BIOPHARMACEUTICAL RESEARCH ON THE CHOICE OF A NON-STEROIDAL ANTI-INFLAMMATORY AGENT IN THE DEVELOPMENT OF COMBINATION GEL FOR MASTOPATHY THERAPY

Zuikina S. S., Vyshnevska L. I.

National University of Pharmacy

Introduction.

Today it can be considered as already established fact, that tumours are genetic diseases whose pathogenetic substrate is damage to specific sections of DNA. These sections are responsible for the mechanisms of control of cell proliferation and differentiation. As a result, this can lead to tumour formation [1].

Over the past 20 years, the breast cancer incidence rate has increased by an average of 40% and pathology has become the most common cancer affecting every tenth woman in the life expectancy range from 13 to 90 years [2].

According to experts from the National Cancer Institute, a diagnosis of breast cancer (BC) by the end of 2020 may become a reality for almost 17% of Ukrainian women, which gives reason to view it as a socially significant epidemiological problem. According to the International Agency for Research on Cancer (IARC) at WHO, more than 1.7 million women are diagnosed with breast cancer annually. In Ukraine, according to the National Cancer Registry, in 2013, BC was first identified in 16,624 women (in 2012 - 16,660), which is 72.2 cases (in 2012 - 67) for every 100,000 women in the country. The global death toll from this disease is growing and is killing more than 500,000 women worldwide annually. In 2013 in Ukraine, 7429 women died with this diagnosis (7558 in 2012) [3].

Uniform classification of breast pathology, including fibrocystic mastopathy (FCM), is presented in sections N60-N64 of the International Statistical Classification of Diseases (ICD-10), which allows comparisons of the prevalence of this pathology in different regions of the world.

The greatest achievement of theoretical oncology in the second half of XX century was the disclosure of the role of chronic inflammation as a precancerous condition. In chronic inflammation, begin to proliferate stem cells, transforming already into the stem cells of a malignant tumour. "Inflammatory cells" allocate growth factors for tumour cells, as well as angiogenic factors that stimulate the formation of tumour in the microcirculatory bloodstream. Due to chronic inflammation, the stroma of the malignant tumour is formed. Permanent inflammation due to ongoing necrosis in the tumour provides the cells with angiostimulating and growth factors.

The path from the origin of the tumour cell to the progression of the disease is determined by the consistent implementation of the patterns inherent in tumour growth (carcinogenesis). It is generally accepted characteristic of the development of cancer in the form of four successive stages: initiation, promotion, evasion of differentiation and progression [5].

The most studied physiological promoters of proliferation in the mammary gland to date are the steroid ovarian hormone estradiol and its metabolites [4].

Homeostasis of breast tissues is the result of equilibrium between proliferation, differentiation and apoptosis of its cellular composition, which depends on the optimal ratio, both in serum and in breast tissues, the concentration of the major sex hormones (estrogen and progesterone), which provide the above effects.

An essential step in a comprehensive approach to reducing the incidence of BC was the unified clinical protocol "Breast Cancer" (Order of the Ministry of Health of Ukraine No. 396 of June 30, 2015), in which mastopathy is organizationally attributed to precancerous pathology, which is detectable in mammological screening programs (physical, ultrasound and radiological with histological verification of the nature of the process in suspected BC), and women with any form of mastopathy are subject to mandatory treatment and preventive recovery [3].

In recent years, the approach to the treatment of precancerous diseases has been gradually changing, due to the emergence of a new unique and promising direction - "targeted therapy", based on the principles of targeted effects on the underlying molecular mechanisms underlying of this or that disease [1].

In the treatment of BC usually used several main classes of targeted drugs that provide control of the cell cycle: 1) affect the cellular estrogen receptors; 2) inhibit aromatase; 3) block the receptor of the human epidermal growth factor; 2 (HER; 2) and PARP; proteins, etc. [1].

The clinical protocol "Prevention and diagnosis of breast hormone diseases" (Order of the Ministry of Health of Ukraine No. 676) contains a list of recommended drugs for the prevention of breast tumours, which include sedative drugs; vitamins B1, B2, C, E, A, folic acid, multivitamins; antioxidants; immunocorrectors, adaptogens, drugs that improve digestive tract function and metabolism in the liver, dopamine receptor antagonists; GnRH analogues.

