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## The Immuno-stimulating Drugs Application for Specific Prophylaxis of Infectious Diseases.

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### ABSTRACT

The aim of this work was to study the use of xymedon and miyelopid hydrochloride to increase the specific prophylaxis effectiveness of rabies and listeriosis in animals. The experiments were carried out on rabbits and guinea pigs immunized with rabies inactivated vaccine from the Shchyolkovo-51 strain and vaccine against listeriosis of farm animals from the AUF strain. The xymedon hydrochloride and polypeptide, B-activin (myelopid), were used as immunostimulating drugs. The immune status assessment of animals due to vaccines and immunostimulating agents was carried out by studying the dynamics of hematological, serological and biochemical blood value, phagocytic activity of leukocytes, bactericidal properties of blood serum according to conventional methods. The increase of protective effect when using an antirabic vaccine in combination with xymedon hydrochloride, which was accompanied by an increase in the number of T-helpers, T-lymphocytes and an increase in the titer of specific virus neutralizing antibodies, was established. The intensity of immune response in immunodeficient animal immunized against rabies twice in the combination of xymedon hydrochloride was higher than in animals immunized once with only one vaccine. The analysis of these rabbits blood tests showed that the double intramuscular mielopid administration and a single administration of xymedon hydrochloride in combination with the vaccine against listeriosis from the AUF strain causes a significant increase in the natural and immunological resistance of experimental animals. It is confirmed by the study results of clinical state, blood parameters and experimental infection of test animals. The generalized analysis of these experimental studies gives grounds to say about the prospects of the xymedon and myeloid (B-activin) hydrochloride use in order to stimulate the immunological process in organism of animals after vaccination against infectious diseases.

**Keywords:** immunostimulation, xymedon hydrochloride, myeloid, vaccination, rabies, listeriosis, laboratory animals.

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## INTRODUCTION

After scientific substantiation of L. Pasteur in the early 80s of the XIX century of the possibilities of specific infectious diseases prevention of animals and humans, the classical theories of cellular and humoral immunity were created, highly effective immune drugs that provide specific prevention of infectious diseases were obtained. However, it is often necessary to state the cases of mass reduction of natural and immunological resistance of humans and animals, caused by the defeat of the immune system and the mechanisms of non-specific body protection, which are in complex regulatory relationships. The contravention of the normal organism functioning principles can be caused genetically (primary immunodeficiency) or occur under the influence of various factors (secondary immune deficiencies) [1-4].

Currently, the development of immunological protection of people and animal organism goes in two directions: the search for highly immunogenic vaccines continues and expands, and the new section of immunology – regulation of immunological reactivity of an organism by means of nonspecific stimulation by medicines – immunostimulators and immunomodulators at the same time quickly develops [5,6].

The literature analysis to study the properties of various immunostimulatory drugs showed that among non-specific immunity stimulators, differing in origin, structure and mechanism of action, only a few ones were used in veterinary practice. This is due to the toxicity of several drugs, the presence of side effects, the lack of standard, as well as the insufficiency or inconsistency of the stimulating effect.

According to R. V. Petrov [7] the choice of immunostimulator for a particular infection should be targeted, and the primary assessment of immunotropy of the used agent should be based on its impact on the activity of macrophages, the absence of toxic effects on T- and B-cells and their impact on cooperation between these cells.

In the last decades derivatives of pyrimidine, purine, adjuvants of polypeptide nature and synthetic interferon inducers are increasingly used as means of increasing the body's resistance to infection. These drugs are low-toxic and have no side effects and at the same time have a polyvalent effect, being anti-inflammatory factors, stimulators of tissue regeneration, and T- and B-immunity systems. One of the mechanisms of increasing the body's resistance under the influence of pyrimidines is the stimulation of plasma cells transformation in the lymphoid organs. The immunostimulatory effect of peptides produced by bone marrow cells is justified by the effect on the T- and B- immunity system, which is most expressed in various immunodeficient states [8-11].

The aim of this work was to study immunostimulation in animal vaccination against listeriosis and rabies, which pose a real danger to humans and animals.

