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CLINICAL AND EXPERIMENTAL SUBSTANTIATION OF THE USE OF MACROLIDE ANTIBIOTIC IN GASTROINTESTINAL AND RESPIRATORY DISEASES

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ABSTRACT

The overpopulation of the planet, especially the increased population density of large cities in developed countries, inevitably leads to the rapid spread of various diseases. Treatment of respiratory and gastrointestinal diseases is almost impossible without the use of antibiotics. Thus, various viruses develop resistance to the action of certain antibiotics. Today, one of the most pressing problems in the market of pharmacological drugs is the development and production of new types of antibiotics. This article discusses the properties of a new macrolide-type antibiotic novomycin. Macrolides are one of the most commonly used antibiotics in adults and children. Their action mechanism is a violation of protein production inside the microbial cell. In scientific work on the example of laboratory animals, studies of acute and chronic toxicity of the drug novomycin, allergic effects on the skin, and the effect of the drug on the course of pregnancy and fetal pathology were carried out. In addition, the antimicrobial activity of novomycin was analyzed. Antimicrobial activity studies have shown that the protection index of novomycin when used 3 hours before infection in white mice was 63% for bordetella infection; 44% for salmonella infection; 56% for pasteurellosis; 80% for staphylococcal infection. Conclusions are made about the effectiveness and safety of the drug.

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Introduction

The increased concentration of people in small areas contributes to a significant spread of gastrointestinal and respiratory diseases, a decrease in general nonspecific and specific resistance, and irrational therapy contributes to the emergence and spread of drug-resistant populations of pathogens [1]. All this ultimately contributes to significant morbidity of both the adult population and children. A decrease in the level of natural resistance and immunobiological reactivity, against which the conditionally pathogenic microflora manifests its effect, makes it difficult to carry out preventive measures for gastrointestinal and respiratory diseases [2, 3]. Moreover, most pathologies of the gastrointestinal and respiratory tracts: gastroenteritis, pneumonia, and with systemic pneumoenteritis, occur with the participation of not one, but several pathogens at the same time [4].

All this implies a significant use of preventive and therapeutic agents, cyclicity, and rotation in their application, the development of new treatment and prevention schemes for various diseases, as well as the creation on their basis of new resistant compounds with a potentiated, synergistic antimicrobial effect, addiction to which will become unlikely, and the therapeutic and preventive effect, is much higher [5, 6]. One of the main directions of creating new pharmacological agents is the design of complex drugs [7].

Macrolides are one of the most commonly used antibiotics in adults and children. The mechanism of their action is to disrupt the production of protein inside the microbial cell. This leads to a halt in their growth and reproduction. These agents have high activity against Staphylococcus, Streptococcus, and gonococcus, the causative agent of whooping cough, diphtheria, intracellular bacteria (legionella, chlamydia, mycoplasma), and other microbes [8].

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Antibiotics of this group are preferred for allergies to penicillins, cephalosporins, and other beta-lactam drugs. These drugs quickly penetrate inflamed tissues and are safe and well tolerated [9, 10].
Macrolides are used for respiratory tract infections, gastrointestinal tract, sexually transmitted diseases, and pelvic inflammatory processes:

- pneumonia caused by intracellular microbes (mycoplasmas, legionella, and Moraxella);
- mild forms of bronchitis, sore throats, otitis, sinusitis;
- streptodermia, erysipelas, mastitis;
- whooping cough;
- diphtheria;
- acne;
- chlamydia, syphilis, gonorrhea, etc.

Medications are used to prevent endocarditis and rheumatism, and are also included in the treatment regimen for gastritis, gastric ulcer, and duodenal ulcer [11].
This article discusses some of the properties of the new macrolide antibiotic Novomycin, as well as its effectiveness in respiratory and gastrointestinal diseases during laboratory studies on animals [12].
Novomycin is a semi-synthetic macrolide broad-spectrum antibiotic. The main active ingredient: is azithromycin dihydrate, dosage in terms of azithromycin 125.0 / 500.0 mg. Excipients: anhydrous calcium hydrophosphate, hypromellose, sodium lauryl sulfate, magnesium stearate, hypromellose, titanium dioxide, and polysorbate [13].