All diffuse forms of mastopathy are subjected to conservative treatment. The general disadvantages of most drugs used today for the treatment of mastopathy can be attributed to the low effectiveness and focus only on alleviating symptoms, not correcting the problem as such.

Not only the estrogens themselves but also their metabolites play an important role in the processes that trigger the proliferative diseases of the female reproductive system. It has been proved that estrone and estradiol make up 10-15% of the total amount of estrogen derivatives, and 85-90% are metabolites of estrogens (mainly hydroxy derivatives).

Many years of searching for chemical compounds that block the development of hyperplastic processes in hormone-dependent tissues have drawn the attention of scientists to indole derivatives. They have been found to enhance the expression of cytochrome P450-CYP1A1 isoforms, which in turn is hydroxylated to hydroxyestrone form. It is established that this metabolite has antiproliferative (antiestrogenic) activity [5, 6].

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Materials and methods

In order to substantiate the use of the indole derivative - indomethacin - as an active pharmaceutical ingredient (API) in the complex gel for the treatment of mastopathy and prevention of malignant breast tumours, the domestic pharmaceutical market was studied for the presence of drugs with indomethacin in different dosage forms, different composition and presented by different countries and manufacturers (Fig. 1 – 3).

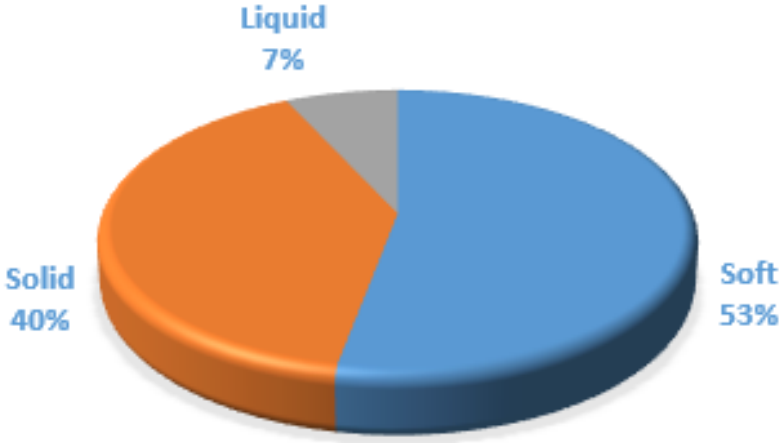

Fig. 1. Distribution of indomethacin drugs by dosage forms

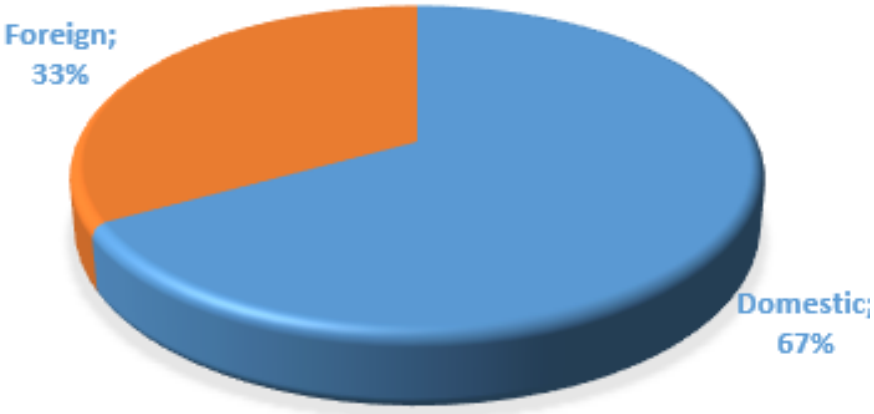

Fig. 2. Distribution of indomethacin drugs by manufacturers

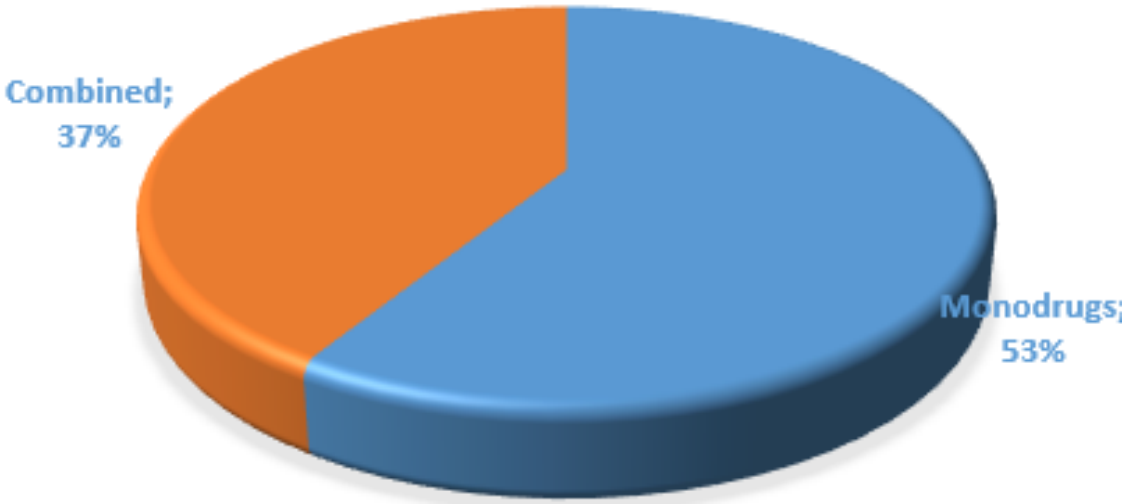

Fig. 3. Distribution of indomethacin drugs by compound

Results & discussion. As it shown in the data of fig. 1-3, most of the medicines available on the Ukrainian pharmaceutical market are complex soft dosage forms, mostly gels, presented by “Indovazin-Teva”, “Indovazine” manufactured by Balkanpharma-Troyan JSC, Bulgaria; “Indovenol” manufactured by PJSC “Borschagov Chemical and Pharmaceutical Plant”, Ukraine; complex

