MICROBIOLOGICAL STUDIES OF ANTI-HISTAMINE SUPPOSITORIES "LORAVIT" FOR CHILDREN

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Abstract: Among the current problems of medicine and pharmacy, attracting the attention of researchers around the world, allergic diseases are on an important place; for example, bronchial asthma, allergic rhinitis, atopic dermatitis, and others. These pathologies affect their abundance not only among adults, but also among children [1]. According to statistics, almost every child had allergic reactions to drugs, food, clothing, hygiene products, etc. at least once [2]. In this regard, we have developed a new anti-allergic drug for children in the form of suppositories under the conditional name "Loravit".

The purpose of this work was to conduct microbiological research of new anti-allergic drug "Loravit" for children in the rectal dosage form.

During researches, the correlation of the growth properties of nutrients was analyzed and the microbiological purity of the drug "Loravit" was studied. A thioglycol semi-liquid substratum, Saburo's substratum, solid nutrient substrata: nutrient agar, Saburo's substratum, Chistovich's substratum, nutrient agar based on blood agar, Endo's substratum, and also test microorganisms – Staphylococcus aureus ATCC 6538, Esherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027, Bacillus subtilis ATCC 6633, Candida albicans ATCC 885/653 were used for carrying out these studies.

In the study of microbiological purity, the method of direct seeding on liquid nutrient substrata and the method of deep sowing were applied.

As a result of the conducted studies it was established that a new medicinal product for children in the form of suppositories for the treatment of allergic diseases meets the requirements of the State Pharmacopoeia of Ukraine and European Pharmacopeia for the indicator "Microbiological purity". The norms of microbiological purity of suppositories "Loravit" are also established. The test results were included in the draft quality control methods for the developed drug.

Keywords: Allergic diseases, children, suppositories, loratadine hydrochloride, microbiological purity, deep sowing, direct sowing.

1. Introduction

Nowadays, the wide prevalence of allergic diseases is a big problem. But the most terrible are its stats among the children. Any disease statistics (ADs) (incl. the allergic diseases) are required to control the corresponding pathology and supply the required services with personnel, healing/diagnostic means, scientific addons etc [3].

Over the past decade there has been an increase in the prevalence of food allergies in children [4]. Earlier researchers defined the ADs spreading is 10 times higher, than the officially registered [5].

Screening epidemiological studies are conducted regularly throughout the world [6]. According to many scientists, the ADs are one of the most spread diseases. In the USA 20 % population are allergic and 40–50 % have the unstable allergy symptoms [7]. In Mexico, Canada, Brazil, Portugal, Cuba, the ADs are mostly equal to the USA [8]. In Germany, the ADs are targeting 20 %, in Serbia – 23 % population, in France the given diseases target 5…6 million people, 75 % of that number obsess the respiratory AD manifestations, mainly in the form of allergic rhinitis [9]. The less spreading of allergic pathology is detected in Columbia, Italy, Turkey and Philippines, the most – in Ukraine, Japan, Bulgaria, GB and Sweden [10]. According to the WHO data the SAR (season allergic rhinitis) spreading in different countries varies from 1 to 40 %, the yearly allergic rhinitis (YAR) – from 1 to 18 %. The AR symptoms are manifested among 40 % Ukrainians [11].

We must define, that such stats work not only with the adults, but also with the children. That's why the modern pharmacy has a task to develop the new anti-allergic drugs for children.

During the investigations we developed the contain and technology of children's "Loravit" suppositories, containing the loratadine hydrochloride (main antihistamine component) and the immunostimulator/antioxidant, α-tocopherol acetate 30 % oil solution.

The work purpose is the holding of microbiological investigations for the "Loravit" suppositories, based on I. I. Mechnikov Institute of microbiology and immunology, guided by the Head of Laboratory, BioD, T. Osolodchenko.

2. Material and Methods

Microbiological clearance testing of "Loravit" suppositories was made using the Petri cup seeding method and antibacterial activity definition with drug diffusion into agar-agar.

To test the drug microbiological clearance we used the standard environment, prepared according to the manufacturer requirements (powder quantity per litre, environment pH, autocloning conditions etc.) Every environment, used in the experiment, was checked for the growth indexes according to the normative documents.

We used the next environments – thioglycol half-liquid environment, liquid Saburo's substratum, solid substrata: nutritive agar, Saburo's substratum, Chistovich's substratum, blood agar, based on the nutritive agar, Endo's substratum.

