EXPERIMENTAL STUDY OF THE CENTRAL COMPONENT OF ANALGESIC ACTIVITY OF NEW TWO-COMPONENT COMPOSITIONS

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Due to the continuous increase in the number of people worldwide, including in Ukraine, suffering from various diseases, including inflammatory conditions characterized by pain syndrome, we consider the development of new, effective, and safe pharmaceutical products with analgesic activity to be a relevant direction in modern medicine and pharmacy. These medications can be used for pain syndromes of various etiologies.

Pain is the body's response that signals harmful effects and potential danger. It is a manifestation of many pathological processes, particularly inflammation. Therefore, pain is considered the most important signal of the human body, indicating a specific problem, namely a pathological process occurring in the patient's body. In light of the above, we emphasize that one of the priority areas of modern pharmacy, especially in today's conditions, is the development of pain-relief agents that can be used to improve the quality of human life. Considering that pain syndromes often accompany inflammatory processes, we believe that analgesic action is a crucial component in the treatment of inflammatory conditions. A well-established and rational approach to treating pain syndromes and inflammatory processes is the use of non-steroidal anti-inflammatory drugs (NSAIDs).

In our opinion, there are two main ways to create new drugs:

- synthesis of new ones;

- rational use of existing drugs, including the creation of new pharmaceutical compositions based on them.

The advantages of combination drugs compared to monotherapy drugs are supported by the results of large-scale studies. It is known that the therapeutic effect of a drug can be potentiated due to the synergy of the mechanisms of action of its components. As a result, this may lead to a reduction in their therapeutic doses, a decrease in the side effects of the drug, or an enhancement of the specific effect of the medication.

Therefore, despite the saturation of the pharmaceutical market with NSAIDs, the search for new safe drugs for the treatment of pain processes that would be more effective and less toxic remains a pressing issue in modern pharmacy and medicine.

Previously, at the Department of Medical and Bioorganic Chemistry of Kharkiv National Medical University (KhNMU), pharmaceutical compositions of well-known NSAIDs with different chemical structures were studied, including 4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazin-3-carboxamide-1,1-dioxide (piroxicam), 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazin-3-carboxamide-1,1-dioxide (meloxicam), 4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide (celecoxib), 4-(4-methylsulfonylphenyl)-3-phenyl-5H-furan-2-one (rofecoxib) in combination with 1,3,7-trimethylxanthine (caffeine). The results showed that caffeine enhanced the central component of the analgesic activity (AA) of the studied NSAIDs with different chemical structures.

Given the established role of caffeine as an adjuvant to rofecoxib, celecoxib, piroxicam, meloxicam, **the aim of our study** was to investigate the central component of the AA of newly developed two-component pharmaceutical compositions based on well-known NSAIDs: N-(2,3-dimethylphenyl)-anthranilic acid (mefenamic acid) and 1,3,7-trimethylxanthine (caffeine), as well as N-(4-nitro-2-phenoxyphenyl)methanesulfonamide (nimesulide) and 1,3,7-trimethylxanthine (caffeine) in laboratory rats. The study also aimed to compare their central AA

component with that of the monocomponent pharmaceutical compositions and the reference drug, 2-[(2,6-dichlorophenyl)amino]phenyl]acetate sodium (diclofenac sodium), under conditions of experimental formalin-induced edema (f.e.) in laboratory animals.

Materials and Methods.

The experiments were conducted, and drug doses were calculated in accordance with existing recommendations. The central component of AA was studied in sexually mature male random-bred WAG rats weighing 180–220 g using an experimental model of f.e. The assessment of AA was performed using a von Frey filament-based tactile sensitivity threshold measurement device with an algesimeter (IITC Life Science, USA). The essence of the experiment involved applying the sensor tip to the central fold area of the animal's hind paw. Paw withdrawal in response to stimulation was recorded as a reaction to nociceptive stimuli. The animals were divided into 8 groups, with 6 rats in each group.

In this experiment, aseptic exudative inflammation was induced in the animals by subplantar injection of 0.1 ml of a 2% formalin solution into the hind paw. The animals in the first group served as the control and received a single intragastric (i.g.) administration of a 3% starch mucilage (2 ml per 200 g of rat body weight). The animals in the second group underwent f.e. modeling and also received a single i.g. administration of a 3% starch mucilage (2 ml per 200 g of rat body weight). The animals in groups 3–8, against the background of f.e., received a single i.g. administration of the test drugs and their pharmaceutical compositions as suspensions in 3% starch mucilage: group 3 - mefenamic acid (50 mg/kg rat body weight), group 4 - nimesulide (15 mg/kg rat body weight), group 5 - caffeine (0.6 mg/kg rat body weight), group 6 - a pharmaceutical composition of mefenamic acid (50 mg/kg) with caffeine (0.6 mg/kg), group 7 - a pharmaceutical composition of nimesulide (15 mg/kg) with caffeine (0.6 mg/kg), group 8 - the reference drug diclofenac sodium (8 mg/kg).