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drugs “Troxovedol” manufactured by PJSC Viola Pharmaceutical Factory, Ukraine; complex medicines in the form of ointments “Indomethacin Plus” manufactured by PJSC “Chemical Factory Red Star”, Ukraine; “Indomethacin Sopharma” manufactured by JSC “Sopharma”, Bulgaria.

Drugs in solid dosage forms are represented by enteric tablets "Indomethacin Zdorovya" manufactured by pharmaceutical company “Zdorovye”, “Indomethacin Sopharma” manufactured by JSC “Sopharma”, Bulgaria; tablets effervescent “Difmetre”, manufactured by “Abbott Labora GmbH”, Germany; suppositories “Indomethacin Sopharma” manufactured by JSC “Sopharma”, Bulgaria. Representative of liquid medicines there are eye drops “Indokolir 0.1%” manufactured by LLC “Valeant Pharmaceuticals”, Chauvin laboratory, France.

The drugs are classified into different groups according to ATC classification: C05CA51 “Vasoprotectives. Capillary stabilizing agents. Bioflavonoids. Rutoside, combinations”, M02AA “Anti-inflammatory preparations, non-steroids for topical use”, M02AA23 “Anti-inflammatory preparations, non-steroids for topical use. Indometacin”, S01BC01 “Ophthalmologicals. Anti-inflammatory agents, non-steroids”, etc.

Given the lack of complex drugs in the Ukrainian market in the form of gels, the composition and mechanism of action of which would make it possible to use them in the scheme of complex treatment of mastopathy, the task was to develop a soft dosage form that would provide a complex mechanism of action, taking into account pathogenetic links of the disease [7 - 10].

Due to the effectiveness of the use of indole derivatives in the treatment of various forms of mastopathy, it was decided to use it as a non-steroidal anti-inflammatory component of the developing gel.

A number of scientists (Drogovoz S.M., Derimedvid L.V., Shchokina K.G.) conducted a comparative study of the types of pharmacological action of NSAIDs and established ED_{50} for indometacin by the types of studied activity (Fig. 4 - 5) [11].