We used the following microorganisms for the test: Staphylococcus aureus ATCC 6538, Esherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027, Bacillus subtilis ATCC 6633, Candida albicans ATCC 885/653.

Before the test of microbiological sample clearance, we analyzed the conformity of environment growth properties. To do this, we inoculated the 5 series of given environments with the proper microorganism test strains ($10–10^2$ colony-forming units per environment ml – CFUs/ml)

The Saburo's substratum was planted with Candida fungi. The nutritive agar – with Pseudomonas aeruginosa and Bacillus subtilis, Endo's substratum – Escherichia coli, Chistovich's substratum – Staphylococcus aureus ATCC 26923. The thioglycol substratum was held in the thermostat at 35°C for three days (**Table 1**).

The table No 1 shows us, that every organism culture complies with the strain taxonomical index and the morphology of cultivated cultures and microscoped cells is typical. The thioglycol substratum complies with the sterility conditions – microorganisms don't grow, the environment is transparent.

After the definition of nutritional substrata growth properties, we defined the "Loravit" drug microbiological clearance.

According to the State Pharmacopoeia of Ukraine part 5.1.4, the suppositories belong to the 3A category of manufactured drug forms. This State Pharmacopoeia of Ukraine part requires

the following overall living aerobic microorganisms' quantity: not more, than 10^2 bacteria and 10^2 fungi per g, not more 10^2 enterobacteria and some other gram-negative bacteria per g.

Table 1
Growth properties of nutritional environments at the microorganism test strains inoculation before the microbiological clearance test

Test strains	Nutritional substrata	Cultivation conditions		Observations
		Temperature	Cultivating time	
Staphylococcus aureus ATCC 6538	Chistovich	35 °C	24–72 hrs.	Colony and cell morphology is typical
Esherichia coli ATCC 25922	Endo	35 °C	24–72 hrs.	Colony and cell morphology is typical
Pseudomonas aeruginosa ATCC 9027	Nutritional agar	35 °C	24–72 hrs.	Colony and cell morphology is typical
Bacillus subtilis ATCC 6633	Nutritional agar	35 °C	24–72 hrs.	Colony and cell morphology is typical
Candida albicans ATCC 885/653	Saburo	35 °C	24–120 hrs.	Colony and cell morphology is typical
X	Thioglycol environment for sterility control	35 °C	24–72 hrs.	Microorganism growth is disabled

***Note**: x – microorganisms weren't planted*

The investigations were held by the direct seeding method at the liquid nutritional substrata. The thioglycol and liquid Saburo's substratum were poured 10 ml per vial in aseptic conditions. We put 1,0 g "Loravit" into every vial. The seedings were incubated 14 days on the thioglycol substratum in the thermostat at 35 °C, the seedings on Saburo's liquid substratum – at 25 °C.

3. Results

The investigation results are given in the **Table 2, 3**.

Table 2
Drug microbiological clearance investigation

Substrata and cultivation conditions	
Thioglycol substratum (14 days at 35 °C)	Saburo's liquid substratum (14 days at 25 °C)
Microorganisms growth	No fungi growth

***Note**: n=3*

According to the **Table 2**, there was no fungal growth on the Saburo's substratum after 14 incubation days. The microorganisms' growth was detected on the thioglycol substratum. The microscopy detected the availability of gram-positive spore bacillus. The confirmation was obtained by the seeding at the differential nutritive substrata.

According to the **Table 3** data, the extracted microorganisms belong to the Bacillus sp. genus by the morphology of colonies and some biologic properties. On the differential substrata (Chistovich and Endo) for the intestine and pathogenic staphylococci extraction there were no other microorganisms.

Table 3
Identification of microorganisms, grown at the nutritional subsrata

"Loravit" sample	Microorganisms growth at the nutritional subsrata				
	Chistovich	Endo	Blood agar	Saburo	Nutritive agar
1	x	x	Dry grey, peasy, non-glowing colonies with rough edges, haemolysis	x	Dry grey, peasy, non-glowing colonies with rough edges
2	x	x	Dry grey, peasy, non-glowing colonies with rough edges, haemolysis	x	Dry grey, peasy, non-glowing colonies with rough edges
3	x	x	Dry grey, peasy, non-glowing colonies with rough edges, haemolysis	x	Dry grey, peasy, non-glowing colonies with rough edges

***Note**: x – microorganism growth unavailable*

By using the deep seeding method, which suggests the addition of drug by 1 g per g substratum (agar), we defined the quantity of living microorganism and fungus cells. The investigation of deep and surficial seeding on Saburo cups showed no fungi growth. When cultivated on the nutritional substratum, the microorganisms grew. The investigation results data are given in the **Table 4**.

