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THE EXPERIMENTAL STUDY OF PHARMACEUTICAL COMPOSITION PIROXICAM AND CAFFEINE

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Abstract. *In an experiment on rats, the effects of a non-steroidal anti-inflammatory agent of the group of oxicams piroxicam, caffeine and their composition on emotional-behavioral reactions under formalin edema were conducted. Evaluation of drug's and their compositions influence on characteristics of animal's behavior was done by comparison remedies with control and with formalin edema. Observation of the parameters of routine research activity in the "open field" test and the multi-parameter method for evaluating anxiety-phobic states was carried out according to the generally accepted method.*

The analysis of the results of experimental studies indicates that caffeine increases the activity of piroxicam relatively to horizontal and vertical motion activity, cognitive activity, grooming and the number of urinations in rats under formalin edema.

The results of research can be the basis for the development of new domestic combined medicines.

Keywords: *piroxicam, caffeine, "open field", formalin edema, pharmaceutical composition.*

Introduction. The problem of pharmacological regulation is one of the most relevant modern medicine's problems of inflammation and pain syndrome [1-3]. There is an arsenal of non-steroidal anti-inflammatory drugs (NSAID) and nonnarcotic analgesics (NNA), but the risk of side effects (gastro-toxicity, nephrotoxicity, hematotoxicity, hepatotoxicity, cardiotoxicity, allergic reactions) decreases the ability of its usage [4-9].

The creation of medical compositions whose pharmacological effect reaches as expands the pharmacological spectrum because of their composition is the current problem of medicine [10, 11]. By experimental and clinical research was shown the advantage of compositions over monotherapy medicines in pharmacotherapy of pain [11-13]. Those compositions enable the addition to medicines active substances in fewer doses that decreases toxicity and the number of side effects [13]. This circumstance stimulates search of new drugs with wider pharmacological spectrum and less toxicity and minimal number of side effects.

It's known that it was studied and NNAs are combined with other remedies and induce complementarity or potentiate each other. Caffeine is famous adjuvant for NSAIDs and NNAs [1, 2, 14-16]. It enhance analgesic and anti-inflammatory effect of NSAIDs and NNAs as increases their bioavailability [5, 2, 14-16]. Literature sources dealing with questions of pharmaceutical activities of drugs indicate that presently there are no compositions based on piroxicam and caffeine. This circumstance disposed us to choose piroxicam as the object of study.

The pharmacological compositions of NSAIDs with caffeine were developed at the Department of medical and biological chemistry of Kharkiv national medical university (KhNMU) under leadership of prof. Syrovaya A.O. There was research of caffeine's influence on pharmacological activity of NAIDs oxicam's group – piroxicam (4-hydroxy-2-methyl-N-(pyridin-2-yl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide) on laboratory animals (mature rats of WAG line) with intragastric injection using an experimental model of formalin edema.

Interaction of animal and the environment is based on mechanisms of nervous and humoral systems and immune regulation. Animals differently react to environmental changes depending on genetic determinations. Because of it we can pick out individual typologically properties [17]. Also, individual features of animal are based on previous experience of solving problem and other features acquired throughout life.

Materials and methods. Studying mechanisms of pharmaceutical drug's action it's important to explore the influence on characterization of animal behavior. Absence of verbal contact limits amount of tests. The most popular and informative is test on "open field" [18].

Animals were divided into 6 groups, 6 in each. 1st group was the control one, 3 % starch mucilage was once orally intragastrically administered to animals of this group (2 ml per 200 g of rat). Exudative inflammation was modeled by subplantar injection of 0,1 ml of 2 % formalin solution in the rat hind paw and intragastrically administered 3 % starch mucilage [19]. Animals from 3-6 groups once got orally intragastrically starch mucilage. Then to animals were administered the explored pharmaceutical compositions: 3th group – piroxicam (1,3 mg/kg), 4th group – caffeine (0,6 mg/kg), 5th group – combination of piroxicam (1,3 mg/kg) and caffeine (0,6 mg/kg), 6th group – reference drug diclofenac sodium (8 mg/kg).

The moment of maximal swelling is 4 hours after administration [19], so pharmaceutical remedies and their compositions, also 3 % starch mucilage (control group) were administrated an hour before this moment considering their pharmacological characteristics.

Evaluation of drug's and their compositions influence on characteristics of animal's behavior was done by comparison 3-6 groups with control (group 1) and with formalin edema (group 2) and with reference drug diclofenac sodium (group 6). Observation of parameters of the orienting-research activities by test "open field" and multiparametric evaluation method of phobic anxiety state by accepted technique were done during 3 minutes [20, 21].

The study was carried out in accordance with the methodological recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine [19]. Number of animals and their distribution in groups were in accordance with economical approach, bioethical rules and statistics requirements. Recalculation of the human doses for rats was done by using the ratio of species sensitivity by Rybolovlev Yu. R. [22]. Laboratory animals employed in the study were kept in experimental biological clinic of KhNMU following the norms of the storage, care and feeding approved by the principles of "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" (Strasbourg, 1986) [23] and the decision of the First national Congress on Bioethics (Kyiv, 2007) [24]: air temperature – 23-25°C, lighting in a room – 100 lx, in a cage – 20-40 lx). The duration of animal's staying – 1,5 months, acclimatization – 2 weeks; basic diet – vegetables, aft beet, source of water – tap water. The study was done in the morning [25-27].

Received data were processed using generally accepted methods of statistical analysis (mean, error of mean, F-test) with the help of MS Excel and Stat Graphics Plus 2.1.[28].

Results of our experiment. *The influence of explored drugs and their compositions on rat's horizontal and vertical motion activity.* The analysis of the orienting-research activities by test "open field" by horizontal motion activity (HMA) and vertical motion activity (VMA) indicators opens the motivation's part of rat's behavior. Wherein they try to contact indirectly with subjects which are located outside "open field".

