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THE STUDY OF MICROBIOLOGICAL PURITY OF "LORAVIT" ANTI-ALLERGIC SUPPOSITORIES

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Key words: microbiological purity; anti-allergic suppositories; "Loravit"

The relevance of creation of anti-allergic drugs in the form of the rectal dosage form - suppositories for the treatment of allergic diseases in young children has been proven as a result of the analysis of the published data. Based on the research conducted the composition of antihistamine suppositories with loratadine hydrochloride has been theoretically and experimentally substantiated. The production technology for the suppositories in pharmacy and industrial conditions has developed as a result of the physical and chemical (the melting points, the total strain time have been determined), rheological (the structural and mechanical properties of the suppository mass and the effect of the active substances on the change of the suppository base viscosity have been studied) and thermogravimetric research (the temperature regimen for drug production has been determined). The methods of qualitative and quantitative analysis have been also developed, the conditions and shelf life of the anti-allergic medicine developed under the conditional name "Loravit" have been found. For the purpose of quality control and development of the quality control methods at the Institute of Microbiology and Immunology named after I.I. Mechnikov the samples of antihistaminic suppositories under the conditional name "Loravit" have been analyzed according to the section "Microbiological purity" of the requirements of the SphU. Suppositories with loratadine hydrochloride for treatment of allergic disease in children meet the requirements of the pharmacopoeia in terms of microbial contamination for rectal dosage forms.

The XXI-th century will be the era of allergies according to forecasts of the WHO because the prevalence of allergic diseases has reached epidemic proportions. The prevalence is increased by 2-3 times every 10 years. Nowadays allergic pathology is included to the six of the most common human diseases. According to statistics, one of five persons in our planet have different forms of allergies. It is a serious social, economic and medical problem. A great number of allergies is common among children [1, 3, 6, 8].

At the same time the use of drugs in childhood has some peculiarities, above all, they should be highly effective and with minimal side effects. Children have some physiological and psychological differences from adults – endocrine, nervous and other systems of the body are not fully formed, the child may refuse to take medicines, etc. Therefore, medicines for children should be developed in such dosage forms that are pleasant or convenient for use by a child and are not harmful for children [4, 5, 7, 9, 10].

Taking into account the abovesaid, antihistamine suppositories with loratadine hydrochloride for the treatment of allergic diseases in children have been developed. One of the important indicators for checking the drug quality is its microbiological purity, the analysis is required by the SPhU [2].

Experimental Part

Microbiological studies of "Loravit" suppositories were performed by the method of the SPhU (1st ed., § 2.6.12, § 2.6.13) under the section "Microbiological purity" at the Institute of Microbiology and Immunology named after I.I. Mechnikov.

Standard media were used for drug testing for microbiological purity; they were prepared in accordance with the manufacturer's requirements (the amount of powder per liter, pH medium, etc.). Every medium used in the experiment was tested on the growth parameters according to normative documents.

The following media were used – semisolid thioglycolate broth, liquid Sabouraud's medium, solid culture media: nutrient agar, Sabouraud's medium, Chistovich medium, blood agar based on the nutrient agar, Endo medium.

Staphylococcus aureus ATCC 6538,
Escherichia coli ATCC 25922,
Pseudomonas aeruginosa ATCC 9027,
Bacillus subtilis ATCC 6633,
Candida albicans ATCC 885/653.

Before the research concerning determination of microbiological purity the conformity of growth properties of nutrient have been analyzed. For this purpose five series of the media mentioned above were inoculated with the appropriate test-resistant strains of microorganisms ($10\text{-}10^2$ colony forming units per 1 ml of the medium – CFU/ml).

Fungi of *Candida* genus were inoculated on the Sabouraud's medium. *Pseudomonas aeruginosa* and *Bacillus subtilis* were inoculated on the nutrient agar, *Escherichia coli* – on the Endo medium, *Staphylococcus aureus* ATCC 26923 – on the Chistovich medium. Thioglycolate broth was kept in an incubator at 35°C for three days (Table 1).

From the table it can be concluded that all cultures of microorganisms are responsible to taxonomic indi-

Table 1

Growth properties of nutrient media when inoculating of microorganisms by test strains before determination of microbiological purity

Test strains	Nutrient medium	Terms of cultivation		Observation
		Temperature	Cultivation time	
Staphylococcus aureus ATCC 6538	Chistovich	35°C	24-72 h	Morphology of colonies and cells is typical
Esherichia coli ATCC 25922	Endo	35°C	24-72 h	Morphology of colonies and cells is typical
Pseudomonas aeruginosa ATCC 9027	Nutrient agar	35°C	24-72 h	Morphology of colonies and cells is typical
Bacillus subtilis ATCC 6633	Nutrient agar	35°C	24-72 h	Morphology of colonies and cells is typical
Candida albicans ATCC 885/653	Sabouraud's medium	35°C	24-120 h	Morphology of colonies and cells is typical
X	Thioglycolate broth for sterility control	35°C	24-72 h	Growth of microorganisms is absent

Note: x – organisms are not inoculated

Table 2

Study of microbiological purity of the medicine

Media and cultivation conditions	
Thioglycolate broth (14 days at 35°C)	Liquid Sabouraud's medium (14 days at 25°C)
Growth of microorganisms	No growth of fungi

Note: n = 3

cation of the strain, and the morphology of colonies when cultured on media and the morphology of cells in microscopy are typical. The thioglycolate broth corresponds to the requirements for sterility – the growth of microorganisms is absent, the medium is clear.