The pain tactile sensitivity threshold was measured in grams both before and 4 hours after subplantar injection of the phlogogen (formalin). The obtained data on

the determination of the central component of AA were converted into a percentage of activity using the following formula:

$$\text{AA} = \frac{\Delta Lc - \Delta Le}{\Delta Lc} \cdot 100\%,$$

where:

AA – analgesic activity (%),

ΔL_c – load on the paw in the control pathology group (g),

ΔL_e – load on the paw in the experimental group (g).

The animals were kept under standard vivarium conditions at the Central Research Laboratory (CRL) of KhNMU in polypropylene cages, on a standard diet with free access to food and water, at a temperature of 19–24 °C, and a relative humidity not exceeding 60%, in compliance with sanitary and hygienic standards. The principles of Directive 210/63/EU of the European Parliament and the EU Council "On the protection of animals used for scientific purposes" (Brussels, 2010), as well as the "General Ethical Principles for Animal Experiments" (Kyiv, 2001), were adhered to. Additionally, compliance was ensured with the Law of Ukraine "On the Protection of Animals from Cruel Treatment" № 3477-IV of 21.02.2006 (as amended) and the Order of the Ministry of Education and Science, Youth and Sports of Ukraine "On Approval of the Procedure for Conducting Experiments and Research on Animals by Scientific Institutions" № 249 of 01.03.2012.

Results and their discussion.

Experimental studies of the central component of AA showed that all the pharmaceutical drugs selected for the study, when administered individually to laboratory rats against the background of f.e., demonstrated biological activity. The leader of our study was nimesulide, which, at a dose of 15 mg/kg in rats, administered once intragastrically (i.g.), exhibited centrally mediated AA of 67.3% (group 4), which was 18.9% higher than that of mefenamic acid (group 3, 50 mg/kg in rats under the same conditions). The potential adjuvant caffeine, administered at a dose of 0.6 mg/kg in rats, exhibited a central component of AA of 57.4% (group 5), which was approximately at the level of the reference drug, diclofenac sodium (group 8). Caffeine exhibited centrally mediated AA that was on 9% higher than that

of mefenamic acid and on 9.9% lower than that of nimesulide under the same experimental conditions (Table 1). Thus, based on their central component of AA, the studied pharmaceutical drugs formed the following series: nimesulide > diclofenac sodium ≥ caffeine > mefenamic acid.

Table 1

Study of the central component of AA of the developed two-component pharmaceutical compositions and their components against the background of formalin-induced edema in rats (n = 6)

№	Animal groups	AA, %
1.	Control	-
2.	Formalin-induced edema (f.e.)	-
3.	Mefenamic acid (f.e.)	48,4
4.	Nimesulide (f.e.)	67,3
5.	Caffeine (f.e.)	57,4
6.	Mefenamic acid + Caffeine (f.e.)	55,1
7.	Nimesulide + Caffeine (f.e.)	71,1
8.	Diclofenac sodium (f.e.)	58,4

The two-component pharmaceutical compositions we developed also proved to be pharmacologically active: the addition of a potential adjuvant, caffeine (group 7), to the leader among monotherapeutic agents, nimesulide, contributed to an increase in the central component of AA by 3.8% compared to the monoadministration of nimesulide (group 4), exceeding sodium diclofenac by 1.2 times (group 8) and nimesulide by 1.1 times (group 4).

Caffeine potentiated the central component of AA of mefenamic acid as well: the developed two-component composition (group 6) also proved to be biologically active concerning the central component of AA (55.1%). However, in terms of effectiveness, it was 1.3 times (16%) lower than the pharmaceutical composition of nimesulide with caffeine (group 7) and 3.3% inferior to the reference drug (group 8).

Therefore, we consider the two-component composition of nimesulide with caffeine, which became the leader of our study (77.1%) in the investigation of the central component of AA, to be appropriate and promising for further research.

Conclusions.

1. Experimental studies have demonstrated the presence of the central component of AA in pharmaceutical preparations against the background of formalin-induced edema in rats. Based on the studied component, the ranking order is as follows:

Nimesulide (67,3%) > Sodium diclofenac (58,4%) ≥ Caffeine (57,4%) > Mefenamic acid (55,1%).

2. Caffeine potentiated the central component of AA of nimesulide and mefenamic acid by 3,8% and 6,7% respectively.

3. The pharmaceutical compositions of mefenamic acid and nimesulide with caffeine that we developed had a more effective impact on the central component of AA than the monoadministration of each individual component.

4. The leader of the experimental study was our developed two-component composition of nimesulide with caffeine, which exhibited AA of central origin at 71,1%, this exceeds by 16% the central component of AA of mefenamic acid with caffeine (55,1%) and by 12,7% the reference drug, sodium diclofenac (58,4%). Therefore, we consider composition of nimesulide with caffeine promising for further research.

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