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CREATION OF NEW DRUG COMPOSITIONS AND PHARMACOLOGICAL SUBSTANTIATION OF THEIR SUITABILITY FOR PAIN SYNDROMES AND INFLAMMATIONS IN EXPERIMENTAL RATS

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Abstract. *Creation of combined drugs whose pharmacological effects are due to the rational combination of ingredients is the urgent problem of modern medicine. Combination of several components in one drug expands its pharmacological range and promotes polytropic activity. The advantage of combined drugs compared with pure drugs is that they more effectively eliminate pain and inflammation than each individual component.*

Combined analgesics often include caffeine. According to literature data caffeine enhances analgesic effect of nonsteroidal anti-inflammatory drugs (NSAIDs) and nonnarcotic analgesics (NNA). However, there are no data on the composition of diclofenac sodium (D-Na), ibuprofen (Ib) with caffeine in the literature. This fact caused experimental studies of influence of caffeine on the analgesic and anti-exudative effects of D-Na and Ib. Experimental studies have been conducted on laboratory animals (white adult rats of the WAG strain) by intragastric administration. Analysis of experimental results clearly indicates that caffeine potentiates analgesic and anti-exudative effects of D-Na and Ib.

Thus, compositions of D-Na and Ib with caffeine were experimentally studied for the first time. Suitability of compositions is proved and application in inflammation and pain of various origins is demonstrated. Results can serve as foundation for development of new domestic combined drugs with analgesic and anti-inflammatory effects.

Keywords: *non-steroidal anti-inflammatory drugs, ibuprofen, diclofenac sodium, caffeine, analgesic activity, anti-inflammatory activity, CNS.*

Combination drug therapy is often used in medical practice. Caffeine (1,3,7-trimetylxanthine) is often introduced to the combined analgesic drugs for enhancing of their pharmacological activity [1, 2]. Derivative of acetic acid – diclofenac sodium

(D-Na) is on of the most effective non-steroidal anti-inflammatory drugs (NSAIDs). It possesses both analgesic and anti-inflammatory effects and also is well tolerated. It has stronger anti-inflammatory and analgesic effects than acetylsalicylic acid, butadion, and others. D-Na serves as the modern world "gold standard" of treatment [3–5]. Propionic acid derivative –Ibuprofen (Ib) also belongs to safe NSAIDs along with D-Na [6–9]. There are many known combinations of NSAIDs and nonnarcotic analgesics (NNA) with caffeine, but there are no compositions Ib + caffeine, D-Na + caffeine in pharmaceutical practice.

The purpose of our study was to create and investigate new compositions comprising caffeine and NSAIDs of different chemical structure and to substantiate pharmacologically their suitability for pain syndromes and inflammatory processes. An opportunity to achieve a stronger pharmacological activity (analgesic and anti-inflammatory) of the composition compared with individual drug was the basis of our study.

Materials and Methods. Effect on the nociceptive system was determined by the influence on the central and peripheral components of the pain response.

Analgesic effect of peripheral origin is studied on peripheral component of the nociceptive response. Comparative characteristics of the analgesic effect of the drug D-Na and pharmacological combination D-Na with caffeine, as well as drug Ib and its pharmacological composition with caffeine was carried out using the screening model “acetic acid-induced cramps”. The mechanism of pathology under the influence of acetic acid includes activation of kallikrein-kinin system, prostaglandins, biogenic amines, and leukotrienes which are endogenous mediators of inflammation and contribute to the development of abdominal muscles cramps, accompanied by stretching of hind limbs and arching of the back. The rats were observed for 20 minutes after administration of acetic acid and number of cramps in rats was counted. Cramps were induced by single intraperitoneal administration of 0.6 % acetic acid at the rate of 1 ml per 100 g weight of the animal. Animals were divided into 5 groups, 6 animals in each group. First group was the control one, 3% starch mucilage was once orally intragastrically administered to animals of this group (2 ml per 200 g of

rat). Investigated NSAIDs and their compositions with caffeine in the form of 3% starch mucilage suspension were once intragastrically administered to animals of groups 2nd – 5th. D-Na (5 mg per 1 kg of body weight of the animal) was administered to the animals of 2nd group. Composition of D-Na (5 mg per 1 kg of body weight of the animal) with caffeine (0.6 mg per 1 kg of body weight of the animal) was administered to the animals of 3rd group. Ib (6 mg per 1 kg of body weight of the animal) was administered to the animals of 4th group. Composition of Ib (6 mg per 1 kg of body weight of the animal) with caffeine (0.6 mg per 1 kg of body weight of the animal) was administered to the animals of 5th group. Examined NSAIDs and their compositions with caffeine as well as 3 % starch mucilage were administered 1 hour before administration of algogenic agent. The animals were observed for 20 minutes and number of cramps was counted. Analgesic activity was estimated by the ability of NSAIDs and their compositions with caffeine to reduce the number of cramps in the experimental groups in comparison with control animals and expressed in percents. Analgesic activities of pure Ib and D-Na were also compared with analgesic activities of their compositions with caffeine.