Materials and Methods

To characterize the clinical condition of the animals, body temperature was measured (rectally), pulse and respiratory rate, and the nature of nasal secretions and feces were determined. Before and after the use of drugs, nasal secretions were taken from 3-5 animals of each group to isolate and identify microorganisms [14].
To study the physiological and biochemical changes in the body of laboratory animals before stress exposure and after 1, 10, 15, 30, 35, and 60 days, blood was taken from blood vessels, in which the content of erythrocytes and leukocytes was examined on the Culter Count particle counter (France), hemoglobin - Sali hemometer and hemoglobin cyanide method, hematocrit on a spiral MPV-310 centrifuge (Poland), SOE - according to Panchenkov, leukogram - by counting 200 cells stained according to Romanovsky-Giemsa, with the calculation of the percentage of each species [15-17].
Antimicrobial activity of the drug against reference strains of mycoplasmas and acholeplasmas, as well as field cultures of Escherichia, Pasteurella, and Staphylococcus aureus, were studied on liquid and dense nutrient media using the 2,3,5 - triphenyltetrazolium chloride indicator with the determination of bactericidal and bacteriostatic effects of the drugs [18].
The assessment of the harmlessness of the drugs was carried out on mongrel white rats with an average body weight of 160-250 g. The test drug was administered in doses and terms three times higher than therapeutic ones [19].
The complex of physiological-biochemical and pharmaco-toxicological studies included the determination of acute and chronic toxicity, teratogenic, embryotoxic, allergenic, and irritant effects [20].
At the beginning, middle, and end of the experiments, the above morphological and immunobiochemical parameters were determined in blood and serum.
The pharmacokinetics of the studied drug was studied on laboratory animals (rats) with the determination of the active substance. The drug was prescribed for 10 days at a therapeutic dose. After 3, 6, 12, 24, and 48 hours after stopping its administration, animals were slaughtered and samples of internal organs (liver, kidney, spleen, lungs, heart, stomach, small and large intestines), tissues (skin, muscle, blood), stomach contents and colon were taken. As a control, homogenates of the same organs from animals that did not receive drugs were used [21].
The resulting digital material was subjected to mathematical processing using methods of mathematical statistics adopted in biology and medicine and the Microsoft Excel application software package.

Results and Discussion

Antimicrobial Activity of the Macrolide Antibiotic Novomycin
Studies of antimicrobial activity in vivo have shown **(Table 1)** that the protection index of novomycin, when used 3 hours before infection in white mice, was 63% for bordetella infection; 44% for salmonella infection; 56% for pasteurellosis; 80% for staphylococcal infection. With a single administration of drugs simultaneously with infection, it was lower and amounted to 50% with bordetellosis; 22% with pasteurellosis; 50% with staphylococcal, and 44% with salmonella infections. The protection index decreased with a single use of drugs 3 hours after infection with bordetellosis infection to 13%, pasteurellosis 0%; staphylococcal 20%; and salmonella up to 11%. The administration of drugs simultaneously with infection and 7 hours after. Subsequent administration twice daily for 7 days provided a protection index for bordetella infection of 33%; staphylococcal 40%; and salmonella 44%. The original cultures were re-isolated from the fallen animals from the blood of the heart, liver, kidneys, and spleen.

Thus, the use of the macrolide antibiotic Novomycin 3 hours before infection provides the highest effect in the studied infections. With a single administration of the drug simultaneously with infection and 3 hours after it, it decreases, and the administration of drugs simultaneously and 7 hours after infection and its subsequent administration for 7 days provides a relatively high protection index.

Toxicological Properties of the Macrolide Antibiotic Novomycin

Acute Toxicity

In the first experiment to study the acute toxicity of novomycin, 40 mongrel white rats weighing 210-250 g were used, divided into four equal groups. Animals of groups 1-3 were orally administered the drug in the form of a 35 percent aqueous suspension in an amount of 6 ml. Their dose for white rats was the maximum possible for administration and was equal to 9-10 g. Group 4 rats served as a control. The animals were monitored for seven days, paying attention to their general condition and appetite. In the second and third experiments, novomycin was administered subcutaneously in the form of aqueous solutions in doses from 1200 to 3200 mg/kg of body weight. Similar animals were controlled, which were injected with distilled water in the maximum volumes for administration. Daily clinical observations were conducted for a week, taking into account the picture of intoxication, the death of animals, and the results of the pathoanatomical autopsy of the deceased. The results of the conducted studies are presented in **Figure 1**.

