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DOSE-DEPENDENT EFFECT OF HEXADECYL ESTER OF IBUPROFEN ON THE MODEL OF FORMALIN-INDUCED INFLAMMATION

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Introduction. Inflammation is a protective reaction of the organism to the damaging agent. Ibuprofen has anti-inflammatory, antipyretic, and anti-edema effect. But ibuprofen has also side effects.

The main goal was to estimate the "dose-effect" dependence of the hexadecyl ester of ibuprofen on the formalin model of inflammation.

Materials and methods. An acute inflammatory reaction was caused by the subplantar injection of 0.1 ml of 2% formalin into the hind limb of experimental rats. After the development of the inflammatory process, rats were treated by transdermal administration of the hexadecyl ester of ibuprofen as an ointment containing the ester in various concentrations – 5%, 2.5%, 1% and 0.5%. The dynamics of change in the inflammatory process determined by measuring the width and volume of the affected limbs.

Results. The hexadecyl ester of ibuprofen reduces the width and volume area of the inflammation in animals and has anti-inflammatory activity at the concentrations 0.5–5%.

Conclusion. The hexadecyl ester of ibuprofen demonstrated the high anti-inflammatory activity. The anti-inflammatory activity was not dependent from the concentration. It is advisable to use the 0.5% ointment of ibuprofen ester, which is almost equal in their efficiency to the 5% ointment.

Key words: pharmacological screening, anti-inflammatory effect, ibuprofen hexadecyl ether, dose-effect, formalin-induced inflammation.

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ЗАЛЕЖНІСТЬ ДОЗА-ЕФЕКТ ГЕКСАДЕЦИЛОВОГО ЕФІРУ ІБУПРОФЕНУ НА МОДЕЛІ ФОРМАЛІНОВОГО ЗАПАЛЕННЯ

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Досліджено залежність доза-ефект гексадецилового ефіру ібупрофену на моделі формалінового запалення. Запальний процес викликали субплантарним введенням 0,1 мл 0,2% формаліну під підошовний апоневроз задньої кінцівки дослідних щурів. Після розвитку процесу запалення проводили аплікацію мазевої основи, яка містила гексадециловий ефір ібупрофену у різних концентраціях – 5%, 2,5%, 1% та 0,5%. Встановлено, що в групах тварин, де гексадециловий ефір ібупрофену використовувався для лікування процесу запалювання, спостерігалось значне зменшення показників ширини та об'єму осередку запалення. Варто зазначити, що для досягнення основного фармакологічного ефекту на організм необхідно використовувати 0,5% мазь гексадецилового ефіру ібупрофену, оскільки його ефективність не поступається 5% мазі.

Ключові слова: фармакологічний скринінг, протизапальна дія, гексадециловий ефір ібупрофену, доза-ефект, формалін індуковане запалення.

Introduction. The vast majority of diseases include general pathological processes, among which inflammation is the most well-known [1].

Inflammation is a protective reaction of the body, which is aimed at destroying the damaging agent and eliminating ensuing changes in tissues. The local effect of the inflammatory process is always accompanied by general changes in the entire body. First of all, it manifests itself in increased temperature, leukocytosis, violation of all types of metabolism, and hypoproteinemia in chronic inflammation [2; 3].

The medicines actively used for the treatment of the inflammatory process include non-steroidal anti-inflammatory drugs (NSAIDs), among which the most famous is ibuprofen [3]. According to modern terminology, ibuprofen belongs to "acidic" anti-inflammatory agents and has a large number of possible side effects, such as an irritating effect on the mucous membrane of the gastrointestinal tract, impaired kidney function, and others [4]. Its local usage as a soft dosage requires frequent application to the skin (3–4 times a day) [5], so it is relevant to create derivatives based on ibuprofen, which would be characterized by an increase in the retention time of the active molecule in the body both due to an increase in lipophility and slow hydrolysis, which leads to prolonged action.

The analysis of literary sources demonstrates a lack of information about the anti-inflammatory properties of ibuprofen esters during transdermal administration. No information was found on the concentration-dependent activity of ibuprofen hexadecyl ester in the formalin model of inflammation too.

One of the ways to solve this problem is to develop methods of chemical modification of the ibuprofen molecule, as a result of which it would be possible to obtain more active analogues. One of these medicinal forms are prodrugs [6]. From a chemical point of view, prodrugs can be qualified as medicinal compounds temporarily containing special biologically inert groups, which are used to change the undesirable properties of the parent molecule: solubility, bioavailability, specific action, instability, long time of release of the substance from the dosage form, toxicity, and inconvenience of use for patient (taste, smell).

The most widely used compound for creating prodrugs is ester. Esters are easily synthesized from active substances containing alcohol groups and carboxylic acids [7].

