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ANTI-EXUDATIVE ACTIVITY OF 7-DISUBSTITUTED 8-METHYLPIPERAZINE-1,3-DIMETHYLXANTHINES

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Summary. It has been established that the greatest anti-exudative action was shown by 7-(3-chlorobutane-2-diol-1)-8-N-ethylpiperazinetheophylline (compound 4). Derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines are a promising group of heterocyclic compounds for more effective anti-exudative substances search.

Keywords: derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines, anti-exudative activity

Introduction. Inflammation in animals and humans is the most frequent symptom of a variety of diseases and represents an important clinical problem. In the development of inflammatory reaction, the central role belongs to the leukocytes migration from the microvasculature into the tissues. The interaction of leukocytes, thrombocytes, and endothelium in the focus of inflammation is mediated by adhesion molecules. In addition, inflammation involves interleukin-1, tumor necrosis factor, thrombocytes activation factor, cytokines, leukotriene B4, as well as prostaglandins (PG). The last participate in the inflammatory reaction development, as well as in pain and fever occurrence. Violation of PG synthesis by not narcotic analgesics leads to the implementation of the anti-inflammatory effect (Hinz, Dormann and Brune, 2006; Kato et al., 2001).

At present, the interest in the treatment of the inflammatory process has increased, which has contributed to the expansion of pathogenesis studies and the search for more effective and safe pharmacological substances for the drug regulation of inflammation at various diseases that are not accompanied by ulcerogenic effects that is typical for modern NSAIDs that cause erosion and gastrointestinal bleeding.

Because of the peculiarities of inflammation development mechanisms, drugs of different pharmacological groups are used for treatment, among which a special place is occupied by drugs of symptomatic therapy — nonsteroidal anti-inflammatory drugs (NSAIDs) (Nasonov, 2002).

The anti-inflammatory effect of NSAIDs is mediated by two independent mechanisms. Low concentrations of NSAIDs, interacting with the arachidonate-COX complex, prevent the stable prostaglandins (PG) formation. High NSAIDs concentrations block the association of arachidonate with G protein and suppress the cellular activation of stable PG formation. It leads to the violation of the class E prostaglandin synthesis in the mucous membrane of the stomach and the development of erosive and ulcerative lesions, and the inhibition of cyclooxygenase-2 (COX-2) in the site of inflammation, a key enzyme in the prostaglandins synthesis of anti-inflammatory activity (Sorotskaya and Karateev, 2005; Goldstein et al., 2000) and selective blockers COX-2 are a threat to the development of thrombotic events, myocardial infarction (Crofford et al., 2000).

The moderate anti-inflammatory effect has theophylline, which inhibits the formation of free oxygen radicals, the synthesis and release of cytokines. When searching for new anti-exudative substances, we used 7-substituted 8-N-methylpiperazin-1,3-dimethylxanthine derivatives (Kornienko, Tarasevičius and Samura, 2013).

The aim of the study was to study the dependence of anti-exudative activity on the chemical structure of the newly synthesized 7-substituted 8-N-methylpiperazine theophylline.

Materials and methods. New derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines (compounds 1–12) were taken as a research object. Synthesis of substances was carried out at the Department of Biological Chemistry of the Zaporizhia State Medical University under the direction of Dr. Pharm. Sci., Prof. N. I. Romanenko (Romanenko et al., 2013).

The structure of the synthesized substances was confirmed by the modern physicochemical methods of elemental analysis, UV-, IR-, PMR-, and mass spectrometry, counter synthesis, and the purity of the synthesized substances was monitored by thin-layer chromatography. Non-linear rats weighing 170–195 g were used for the study. The anti-exudative activity of 7-substituted 8-N-methylpiperazine theophylline was studied on the acute inflammatory edema model caused by the subplantar administration of a 1% carrageenan solution. The investigated substances in the form of a finely dispersed aqueous suspension, stabilized by Tween-80, at the volume of 0.5 ml were administered intraperitoneally at doses of 0.05 LD_{50}. The control group of animals received in the same way an isotonic 0.9% solution of sodium chloride and Tween-80 in the appropriate volume and appropriate doses (Mokhort, Yakovleva and Shapoval, 2001).

After 30 min, 0.1 ml of a 1% aqueous suspension of carrageenan was injected under the aponeurosis of the hind paw of the rat. Using the oncometer, the paw volumes

were measured in rats prior to the beginning of the experiment and hourly for 4 hours.

The anti-exudative activity was determined by the degree of reduction of the experimental edema in the experimental rats in comparison with the control animals. It was expressed as a percentage of the control. The drug of comparison was Diclofenac sodium at a dose of 8 mg/kg. The degree of edema oppression was calculated by the formula (1):

$$\% \text{ oppression} = \frac{Yc-Ye}{Yc} \times 100, \quad (1)$$

where Yc and Ye, respectively, the volume of the paw in the control and in the experiment (Mokhort et al, 2001; Sernov and Gatsura, 2000).