Table 1. Antimicrobial activity of novomycin

№	Schedule of drug administration	Died, individuals	Survived, individuals	Protection index
	Bordetella infection			
1	Control	8	2	
2	Infection 3 hours after administration of the drug	3	7	63%
3	Infection simultaneously with the administration of the drug	4	6	50%
4	Administration of the drug 3 hours after infection	7	3	13%
5	Infection and administration of the drug twice a day	5	5	38%
	Pasteurellosis infection			
1	Control	9	1	
2	Infection 3 hours after administration of the drug	5	5	44%
3	Infection simultaneously with the administration of the drug	7	3	22%
4	Administration of the drug 3 hours after infection	9	1	0
5	Infection and administration of the drug twice a day	6	4	33%
	Salmonella infection			
1	Control	9	1	
2	Infection 3 hours after administration of the drug	4	6	56%
3	Infection simultaneously with the administration of the drug	5	5	44%
4	Administration of the drug 3 hours after infection	8	2	11%
5	Infection and administration of the drug twice a day	5	5	44%
	Staphylococcal infection			
1	Control	10	0	
2	Infection 3 hours after administration of the drug	2	8	80%
3	Infection simultaneously with the administration of the drug	5	5	50%
4	Administration of the drug 3 hours after infection	8	2	20%
5	Infection and administration of the drug twice a day	6	4	40%

The pattern of intoxication in all experiments was approximately identical and was characterized by depression after the introduction of small doses, the animals gathered in a heap and huddled in a corner for 2-6 hours. His breathing was rapid and shallow. Tactile sensitivity increased, and reflexes were preserved. After the specified time, the condition of the animals was normalized, however, the appetite was reduced during the day.

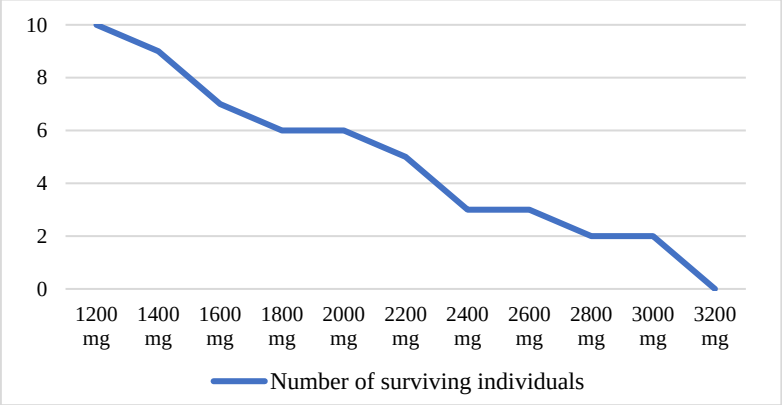

Figure 1. Results of an experiment on the study of acute toxicity of novomycin with subcutaneous administration

Chronic Toxicity

The scheme of experiments to study the chronic toxicity of the macrolide antibiotic novomycin provided for the use of 5 rats in the experimental and control groups. The test method provided for a long-term, for one month, feeding of three-fold increased therapeutic doses of drugs, accompanied by clinical studies, body weight control, and blood collection on the 1st, 15th, and 30th days of the experiment.

The conducted studies have established that novomycin does not have a negative effect on animals. When they were opened, erosions and ulcers on the mucous membranes of various parts of the gastrointestinal tract and other pathoanatomic changes were not detected.

When studying the effect of drugs on the growth dynamics of animals, it was found that their prolonged use contributed to an increase in body weight on the 15th and 30th days of the experiment **(Table 2)**.