The main goal was to establish the "dose-effect" dependence of the hexadecyl ester of ibuprofen on the formalin model of inflammation.

Materials and methods.

Animal groups

Experiments involved 96 purebred white rats of the Wistar line, weighing 150–180 g, obtained from the vivarium of the Odesa National Medical University. Animals were subject to a standard diet with water and food ad libitum. Experimental studies were conducted following the methodological recommendations of the State Pharmacological Center, Ministry of Health of Ukraine, and the bioethics commission of the Odesa I.I. Mechnikov National University, Ministry of Education of Ukraine.

Synthesis

The hexadecyl esters of ibuprofen were synthesized by the azeotropic water distillation with benzene using a Dean-Stark nozzle (Fig. 1).

The purity of the obtained esters was verified by the method of thin layer chromatography (TLC) in the system – heptane-chloroform-ethyl acetate 1:1:0.5.

Hexadecyl ester of ibuprofen was synthesized at the Pharmaceutical Chemistry Department of Odesa I.I. Mechnikov National University ($C_{16}H_{33}$) (Fig. 2).

Design of the experiment. An acute inflammatory reaction was induced by the method of subplantar injection of 0.1 ml of 2% formalin [8; 9] into the hind limb of experimental rats. After 24 hours, rats were treated by transdermal administration of the hexadecyl ester of ibuprofen (see Fig. 2) with an ointment containing the ester at various concentrations – 5%, 2.5%, 1% and 0.5%.

The hydrophilic base, consisted of PEG-1500:PEO-400:1,2-propylene glycol, in a ratio of 4:2:3, respectively, is often used in medical practice and does not cause allergic reactions and has no contraindications for use [10].

The results of the experimental study were evaluated on the basis of the dynamics of changes in the width and volume of the affected limb (the width was determined by an electronic calliper, and the volume by the volumetric method).

Results and discussion.

The study of dose-effect response to the model of formalin inflammation showed that the use of ibuprofen hexadecyl ester for treating the inflammatory process demonstrates significant anti-inflammatory activity (Tables 1, 2).

The formalin model leads to the development of chronic inflammation, which can cause tissue necrosis. This is a classic model of the chronic inflammatory process [9]. Therefore, we studied the dose-effect relationship of the hexadecyl ester of ibuprofen with transdermal administration specifically on the formalin model of inflammation.

The obtained results reveal that on the 1st day (24 hrs) of treatment, the parameters of the width and volume of the affected limbs of the experimental rats in the groups where ibuprofen hexadecyl ester was used did not differ from the control group, where the treatment was not carried out.

Starting from the second day of treatment, there is a tendency to a significant decrease in inflammation indicators in the groups that were injected with ibuprofen hexadecyl ester, and there is a return of the width indicators of the affected limbs to the initial ones on the 10th day, and the volume indicators – on the 9th day of the experiment.

In the control group of animals, the width and volume of the inflammation at the end of the experiment did not reach the intact ones and exceeded the indicators of the other groups by 48% (width of the affected limbs) and 35% (volume of the affected limbs), respectively.

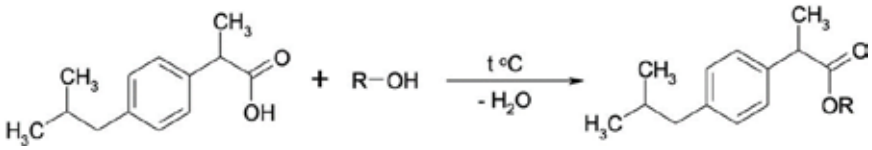

Fig. 1. Synthesis reaction of hexadecyl ester ibuprofen

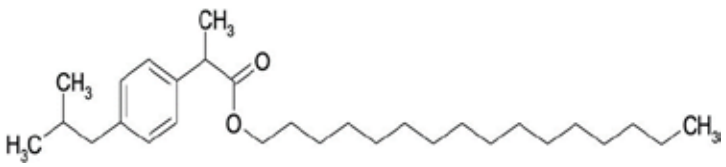

Fig. 2. Molecular structure of hexadecyl ester ibuprofen (IBP-$C_{16}H_{33}$)

Table 1

Anti-inflammatory activity of the hexadecyl ester of ibuprofen on the model of formalin inflammation depending on the concentration (width of swelling of the affected limbs of rats, in % of intact ones, (M ± m) (n=10))