Experimental studies were conducted according to the regulations on the use of animals in biomedical research (Strasbourg, 1986) and the 'General Ethical Principles of Experiments in Animals' (Kiev, 2001), and agreed with the requirements of the 'European Convention for the Protection of Vertebrate Animals, used for experimental and scientific goals'.

The statistical verification of the data was carried out using a standard analysis package for the statistical processing of the results of the version of Microsoft Office Excel 2003. The results are presented as a sample mean and a standard error of the mean value. The reliability of the differences between the experimental groups was assessed using the Student's *t*-criterion and the Mann-Whitney U test of the computer program Statistica® for Windows 7.0 (Statsoft Inc. No.AXXR712D833214 Fan5). Differences at a significance level of < 0.05 (Lapach, Chubenko and Babich, 2001) were considered statistically significant for all types of analysis.

Results and discussion. Table 1 shows the results of studying the anti-exudative activity of heterocyclic derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines.

It has been found that the most pronounced anti-exudative effect was shown by compound 4 — 7-(3-chlorobutane-2-diol-1)-8-N-ethylpiperazine theophylline which, at a dose of 19.3 mg/kg in 4 hours after administration, caused a decrease in edema of the paw in rats by 45.2%. Replacement in the 7th position of 7-(3-3-chlorobutane-2-diol-1)-8-N-ethylpiperazine teophylline 3-chlorobutane-2-diol radical (compound 4) molecule with *м*-bromobenzyl (compound 1), β-phenylethyl, (compound 2), α-methylbenzyl (compound 3), α-naphthylmethyl (compound 5), β-phenylethyl (compound 6) led to decreasing in the development of experimental carrageenan paw edema in rats from 45.2% to 28.9%.

Replacement of the ethyl radical in the 8th position of the piperazine fragment of the molecule 1-*n*-fluorobenzyl-8-(4-ethylpiperazinyl-1)-theobromine (compound 9) by methyl (compound 12) led to a decrease in anti-nociceptive activity by 3.5%.

Table 1 — Anti-exudative activity of the derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines

Compound No.	Code	Dose, mg/kg	Anti-exudative activity	
			paw volume after 4 hours, ml	paw volume after 4 hours, %
1	α-2466	23.0	1.48±0.14*	35.1
2	α-2660	25.5	1.59±0.12*	32.5
3	α-4253	27.0	1.62±0.19*	28.9
4	α-4255	19.3	1.25±0.06*	45.2
5	α-4256	15.5	1.45±0.08*	36.4
6	α-4258	34.0	1.64±0.16*	28.1
7	α-4259	27.5	1.59±0.17*	30.3
8	α-4260	23.7	1.68±0.18*	26.3
9	α-8431	12.3	1.28±0.15*	43.9
10	α-8319	37.5	1.86±0.07	18.4
11	α-8314	39.0	1.63±0.13*	28.5
12	α-8430	13.0	1.36±0.15*	40.4
Diclofenac sodium		8.0	1.22±0.14*	46.5
Control		—	2.28±0.11	100.0

Note: * — for $p < 0.05$ compared with the control.

Introduction of 7-β-phenylethyl-8-N-ethylpiperazine theophylline molecule instead of the methyl radical of the hydrogen atom to the first position, and to the 7th position instead of phenylethyl *н*-heptyl (compound 7) or *н*-docyl (compound 8) fragments, and anti-exudative activity was 30.3% and 26.3%, respectively.

Compounds 10 and 11 revealed a tendency to suppress the development of paw edema in rats by an average of 18.4 and 28.5%, respectively.

The anti-exudative activity of the comparative drug — diclofenac sodium at a dose of 8 mg/kg was 46.5%.

It can be assumed that the anti-inflammatory effect of the newly synthesized derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines is realized by reducing the release of inflammatory mediators from mast cells and inhibiting the expression of genes responsible for the synthesis of anti-inflammatory cytokines (Kato et al., 2001).

Thus, among the studied derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines, the anti-exudative activity of compound 4 is comparable to the anti-inflammatory effect of the diclofenac sodium comparator.

Conclusions. 1. Expressed anti-exudative activity was shown by the compound 4 — 7-(3-chlorobutane-2-diol-1)-8-N-ethylpiperazinetheophylline, which caused a decrease in the development of experimental carrageenan edema in rats by 45.2%.

2. Derivatives of 7-substituted 8-methylpiperazin-1,3-dimethylxanthines are the promising group of organic substances for the subsequent purposeful synthesis and pharmacological screening in order to create new agents with anti-inflammatory activity on their basis.

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