Analgesic activity of central origin was studied on central component of the nociceptive response. Analgesic effect of investigated NSAIDs and their compositions was studied on influence on the central component of pain response using summation-threshold index (STI) which reflects the functional state of the CNS. STI was determined by the criterion of unconditioned-reflex motor reaction of animals in response to electric stimulation with frequency of 2 impulses per second with increasing voltage 1 V per second by S.V. Speranskiy. Impulse stimulator was employed for this purpose. The distribution of animals in groups and doses of drugs and their combinations with caffeine were similar to research study of analgesic activity of peripheral origin. STI was determined at the beginning of the experiment (initial pain threshold), in 30, 60 and 90 minutes after administration of suspensions of investigated substances D-Na and Ib and their compositions.

The effect on the inflammatory process. Anti-exudative effect of the examined compounds and their compositions was studied using an experimental model of

formalin edema. NSAIDs, their combinations with caffeine, and starch mucilage (control group) were administered 1 hour before the maximum experimental edema. Exudative inflammation was modeled by subplantar injection of 0.1 ml of 2% formalin solution in the rat hind paw. Paw volume was measured using oncometer before the experiment and at the moment of maximal swelling i.e. 4 hours after administration of flogogenic agent. Increasing of edema was expressed in reference units. The percent of inhibition of inflammation was calculated by the formula [10]:

$$\% \text{ inhibition of inflammation} = \frac{(V_k - V_r)}{V_k} \cdot 100\%, \quad \text{where:}$$

V_k – volume of paw in control less the initial volume of this paw before swelling;

V_r – volume of swelled paw in research less the initial volume of this paw.

Animals were divided into 5 groups, 6 animals in each group. First group was the control one, 3% starch mucilage was once orally intragastrically administered to animals of this group (2 ml per 200 g of rat). Investigated NSAIDs and their compositions with caffeine in the form of 3% starch mucilage suspension were once intragastrically administered to animals of groups 2nd – 5th. D-Na (8 mg per 1 kg of body weight of the animal) was administered to the animals of 2nd group. Composition of D-Na (8 mg per 1 kg of body weight of the animal) with caffeine (0,6 mg per 1 kg of body weight of the animal) was administered to the animals of 3rd group. Ib (6 mg per 1 kg of body weight of the animal) was administered to the animals of 4th group. Composition of Ib (6 mg per 1 kg of body weight of the animal) with caffeine (0,6 mg per 1 kg of body weight of the animal) was administered to the animals of 5th group.

The study was carried out in accordance with the methodological recommendations of the State Pharmacological Center MoH Ukraine [10]. Number of animals and their distribution in groups were in accordance with economical approach, bioethical rules and statistics requirements. Recalculation of the human

doses for rats was done by using the ratio of species sensitivity by Rybolovlev Yu. R. [10]. Statistical calculations were performed by conventional methods [11].

Laboratory animals employed in the study were kept in experimental biological clinic of KhNMU following the norms of the storage, care and feeding approved by the principles of "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" (Strasbourg, 1986) [12] and the decision of the First national Congress on Bioethics (Kyiv, 2007) [13]. Experiments were carried out in the morning, which according to the literature data corresponds to the dependence of the main pharmacological parameters and pharmacological activity of investigated drugs on circadian rhythms [14, 15].

Results and Discussion. Effect on the nociceptive system. Studying of peripheral component of analgesic activity.

