

ASSESSING DEPRESSIVE EFFECT OF KETOPROFEN AND ITS MECHANISM OF ACTION USING THE FORCED SWIMMING TEST IN MICE

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Abstract

The mood effect of non-steroidal anti-inflammatory drugs (NSAIDs) is a matter of debate concerning clinical and experimental data as well. This paper presents the mood effect of ketoprofen in mice during the forced swimming test. In three series of laboratory animal groups, the mobility time was significantly shorter than that in controls supporting a ketoprofen depressant effect. Administering amitriptyline and fluoxetine, but not mianserin, antagonized the depressant effect of ketoprofen. These data point out that ketoprofen when administered on longer term has an effect on mood acting only at the serotonin level (receptors or metabolism).

Rezumat

Efectul antiinflamatoarelor nesteroidiene (AINS) asupra dispoziției este controversat, atât pentru datele clinice, cât și pentru cele experimentale. Lucrarea de față a studiat efectul ketoprofenului asupra dispoziției la șoareci, utilizând testul de înot forțat. În trei serii de experimente timpul de mișcare a fost semnificativ mai mic față de martor, demonstrând efectul depresiv al acestuia. Administrarea de amitriptilina, și respectiv fluoxetină, a antagonizat efectul depresiv al ketoprofenului, dar nu și administrarea de mianserină. Aceasta demonstrează că administrarea cronică de ketoprofen are efect asupra dispoziției, acționând doar la nivelul căii serotoninergice (receptori sau metabolism).

Keywords: non-steroidal anti-inflammatory drugs, mood, ketoprofen, serotonin pathway

Introduction

It is estimated that NSAIDs represent approximately 5-10% of all drug prescriptions [12]. These drugs have many clinical indications because of their analgesic, anti-inflammatory and antipyretic effects. The new possible actions of NSAIDs, lately debated are: a neuro-protective effect in Alzheimer disease [16] that is not fully supported by certain results [1,3,7]; an antidepressant effect due to immunological mechanisms involved in the pathogenesis of depression [8,9] as supported by some clinical evidences [2,4] but refuted by results of other studies [5,17].

On the contrary, other data have shown that using NSAIDs, analgesics or combinations of aforementioned drugs decreased significantly the treatment response of depressive patients to antidepressives compared with those not having used these drugs [17]. In a previous paper we showed similar results in older patients: people using on long term NSAIDs had significantly higher scores on a geriatric depression scale. We found out dissimilar results when patients used analgesics and suggested that there could be a direct relationship between long-term use of NSAIDs and depression [15].

Regarding experimental studies, data were shown suggesting that COX2 selective NSAIDs have antidepressant effects [6,10,11]. Other experimental research pointed out that some NSAIDs (naproxen, ibuprofen, and acetylsalicylic acid) diminished effects of selective serotonin reuptake inhibitors (SSRIs) [17].

Considering these conflicting clinical and experimental data this paper aimed to test the depressant or antidepressant effect of ketoprofen in mice. This NSAID was the choice in view of conducting research, as it has been one of most used NSAIDs by our patients during the clinical investigations we carried out and few other experimental data were found out regarding the mood effect of ketoprofen.

Materials and Methods

Male mice were supplied by the University of Medicine and Pharmacy "Carol Davila" (Bucharest, Romania) biobase. Animals were housed in groups of maximum 12 mice, in plexiglass cages with sawdust litter, with *ad libitum* access to food and water. The temperature was between 21°C and 24°C and the relative humidity was generally maintained at 45-60%. All researches were conducted in accordance with The European Directive 86/609/EEC/24.11.1986 and The Romanian Government Ordinance 37/30.01.2002 regarding the protection of animals used for experimental and other scientific purposes.

The forced swimming test was used; it was described and used the first time by Porsolt and at present it is recognized as a valid test to assess the antidepressant effects in laboratory rats and mice [13,14]. The mice received two times per day, 7 days the tested substances (intraperitoneal or by oral gavage). After that, the animals were tested; by dropping the mice into cylinders with water (height 15 cm, diameter 9 cm, 6 cm of water at 25–28°C) and left for 6 minutes. The mobility time was manually recorded only in the finally four minutes of the test. Forced swimming test was

performed two hours after the last administration of substances and the mobility time was the variable that was statistical analyzed.

Three series of mice groups were used, each of four groups of mice: controls and mice treated with NSAID, an antidepressant, and a combination of NSAID and antidepressant. The NSAID was ketoprofen, the antidepressives given successively were amitriptyline, fluoxetine and mianserin, respectively. The used doses utilized were 50 mg/kg body weight for ketoprofen, 10 mg/kg body weight for amitriptyline, fluoxetine and mianserin. Treatments were administered twice, daily for 7 days, intraperitoneally for ketoprofen and amitriptyline and by oral gavage for fluoxetine and mianserin. The control group was treated with methyl cellulose (by oral gavage) and physiological saline intraperitoneally.