## MATERIALS AND METHODS

Guinea pigs (250.0-350.0) and rabbits of breed "Chinchilla" (2.5-3.0 kg) were used in the experiments. The experimental and control groups of animals were formed taking into account age, sex, live weight. The same conditions of feeding and maintenance corresponding to zootechnic norms were maintained.

A dry live vaccine against listeriosis of farm animals from the strain "AUF" and an antirabic inactivated dry culture vaccine from the strain "Schelkovo-51" were used according to instructions of their application to immunize the animals.

The xymedon hydrochloride, medicine of pyrimidine derivatives group, synthesized at A.E. Arbuzov institute of organic and physical chemistry (Kazan) and polypeptide, B-activin (myelopid), developed at the Institute of immunology of the Russian Ministry of Health were used as Immunostimulating drugs. Dry vaccines were suspended with a diluent provided by the manual, or a solution of xymedon hydrochloride. Myelopid (B-activin) was added directly to the vaccine suspension. Clinical observation of experimental animals was carried out within 14-180 days after vaccination and within 15-20 days after control infection with virulent strain of the listeriosis causative agent.

Morphological blood values of animals were determined by the hematological analyzer "Mythic-18" (France). The assessment of specific immune status was carried out by standard methods, including determination of the content of T- and B-blood lymphocytes in the reaction of E-POK with erythrocytes of sheep and white mouse, the number of T-helpers and T-suppressors in the reaction of E-POK with theophiline by the method of A. Shose. The functional-metabolic activity of neutrophils was evaluated in the reduction reaction of nitroblue tetrazolium (NBT test) in spontaneous and induced variants according to the V.N. Paketal method.

The artificially immunodeficient disease was created by intramuscular injection of an immunodepressant, cyclophosphan-LANS, at a dose of 50 mg in animals in the quantity  $1 \text{ cm}^3$  in order to study the immunostimulating activity of xymedon hydrochloride in vaccination against rabies. The rabies vaccine was administered to rabbits in combination with an immunostimulant twice for 0.5 doses throughout  $1 \text{ cm}^3$  apart 10 days, and an immunostimulator for 5 mg/kg in a volume of  $1 \text{ cm}^3$ .

To determine the immunostimulant activity of xymedon and mielopid hydrochloride after vaccination against listeriosis, they were also introduced in conjunction or combination with an appropriate vaccine. The immunity stress was tested by experimental infection of test animals with different lethal doses of the virulent pathogen of listeriosis according to the method developed by O. A. Kotylev.

The virulent strains of listeriosis causative agent intended for targeted use in the main experiments were refreshed before each experiment, the purity was determined, the culture-morphological properties were studied, and DLM was determined in animal analogues.

## RESULTS OF RESEARCH

12 rabbits were divided into 3 groups of 4 animals each to study the immune status in rabies vaccination. Rabbits of the 1st and 2nd groups were subjected to immune suppression. Four days later, the animals of the 1st group were vaccinated twice with an interval of 10 days at doses of  $1 \text{ cm}^3$  in combination with xymedon hydrochloride, and rabbits of the 2nd group were vaccinated without the stimulant in the same regime. The animals of the 3rd group were not subjected to immunocomplementation and vaccinated without stimulator once at a dose of  $2 \text{ cm}^3$ . Blood serum was examined in 3 days after immunosuppression, as well as 10 days after the first vaccination and 7, 14, 21 and 28 days after revaccination.

Serologic studies, enzyme-linked immunoassay, showed that rabbits of the 1st and 3rd groups have the highest antibody content for 28 days of the experiment (1:12800), and the antibody titer of the rabbits blood of the 2nd group did not exceed 1:3200.

At the end of 6 months after vaccination, the level of antibodies of the 1st group animals was recorded at a dilution of  $1:640 \pm 72.6$ , and the titer of the 3rd group rabbits is  $1:225 \pm 22.6$ , and the antibody titer of the 2nd group immunosuppressed rabbits did not exceed  $1:93.7 \pm 24.7$ , which indicates a weakening of humoral immunity. Consequently, the average serum antibody concentrations were significantly higher in animals immunized with xymedon hydrochloride.

