PHARMACEUTICAL SCIENCES

INVESTIGATION OF THE PERIPHERAL COMPONENT OF THE ANALGESIC ACTIVITY OF A NEW TWO-COMPONENT PHARMACEUTICAL COMPOSITION IN AN EXPERIMENTAL MODEL

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Introduction. Nonsteroidal anti-inflammatory drugs (NSAIDs) and non-narcotic analgesics (NNAs) are widely used across various fields of medicine for the treatment of inflammatory processes of different etiologies and pain syndromes. These agents represent one of the most clinically significant groups of pharmaceutical preparations. Annually, more than 300 million people worldwide use NSAIDs and NNAs, with approximately two-thirds consuming them without a prescription. The arsenal of NSAIDs includes a substantial number of pharmaceutical substances and nearly a thousand dosage forms developed on their basis.

Among modern pharmaceutical agents, the most commonly used NSAIDs include classical representatives such as derivatives of arylcarboxylic, salicylic, anthranilic, arylacetic, heteroacetic, indoleacetic, arylpropionic, and enolic acids, as well as pyrazolone derivatives, methanesulfonanilides (e.g., nimesulide), coxibs, and oxicams.

NSAIDs are employed in the prevention and treatment of inflammatory connective tissue diseases (e.g., rheumatoid arthritis, osteoarthritis), pain syndromes (e.g., myalgia, arthralgia, headache, postoperative pain, neuralgia, dysmenorrhea), thrombosis prevention (e.g., hypercoagulation syndrome, thrombophlebitis), and in the management of fever, among other conditions.

However, the use of NSAIDs is frequently associated with adverse reactions, the most common of which involve damage to the gastrointestinal (GI) tract. For this reason, NSAIDs remain a central focus of scientific research.

According to the literature, non-selective NSAIDs exhibit a high ulcerogenic potential on the mucosal lining of the gastroduodenal region. The use of highly selective cyclooxygenase-2 (COX-2) inhibitors, such as celecoxib, rofecoxib, and nimesulide, reduces the incidence of gastrointestinal complications. However, by inhibiting prostacyclin synthesis, these agents may disrupt the balance between prostacyclin and thromboxane levels in favor of thromboxane, a prothrombotic factor, thereby contributing to the development of cardiovascular disorders, including myocardial infarction. At the same time, selective COX-2 inhibitors increase the risk of renal insufficiency due to a reduction in glomerular filtration and a delay in sodium reabsorption.

Therefore, the widespread use of NSAIDs is often limited by their insufficient efficacy and a range of serious adverse effects, primarily their ulcerogenic potential, which frequently necessitates the discontinuation of treatment. The most significant among these adverse effects are gastrotoxicity, nephrotoxicity and hepatotoxicity.

Currently, there is an active global effort aimed at enhancing the therapeutic effects of NSAIDs, particularly their anti-inflammatory and analgesic properties, as well as improving their safety profile.

A promising approach to improving the safety of NSAIDs is the use of combined analgesic agents (CAAs) that demonstrate effective analgesic activity while minimizing adverse effects in modern pharmacotherapy. In the development of such combination drugs, preference is given to domestic active pharmaceutical ingredients that are already well-established on the pharmaceutical market and widely

recognized by the general population.

An important stage in the search for new analgesic agents involves screening studies of the peripheral component of the analgesic activity of active pharmaceutical substances. It is well known that a number of experimental models are employed to investigate the mechanisms of peripheral analgesic activity, including acetic acid-, acetylcholine-, and kaolin-induced writhing tests, all of which are based on chemically induced nociceptive stimulation. The classical screening model is the "acetic acid writhing". Intraperitoneal administration of an acetic acid solution leads to generalized activation of the nociceptive system and local release of bradykinin, histamine, serotonin, prostaglandins and leukotrienes. This cascade results in the development of involuntary contractions of the abdominal muscles, referred to as "writhes", which are accompanied by extension of the hind limbs and arching of the back in experimental animals. According to literature data, caffeine positively influences the bioavailability of NSAIDs and NNAs. The enhancement of analgesic activity of NSAIDs and NNAs is associated with caffeine-induced central cholinergic analgesia, as well as the structural similarity between caffeine and adenosine molecules, which underlies its neurochemical mechanism of action through the blockade of purine P1 receptors in the brain.

Previous experimental studies conducted at the Department of Medical and Bioorganic Chemistry on the effects of caffeine on the anti-exudative (AEA), analgesic (ANA), and antipyretic activities of well-known NSAIDs with various chemical structures (paracetamol, diclofenac, ibuprofen, meloxicam, piroxicam, celecoxib, rofecoxib, mefenamic acid) demonstrated that caffeine enhances both AEA and ANA of the investigated NSAIDs. In our earlier studies, caffeine confirmed its role as an adjuvant to nimesulide (N-(4-Nitro-2-phenoxyphenyl)methanesulfonamide) with respect to the central component of ANA and AEA. The mechanism of its action is based on the inhibition of the phosphodiesterase enzyme by caffeine, leading to the accumulation of intracellular cyclic adenosine monophosphate.

The aim of this study was to investigate the peripheral component of the analgesic activity of a new domestic two-component pharmaceutical composition

based on nimesulide and 1,3,7-trimethylxanthine (caffeine), in comparison with the individual administration of the composition's components and the reference drug 2-[(2,6-dichlorophenyl)amino]phenyl]acetate sodium (diclofenac sodium).