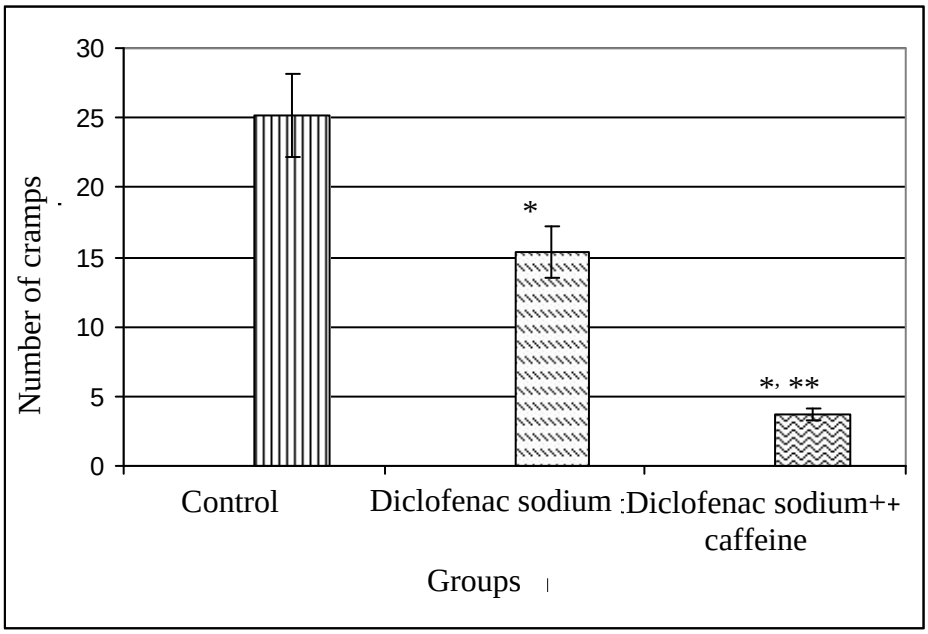
Experimental studies have shown that administration of pure D-Na significantly reduces the number of cramps to 15,33 ± 0,21 as compared to the control group (25,17 ± 0,17), ($P < 0,001$). Analgesic potential is 39 %. Administration of pharmacological composition of D-Na with caffeine significantly reduces the number of cramps to 3,67 ± 0,61 as compared to the control group (25,17 ± 0,17), ($P < 0,001$), as well as compared to the group with administration of pure drug (15,33 ± 0,21), ($P<0,001$). Analgesic potential is 85 % (Fig 1).

Administration of pure Ib also significantly reduces the number of cramps to 10,83 ± 0,31 as compared to the control group (25,17 ± 0,17), ($P < 0,001$). Analgesic activity is 57%.

Addition of caffeine to Ib leads to significant reduce of cramps to 7,5 ± 0,34 as compared to the control group (25,17 ± 0,17), ($P < 0,001$), as well as compared to the group with administration of pure drug (10,83 ± 0,31), ($P<0,001$). Analgesic activity is 70% (Fig 2).

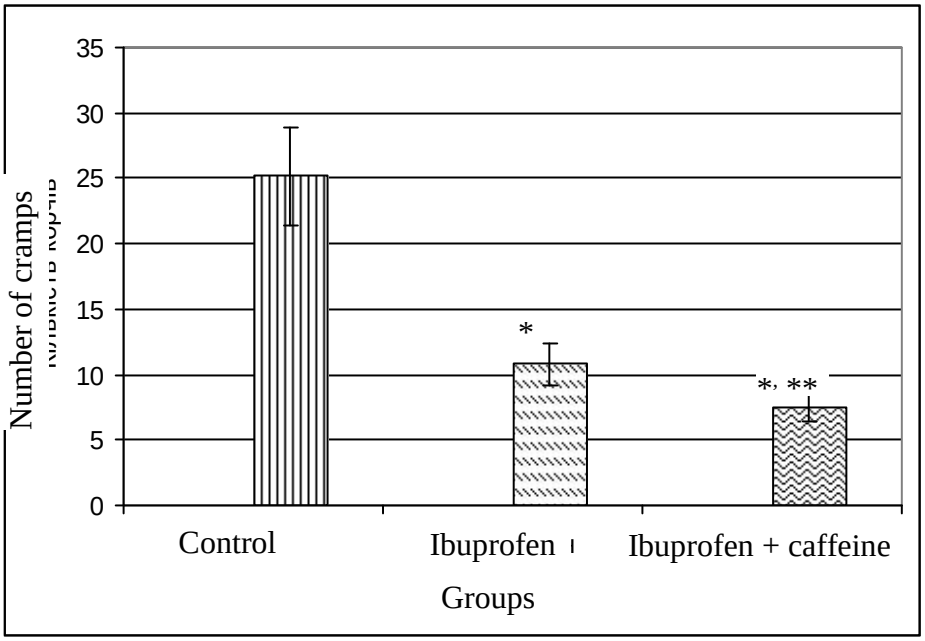
Analysis of experimental results indicates that caffeine potentiates analgesic activity of NSAIDs of different chemical structure – D-Na (phenylacetic acid derivative) and Ib (propionic acid derivative).