For the first two series (to which amitriptyline and respectively, fluoxetine were administered) each group of the four included 12 laboratory animals. In these eight groups, the statistical analysis showed that in the case of the control groups, the standard deviation was approximately 40% of the mean value, whereas for the study - groups treated with the mentioned compounds, the standard deviation was approximately 50%. For this reason, it was calculated again the necessary number of mice per group for the final series, where mianserine was administrated. It was used the Piface-Russell Lenth software from the University of Iowa, in which two sample t test general case was selected to estimate the number of laboratory animals necessary for the control group and the study group to test. Sigma 1 for the control group was considered 0.4 (40%) and Sigma 2 for the study group to test, 0.5 (50%). Difference between means was established at 0.4 (40%), alpha at 0.05, while the resolution power at 0.81 (81%), as tests conducted on laboratory animals enable for a resolution power of the analysis of 0.8 - 0.9. Number of laboratory animals resulted were 18 for the control group and respectively, 23 for the study group to test. So, for mianserin series, the number of laboratory animals was 18 mice for control group and 24 mice per group for the three groups which received active substances.

Results were expressed as mean \pm standard error of the mean (SEM). Differences between groups were analysed using Student's t-test and two-tailed p-values < 0.05 were considered as statistically significant. Data were analysed using the Microsoft Excel program.

Results and Discussion

Three different series of groups of mice were used for two reasons: one reason was to show whether the effect of ketoprofen on mobility time was not obtained by chance and the results validated once again the mood

effect (depressant/antidepressant effect) of administering ketoprofen; the second reason was to find out, by treating with antidepressants which act either on serotonin or norepinephrine, which antidepressive antagonized the effect of ketoprofen and further to establish the mechanism involved in its mood effect.

Amitriptyline was administered in the first series of groups and the results recorded during the forced swimming test were shown in figure 1. The statistical analysis indicated that in the control group the mean mobility time was 75.3 ± 11.1 s. The mean of mobility time was 40.8 ± 7.8 s in the ketoprofen group, the mobility time being significantly shorter in this group compared with the control group [$t(22)=2.550$; $p=0.018$].

In the amitriptyline group the mean mobility time was 117.5 ± 16.5 s. The mobility time was significant longer than that of controls [$t(22)=-2.123$; $p=0.045$]. On the other hand, in the ketoprofen and amitriptyline group the mean mobility time was 76.4 ± 15 s and the mobility time was not significantly different compared with the control group [$t(21)=-0.056$; $p=0.956$].

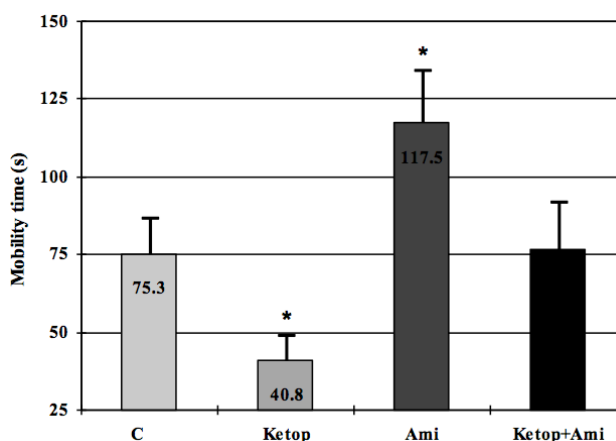


Figure 1

The mobility time at the forced swimming test for the amitriptyline series. control group (C); ketoprofen group (Ketop); amitriptyline group (Ami); ketoprofen and amitriptyline group (Ketop+Ami). All data are presented as mean \pm SEM. Statistically significant effect in the treated groups compared with the control group are noted ($*p<0.05$).

These data pointed out a depressant effect subsequent to a longer administration of ketoprofen. The association of amitriptyline counteracted (antagonized) this depressant effect, as we could compare the mobility time for the group treated with ketoprofen and amitriptyline with that of the control group.

For the second series of groups, a selective serotonin reuptake inhibitor, namely fluoxetine, was chosen. The mobility time for each of the four groups of this second series, is shown in figure 2. When recording data for fluoxetine series the results showed that in control group the mobility time was 55.8 ± 6.8 s. The mean was 31.4 s and SEM 7.6 for mobility time, in the ketoprofen group with a shortened significantly mobility time during forced swimming [$t(20)=2.403$; $p=0.026$].

In the fluoxetine group the mean of mobility time was 43.2 ± 11.1 s; the administration of fluoxetine did not significantly increase the mobility time during the test [$t(22)=0.975$; $p=0.340$] as it was in the case of amitriptyline administration. The mean of mobility time was 34.8 ± 9.5 s in the ketoprofen associated with fluoxetine group. The fluoxetine antagonized the depressant effect of ketoprofen, because the mobility time was comparable between ketoprofen associated with fluoxetine and respectively, control groups [$t(22)=1.800$; $p=0.086$].

