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EXPERIMENTAL INVESTIGATION OF THE INFLUENCE OF PHARMACEUTICAL COMPOSITION ON EMOTIONAL BEHAVIORAL REACTIONS UNDER CONDITIONS OF FORMALIN EDEMA

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Abstract. The analysis of the results of experimental studies of the effect of celecoxib, licopid and their pharmaceutical composition on the central nervous system by emotional-behavioral reactions in the test "open field" in the conditions of formalin edema indicates that the addition of licopid to celecoxib increased rat's locomotor and orienting-research activities, the indicators of their emotional reactions (washings and urinations) in comparison with the mono-administration of celecoxib. The pharmaceutical composition of celecoxib and licopid is appropriate and promising for the study of anti-inflammatory and analgesic effects.

Keywords: celecoxib, licopid, pharmaceutical composition, "open field", formalin edema.

Introduction. One of the most pressing problems of modern medicine is the problem of pharmacological regulation of pain and inflammation [1]. Therefore, an urgent

problem at the present stage of pharmacy development is the creation of new effective national combination medicines based on known non-steroidal anti-inflammatory drugs (NSAIDs), which have advantages over monopreparations [2-10].A literary search conducted by scientists of the Department of Medical and Bioorganic Chemistry of Kharkiv National Medical University (KhNMU) shows that in modern pharmacy there are no combined medicines based on coxibs. Therefore, we chose celecoxib as a research subject.

Celecoxib (4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide) is a synthetic NSAID of the coxib group, a highly selective (specific) cyclooxygenase-2 (COX-2) inhibitor (Fig. 1).

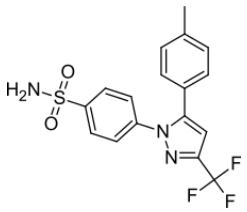

Fig. 1. Celecoxib (4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide)

Celecoxib is a new class drug that inhibits prostaglandin (PG) synthesis by specific inhibition of COX-2, which is activated in humans in response to inflammatory stimuli. Celecoxib has anti-inflammatory and analgesic effects, blocking the production of inflammatory prostanoids by inhibiting COX-2 [11-13].

In vivo and *ex vivo* studies have shown that celecoxib has little affinity for COX-1 [14, 15]. The activity of celecoxib, like other preparations of the coxib group, to the COX-2 isoform is explained by the peculiarity of the structure of the COX-2 active center and the coxib molecule: molecules of coxibs have a rigid side chain [16]. Celecoxib is used in symptomatic therapy for inflammation and pain in osteoarthritis and rheumatoid arthritis [17].

As the second component of a potential painkiller, we have selected the known immunomodulator licopid (glucosaminylmuramildipeptide (GMDP),

Glucoseminylmuramildipeptidum [4-O-(2-Acetylamino-2-deoxy-beta-D-glycopyran) D-α-glutamylamide, $C_{25}H_{43}N_5O_{15}$), which belongs to the group of cytokines and immunostimulants [18]. The mechanism of its action resembles the process of natural immunoregulation [19, 20]. Licopid exhibits antibacterial, antiviral activity and has a leukopoietic effect [16, 18]. Licopid stimulates all parts of the immune system, but primarily activates phagocytes, that is, the ability of cells to absorb foreign cells, bacteria and fungi [20, 21]. Licopid is a highly effective and safe immunotropic drug; it is easy to use, well tolerated [16, 20].

The aim of our study was to seart, create and study a new pharmaceutical composition containing NSAIDs from the group of coxibs (celecoxib) and licopid by influencing the central nervous system (CNS) by emotional-behavioral reactions (EBR) in an experiment on laboratory animals in an «open field» test.

Subject of study. Experimental justification for the use of a new pharmaceutical composition containing NSAIDs of the coxib group (celecoxib) and licopid.

Research methods. Experimental studies of pharmacological activity were carried out on laboratory animals (36 sexually mature WAG rats of the Wistar population weighing 180-280 g of both sexes) at the Department of Medical and Bioorganic Chemistry of the KhNMU. The research was performed in accordance with the methodological recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine [22]. The conversion from human doses to rats was performed using the species sensitivity coefficient of Rybolovlev Yu. R. [23].