The microbiological purity of "Loravit" medicine was carried out after determination of the growth properties of the culture media.

"Loravit" suppositories are standardized according to section 5.1.4 of the SPhU as a drug of 3A category. According to this section of the SPhU the total count of viable aerobic microorganisms (not more than 10^3 bacteria and not more than 10^2 fungi per 1 g; not more than 10^2 enterobacteria and some other gram-negative bacteria per 1 g) is permitted in the medicine.

These studies were carried out by direct inoculation on the liquid culture media. Into sterile test-tubes 10.0 ml of thioglycolate broth and 10.0 ml of the liquid Sabouraud's medium were poured out. To each test-tube 1 ml (1 g) of "Loravit" medicine developed was added. Inoculations were incubated for 14 days on the thioglycolate broth in a thermostat at 35°C, inoculations on the liquid Sabouraud's medium – at 25°C. The results are shown in Tables 2 and 3.

As the data in Table 2 indicate, the growth of fungi is not observed within 14 days of incubation on the Sabouraud's medium. The growth of microorganisms on the thioglycolate broth was recorded. Microscopy showed the presence of a gram-positive spore bacillus. The confirmation was obtained by inoculating on differential nutrient media.

As can be seen from the data presented in Table 3, by morphology of the colonies and some biological properties of the microorganisms selected they belong to *Bacillus sp.* genus. On differential media (Chistovich medium and Endo medium) the growth of intestinal and pathogenic staphylococci among other types of microorganisms was not observed.

The count of viable cells of microorganisms and fungi was determined when studying by the method of

Table 3

Identification of microorganisms grown on the thioglycolate broth

The sample of "Loravit"	The growth of microorganisms in nutrient media				
	Chistovich	Endo	Blood agar	Sabouraud's	Nutrient agar
1	x	x	Dry grey colonies with irregular edges, do not shine, hemolysis	x	Dry grey colonies with irregular edges, do not shine
2	x	x	Dry grey colonies with irregular edges, do not shine, hemolysis	x	Dry grey colonies with irregular edges, do not shine
3	x	x	Dry grey colonies with irregular edges, do not shine, hemolysis	x	Dry grey colonies with irregular edges, do not shine

Note: x – no growth of microorganisms

Table 4

Research of microbiological purity by the method of dish direct inoculation

The sample of "Loravit"	Number of microorganisms by decadic logarithm of the degree of growth when cultured on solid nutrient media			
	Method of deep plating of 1 g of the medicine		Method of surface inoculation of 1 g of the medicine	
	Nutrient agar 35°C for 3 days	Sabouraud's medium 25°C for 5 days	Nutrient agar 35°C for 3 days	Sabouraud's medium 25°C for 5 days
1	1.7±0.7	No growth of fungi	1.5±0.4	No growth of fungi
2	1.7±0.6	No growth of fungi	1.8±0.5	No growth of fungi
3	1.7±0.5	No growth of fungi	1.7±0.6	No growth of fungi

Table 5

Effectiveness of "Loravit" sample

Exposition	Requirements of the SPhU		Logarithm of the microorganisms count (CFU/ml)			
	Bacterial count, CFU/ml, Lg reduction	Fungi count CFU/ml, Lg reduction	*Staphylococcus aureus* ATCC 6538	*Pseudomonas aeruginosa* ATCC 9027	*Candida albicans* ATCC 885/653	*Aspergillus niger* ATCC 16404
Microbial load	10^6	10^6	$3.5x10^5$ (5.54)	$4.5x10^5$ (5.66)	$2.2x10^5$ (5.34)	$2.5x10^5$ (5.39)
Primary inoculation, Lg	-	-	$4.9x10^4$ (0.85)	$5.1x10^4$ (0.96)	$5.2x10^4$ (0.63)	$5.2x10^4$ (0.68)
2 days	2	-	$3.3x10^3$ (2.02)	$2.7x10^3$ (2.23)	$1.3x10^4$ (1.23)	$2.1x10^4$ (1.7)
7 days	3	-	$1.2x10^2$ (3.22)	$1.8x10^2$ (3.41)	$2.2x10^2$ (3.0)	$1.9x10^2$ (3.12)
14 days	-	2	**	**	**	**
28 days	*	*	**	**	**	**

Notes: * – organisms do not grow; ** – bacteria or fungi are not isolated.

deep plating consisted in adding the medicine in the amount of 1 g into the agar and by surface inoculation (1 g) on the agar. Investigations of deep plating and surface inoculation of the drug sample on the Sabouraud's medium dishes showed no fungal growth. The growth of microorganisms was observed. These results of the research are presented in Table 4.

