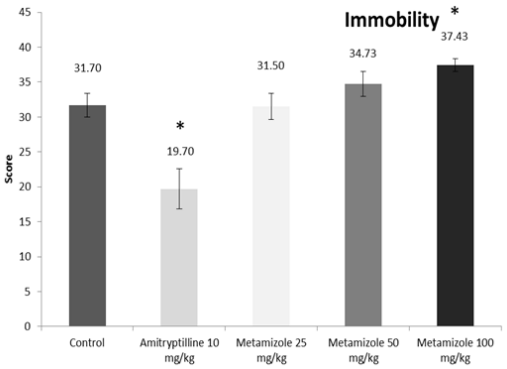

Fig. 3 Immobility score in forced swimming test after administration of metamizole (3 doses) and after administration of amitryptilline 10 mg/kg. Each column shows the score recorded in the final 4 minutes of the test, for each agent administered. *p<0.05 vs. the control group

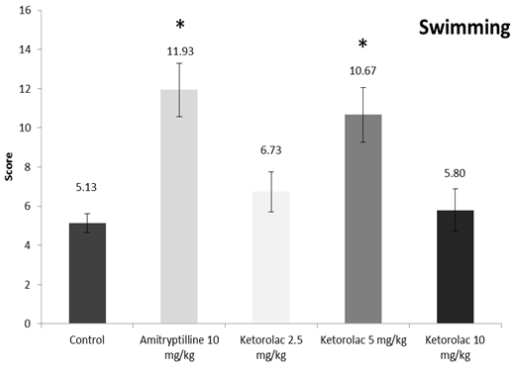

Fig. 4 Swimming score in forced swimming test after administration of ketorolac (3 doses) and after administration of amitryptilline 10 mg/kg. Each column shows the score recorded in the final 4 minutes of the test, for each agent administered. *p<0.05 vs. the control group

IV. DISCUSSION

Meloxicam and lornoxicam did not alter the measured endpoints in forced swimming test, while sodium metamizole 100 mg/kg significantly ($p<0.05$ vs. control) increased the immobility score (depressant effect). Ketorolac 5 mg/kg showed an antidepressant effect, increasing the swimming score ($p<0.05$ vs. control) in forced swimming test. None of drugs altered the cage locomotor activity. The depressant or antidepressant effect of these drugs was not due to locomotor impairment (sedation) or psychomotor stimulation.

Certain NSAIDs do not cause any mood changes per se; other NSAIDs cause positive mood changes, showing antidepressant effects; and other NSAIDs cause negative mood changes, showing depressant effects. NSAID-induced mood changes are not a class effect of NSAIDs. The prostaglandin system is not involved in the NSAID-induced mood changes. For ketorolac, no antidepressant effect has yet been described.

Therefore, the question arises regarding the mechanism by which certain NSAIDs induce mood changes. According to the monoaminergic theory of depression, this mechanism may involve either serotonin or norepinephrine transmission, or

both. This mechanism may be further investigated by looking at the way NSAID-induced mood changes are influenced by either SSRI antidepressants (serotonin transmission), NRI antidepressants (norepinephrine transmission).

TABLE II
RESULTS OF THE FORCED SWIMMING TESTS

Agent used	Dosages	Effect				
		Immobility latency	Immobility score	Swimming score	Climbing score	Type of effect
Meloxicam	5 mg/kg	0	0	0	0	
	10 mg/kg	0	0	0	0	
Lornoxicam	2.5 mg/kg	0	0	0	0	
	5 mg/kg	0	0	0	0	
Amitryptilline	10 mg/kg	↑	↓	↑	0	Reference antidepressant
Meloxicam	1.25 mg/kg	0	0	0	0	
	2.5 mg/kg	0	0	0	0	
	5 mg/kg	0	0	0	0	
Amitryptilline	10 mg/kg	↑	↓	↑	0	Reference antidepressant
Metamizole	25 mg/kg	0	0	0	0	
	50 mg/kg	0	0	0	0	
	100 mg/kg	0	↑	0	0	Depressant
Amitryptilline	10 mg/kg	↑	↓	↑	0	Reference antidepressant
Ketorolac	2.5 mg/kg	0	0	0	0	
	5 mg/kg	0	0	↑	0	Antidepressant
	10 mg/kg	0	0	0	0	
Amitryptilline	10 mg/kg	↑	0	↑	0	Reference antidepressant