As evidenced by the figure, the expressed level of antiproliferative, anti-exudative activity in the presence of inflammatory process, analgesic, infectious activity makes it possible to state that the use of indomethacin will provide the necessary pharmacological action in the treatment of mastopathy, taking into account polyethiology and pathogenicity.

When studying the composition of drugs in the form of gels, presented on the domestic pharmaceutical market, it was found that the concentration of indometacin in their composition is 3% (gel “Indovazine”, “Indovazine Teva” and “Troxevenol”), 5% (ointment “Indomethacin plus”) and 10 % (ointment “Indomethacin Sopharma”). The last representative is used as an anti-rheumatic agent for joint diseases, so, in our opinion, in the further studies to consider the use of the specified NSAIDs is impractical.

Given the sensitivity of the breast skin, as well as experimental studies to determine the therapeutic dose of the substance of other scientists, in the next stage it was rational to study the possibility of developing experimental samples of gel with an indomethacin concentration of 2.5 to 5.5 %. The concentration interval of NSAIDs in the test samples was 0.5 % (Tab. 1). Previously, to select the concentration of indometacin in the developing of gel composition, biopharmaceutical studies were conducted to study the degree of its release from the developed gel base using the agar plate method and dialysis through a semipermeable membrane [8, 12].

The diameter of the coloured zones was determined by colour reaction with hydroxylamine hydrochloride and hydrochloric acid. The reagent was added to the agar gel, diffusing into which, indomethacin interacted, resulting in the formation of coloured areas of different diameter, having a purple-pink colour.

The kinetics of indometacin release from the gel compositions were determined by the diffusion method through a semipermeable cellophane membrane (according to Kruvchinsky). Sampling of dialysate (5 ml) was performed at regular intervals, filling the environment with an equal amount of ethanol 95% (taking into account the solubility of indomethacin) (Fig. 6).

In the course of the experiment we closely monitored the tightness of dialysis devices. All experiments were performed in 5 replicates, the results of the experiments were statistically processed using the Student's t-test with a confidence probability of 0.95.

The quantitative content of indometacin was determined by the titrimetric method according to the requirements of SPhU v.2 [12].

The kinetic curves of indometacin release are shown in Fig. 7.

According to the experimental data, the dynamics of indomethacin release from sample #4 are almost inferior to that from sample #3, and are not significantly different from that from sample #2, while the indomethacin release rate from samples #6 and #7 is much lower, similar to sample #4. This may be explained by the deterioration of the solubility of indometacin in the components of the substrate with increasing its concentration. More dynamic release of the active ingredient from sample # 4 will provide a prolonged and mild effect of the drug, reducing the possibility of side effects.

Thus, we can consider a rational concentration of indomethacin 4% in terms of pharmacological and economic feasibility