As the data of Table 4 indicate, the growth of fungi was not observed while studying all samples. The count of microorganisms grown per 1 g of the drug sample did not exceed 103 CFU/ml complying with the requirements of the SPhU.

The criterion for efficiency evaluation was reduction of the count of viable cell colonies of microorganisms in the period after contamination. In accordance to the requirements of the SPhU in medicines for topical application the log reduction in the viable bacteria colonies count in 2 days at least 2, in 7 days – at least 3, and further the count of viable bacterial cells should not increase. Logarithms of reduction of the viable cells count of fungi in 7 days were not less than 2. These figures correspond to the criterion "A".

As shown in Table 5, after the 7-day cultivation the logarithm the viable cells count for *Candida albicans* was 3.0 and it was 3.12 for *Aspergillus niger*. Cells of fungi did not isolate in the 14-th and 28-th days. In two days after cultivation the logarithm of the viable cells count for *Staphylococcus aureus* was 2.02 and for *Pseudomonas aeruginosa* it was 3.41. On the 14th and 28th day of incubation the microorganism was not recorded. The study of the samples of "Loravit" suppositories has shown that they meet the criteria "A" according to the SPhU.

On the basis of these studies standardization of microbiological purity of "Loravit" suppositories has been determined and the data obtained has been included in the Project of the quality control methods for the suppositories.

CONCLUSIONS

1. It has been proven that the medicine studied conforms to the requirements of the State Pharmacopeia of Ukraine as for medicines for rectal use by the level of microbial contamination.

2. Standardization of microbiological purity of "Loravit" suppositories as finished products of 3A category has been determined.

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ВИВЧЕННЯ МІКРОБІОЛОГІЧНОЇ ЧИСТОТИ ПРОТИАЛЕРГІЙНИХ СУПОЗИТОРІЇВ «ЛОРАВІТ»

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Ключові слова: *мікробіологічна чистота; протиалергійні супозиторії; «Лоравіт»*

Раніше проведеним аналізом літературних даних було встановлено актуальність створення протиалергійних препаратів у вигляді ректальної лікарської форми – супозиторіїв для лікування алергійних захворювань у дітей молодшого віку. На основі проведених досліджень теоретично та експериментально обґрунтовано склад антигістамінних супозиторіїв з лоратадину гідрохлоридом. У результаті проведених фізико-хімічних (визначені температура плавлення, час повної деформації), реологічних (вивчені структурно-механічні властивості супозиторної маси та вплив діючих речовин на зміну в'язкості супозиторної основи) та термогравіметричних (визначення температурного режиму виробництва лікарського препарату) досліджень було розроблено технологію виробництва супозиторіїв як в аптечних, так і в промислових умовах. Також були розроблені методики кількісного та якісного аналізу, встановлені умови та термін зберігання розробленого протиалергійного препарату під умовною назвою «Лоравіт». З метою контролю якості та розробки методів контролю якості (МКЯ) на базі Інституту мікробіології та імунології ім. І.І.Мечникова зразки антигістамінних супозиторіїв під умовною назвою «Лоравіт» піддавали аналізу відповідно до вимог ДФУ за розділом «Мікробіологічна чистота». Встановлено, що супозиторії з лоратадину гідрохлоридом для лікування алергійних захворювань у дітей за рівнем мікробної контамінації відповідали вимогам ДФУ для ректальних лікарських форм.

ИЗУЧЕНИЕ МИКРОБИОЛОГИЧЕСКОЙ ЧИСТОТЫ ПРОТИВОАЛЛЕРГИЧЕСКИХ СУППОЗИТОРИЕВ «ЛОРАВИТ»

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Ключевые слова: *микробиологическая чистота; противоаллергические суппозитории; «Лоравит»*

Ранее проводившимся анализом литературных данных была установлена актуальность создания противоаллергических препаратов в виде ректальной лекарственной формы – суппозитории для лечения аллергических заболеваний у детей младшего возраста. На основе проведенных исследований теоретически и экспериментально обоснован состав антигистаминных суппозиториев с лоратадина гидрохлоридом. В результате проведенных физико-химических (определены температуры плавления, время полной деформации), реологических (изучены структурно-механические свойства суппозиторной массы и влияние действующих веществ на изменение вязкости суппозиторной основы) и термогравиметрических (определение температурного режима производства лекарственного препарата) исследований была разработана технология производства суппозиториев как в аптечных, так и в промышленных условиях. Также были разработаны методики качественного и количественного анализа, установлены условия и срок хранения разработанного противоаллергического препарата под условным названием «Лоравит». С целью контроля качества и разработки методов контроля качества (МКК) на базе Института микробиологии и иммунологии им. И.И.Мечникова образцы антигистаминных суппозиториев под условным названием «Лоравит» подвергались анализу согласно требованиям ГФУ за разделом «Микробиологическая чистота». Установлено, что суппозитории с лоратадина гидрохлоридом для лечения аллергических заболеваний у детей по уровню микробной контаминации отвечали требованиям ГФУ для ректальных лекарственных форм.