PHARMACEUTICAL SCIENCES

EXPERIMENTAL STUDY OF THE PERIPHERAL COMPONENT OF ANALGESIC ACTIVITY OF A NEW PHARMACEUTICAL COMPOSITION

Syrova Ganna,
Doctor of Pharmaceutical Sciences, Professor
Kozub Svetlana,
PhD (Technical Sciences), Associate Professor
Chalenko Natalya,
PhD (Pharmaceutical Sciences), Senior teacher
Kharkiv National Medical University
Kharkiv, Ukraine

Introduction. The problem of pain and analgesia is one of the central issues in modern medicine and pharmacy and is the subject of large-scale multidisciplinary research. Pain is not only a symptom accompanying acute and chronic diseases but also a complex psychophysiological phenomenon that involves regulatory mechanisms, emotional formation, as well as motor, humoral, and hemodynamic responses.

Specific pharmacocorrection of pain is carried out by drugs from the groups of narcotic and non-narcotic analgesics (NNA) and nonsteroidal anti-inflammatory drugs (NSAIDs). Narcotic analgesics (NA) have a pronounced central analgesic effect, which allows them to be used to treat life-threatening high-intensity pain. The action of NNA and NSAIDs is dominated by peripheral analgesic action, due to which the drugs are effective in moderate pain that does not pose a threat to life.

The use of NNA and NSAIDs is often accompanied by the development of side effects (gastro-, hepato-, nephrotoxicity, and others related to the specifics of their mechanisms of action). Non-selective cyclooxygenase inhibitors, along with their pharmacological effects, exhibit characteristic side effects that can cause damage to the liver, kidneys, gastric mucosa, etc. Therefore, several ways exist to search for and

develop new drugs with analgesic effects today. One is the search for and creation of combined pharmaceutical compositions with analgesic activity and a minimum of side effects.

An essential stage in the search for new painkillers is a screening study of the peripheral component of the analgesic effect of active pharmaceutical substances. It is known that several models are used to study the mechanisms of peripheral analgesic activity of drugs, such as acetic acid, acetylcholine and kaolin “writhes”, based on chemical pain irritation. The classic screening model is the “acetic acid writhing”. Intraperitoneal injection of acetic acid solution promotes general activation of the nociceptive system and local release of bradykinin, histamine, serotonin, prostaglandins and leukotrienes, which leads to the development of involuntary contractions of the abdominal musculature - “writhes”, accompanied by extension of the hind limbs and an arching of back of experimental animals.

According to the literature, caffeine is known to have a positive effect on the bioavailability of NSAIDs and NNA. The enhanced analgesic effect of NNA is associated with the induction of central cholinergic analgesia by caffeine, the structural similarity of adenosine and caffeine molecules, which contributes to the neurochemical mechanism of action of the latter in the form of blocking «purine» P1 receptors in the brain.

Preliminary experimental studies have been carried out at the Department of Medical and Bioorganic Chemistry to study the effect of caffeine on the antiexudative (AEA), analgesic (ANA), antipyretic effects of known NSAIDs of different chemical structures (paracetamol, diclofenac, ibuprofen, meloxicam, piroxicam, celecoxib, rofecoxib) have shown that caffeine potentiates the AEA and ANA of the studied NSAIDs. Its mechanism of action is based on caffeine inhibition of the enzyme phosphodiesterase, which leads to the accumulation of cyclic adenosine monophosphate inside the cells. The latter enhances glycogenolysis and stimulates metabolism in organs and tissues, including the central nervous system and muscles. An important link in the mechanism of caffeine's stimulant effect is its binding to purine receptors in the brain. Caffeine enhances and regulates excitation processes in

the cerebral cortex, strengthens positive reflexes, and increases motor activity. These effects are dose-dependent, contribute to increased mental and physical performance, reduce fatigue and drowsiness. It is known that in large doses, caffeine can lead to nerve cell depletion.

The work aimed to create a new domestic two-component pharmaceutical composition based on N-(2,3-dimethylphenyl)-anthranilic acid (mefenamic acid) and 1,3,7-trimethylxanthine (caffeine) and study its peripheral component ANA in comparison with the mono-administration of the composition components and with the reference drug 2-[(2,6-dichlorophenyl)amino]phenyl]sodium acetate (diclofenac sodium).

Materials and Methods. The experimental study was conducted using the "acetic acid writhing" model. The writhes was induced by a 0.6% acetic acid solution at a dose of 0.1 ml per 10 g of rat body weight, administered intraperitoneally 1 hour after the oral administration of the test substances and their pharmaceutical composition prepared on a starch mucilage base. The animals were observed for 20 minutes after the administration of acetic acid, and the number of writhes in the rats was recorded. ANA was assessed by the ability of the test substances and their composition to reduce the number of writhes compared to the control group and was expressed as a percentage, calculated using the formula below:

$$\mathrm{ANA} = \frac{\mathrm{Cav} - \mathrm{Eav}}{\mathrm{Cav}} \cdot 100\%$$

where ANA is analgesic activity, %;

Cav is the average number of writhes in the control group;

Eav is the average number of writhes in the experimental group.