Day	Control	Ibuprofen	IBP-$C_{16}H_{33}$ 0.5%	IBP-$C_{16}H_{33}$ 1 %	IBP-$C_{16}H_{33}$ 2.5%	IBP-$C_{16}H_{33}$ 5.0%
Intact indicators	100±4.2					
24 hrs	155±5.3					
2	161.2±2.2	145.6±4.2*	149.6±3*	145.3±4*	142.5±3*	144 .1±5.8*
3	152.6±2.3	137.0±3.8*	133.0±2.7*	132.8±3.2 *	134.8±2.1*	131.3±3.6*
4	160.0±1.5	134.0±2.1*	135.5±2.4*	130.1±3.1 *	132.7±3.5*	129.0±4.1*
5	159.7±3.1	131.8±2.4*	136.6±3.5 *	132.8±1.9 *	125.3±2.6*	124.2±3.4*
6	153.9±3.2	128.1±1.8*	120.1±2.9 *	126.4±2.7 *	121.1±3.9*	122.6±2.6*
7	152.4±2.1	124.9±2.9*	118.5±2.1 *	121.2±1.5 *	119.5±3.2*	118.2±4.3*
8	147.2±1.3	122.5±1.7*	115.3±4.7 *	119.8±3.7*	113.9±2.7*	116.1±4.0*
9	162.3±3.2	125.7±3.5*	121.1±3.6 *	122.2±2.8 *	118.4±3.6*	119.2±2.4*
10	160.3±2.6	120.7±3.7*	116.8±2.1 *	115.8±2.2 *	114.2±3.0*	113 .7±2.5*
11	150.0±2.4	115.4±3.1*	111.5±3.1 *	110.8±1.4 *	108.2±2.4*	106.0±2.0*
12	148.0±1.2	111.3±4.5*	107.0±2.6 *	105.1±2.4 *	104.3±1.2*	105.0±1.1*

Note: * – $p < 0.05$ – matched with the control group

Table 2

Anti-inflammatory activity of the hexadecyl ester of ibuprofen on the formalin inflammation model depending on the concentration (volume of edema of the affected limbs of rats, in % of intact ones, (M ± m) (n=10))

Day	Control	Ibuprofen	IBP-$C_{16}H_{33}$ 0.5%	IBP-$C_{16}H_{33}$ 1 %	IBP-$C_{16}H_{33}$ 2.5%	IBP-$C_{16}H_{33}$ 5.0%
Intact indicators	100±6.4					
24 hrs	162±7.1					
2	164.9±3.1	160.8±3.4*	162.1±4.3*	158.6±3.1*	154.2±4.8*	155.9±3.2*
3	167.5±4.2	153.2±4.1*	149.5±3.0*	147.5±2.8*	1 49 .8±4.0*	147.3±2.1*
4	175.3±2.1	142.3±4.7*	135.5±4.1*	136.8±3.9*	130.2±3.7*	132.0±2.8*
5	162.4±4.2	138.1±3.5*	130.1±3.9*	137.2±4.2*	132.5±3.0*	127.8±1.8*
6	156.4±3.1	138.7±3.1*	127.6±2.7*	129.2±3.7*	130.4±1.8*	125.3±2.0*
7	157.1±3.8	126.4±2.4*	122.0±3.8*	128.3±4.0*	125.6±2.7*	123.2±1.2*
8	155.1±4.0	120.6±1.8*	118.7±2.5*	122.4±3.2*	115.4±2.3*	114.3±3.4*
9	163.8±3.2	126.6±3.4*	123.1±3.1*	128.8±2.9*	125.1±1.2*	121.1±2.2*
10	158.9±2.4	121.7±2.1*	119.0±1.8*	121.0±2.4*	118.0±3.4*	119,5±3.4*
11	145.6±1.8	116.0±3.2*	112.6±2.7*	114.0±3.4*	111.3±3.1*	114.0±2.8*
12	136.0±3.3	109.8±4.2*	106,2±3.5*	105.0±3.3*	10 3.4±1.8*	107,5±2.8*

Note: * – $p < 0.05$ – matched with the control group

The anti-inflammatory activity in the group with 5% hexadecyl ester of ibuprofen was slightly better than in the groups where 2.5%, 1% and 0.5% ointment was used.

Conclusions. Consequently, referring to the results of indicators of the inflammation of the hind paw on the formalin model of inflammation, it can be stated that the use of transdermal administration of the hexadecyl ester of ibuprofen demonstrates high anti-inflammatory activity in a wide range of concentrations. It means that in order to achieve the desired pharmacological response to the inflammatory process, it is advisable to use 0.5% ibuprofen ester ointment, which is almost as effective as 5% ointment.

Such a phenomenon can be explained by the fact that during transdermal administration the amount of the active substance penetrating through the skin barrier is limited by the permeability of the stratum corneum and the release of the active substance from the skin depot. These parameters lead to slight differences in the manifestation of anti-inflammatory activity at different concentrations of the ester in the soft dosage form.

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