The virus neutralizing activity of blood sera of rabbits immunized against rabies in combination with xymedon hydrochloride was also higher than in animals of the 3rd group in 2 times, and the 2nd group was higher in 2.7 times.

During the entire period of the immunosuppressive rabbits blood studies immunized with rabies vaccine in combination with xymedone hydrochloride, the lymphocyte count increased 1.4-fold; T-lymphocytes - 3.8; B-lymphocytes - 2.1; T-helpers - 7.9; T-suppressors in 24 times as compared with the same indicators in the blood of vaccinated with one vaccine rabbits who had not have immunosuppression.

The observed protective effect with the use of an antirabic vaccine in combination with xymedon hydrochloride was accompanied by an increase in the number of T-helpers, T-lymphocytes with cytotoxic suppressor activity and an increase in the titer of specific virus neutralizing antibodies. The intensity of the immune response in immunodeficient animals against rabies is doubled in combination with xymedone hydrochloride, and is higher than in animals immunized once with only one vaccine.

Therefore, to ensure the formation of an adequate immune response in the immunodeficient state of animals, it is advisable to use twice the rabies vaccination with an immunostimulant, in particular, xymedon hydrochloride.

The schemes and results of experiments on the immunostimulatory activity of myelopid and xymedon hydrochloride in vaccination of guinea-pigs against listeriosis are summarized in Table 1.

**Table 1: The immunostimulatory activity of myelopid and xymedon hydrochloride in vaccination of guinea-pigs against listeriosis**

Group number	Vaccination regimen	Method and timing of administration of xymedon hydrochloride	Mode of infection	Results of infection		
				infected	died	live
1	2	3	4	5	6	7
1	intraperitoneally at a dose of 10 billion m <sup>3</sup> once, according to the instructions for monitoring the activity of the vaccine	In combination with 5mg/kg vaccine once	Intraperitoneally, 21 days after vaccination 2 DLM 4 DLM 6 DLM	5 5 5	0 2 3	5 3 2
2	_*_	In combination with 10mg/kg vaccine once	2 DLM 4 DLM 6 DLM	5 5 5	0 0 1	5 5 4
3	_*_	In combination with 20mg/kg vaccine once	2 DLM 4 DLM 6 DLM	5 5 5	0 0 1	5 5 4
4	_*_	not administered	2 DLM 4 DLM	5 5	0 2	5 3
1	2	3	4	5	6	7
5	not vaccinated	Intramuscularly at a dose of 20 mg/kg, once	2 DLM	5	3	2
6	intraperitoneally at a dose of 10 billion m <sup>3</sup> once, according to the vaccine control instructions	Myelopid, intramuscularly once in combination with the vaccine 25 mg/kg	Intraperitoneally, 21 days after vaccination 2 DLM 4 DLM	5 5	0 3	5 2
7	_*_	Intramuscularly twice in 25 mg/kg I-concurrent with the vaccine II-3 days after vaccination	_*_ 2 DLM 4 DLM	5 5	1 2	4 3

8	-*_	Intramuscularly twice in 25 mg/kg	2 DLM	5	0	5
		I-3 days before vaccination	4 DLM	5	0	5
9	Not vaccinated	Intramuscularly twice in 25 mg/kg with an interval of 3 days	2 DLM	5	1	4
			4 DLM	5	3	2
10	Vaccinated in a dose of 10 billion m <sup>3</sup> according to the vaccine control instructions	not administered	2 DLM	5	0	5
			4 DLM	5	2	3
11	Not vaccinated	not administered	2 DLM	5	5	0

The results analysis showed that the use of xymedon and myelopide hydrochloride in combination with a vaccine against listeriosis causes the formation of strong immunity.

It should be noted that a single administration of myelopid at a dose of 25 mg/kg and xymedon hydrochloride at a dose of 5 mg/kg does not significantly affect immunity. With the double administration of myelopid before and on the day of vaccination at doses of 25 mg/kg, the resistance to infection of the test animals was recorded in 4 DLM of virulent listeria.

The most positive results were obtained with the combined use of xymedon hydrochloride and vaccine. The single administration of this compound in combination with vaccine at doses of 10 and 20 mg/kg caused 100% protection of animals in case of infection of 4 DLM virulent listeria.