Formalin edema modeling (group 2) contributed to reducing HMA in 1,1 times and VMA in 1,5 times relatively to rats from control (group 1). Under single administrating of piroxicam (group 3) HMA increased in 1,2 times and VMA in 1,3 times relatively to 2nd group. Received data have no statistically veritable difference from control value (group 1) and reference drug (group 6). Under single administrating of caffeine (group 4) HMA increased in 1,2 times and VMA in 1,4 times relatively to 2nd group. Received data have no statistically veritable difference from control value (group 1) and reference drug (group 6). Under administrating piroxicam-caffeine composition (group 5) HMA increased in 1,4 times and VMA in 1,3 times relatively to 2nd group, and also we could observe increase of HMA of rats from 1-6 explored groups, but VMA of all rats from 1-6 groups not differ from reference quantities statistically veritably from reference drug (decreasing VMA in 1,5 times) (see table 1).

The number of explored holes. The number of explored holes is a kind of orienting-research behavior's activities – rate of hole reflexes, which shows animal's ability to explore the open field and specifically look into holes. The number of explored holes characterizes rat's cognitive activities.

Formalin edema modeling (group 2) contributed to reducing the number of explored holes in 2,7 times relatively to rats from control (group 1).

Under single administrating of piroxicam (group 3) and caffeine (group 4) the number of explored holes increased relatively to 2nd group in 2,3 and 2 (respectively) and didn't reach control rates. Administrating of piroxicam and caffeine contributed statistically probable reducing of number of explored holes in 2,7 and 3,2 times respectively relatively to reference drug.

Adding caffeine to piroxicam contributed rising of animal's ability to explore in 3 times relatively to 2nd group. Received data have no statistically veritable difference from control value (group 1) and reference drug (group 6). (see table 1).

Table 1. Indices of the rats' behavioral activity under formalin edema according to "the open field" method (n = 6)

№	Groups of animals	Number of interjections (HMA)	Number of sets (VMA)	Number of explored hols	Number of washing (grooming)	Number of urination	Number of defecation
1.	Control	45,33±7,03	6,67 ± 1,09	1,33 ± 0,49	7,50 ± 2,58	1,00 ± 0,00	2,0 ± 0,78
2.	Formalin edema	41,50±0,50	4,50±0,76	0,50 ± 0,22	3,50 ± 0,56	0,50±0,23	1,00±0,45
3.	Peroxicam	50,50±4,37	5,83 ± 1,40	1,17 ± 0,17	4,83 ± 1,82	1,17 ± 0,17	1,50±0,50
4.	Caffeine	50,67± 12,62	6,33 ± 2,32	1,00 ± 0,45	8,00 ± 3,67	3,50±0,44	1,17±0,17
5.	Caffeine + piroxicam	56,33±5,64	5,83 ± 1,28	1,50 ± 0,67	5,67 ± 3,21	1,33± 0,21	0,50±0,34
6.	Sodium diclofenac	48,33± 3,12	8,50±0,72	3,17±0,60	4,50 ± 2,31	1,00 ± 0,00	1,33±0,88

Notes (average ± error of average):

* – result's probability relatively to control group, P < 0,05;

** – to rats under formalin edema, P < 0,05;

*** — to rats with single administering of piroxicam , P < 0,05;

**** – to rats with single administering of caffeine, P < 0,05.

***** – to rats with administering of caffeine's and piroxicam's composition , P < 0,05;

***** – to rats with single administering of diclofenac sodium, P < 0,05.

Rat's cosmetic behavior. Rat's grooming (cosmetic behavior) is important rate of animal's conduct in "open field". Traditionally rats spend more time by combing their hair than running. Grooming closely correlates with physical activity. So it's important to study this rate of behavior.

Formalin edema modeling (group 2) contributed to reducing the number of washings in 2,1 times relatively to control (group 1). Under single administrating of piroxicam (group 3) and caffeine (group 4) the number of washings increased relatively to 2nd group in 1,4 times and 2,3 times (respectively) and there was no statistically veritable difference from reference drug. Administering piroxicam-caffeine composition against the background of formalin edema was observed the growth of grooming 1,6 times relatively to group 2 and there was no statistically veritable difference from reference drug. (see table 1).

Diuresis and defecations. As rate of emotional stress there is an importance of ureation's and defecation's number. Level of rat's emotional state is evaluated by urination's and defecation's number.

Formalin edema modeling (group 2) contributed to reducing the number of urinations and defecations in 2 times relatively to control (group 1). Under single administrating of piroxicam (group 3) and caffeine (group 4) the number of urinations and defecations increased relatively to 2nd group in 2,3 times and 1,5 times respectively. A single administrating of caffeine contributed statistically probable increasing of urination's number relatively to group 2 in 7 times and relatively to control group in 3,5 times and there was no statistically veritable difference from reference drug. Administering the piroxicam's and caffeine's composition contributed statistically probable increasing of diuresis in 2,7 times relatively to 2nd group and there was no statistically veritable difference from reference drug. Number of defecations decreased in 2 times relatively to group 2 and there was no statistically veritable difference from reference drug.

Conclusions.

1. It was explored the influence of NAIDs from oxicams group piroxicam, caffeine and their composition on rat's EPR under formalin edema.

2. Under formalin edema caffeine increases piroxicam's activity relatively to rat's HMA, cognitive activities, grooming and number of ureations.

3. Administering piroxicam, caffeine and their composition increases HMA and VMA, cognitive activities, grooming and number of ureations.

4. Administering piroxicam-caffeine composition under formalin edema decreases defecation's number.

The results of research can become the basis for the production of combined domestic drugs.

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