EXPERIMENTAL RESEARCH ON THE ACTION OF KETOPROFEN AND THE ASSOCIATION OF KETOPROFEN AND ENALAPRILAT ON THE THERMALGALGESIC SENSITIVITY IN NAÏVE AND ETHANOL-TREATED RATS

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Abstract. The aim of this study is the experimental research of the influence of Ketonal and combination of Ketonal and Enap on nociception in naïve and ethanol treated rats. Materials and methods: We used male Wistar rats divided in two groups: naïve rats and ethanol treated rats (10% solution of alcohol, for three weeks). Both groups received intraperitoneal Ketonal 1 mg/kbw, and Ketonal (the same dose) in combination with Enap (20 μg/kbw). The control group received distilled water. Nociception was investigated through the hot plate assay, at 15, 30, 60, 90 minutes after the administration of substances. The results were statistically analyzed using the Anova test, followed by the post-hoc test. In conclusion, we have found Ketonal antinociceptive effect, both in naïve and in ethanol treated rats, more evident in the first case. The association of Ketonal with Enap produced analgesia in naïve animals and nociception in the case of alcohol fed rats.

Keywords: analgesia, nociception, Ketoprofen, Enalaprilat

Aim

The aim of this study is the experimental research on the action of Ketonal, administered alone or in combination with Enap, on nociception in naïve and ethanol treated rats.

Introduction

Although most clinical practice guides warn against the adverse effects of ethylic alcohol ingestion in patients with rheumatic and cardiovascular pathologies, alcohol consumption is still present in this category of patients.

This is why we have considered it necessary to investigate the effects of an analgesic substance belonging to the class of nonsteroidal anti-inflammatory drugs (ketonal) and of its association with an antihypertensive agent (Enap) on the thermically induced somatic nociception, in animals which had received ethanol.

Nonsteroidal anti-inflammatory drugs are indicated particularly in the treatment of mild to moderate pain, in chronic arthritis pain (Burke, 2006) or in severe pain, in combination with opioids (Kokki, 2003). The mechanisms of the antinociception determined by nonsteroidal anti-inflammatory drugs are mainly mediated through the inhibition of cyclooxygenases 1 and 2 (Mitchell, 1999); (Langford, 2002), key enzymes in prostaglandin synthesis (Smyth, 2006). Ketoprofen is different compared to other nonsteroidal anti-inflammatory drugs because it additionally inhibits the lipoxygenase pathway, together with that of the cyclooxygenase, thus preventing the production of leukotrienes and of prostaglandins (Sigurdsonn, 1994). Known deter-
minants of hyperalgesia in animals (Levine, 1984) and in humans (Soter, 1983), leukotrienes play an important role in sustained nociceptive responses (Tonussi, 1999).

Angiotensin II, a product of the renin-angiotensin-aldosterone system, is a vasoconstricting agent which determines arterial hypertension. Experimental studies show that angiotensin II also is a potent proinflammatory mediator (with growth and remodeling effects). The inhibition of angiotensin II by the conversion enzyme inhibitors and by the angiotensin II receptor blockers has generated significant benefit for patients with inflammatory diseases, and most of these positive effects have been shown to be independent from the antihypertensive effect of blocking angiotensin II, which suggests a distinct mechanism of action. Literature data mention the fact that angiotensin conversion enzyme inhibitors could have anti-inflammatory effects. (Peeters, 1998), (Kranzhofer, 1999).

The angiotensin conversion enzyme has two main actions: the conversion of angiotensin I to the vasopressor peptide called angiotensin II and the breakdown of bradykinin, a potent vasodilating agent. Thus, angiotensin conversion enzyme inhibitors generate the lowering of angiotensin II levels and the increase of bradykinin levels (Brown, 2000).

Material and methods

1. Test animals: male adult Wistar rats
2. Substances used for experiments: injectable Enalaprilat, 1ml vial (Enap®iv, produced by KRKA), the dose used for the experiment was 20 μg/kbw; injectable Ketoprofen, 2ml vials (Ketonal ®, produced by Lek, Slovenia), the dose used for the experiment was: 1mg/kbw; distilled water (DW) (SC ''Sicomed'' SA, Romania); ethyllic alcohol 96º (Farmachim S.A.), Romania;
3. Method for nociception measuring in mice:
   • the hotplate device is a metallic surface heated up to temperatures of 55±0.5ºC. The nociceptive response is represented by the time interval elapsed from the moment when placing the animal on the surface of the hotplate up to the occurrence of the first signs of pain (licking or shaking of the paws, cries, escape attempt).
   The device’s timer is stopped by pressing on a pedal at the time of removing the rat from the hot surface (Mungiu, 2001), (Mungiu, 2008).
   The experiments have taken place in concordance with the international ethical codes (Zimmerman, 1983).

For the experiment, 24 male adult Wistar rats were used, acquired from the Hatchery of the Central Laboratory for Drug Testing of the „Gr. T. Popa“ University of Medicine and Pharmacy, Iași, with weights between 165 and 215 grams. The rats were kept in an isolated room, free from noise, at a constant temperature of 23±1ºC and under controlled humidity conditions (55-65%). A light/dark cycle of 12h/12h was maintained.

