THE STUDYING OF ANALGESIC ACTIVITY OF NEW PHARMACEUTICAL COMPOSITION IN THE EXPERIMENT

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Nonsteroidal anti-inflammatory drugs (NSAIDs) – also known as medications, which provide analgesic and anti-inflammatory effects and don't cause an addiction. NSAIDs are indicated to relieve the symptoms that occur in the states like rheumatoid arthritis, inflammatory arthropathies (ankylosing spondyloarthritis, psoriatic arthritis, Reiter's syndrome), acute podagra attack, dysmenorrhea with pain feeling, metastases, headache and migraine, postoperative pain, mild and moderate pain due to the inflammation and damage of the tissue, rigidity and pain in muscle as a consequence of Parkinson's disease, fever, intestinal obstruction, renal colic etc. [1-5]. It is established, that the main mechanism of therapeutic effect of NSAIDs lies in the interruption of cyclooxygenase pathway of arachidonic acid metabolism. As a result, the synthesis of prostaglandins is inhibited, which are important factors of inflammatory process development [2]. There are two forms of cyclooxygenase (COX) that are well-known: structural (COX-1) and induced (COX-2). COX-1 is responsible for protective properties of mucous membranes of the gastrointestinal tract, and COX-2 participates in prostaglandins formation in the area of inflammation. Moreover, NSAIDs inhibit the formation of prostaglandin not only in the area of inflammation, but also on the systemic level, so that well-known side effect of gastropathy often occurs during the administration of non-selective NSAIDs. Even during short-term administration, the alteration of mucous membranes develops, that has a dose-dependent character and is characterized by an ultrastructure damage of superficial epithelium in a few minutes and endoscopic visible submucosal bleedings and erosions in a few hours after its administration (acute NSAIDs-gastropathy) [5-7].

That is why modern pharmacy sets a goal to minimize the toxicity of medications and draw attention on the establishment of the new modern domestic pharmaceutical medications, that is more effective and have minimal quantity of side effects [5-7].

During the last decades the new group of NSAIDs was included in medico pharmaceutical practice – it is coxibs, the most popular representative of them on pharmaceutical market of Ukraine is 4-(4-(methylsulfonyl)phenyl)-3-phenyl-2(5H)-furanone (rofecoxib) – highly selective inhibitor of COX-2, its chemical structure is the sulfonamide derivative. However, the creation of a highly selective inhibitors doesn't completely solve the problem of safety during NSAIDs administration, because by replacing classical side effects, the new ones occur (cardiotoxicity etc.) [8-11].

To increase the effectiveness of pharmacotherapy in medical practice, pharmaceutical compositions are used. Combined effect is observed during applying different combinations of medications with the purpose of increasing the therapeutic effect, of reducing the time of treatment and preventing complications. During applying combined, the pharmaceutical components influence each other and, as a result, the efficiency of pharmacological effects changes. It can be due to their interaction on the level of pharmacokinetic processes (absorption, distribution, metabolism, excretion) or pharmacodynamic reactions, which are manifested as a synergism or antagonism. The opportunity of obtaining more significant pharmacological activity from the pharmaceutical combination, compared to every individual medical component, became the basis of creating a new two-component pharmaceutical composition [12].

Earlier in the medical and bioorganic chemistry department of Kharkiv national medical university (KNMU) we studied the pharmaceutical composition of NSAIDs different chemical structure: 2,4-dichlorobenzoic acid, sodium (2-[(2,6-dichlorophenyl)amino]phenyl] acetate, N-(4-hydroxyphenyl) acetamide, (2RS)-2- [4-(2-Methylpropyl)phenyl] propanoic acid, 4-Hydroxy-2-methyl-N-pyrid-2-yl-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide, (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide with the adjuvant 1,3,7-trimethylxanthine (caffeine). The results of our investigation showed us that the caffeine enhances the analgesic and antiexudative effects of NSAIDs different chemical structures [13-22]. But its influence on pharmacological activity of coxibs, in particular the rofecoxib, has not been examined earlier. Therefore, in our investigation we set a goal to create the pharmaceutical composition of 4-(4-(methylsulfonyl)phenyl)-3-phenyl-2(5H)-furanone (rofecoxib) with 1,3,7-trimethylxanthine (caffeine) and examine its analgesic activity (AA) in the experiment on rats with the formalin edema conditions.

Materials and methods of investigation.

The study was made, and doses of preparation were selected due to the existing recommendations. AA of pharmaceutical preparations was studied on white male rats by using the experimental model of formalin edema. The animals were divided on 5 groups of 6 animals each. In this experiment, aseptic exudative inflammation was induced in animals by subplantar injection of 0.1 ml of 2% formalin solution into the hind paw [23-24]. The animals of the 1st group were the control. They were given a

single intragastric injection of 3% starch mucus (2 ml per 200 g of the animal's body weight) 1 hour before the development of maximum edema. Analogically, in form of suspension on starch mucus the rofecoxib, caffeine and its pharmaceutical composition and a reference drug were injected: the 2nd group of rofecoxib in dose of 1,5 mg per a 1 kg of animal's body weight; the 3rd group of caffeine by the calculation of 0,6 mg per 1 kg of animal's body weight; 4th group of pharmaceutical composition of rofecoxib (1,5 mg per kg of animal's body weight) and caffeine (0,6 mg per a 1 kg of animal's body weight), the animals of 5th group – the reference drug diclofenac sodium by calculation of 8 mg per 1 kg of rat's body weight.