At the same time, the vaccinated guinea pigs, when infected with 4 DLM of virulent listeria without the introduction of immunostimulants, survived only 60% of animals with loss of all intact guinea pigs after infection with 2 DLM pathogenic listeria.

Thus, it is experimentally found that a double intramuscular administration of myelopid and a single one of xymedon hydrochloride in combination with the vaccine against listeriosis of the AUF strain causes a significant increase in the natural and immunological resistance of test animals. The optimal immunostimulating doses of myelopid (B-activin) are 25 mg/kg when administered 3 days before and on the day of vaccination. The xymedon hydrochloride should be administered once in combination with the vaccine at doses of 10-20 mg/kg. At the same time, a dry vaccine should be suspended in a sterile solution of xymedon hydrochloride.

The scheme of experience, the results of blood tests and the control infection of rabbits in the comparative study of immunostimulating activity of xymedon and myelopid hydrochloride in vaccination against listeriosis are summarized in Table 2.

**Table 2: Comparative assessment of immunostimulating activity of xymedon and myelopide hydrochloride in vaccinating rabbits against listeriosis (the dose of the vaccine is 10 billion cubic meters once, intramuscularly)**

Group number	Number of animals in the group	Mode of application of immunostimulant	Terms of blood tests (days)	Titer of agglutinins	Number of leukocytes (ths.)	The phagocyte activity of leukocytes in %	Q-ty of T-lymphocytes	Q-ty Of B-lymphocytes	Method of infection	Result of infection		
										Infected	Died	Live
1	10	The xymedon hydrochloride 20 mg/kg in combination with the vaccine, intramuscularly	0	1:20	9.5	48	34	24	Intravenously, 3 DLM, 17 days after vaccination	4	0	4
			5	1:320	12.4	52	36	27				
			10	1:480	15.8	68	42	29				
			15	1:640	15.6	81	44	34				
2	10	Myelopid intramuscularly twice at doses of -15 mg/kg 3 days before vaccination  -15 mg/kg in combination with vaccine	0	1:20	8.9	38	29	17	-*-	4	0	4
			5	1:210	11.6	48	32	24				
			10	1:640	14.7	68	38	34				
			15	1:640	15.8	72	41	36				
3	10	Vaccinated without the stimulator	0	1:20	9.9	41	29	19	-*-	4	2	2
			5	1:120	12.1	45	32	26				
			10	1:240	14.0	51	28	21				
			15	1:480	13.2	62	32	17				
4	10	Not vaccinated, no stimulant was administered	0	1:20	9.2	41	29	19	-*-	4	4	0
			5	1:40	11.7	43	31	26				
			10	1:20	10.1	42	27	21				
			15	1:20	9.8	43	29	17				

The clinical observation and laboratory studies of rabbit blood analysis showed that the use of xymedon hydrochloride in combination with the vaccine against listeriosis causes an earlier and intensive accumulation of specific agglutinins in the blood serum of rabbits, a significant increase in the number of leukocytes and their phagocytic activity and the content of T and B lymphocytes. When using myelopid only for 10 days after vaccination a sharp increase in the titer of specific antibodies, the number of leukocytes and the relative level of B-lymphocytes compared to similar data in rabbits of the 3rd group vaccinated without immunostimulators noted.

The results of blood tests were correlated with the control infection data. The survival rate of rabbits, vaccinated with the combination of xymedon hydrochloride and in combination with mielopid after intravenous injection of a virulent listeria strain at a dose of 3 DLM was maximal, and without immunostimulants it was 50% at 100% death of unvaccinated animals.

### **CONCLUSION**

The obtained data make it possible to state that when xymedone hydrochloride was administered, the increase in the number of leukocytes, their phagocytic activity level and the content of T-lymphocytes in peripheral blood was more pronounced while the moderate accumulation of specific agglutinins was registered with the injection of myelopid, the ratio of T- and B-lymphocytes changed in the direction of increasing the last one.

The generalized analysis of the research results, the blood values and experimental infection of test animals gives grounds to state the prospects of using xymedon and myelopide hydrochloride (B-activin) in order to stimulate the immunological process of animals when vaccinating them against infectious diseases.

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