Materials and Methods. The experimental study was conducted using the "acetic acid writhing" model. Writhing was induced by intraperitoneal injection of a 0.6% acetic acid solution at a dose of 0.1 ml per 10 g of rat body weight, administered 1 hour after oral administration of the test substances and their pharmaceutical composition prepared in starch mucilage. Animals were observed for 20 minutes following the acetic acid injection, and the number of writhes was recorded for each rat. The peripheral component (PC) of ANA was assessed based on the ability of the tested substances and their composition to reduce the number of writhes compared to the control group. The effect was expressed as a percentage and calculated using the following formula:

PC ANA = (Cav-Eav) / Cav·100%, where

PC ANA is the peripheral component of 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 five groups, with six rats in each group. Animals in Group 1 served as the control and received a single intragastric (i/g) administration of 3% starch mucilage (2 ml per 200 g of rat body weight). Animals in Groups 2 to 5 received a single i/g administration of the test pharmaceutical substances or their compositions, suspended in 3% starch mucilage, as follows:

Group 2 – nimesulide (15.0 mg/kg of rat body weight);

Group 3 – caffeine (0.6 mg/kg of rat body weight);

Group 4 – a combination of nimesulide (15.0 mg/kg of rat body weight) and caffeine (0.6 mg/kg of rat body weight);

Group 5 – the reference drug diclofenac sodium (8.0 mg/kg of rat body weight).

The study was conducted on WAG laboratory rats obtained from the Experimental Biological Clinic of KhNMU, in compliance with the standards for

housing, care, and feeding. Environmental conditions included an ambient temperature of 23–25°C, lighting levels of 100 lux in the room and 20–40 lux inside the cages. The animals were housed for 1.5 months, including a two-week acclimatization period. The standard diet consisted of vegetables and forage beets, while the water source was settled tap water. The rats were maintained under vivarium conditions in accordance with ethical guidelines for the humane treatment of laboratory animals. The study was carried out in accordance 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). All experiments were conducted in the first half of the day, which, according to literature data, corresponds to the circadian dependence of the main pharmacological parameters and the pharmacological activity of the investigated compounds.

The study was conducted in accordance with the methodological guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine. The selection of the number of animals and their distribution into experimental groups was guided by a resource-efficient approach, bioethical principles, and statistical requirements.

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

Results and Discussion. Our experimental studies demonstrated that monotherapy with nimesulide (Group 2) and caffeine (Group 3) led to a statistically significant reduction in the number of writhing responses in rats compared to the control Group 1: 20,5 ± 0,428. The reduction was 2.2-fold in Group 2 and 2.4-fold in Group 3 (Table 1). Nimesulide also significantly decreased the number of writhing episodes compared to both caffeine (Group 3) and sodium diclofenac (Group 5).

The addition of caffeine to nimesulide (Group 4) resulted in an even more pronounced and statistically significant reduction in the number of writhing responses in rats – by 4,4-fold compared to the control group (Group 1), 1,5-fold compared to the group receiving nimesulide (Group 2) and 2,7-fold compared to the reference drug (Group 5). This indicates that caffeine potentiated the PC of the analgesic effect

of nimesulide.

Table 1

Evaluation of the Peripheral Component of the Analgesic Effect of Nimesulide, Caffeine and Their Pharmaceutical Combination in Rats Using the "Acetic Acid Writhing" Model

№	Animal Groups	Number of Writhing Responses	PC ANA, %
1.	Control	20,5 ± 0,428	--
2.	Nimesulide	6,2± 0,307 */***/****/*****	69,8
3.	Caffeine	8,5 ± 0,224 */**/****/*****	58,5
4.	Nimesulide + Caffeine	4,67 ± 0,211 */**/***/*****	77,2
5.	Sodium Diclofenac	7,33 ± 0,211 */**/***/****	64,2

Note 1. (mean ± standard error) * – statistically significant difference compared to the control group, P < 0,05;

Note 2. (mean ± standard error) ** – statistically significant difference compared to nimesulide monotherapy, P < 0,05;

Note 3. (mean ± standard error) *** – statistically significant difference compared to caffeine monotherapy, P < 0,05;

Note 4. (mean ± standard error) **** – statistically significant difference compared to the combination of nimesulide and caffeine, P < 0,05;

Note 5. (mean ± standard error) ***** – statistically significant difference compared to sodium diclofenac monotherapy, P < 0,05.

In terms of impact on the PC of ANA effect, the two-component composition of nimesulide and caffeine developed by us demonstrated the highest efficacy (77,2%), surpassing that of nimesulide (69,8%), caffeine (58,5%) and sodium diclofenac (64,2%).

Conclusions.

1. All pharmaceutical agents investigated in this study, including the newly developed two-component pharmaceutical composition, demonstrated pharmacological activity with respect to the peripheral component of ANA. The efficacy ranking was as follows: the nimesulide + caffeine composition (77,2%) >

nimesulide (69,8%) > reference drug sodium diclofenac (64,2%) > caffeine (58,5%).

2. The pharmaceutical composition of nimesulide and caffeine developed in our study exhibited greater efficacy on the peripheral component of ANA compared to monotherapy with either component. Caffeine effectively potentiated the peripheral analgesic effect of nimesulide, supporting the rationale for the use of such a pharmaceutical combination in cases of peripheral genesis.

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