The animals were distributed into 2 batches, as follows: batch 1 (rats which did not undergo alcohol administration) – naïve rats; batch 2 (alcoholic rats, which underwent 10% alcohol administration). Batch 1 is considered the witness batch, these rats receiving water as the only fluid available for drinking. The rats in batch 2 underwent induction of alcohol addiction through gavage administration by means of an esogastric tube. We used a 10% alcoholic solution for three weeks. The administration respected the same time for each procedure – 9 a.m. The animals in the two batches were divided into three groups of 6 animals each, which were injected intraperitoneally with the same doses of the researched agents, (1mg/kbw), Ketonal with Enap (20μg/ kbw) and distilled water and subsequently underwent the thermoalgesic test. The tests were performed at 15, 30, 60 and 90 minutes after administration of the investigated substances or of the witness administration (distilled water).

Data analysis

The obtained results were expressed as arithmetic mean of the latencies determined for each time span and they were statistically processed through Anova, followed by the post-hoc Tukey HSD test (for multiple comparisons). Values of under 0.05 of the p coefficient were considered statistically significant.

Results and discussions

The results of the hotplate test for naïve rats treated with distilled water, Ketonal and Ketonal + Enap association are graphically represented in figure 1.

Statistically, the differences proved to be significant between the batch treated with Ketonal and the witness one, at 30 and 60 minutes from the injection (values of the p coefficient of under 0.05) (table 1). Ketoprofen has displayed a powerful analgesic effect which initially occurred at 15 minutes, and subsequently reached a maximal value at one hour.
Our results are in concordance with the literature data, confirming the analgesic effect of the nonsteroidal anti-inflammatory agent used in this experiment (Miranda, 2001).

Regarding the association of the two substances, our experimental data for the hotplate test have identified the same tendency of prolonging the latency times compared to the witness batch, 15 minutes after the intraperitoneal administration, the effect being present for one hour (figure 1).

The statistical analysis of the results did not result in statistically significant differences between the batch injected with the association of the two substances and the witness batch (p coefficient over 0.05). In the field literature, we did not find data regarding the action on the nociception of laboratory animals of an association between the angiotensin II conversion enzyme inhibitor and the analgesic that we used. We would like to draw attention to the results of Czarnecka et al. which note an antinociceptive action of the conversion enzyme inhibitors (in extremely high doses) in the thermoalgesic and mechanoalgesic tests (Czarnecka, 2000). This effect was considered surprising and the authors did not offer an explanation for a possible mechanism of action.

The hotplate test performed in rats which had received ethanol revealed the following data (figure 2), according to the analysis of the mean values of the latency times:

- the highest values suggesting an antinociceptive action were observed for the batch treated with Ketonal, the differences from the witness substance not being significant for any of the time spans when the test was performed (respectively 15, 30, 60 and 90 minutes). The analgesic effect displayed an onset at 15 minutes and remained situated at close values until up to 90 minutes after administration. The action of Ketoprofen was less evident in the batch of rats treated with alcohol than in the case of naïve rats which had received water as the only fluid available for drinking (figure 1, figure.2).

In the field literature, we did not find data, apart from those already mentioned, with a potential to aid in interpreting the results that we have obtained in the test for the use of a nonsteroidal anti-inflammatory drug with antinociceptive role

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<th>(I) Substance</th>
<th>(J) Substance</th>
<th>Subtraction of means (I-J)</th>
<th>Sig.</th>
<th>Confidence interval 95%</th>
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Table 1. Tukey HSD test for multiple comparisons at 60 minutes; hotplate test in naïve rats
in algesia tests in alcohol-treated rats.

- a nociceptive tendency was observed for the associated administration of the two substances (analgesic plus antihypertensive agent) in the batch of rats treated with 10% alcohol, tested through the hotplate assay. The decrease in latency times was more evident at 15 and respectively at 30 minutes from substance injection. We did not notice statistically significant differences between the two animal groups (alcoholic and witness group). In the field literature, we did not find data regarding the manner in which the association Ketonal - Enap could influence nociception in the alcoholic rats.

Conclusions

Ketonal, in the dose we have used (1mg/kbw), generated antinociceptive effects in both animal batches, more evident in the case of naïve rats.

The concomitant administration of Ketonal and Enap has increased the latency times for naïve rats and has displayed an opposite effect in those treated with 10% ethanol. In this case, the analgesic action of ketoprofen was no longer present, being countered by the administration of enalaprilat. A possible explanation relies in the possibility of enalapril to block the metabolizing of bradykinin. This would lead to the accumulation of this known algogenic mediator, which could explain the attenuation (counteraction) displayed on the analgesic effect of ketonal, evident in alcohol-addicted rats.

References

7. Levine JD, Lau W, Kwiat G etc. Leukotriene B4 produces hyperalgesia that is dependent on polymorphonuclear leukocytes, Science, 1984, 225: 743-745