The work was carried out on laboratory animals from the experimental-biological clinic of KhNMU, taking into account the standards of storage, care and feeding [24]. The rats were kept under vivarium according to the rules of humane treatment for laboratory animals. The studies have been carried out in compliance with the principles of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes" (Strasbourg, 1986) [25], Directive 2010/63/EU of the European Parliament and of the Council on the European Union «On the Protection of Animals Used for Scientific Purposes» (Brussels, 2010) [26] and «General Ethical Principles for Experiments on Animals» (Kyiv, 2001), the

Decree of the First National Bioethics Congress (Kiev, 2009) [27]. Experiments were conducted in the first half of the day, which according to the literature agrees with the dependence of the basic pharmacological parameters and pharmacological activity of the drugs taken from the circadian rhythms [28, 29].

Statistical processing of the data was carried out using generally accepted methods of statistical analysis (mean, mean average error, Fisher Student's exact probability test) using MS Excel and Stat Graphics Plus 2.1 programs [30].

The animals were divided into 6 groups of 6 animals each. Animals of the 1st intact group intragastrically (i/g) received single dose of 3 % starch mucus (2 ml per 200 g of rat's weight). Animals of the 2nd to 6th groups received 3 % starch mucus and the formalin induced edema (f. i. e.) was modeled by sub-plantar administration 0.1 ml of 2 % formalin solution in hind paw of rat [16]. The experimental drugs and their pharmaceutical composition were administered i/g in a form of suspension of 3 % starch mucus: animals of the 3rd group – celecoxib (5.0 mg/kg), 4th group – licopid (0.6 mg/kg), 5th group – received composition of celecoxib (5.0 mg/kg) with licopid (0.6 mg/kg), 6th group – reference drug sodium diclofenac (8 mg/kg)). Maximum development of f. i. e. was observed 4 hours after its modeling [22]. Drugs and their pharmaceutical composition, as well as 3% starch mucus (groups 1 and 2) were administered 1 hour before, taking into account their pharmacokinetic characteristics.

In studying the mechanisms of pharmaceutical action of drug's, it is important to study their effects on characteristics of animal behavior. Absence of verbal contact limits number of possible tests. The most common and informative test in the study of the effect of drugs on EBR is the "open field" test [25, 26].

The effect of the drugs and their compositions on the characteristics of animal's behavior was carried out by comparing groups 3-5 with intact (group 1), and with formalin edema (group 2) and with a reference drug (group 6). Observation of the parameters of orienting-research activity of the rats in the "open field" test [25, 26] and multi-parameter method of estimation of anxiety-phobic states by the conventional method were carried out for 3 minutes [27].

Research results. F.-i. e. (group 2) reduced the rat's locomotor activity (the number of squares crossed) by 1.9 times relative to the intact group (Table 1). Mono-administration of celecoxib (group 3) showed a increase of locomotor activity of rats relative to group 2 and not statistically significantly different from the control group.

Table 1

The study of the effect of celecoxib, licopid and their pharmaceutical composition on the quantitative parameters of EBR of rats under conditions of f.-i. e. by «open field» method (n = 6)

№	Groups of rats	Locomotor activity	Orientation-research activity		Emotional reactions		
		number of crossed squares	number of rearings	number of examined openings	number of washing (grooming)	number of urinations	number of defecations
1.	Control	18,50±0,62	4,83±0,65	7,17±1,01	8,50±2,99	1,17±0,31	4,17±0,48
2.	F.-i. e.	10,00±0,52*	2,17±0,79*	2,17±0,65*	1,67±1,05	1,00±0,00	2,67±0,42
3.	Celecoxib (f.-i. e.)	19,25±1,89**	3,75±0,75	3,75±1,75	4,50±0,65	1,00±0,48	3,75±0,48
4.	Licopid (f.-i. e.)	11,67±2,28*	2,67±0,62	2,17±0,48	1,50±0,96	1,17±0,17	2,83±0,95
5.	Celeco	21,00±2,05$^{***/****}$	6,67±1,43$^{**/***}$	5,00±1,48	5,50±2,77	1,17±0,17	1,33

	xib + licopid (f.-i. e.)						±0,49*/***
6.	Sodium diclofenac (f.-i. e.)	18,00±0,82**	6,25±0,25**/***	6,50±0,65	5,75±2,10	1,50±0,29	2,50±0,87

Notes (mean ± error in mean):