Table 2. The effect of long-term administration of three-fold increased therapeutic doses of drugs on the dynamics of animal body weight

Name of groups	Average daily body weight gain, g			
	After 15 days		After 30 days	
	Gram	%	Gram	%
Control	2.3	100	2.4	100
Experience	3.2	139	3.9	162

Study of the Embryotoxic and Teratogenic Effects of the Macrolide Antibiotic Novomycin

Experiments to study the effect of novomycin on the embryonic development and generative function of the ovaries of animals were carried out on 48 mature white female rats, weighing 200-250 g **(Table 3)**. In experimental groups from the day of planting to the females of males (in the ratio of 1:1), novomycin was administered daily with feed in doses of 0.06 g / kg of body weight. No drugs were used in the control group. On the 17th-20th day of pregnancy, half of the females were killed and morphological examination of internal organs and fetuses was carried out according to the protocol described by Lyashenko *et al*. (2023) [22].

The conducted studies have not revealed the toxic effect of novomycin on the course of pregnancy. The number of yellow bodies in the ovaries as well as the number of implanted embryos in the rats of the experimental group was not less than in the control. Preimplantation death in the experimental groups did not exceed a similar indicator in the control. Fetal death was not recorded throughout the experiment, and the number of resorbed embryos in the experimental groups differed little from the control.

The results of the study of the morphology of the external and internal organs of rat fetuses indicate the absence of teratogenic and embryotoxic effects of the studied drugs. The average weight of embryos and baby rats in the experimental groups was slightly higher than in the control.

Thus, the administration of novomycin does not adversely affect the embryonic and postnatal development of fetuses. The drugs promote better growth of fetuses, as well as reduce their pre- and post-implantation death.

Table 3. Results of the study of the teratogenic effect of the macrolide antibiotic novomycin

Indicators	Experience	Control
Intrauterine period:		
The number of yellow bodies in the ovary	13.3	12.3
Number of implantation sites in the uterus	13.1	11.7
Percentage of preimplantation death	2.23	4.9
Number of resorbed embryos (per female)	0.3	0.3

The number of dead fetuses (per 1 female)	-	-
Embryo body weight (g)	5.7	5.6
Abnormalities of the development of internal organs and skeleton	-	-
Lactation period:		
Number of live baby rats (per 1 female)	12.3	12.3
Average body weight of 1 baby rat (g)	32.5	30.2
Deformities and anomalies of the skeleton and internal organs	-	-

Skin-Resorptive, Allergenic, and Irritating Properties of the Macrolide Antibiotic Novomycin

The study of the skin-resorptive and irritating effects of drugs was carried out by single and multiple applications: in rats – by immersing the tail in a solution of the studied drugs as described by Blino *et al*. (2022) [23]. The conducted studies did not reveal the skin-resorptive and irritating effects of novomycin.

The study of the allergenic properties of the drug was carried out by the method of epicutaneous applications and detection of skin reactions (redness, itching, swelling of the skin). The conducted studies have not revealed the allergenic properties of the drug novomycin.

Thus, the drug novomycin is a low-toxic agent, belongs to the 4th class of toxicity, and does not have a skin-resorptive and allergenic effect.

The Effect of Toxic Doses of the Macrolide Antibiotic Novomycin on the Functions of the Digestive Organs

When prescribing toxic doses of the macrolide antibiotic novomycin (30 mg/kg), the feces of experimental and control animals were the same in quantity, color, odor, shape, consistency, surface characteristics, absence of impurities, and the corresponding type and age of digestibility. The stool reaction was neutral, and blood and bile pigments were absent. Single fat droplets and starch grains were found in the feces of all animals. In animals treated with the drugs, a slight increase in the protein content in feces was noted compared to the control. The same trends were declared in other works [24-28].

The Effect of Novomycin on the Biochemical Parameters of the Blood of Laboratory Animals

When studying the long-term effect (for 30 days) of toxic doses of novomycin (600 mg per kg of body weight) on blood parameters of clinically healthy rats, it was found that under its influence there is an increase in the content of total protein and urea, which indicates the activation of protein metabolism **(Table 4)**. The results obtained correspond to the results of another research [29].