The animals were divided into 5 groups, with 6 rats in each group. The animals in the 1st group served as the control and were administered a single intragastric dose of 3% starch mucilage (2 ml per 200 g of rat). Animals in the 2nd to 5th groups were given a single intragastric dose of the test drugs and their compositions as suspensions in 3% starch mucilage: group 2 – mefenamic acid (50.0 mg per 1 kg of animal weight), group 3 – caffeine (0.6 mg per 1 kg of animal weight), group 4 – a

combination of mefenamic acid (50.0 mg per 1 kg of animal weight) and caffeine (0.6 mg per 1 kg of animal weight), group 5 – the reference drug diclofenac sodium (8.0 mg per 1 kg of animal weight).

The study was conducted on laboratory rats of the WAG strain from the Experimental Biological Clinic of KhNMU, following standards for housing, care, and feeding (air temperature: 23–25°C, lighting: 100 lx in the room, 20–40 lx in the cages). The animals were kept for 1.5 months, including a 2-week acclimatization period. The primary diet consisted of vegetables and fodder beets, with dechlorinated tap water as the water source. The rats were housed under vivarium conditions in accordance with the principles of humane treatment of laboratory animals. The study complied with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the resolution of the First National Congress on Bioethics (Kyiv, 2007). Experiments were conducted in the first half of the day, consistent with literature data indicating the dependence of key pharmacological parameters and pharmacological activity of the studied drugs on circadian rhythms.

The study was conducted in accordance with the methodological recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine. An economical approach, bioethical principles, and statistical requirements were considered when determining the number of animals and their distribution among groups.

Statistical processing of the obtained data was performed using standard statistical analysis methods with the help of MS Excel and Statistica software.

Results and Discussion. Our experimental studies demonstrated that the mono- administration of mefenamic acid (group 2) and caffeine (group 3) resulted in a statistically significant reduction in the number of writhes in rats compared to the control group 1 (20.5 ± 0.428): in 2.2 (group 2) and in 2.4 (group 3) times (Table 1).

The addition of caffeine to mefenamic acid (group 4) contributed to an even more intense, statistically significant reduction in the number of writhes in the experimental group - by 2.6 times compared to the control group, i.e. caffeine

enhanced the ANA of mefenamic acid, however, the data obtained in experimental groups 2-4 were statistically significantly different from the reference drug (7.33 ± 0.211), which reduced the number of writhes in rats by 2.8 times (see Table 1).

Table 1

Study of the peripheral component of analgesic activity of mefenamic acid, caffeine and their pharmaceutical composition in rats using the "acetic acid writhing" model

№	Animal groups	Number of writhes	ANA, %
1.	Control	20,5 ± 0,428	--
2.	Mefenamic acid	9,67 ± 0,211 */***/****/*****	52,8
3.	Caffeine	8,5 ± 0,224 */**/***/*****	58,5
4.	Mefenamic acid + Caffeine	7,67 ± 0,333 */**/****/*****	62,6
5.	Diclofenac sodium	7,33 ± 0,211 */**/***/****	64,2

Note 1. (mean ± standard error of the mean) * – significance of the results relative to the control group, P < 0,05;

Note 2. (mean ± standard error of the mean) ** – significance of the results relative to the mono administration of mefenamic acid, P < 0,05;

Note 3. (mean ± standard error of the mean) *** – significance of the results relative to the administration of the composition of mefenamic acid with caffeine, P<0,05;

Note 4. (mean ± standard error of the mean) **** – significance of the results relative to the single administration of caffeine, P < 0.05;

Note 5. (mean ± standard error of the mean) ***** – significance of the results relative to the mono administration of diclofenac sodium, P < 0.05.

In terms of the effect on the peripheral component of analgesic activity the two-component composition of mefenamic acid with caffeine (62.6%) developed by us proved to be the most effective. Its ANA exceeded that of mefenamic acid (group 2) and caffeine (group 3) but did not reach the level of ANA demonstrated by

diclofenac sodium (64.2%).

Conclusions

1. All the pharmaceutical preparations studied, as well as the two-component pharmaceutical composition we developed, are pharmacologically active concerning the peripheral component of ANA: mefenamic acid (52.8%) < caffeine (58.5%) < the pharmaceutical composition of mefenamic acid with caffeine (62.6%) < the reference drug diclofenac sodium (64.2%).

2. The pharmaceutical composition of mefenamic acid with caffeine that we developed demonstrated a more effective effect on the peripheral component of analgesic activity (ANA) than the mono administration of each individual component. Caffeine effectively potentiated mefenamic acid's analgesic activity, leading us to consider this pharmaceutical composition appropriate for addressing the analgesic activity of peripheral genesis.

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