Fig. 1. Influence of caffeine on analgesic activity of D-Na



Note: * – difference is statistically significant versus control
** – difference is statistically significant versus pure D-Na

Fig. 2. Influence of caffeine on analgesic activity of Ib



Note: * – difference is statistically significant versus control
** – difference is statistically significant versus pure Ib

Research of the central component of the analgesic activity. Analysis of the results of center link of analgesic activity research indicates that administration of

pure Ib does not cause significant difference between the derived parameters compared with the control and the initial level that does not correspond to the central mechanism of the analgesic activity of the drug (Table 1). Administration of pharmacological composition of Ib with caffeine causes significant decrease in the summation ability of CNS in rats as compared to the initial level as well as compared to the group of pure drug administration in 30 and 60 minutes. This fact confirms the central mechanism of analgesic activity of pharmacological composition (Table 1). Pure D-Na as well in a pharmacological composition with caffeine does not cause significant shift of studied parameter (Table 1.).

Table 1.

Parameters of STI (sec) in case of administration of pure D-Na and Ib and pharmacological compositions with caffeine (n=6)

№ gr.	Research conditions	Initial level	30 min	60 min	90 min
1.	Control	8,62±0,20	8,58±0,18	8,60±0,21	8,65±0,24
2.	D-Na	8,17±0,24	9,58±0,47	9,30±0,49	9,57±0,38
3.	D-Na+ caffeine	8,17±0,24	9,68±0,45	8,88±0,39	9,27±0,28
4.	Ib	8,75±0,17	8,92±0,25	8,73±0,26	8,28±0,20
5.	Ib+ caffeine	8,75±0,17	8,03±0,27*,**,***	7,90±0,26*,**,***	8,43±0,27

Note: difference is statistically significant: * – versus control;
** – versus initial level; *** – versus administration of pure NSAID.

Influence on the process of exudation. Analysis of experimental results shows that D-Na and Ib reduce swelling by 33% and 37 % respectively. The compositions of NSAIDs with caffeine show more intense anti-exudative activity: D-Na + caffeine significantly reduces the formalin edema as compared with control group ($P < 0,01$). Activity of D-Na composition with caffeine is 47%, which is significantly different from the activity of pure D-Na ($P < 0.05$ – 33%). This result allows making conclusion that caffeine is able to potentiate anti-exudative activity of D-Na (Table 2).

Composition of Ib with caffeine appears to be the most effective among the studied compositions. This combination significantly reduces development of formalin edema ($P < 0,001$), as testified by paw volume which almost coincides with the initial volume (inhibition – 95%). Pure Ib reduces edema by 37% (the difference between activity of pure Ib and its combination with caffeine is statistically significant – $P < 0,001$) (Table 3).

Table 2.

Anti-exudative activity of D-Na and its compositions with caffeine (n=6)

Parameters	Control		D-Na		D-Na + caffeine	
	Initial volume of paw (mm)	Volume of paw in 4 hours after edema inducing (mm)	Initial volume of paw (mm)	Volume of paw in 4 hours after edema inducing (mm)	Initial volume of paw (mm)	Volume of paw in 4 hours after edema inducing (mm)
X±Sx	17,75±1,03	30,5±1,19	19,25±0,85	27,75±0,85 *	17,00±0,32	23,8±1,20 *, **
Inhibition of edema, %				33		47

Note: * – difference is statistically significant: D-Na versus control;

** – difference is statistically significant: pure D-Na versus composition with caffeine

Table 3.

Anti-exudative activity of Ib and its compositions with caffeine (n=6)

Parameters	Control		Ib		Ib + caffeine	
	Initial volume of paw (mm)	Volume of paw in 4 hours after edema inducing (mm)	Initial volume of paw (mm)	Volume of paw in 4 hours after edema inducing (mm)	Initial volume of paw (mm)	Volume of paw in 4 hours after edema inducing (mm)
X±Sx	17,75±1,03	30,5±1,19	18,75±1,31	26,75±1,03 *	17,8±0,2	18,4±0,51 *, **
Inhibition of edema, %				37		95

Note: * – difference is statistically significant: Ib versus control;

** – difference is statistically significant: pure Ib versus composition with caffeine.

Thus, the compositions of the investigated NSAIDs with caffeine have pronounced anti-exudative activity, so caffeine potentiates anti-exudative activity of the studied NSAIDs – D-Na and Ib. The composition of caffeine with Ib shows more intensive anti-exudative effect than composition of caffeine with D-Na (Table 2 and 3).

Conclusions. 1. Compositions of studied NSAIDs with caffeine affect the peripheral component of pain reaction and have pronounced analgesic activity of peripheral origin, thus caffeine potentiates the analgesic activity of D-Na and Ib. As for analgesic activity of central origin it is found that caffeine potentiates the central mechanism of analgesic activity of Ib only [16].

2. Compositions of studied NSAIDs with caffeine have pronounced anti-exudative activity, so caffeine potentiates anti-exudative activity of D-Na and Ib. The composition of caffeine with propionic acid derivative (Ib) has more intense anti-exudative effect than caffeine composition with phenylacetic acid derivative (D-Na) [17].

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