Table 4
Microbiological clearance investigation using the direct cup seeding method

"Loravit" sample	The microorganism quantity by lg growth ratio when cultie vated on the solid nutritional substratum			
	Deep seeding method (1,0 g drug)		Surficial seeding method (1,0 g drug)	
	Nutritional agar (35 °C) 3 days	Saburo (25 °C) 5 days	Nutritional agar (35 °C) 3 days	Saburo (25 °C) 5 days
1	1,7±0,7	No fungal growth	1,5±0,4	No fungal growth
2	1,7±0,6	No fungal growth	1,8±0,5	No fungal growth
3	1,7±0,5	No fungal growth	1,7±0,6	No fungal growth

According to the 4 table data, there was no fungal growth during every sample investigation. The microorganisms' quantity per drug g was not more, than 10^3 CFUs/ml, which complies with the State Pharmacopoeia of Ukraine requirements.

According to the State Pharmacopoeia of Ukraine requirements for topical drug forms, the logarithm of living bacteria colonies quantity reduction in 2 days is not less, than 2, in 7 – not less, than 3, further the living bacteria quantity must not grow. The corresponding logarithm for fungi must be not lower, than 2 in 7 days. Those indexes correspond to the "A" criterion.

The results of these researches are given in **Table 5**.

According to the 5 table data, after the 7^{th} cultivation day, the living Candida albicans cell algorithm was 3,0 and 3,12 for Aspergillus niger. The fungi cells weren't being extracted after 14 and 28 cultivation days. After two cultivation days, the Staphylococcus aureus living cell logarithm was 2,02 and 3,41 for Pseudomonas aeruginosa. At the 14^{th} and 28^{th} incubation days, microorganism growth hasn't been registered. The "Loravit" suppository samples investigation showed their compliance with the State Pharmacopoeia of Ukraine requirements "A" criterion [12, 13].

Basing on the investigations held, we defined the microbiological purity norming for "Loravit" suppositories and saved the obtained data to the drug QC methods project.

4. Discussion

Summarizing the obtained results of studies on the study of microbiological purity of "Loravit" suppositories, it can be concluded that the level of microbial contamination corresponds to the requirements of the State Pharmacopoeia of Ukraine for preparations for rectal administration.

Since the drug is new and is in the process of development, the results can be used for further researches. They can also be used to develop methods for quality control of this drug and to apply the study of the microbiological purity of suppositories when they are manufactured under pharmacy conditions.

Based on the conducted studies, the standardization of microbiological purity of suppositories "Loravit" as a ready-made medicinal product of category 3A was established.

Table 5

Living bacteria and fungi cell colonies quantity reduction logarithm

Exposition	State Pharmacopoeia of Ukraine requirements		Microorganism number logarithm (CFU/ml)			
	Bacteria quantity CFUs/ml reduction lg	Fungi quantity CFUs/mlreduction lg	Staphylo-coccus aureus ATCC 6538	Pseudo-monas aeruginosa ATCC 9027	Candida albicans ATCC 885/653	Asper-gillus niger ATCC 16404
Microbial load	10^6	10^6	$3,5\times10^5$ (5,54)	$4,5\times10^5$ (5,66)	$2,2\times10^5$ (5,34)	$2,5\times10^5$ (5,39)
Primary seeding lg	–	–	$4,9\times10^4$ (0,85)	$5,1\times10^4$ (0,96)	$5,2\times10^4$ (0,63)	$5,2\times10^4$ (0,68)
2 days	2	–	$3,3\times10^3$ (2,02)	$2,7\times10^3$ (2,23)	$1,3\times10^4$ (1,23)	$2,1\times10^4$ (1,7)
7 days	3	–	$1,2\times10^2$ (3,22)	$1,8\times10^2$ (3,41)	$2,2\times10^2$ (3,0)	$1,9\times10^2$ (3,12)
14 days	–	2	*NE	NE	NE	NE
28 days	*NI	NI	NE	NE	NE	NE

***Note**: *NI – no microorganisms quantity growth; *NE – the microorganisms or fungi are not being extracted*

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