* - the difference is significant as compared to the control group, P <0.05;

** - the difference is significant as compared to f.-i. e., P <0.05;

*** - the difference is significant as compared to the mono-administration of celecoxib, P <0.05;

**** - the difference is significant as compared to the mono-administration of licopid, P <0.05;

***** - the difference is significant as compared to the administration of the celecoxib and licopid composition, P <0.05;

****** - the difference is significant as compared to the mono-administration of sodium diclofenac, P <0.05.

Mono-administration of licopid (group 4) also a slight increase of locomotor activity of rats relative to group 2 (f.-i. e.) and the data obtained were statistically significantly different from the control values (group 1). The composition of celecoxib with licopid, increased the locomotor activity of rats relative to all groups, which was statistically not significantly different from the control group and mono-administration of celecoxib (group 3) and licopid (group 4) (see Table 1). According to the influence of locomotor activity, the investigated agents were arranged in a row: licopid < sodium diclofenac < celecoxib < composition celecoxib + licopid.

An analysis of the rat's orienting-research behavior in "open field" test in terms of the number of sets and surveyed openings reveals the motivational component of the animal's characteristics.

F.-i. e. (group 2) contributed to statistically significant reduction in the number of sets to the control group (see Table 1). The mono-administration of celecoxib (group 3) there was an increase the number of rearings by 1.7 times relative to group 2, which did not statistically significantly different from either the control and the reference drug. The mono-administration of licopid a slight increased the number of rearings (1.2 times) relative to group 2. Composition of celecoxib and licopid showed a statistically significant increase in the number of rearings relative to group 3, which was stay on level of reference drug.

The number of examined openings characterizes cognitive activity of rats. F.-i. e. there was a statistically significant decrease in the number of examined opening by 3.3 times relative to the control group. Mono-administration of celecoxib (group 3), licopid (group 4) contributed to the increase in the number of examined opening in rats relative to group 2, but did not reach the level of control values. The administration of the pharmaceutical composition of celecoxib with licopid (group 5) increased the cognitive activity of rats in 3.3 times relative to group 2, in 1.2 times relative to group 3 (mono-administration of celecoxib). These indicators were not statistically significantly different from the control group and those of the reference drug, that is, the introduction of this composition contributed to the normalization of vertical activity (see table. 1).

Emotional reactions in rats are an important characteristic of animal behavior in the «open field» test. The level of emotional state of rats is estimated by the number of washings (grooming), urinations and boluses (defecation).

F.-i. e. shows that there was a decrease in the amount of washings by 5.1 times relative to control (group 1). The mono-administration of celecoxib (group 3) increased in the amount of washing by 2.7 times relative to group 2, but did not reach the control values. The mono-administration of licopid did not affect the grooming under conditions of f.-i. e. (see Table 1). Addition of licopid to celecoxib contributed to an increase in grooming by 3.3 times relative to group 2 and 1.2 times relative to group 3 (mono-administration of celecoxib). These indicators were not statistically significantly different from the control group and those of the reference drug, that is,

the introduction of this composition contributed to the normalization of cosmetic behavior of rats (see table. 1).

It should be noted that as an indicator of the emotional status of rats are significant number of urinations and boluses. F.-i. e. (group 2) contributed to the reduction diuresis and defecation by 1.2 times and 1.6 times, respectively, relative to the control group. Mono-administration of celecoxib in the condition's formalin edema did not affect the number of urinations, mono-administration of licopid (group 4) and its composition with celecoxib (group 5) contributed to normalization of the number of urinations, which was not statistically significantly different from control (group 1) (see Table 1).

F.-i. e. contributed to the reduction the number of boluses. Mono-administration of celecoxib contributed to the normalization of this process, unlike the mono-administration of licopid, its pharmaceutical composition with celecoxib and sodium diclofenac.

Conclusions. A study was conducted to evaluate the effect of celecoxib, licopid and their pharmaceutical composition on EBR indices in rats by the method of "open field" method under the conditions of formalin edema, which showed that the administration of the pharmaceutical composition of celecoxib and licopid promoted to an increase of rat's locomotor and orientation-research activity, its indicators of emotional reactions (washings and urinations) compared to mono-administration of celecoxib.

The pharmaceutical composition of celecoxib with licopid is appropriate and promising for the study of anti-inflammatory and analgesic effects.

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