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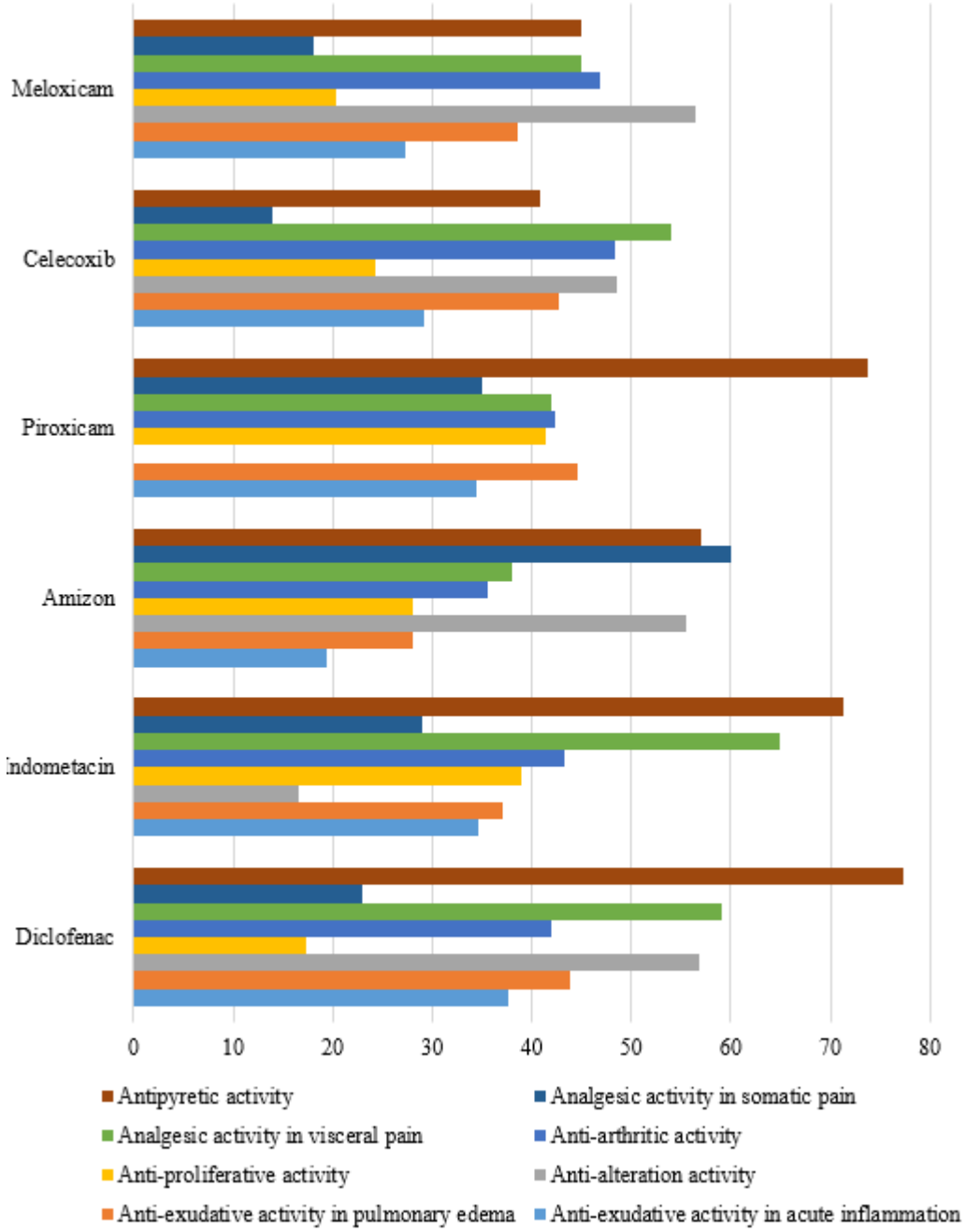

Fig. 4. Comparison of types of pharmacological activity of representatives of NSAIDs

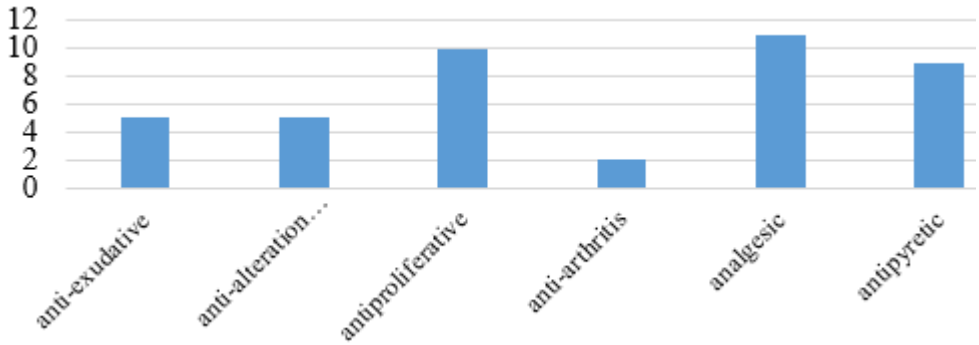

Fig. 5. ED_{50} for indometacin by type of activity

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Table 1. **Quantitative content of indometacin in experimental gel samples**