Table 4. Effect of long-term use of novomycin on biochemical parameters of rat blood

Indicators	Background	Day 1	Day 15	Day 30
Gamma Globulin(%)	42.18+4.79	39.33+7.88	29.67+3.77	50.17+4.11
Total protein (g%)	6.67+0.31	7.38+0.28	7.58+0.41**	7.17+2.06
Urea (mg%)	27.36+2.2	37.21+4.45*	32.39+5.14	30.29+3.7
RNA (mg%)	2.80+0.48*	3.09+0	3.00+0.38*	3.37+0.37
DNA (mg%)	0.67+0.05	0.48+0.04	0.58+0.11	0.78+0.04
Total lipids (mg%)	718+20.55	573+82.19	500+34.24	632+20.55
Lipoproteins (mg%)	55+3.08	39.6+4.11*	41.6+0.34	46.0+1.3
Cholesterol (mg%)	98+3.42	93.3+16.44	68.0+8.22	80+2.74
Glucose (mg%)	327.5+9.59	372.5+51.36	314.1+51.36	270+65.06

The Effect of Drugs on the Structure of Organs and Tissues of Animals

The experiments were carried out on mongrel white rats (with an average body weight of 125-250 g). The effect on the organ structure of the macrolide antibiotic novomycin was studied at doses three times higher than therapeutic and compared with the control that did not receive the drugs. On the 1st, 15th, and 30th days of the experiments, blood was taken from animals for morphological studies. At the end of the experiment, rats were killed for histological studies. The internal organs and the laboratory animals themselves were weighed. The results of weighing the internal organs of white rats are presented in **Table 5**.

Table 5. Effect of long-term use of the macrolide antibiotic novomycin on the mass of the internal organs of white rats

Name of the group	№	Dose, mg/kg	Mass of organs, mg				
			Heart	Lungs	Liver	Kidneys	Spleen
Control	1	-	2.11	3.43	7.56	1.65	2.55
	2	-	2.01	3.39	7.52	1.61	2.66
	3	-	1.71	3.12	7.48	1.76	2.12

Experiment	4	100	2.03	2.21	7.59	1.87	1.87
	5	100	2.02	2.33	7.92	1.83	2.02
	6	100	1.93	2.33	8.15	1.53	2.02

Table 5 shows that in white rats, under the influence of novomycin, there was a tendency to increase the mass of the liver, kidneys, and heart.

During autopsy and histo-examination in the organs and tissues of all experimental animals, no differences were found in the morpho-functional state of all components of the mucous membrane and other membranes of the gastrointestinal tract, specific and connective tissue structures of parenchymal organs - liver, kidneys, adrenal glands, spleen, mesenteric lymph nodes, lungs [30-33].

Macroscopic examination revealed no changes in the anatomy and topography of the internal organs of all experimental animals. Pathomorphological studies were also carried out on animals used in the study of chronic toxicity and killed immediately after the prescription of the drug. In all animals, the epithelium of the gastrointestinal tract and renal tubules is mostly preserved. The general structure, as well as the condition of the blood and lymphatic vessels of the above-mentioned organs, corresponds to the normal morphofunctional status. Thus, the conducted studies have not established differences in the anatomy of animal organs of control and experimental groups.

Conclusion

This article discussed some of the properties of the new macrolide antibiotic novomycin, as well as its effectiveness in respiratory and gastrointestinal diseases during laboratory studies on animals. Studies of antimicrobial activity in vivo have shown that the protection index of novomycin, when used 3 hours before infection in white mice, was 63% for bordetella infection; 44% for salmonella infection; 56% for pasteurellosis; 80% for staphylococcal infection. Thus, the use of the macrolide antibiotic novomycin 3 hours before infection provides the highest effect in the studied infections.

The conducted studies have not revealed the toxic effect of novomycin on the course of pregnancy. The number of yellow bodies in the ovaries as well as the number of implanted embryos in the rats of the experimental group was not less than in the control. Preimplantation death in the experimental groups did not exceed a similar indicator in the control. Fetal death was not recorded throughout the experiment, and the number of resorbed embryos in the experimental groups differed little from the control. The conducted studies did not reveal the skin-resorptive and irritating effects of novomycin. Thus, the drug novomycin is a low-toxic agent, belongs to the 4th class of toxicity, and does not have a skin-resorptive and allergenic effect. Moreover, the conducted studies have not established differences in the anatomy of animal organs of control and experimental groups.

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Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

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