Results Are Statistically Significant ($p < 0.05$ vs. The Control Group), 0-no Effect, ↑-Increase, ↓-Decrease

V. CONCLUSIONS

These results lead to two conclusions: NSAID-induced mood changes are not a class effect of NSAIDs. The prostaglandin system is not involved in the mood changes induced by certain NSAIDs.

ACKNOWLEDGMENT

This paper was co-financed from the European Social Fund, through the Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU/159/1.5/S/138907 "Excellence in scientific interdisciplinary research, doctoral and postdoctoral, in the economic, social and medical fields -EXCELIS", coordinator The Bucharest University of Economic Studies.

REFERENCES

[1] X.T. Guan, F. Shao, X. Xie, L. Chen, W. Wang. „Effects of aspirin on immobile behavior and endocrine and immune changes in the forced swimming test: comparison to fluoxetine and imipramine," *Pharmacol. Biochem. Behav.*, vol. 124, pp. 361-366, Sep. 2014.

[2] R.M. Santiago, J. Barbiero, B.J. Martynhak, S.L. Boschen, L.M. da Silva, M.F. Werner, et al., „Antidepressant-like effect of celecoxib piroxicam in rat models of depression," *J. Neural. Transm.*, vol 121(6), pp. 671-682, Jun. 2014.

[3] T. Zaminelli, R.W. Gradowski, T.B. Bassani, J.K. Barbiero, R.M. Santiago, D. Maria-Ferreira, et al., „Antidepressant and antioxidative effect of Ibuprofen in the rotenone model of Parkinson's disease", *Neurotox. Res.*, vol 26(4), pp. 351-362, Nov. 2014.

[4] L.A. Saleh, M. Hamza, N.H. El Gayar, A.A. Abd El-Samad, E.A. Nasr, S.I. Masoud, „Ibuprofen suppresses depressive like behavior induced by BCG inoculation in mice: role of nitric oxide and prostaglandin," *Pharmacol. Biochem. Behav.*, vol. 125, pp. 29-39, Oct. 2014.

[5] V.N. de Paiva, S.N. Lima, M.M. Fernandes, R. Soncini, C.A. Andrade, A. Giusti-Paiva, „Prostaglandins mediate depressive-like behaviour induced by endotoxin in mice," *Behav. Brain Res.*, vol. 215(1), pp. 146-151, Dec. 2010.

[6] N.K. Jain, S.K. Kulkarni, A. Singh, „Lipopolysaccharide-mediated immobility in mice: reversal by cyclooxygenase enzyme inhibitors," *Methods Find. Exp. Clin. Pharmacol.*, vol. 23(8), pp. 441-444, Oct. 2001.

[7] J.L. Warner-Schmidt, K.E. Vanover, E.Y. Chen, J.J. Marshall, P. Greengard, „Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated by anti inflammatory drugs in mice and humans," *Proc. Natl. Acad. Sci. U S A*, vol 108(22), pp. 9262-9267, May. 2011) Erratum in: *Proc. Natl. Acad. Sci. U S A.*, vol. 108(27), pp. 11297, Jul. 2011.

[8] I. Răducanu, A. Segărceanu, G. Prada, C. Ionescu, A. Arsene, I. Fulga, "Assessing depressive effect of ketoprofen and its mechanism of action using the forced swimming test in mice," *Farmacia*, vol. 60(5), pp. 759-766, 2012.

[9] AP. Costa, C. Vieira, L.O. Bohner, C.F. Silva, E.C. Santos, T.C. De Lima, C. Lino-de-Oliveira, „A proposal for refining the forced swim test in Swiss mice," *Prog. Neuropsychopharmacol. Biol. Psychiatry*, vol. 45, pp 150-155, Aug. 2013.