Sample №	Indometacin, %
1	2.5
2	3
3	3.5
4	4
5	4.5
6	5
7	5.5

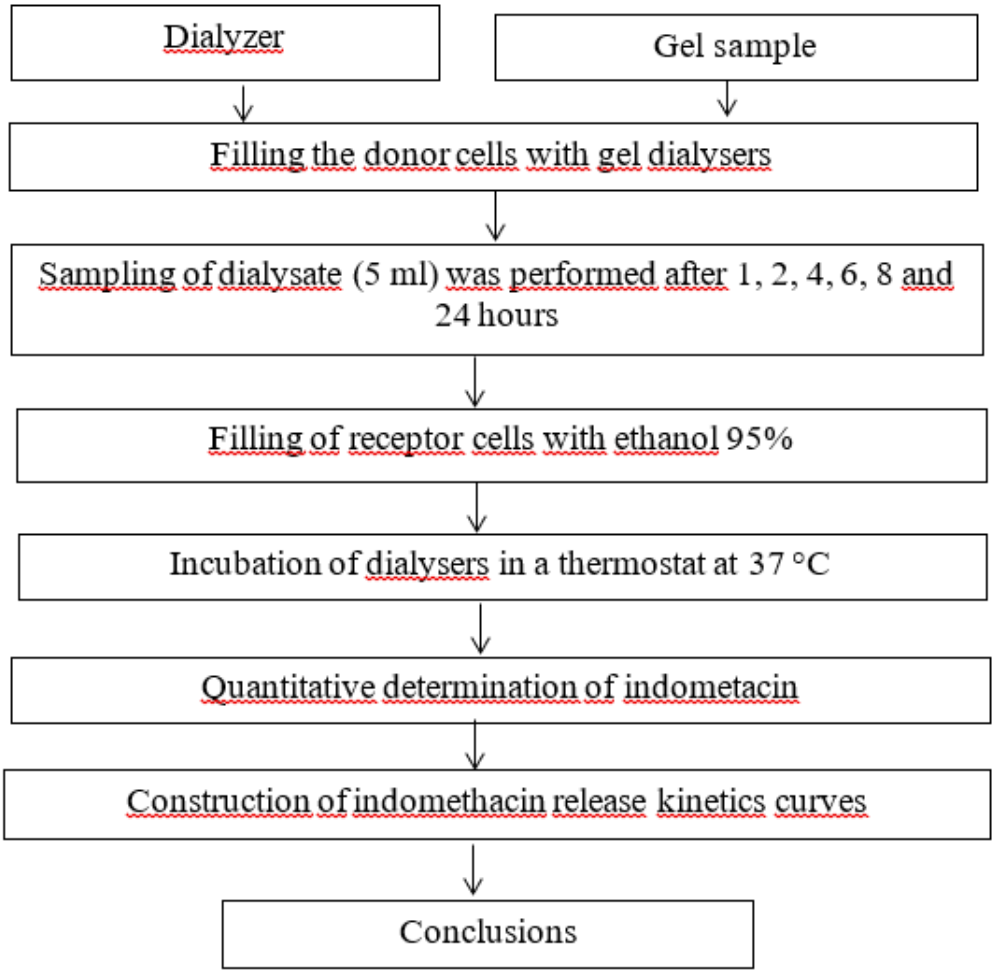

Fig. 6. An algorithm for experimental work to determine the effect of indomethacin concentration on the gel release process by dialysis through a semipermeable membrane