The AA evaluation was carried out on the device for measuring the threshold of tactile sensitivity by the von Frey method by using an algesimeter (IITC Life Science (USA)). The essence of the experiment lies in the impact on the central fold of the animal's hind paw with of the sensor tip. Paw withdrawal is recorded as a response to irritation. Measurements of the threshold of tactile sensitivity of pain were carried out in grams before and 4 hours after the subplantar administration of formalin. The obtained data was recalculated in percentage of activity by using a formula:

$$AA = \frac{\Delta Hc - \Delta He}{\Delta Hc} \cdot 100\%,$$

where AA – the analgesic activity (%);

ΔH_c – the load on a paw in control pathology group (g);

ΔH_e – the load on a paw in examined group (g).

The animals were kept in standard conditions of vivarium CSRL KNMU, according to SOP CSRL in polypropylene cages on standard food ration with free access to water and food, by a temperature 19-24° C, by a relative air humidity no more than 60%, by the implementation of sanitary-hygienic norms. Following the principles of Directive 210/63/EU of the European Parliament and The Council of EU "On the protection of animals used for scientific purposes" (Brussels, 2010) and "General ethical principles of experiments on animals" (Kyiv, 2001), the Law of Ukraine "On protection of animals from cruel treatment" №3477-IV from 21.02.2006 with changes and The Order of the MES of Youth and Sport of Ukraine "On approval of the procedure for conducting tests, experiments on animals by research institutions" №249 from 01.03.2012.

The results and discussion.

The results of experimental study of AA proved that all examined pharmaceutical preparations are pharmacologically effective during the single dose injection: rofecoxib revealed AA 51,3%, that can be explained by its classical mechanism of action. AA of caffeine was 57,9%, that can be explained by its purine structure (it can influence on that target structures, on which adenosine does, which bounds with nucleosides and nucleotides). The caffeine molecule has structure similarity to adenosine, the caffeine quickly penetrates to the brain and acts as a substance-antagonist to adenosine receptors, which are in the brain. The caffeine bounds with adenosine receptors as a competitive inhibitor. It is known that the caffeine is a competitor to adenosine for purinergic A1 and A2 – receptors [25-26]. However, the AA of rofecoxib and caffeine yield to AA of reference drug (59,6%).

The pharmaceutical composition of rofecoxib with caffeine showed higher AA (60,1%) than during injection of monocomponents and higher than during injection of comparison drug – diclofenac sodium. The mechanism of caffeine potentiation of analgesic effect of rofecoxib can be associated with the improvement of bioavailability of NSAIDs in case of its combination with the caffeine, caffeine induction of central cholinergic analgesia, the structure similarity of adenosine and caffeine molecules, that promotes the neurochemical mechanism of action of 1,3,7-trimethylxanthine (caffeine) in form of blocking specific P1"purine" receptors in the brain, also it can be explained by the well-known fact, that was above-mentioned (caffeine is an adenosine competitor for purinergic A1 and A2-receptors, and "purine analgesia" is mediated by an activation of inhibitory A1-receptors on the peripheral, central and supersegmental levels) [25-26].

The results of study of AA are showed in the table 1.

Table 1

Analgesic activity of composition of 4-(4-(methylsulfonyl)phenyl)-3-phenyl-2(5H)-furanone (rofecoxib) with 1,3,7-trimethylxanthine (caffeine) on the background of formalin edema in the rats.

№	Group	Analgesic activity, %
1.	Control	0
2.	Rofecoxib	51,3
3.	Caffeine	57,9
4.	Rofecoxib+caffeine	60,1
5.	Diclofenac sodium	59,6

Conclusions

1. The analysis of results, which were experimental studied, indicates that all examined pharmaceutical preparations are analgesic active. According to the AA, the investigated preparations can be arranged in a series:

Diclofenac sodium (sodium 2-[(2,6-dichlorophenyl)amino]phenyl] acetate) > Caffeine (1,3,7-trimethylxanthine) > Rofecoxib (4-(4-(methylsulfonyl)phenyl)-3-phenyl-2(5H)-furanone)

2. The pharmaceutical composition of rofecoxib with caffeine in the experiment on laboratory rats proved to be more analgesic effective (60,1%), than the reference drug (59,6%).

3. The 1,3,7-trimethylxanthine (caffeine) potentiates the AA of 4-(4-(methylsulfonyl)phenyl)-3-phenyl-2(5H)-furanone (rofecoxib), so we consider this composition is perspective to further studying.

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