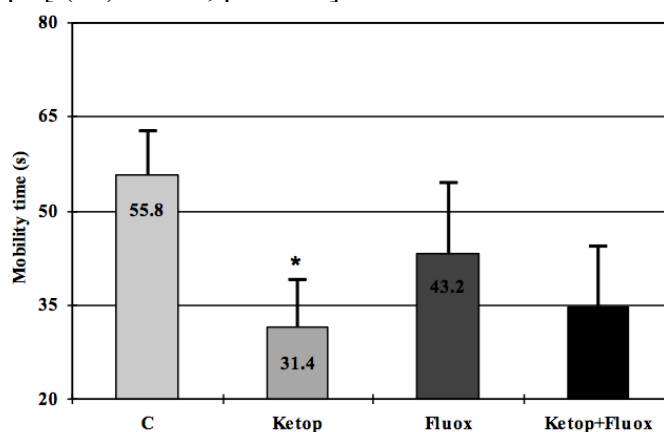


Figure 2

The mobility time at the forced swimming test for the fluoxetine series control group (C); ketoprofen group (Ketop); fluoxetine group (Fluox); ketoprofen and fluoxetine group (Ketop+Fluox). All data are presented as mean \pm SEM.

Statistically significant effect in the treated groups compared with the control group are noted ($*p < 0.05$).

In the third series of groups, the used antidepressive was mianserin (figure 3). At this time, the value for the mobility time in the control group was 61.7 ± 5.8 s. The mean was 39.7 s and SEM 5 for mobility time, in the ketoprofen group. One more time the depressant effect of ketoprofen was proved, as the mobility time at the forced swimming test was significantly shorter than that of control [$t(35)=2.883$; $p=0.007$].

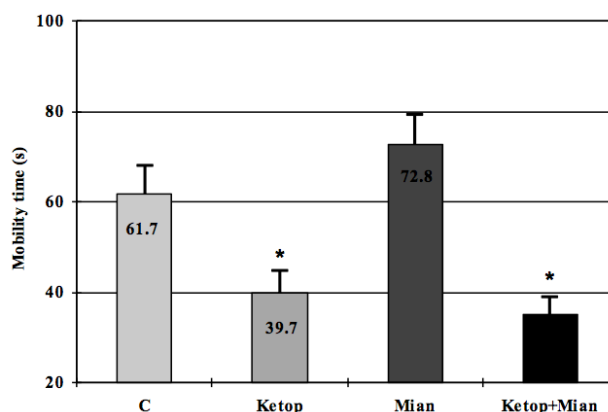


Figure 3

The mobility time at the forced swimming test for the mianserin series control group (C); ketoprofen group (Ketop); mianserin group (Mian); ketoprofen and mianserin group (Ketop+Mian). All data are presented as mean±SEM. Statistically significant effect in the treated groups compared with the control group are noted ($*p<0.05$).

In the mianserin group, the mobility time was 72.8 ± 6.4 s; this antidepressant did not significantly increase the mobility time during the test [$t(22)=-1.241$; $p=0.222$] as it was the case of fluoxetine. When ketoprofen and mianserin were simultaneously administered, the value for mobility time recorded was 35.2 ± 3.5 s. This time, when associating mianserin with ketoprofen the mobility time was significantly lower than in controls [$t(37)=4.052$; $p=0.0001$]. The association of antidepressant with NSAID did not antagonize the depressant effect of the NSAID and we could compare the mobility time of this group with that of the ketoprofen group [$t(38)=0.747$; $p=0.460$].

In conclusion, the ketoprofen systematically decreased significantly the mobility time at the forced swimming test and only amitryptiline and fluoxetine antagonized this effect, by increasing the mobility time when associating it with these antidepressants. In contrast, mianserin did not influence the mobility time decreased by ketoprofen at the forced swimming test.

Conclusions

Long term administration of ketoprofen shortened significantly the mobility time at the forced swimming test in each group of the three experimental series. This proved that ketoprofen had a depressant effect in mice when it was administered on long term (7 days).

Amitriptyline, which acts on both serotonin and norepinephrine pathways, and also fluoxetine (SSRI) that acts on the serotonin reuptake transporter, increasing only the serotonin in the cleft, antagonized the depressant effects of ketoprofen. These data suggest that ketoprofen may act only at the serotonin level (receptors or metabolism). This is the reason for which, in the final series treated with mianserin, the number of laboratory animals had to be doubled. Mianserin, which acts on the release of norepinephrine only, did not antagonize the depressant effect of ketoprofen supporting as well the theory of serotonin pathway.

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