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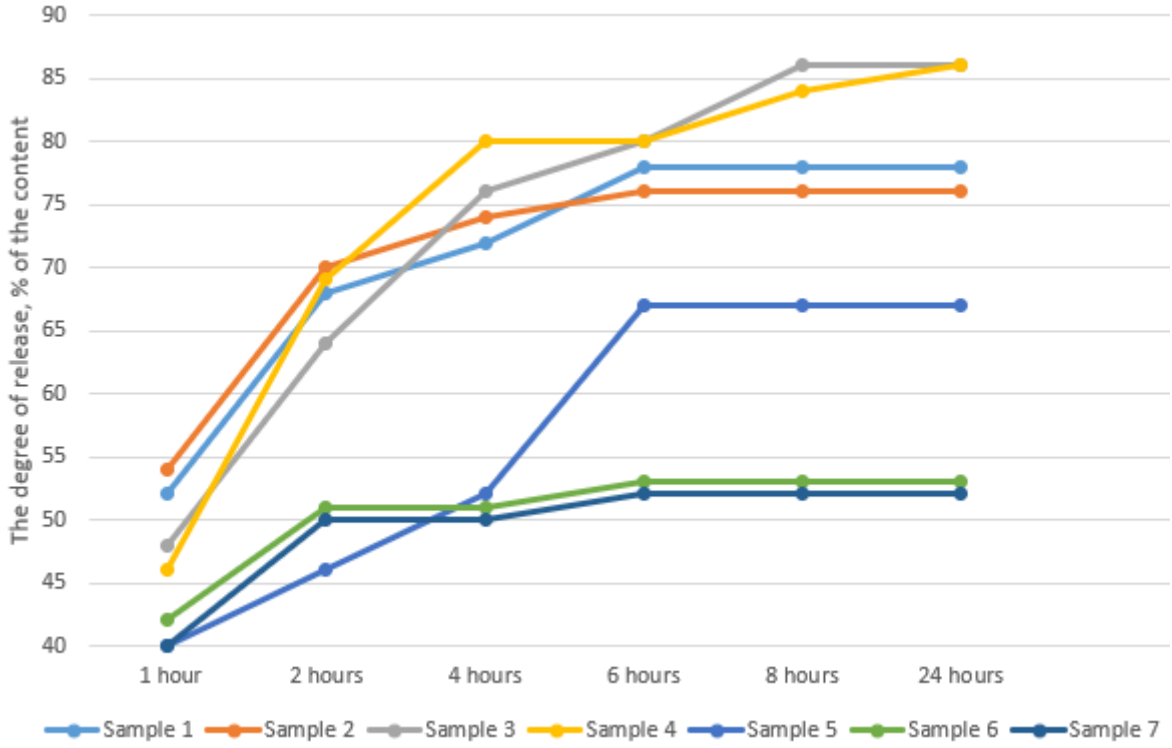

Fig. 7. Kinetics of indomethacin release from experimental gel samples

Conclusions

1. The etiology of mastopathy, strategic directions of pharmaco-correction and mechanisms of action of drugs for its complex therapy have been investigated.
2. The domestic pharmaceutical market was analysed for the presence of indomethacin medicines. The types of dosage forms, the countries of origin, the composition of drugs and the concentration of indometacin in them were determined.
3. The spectrum and level of different types of indometacin activity have been studied, with which the expediency of its use for pathogenetic correction of mastopathy is substantiated.
4. Biopharmaceutical studies have been conducted to study the degree of indometacin release from experimental combination gel samples for the treatment of mastopathy by agar gel diffusion and dialysis through a semi-permeable membrane.
5. According to the results of the release kinetics analysis, the concentration of indometacin as the active pharmaceutical ingredient of the gel is proposed.

Biopharmaceutical research on the choice of a non-steroidal anti-inflammatory agent in the development of combination gel for mastopathy therapy

Zuikina S. S, Vyshnevska L. I.

According to the International Agency for Research on Cancer (IARC) at WHO, more than 1.7 million women are diagnosed with breast cancer annually. In Ukraine, according to the National Cancer Registry, in 2013, BC was first identified in 16,624 women (in 2012 – 16,660), which is 72.2 cases (in 2012 – 67) for every 100,000 women in the country. The global death toll from this disease is growing and is killing more than 500,000 women worldwide annually. According to experts from the National Cancer Institute, a diagnosis of breast cancer by the end of 2020 may become a reality for almost 17% of Ukrainian women, which gives reason to view it as a socially significant epidemiological problem.The aim of the study was to substantiate the choice of NSAIDs and their concentration for use in the development of combination gel for the treatment of mastopathy and prevention of breast cancer. The methods of literary analysis, marketing research and biopharmaceutical methods in vitro were used. The results of marketing studies and pharmacological studies of the level and types of activity of NSAIDs allowed indometacin to be selected as the active pharmaceutical ingredient (API). According to the results of biopharmaceutical studies, the concentration of the active substance in the composition of the gel is established.

Key words: breast cancer, mastopathy, gel, indometacin, release.

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