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The Impact Study of Poline-Rich Polypeptide Galarmin D-15 Derivative on the Streptococci

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ABSTRACT

The antibody titer of the blood serum in the experimental animals treated with galarmin d-15 derivative of proline-rich polypeptide combined with inactivated streptococci has been evaluated according to the serological (precipitation) reaction. The investigated blood sera have been diluted in the physiological solution with the ratio of 1:5-1:20. It is worth mentioning that as a result of interaction with the antigen, blood sera, treated with galarmin derivative and inactivated streptococci, have generated precipitate in case of 1:5-1:20 dilution ratio, while in the control variant it has been marked in case of 1:5-1:10 dilution ratio. As a result of numerous experiments it has been found out that the galarmin derivatives are modulators for the activation of humoral and cellular immunities.

Introduction

Preventive measures taken against the contagious diseases of farm animals and socio- economic policy developed within the framework of combating such diseases have become urgent issues. The abovementioned is particularly true for streptococcal disease, since it is common for humans and animals.

Streptococci are widely spread in our environment, in the soil, as well as in the gastrointestinal tract of the sick and healthy animals. All mammals, birds and men are susceptible to the mentioned pathogenic bacterium (Grigoryan, 2002, Bessarabov, Vatutin, Voronin, 2007). Due to the stability and viability of the pathogens, they survive in the environment for 3-4 months, while in the purulence they maintain for 6 months.

Streptococci (chain of cocci) are found with great amounts in the internal organs of the animals stricken with streptostaphylococcosis, in the lungs of the animals sick with infectious and contagious pleuropneumonia, in the mammary glands of the mother animals infected with mastitis, in the lymphatic glands of the horses stricken with infectious lymphadenitis, in the muscular tissue, internal organs and blood of the animals infected with

foot rot (Paronychia contagiosa) and cause purulent foci.

The dairy and meat products, as well as internal organs and intestines of the animals afflicted with the aforementioned pathogens pose serious danger for humans.

The identification of the animals infected with streptococcus is implemented by means of serological (precipitation) reaction.

To promote the immunoreactivity in the organisms of the experimental animals there was a need to use the proline-rich polypeptide (PRP) galarmin secreted by the hypothalamus cells and its derivatives (Ala-Gly-ALg-Pro-Glu-Pro-Aala-Glu-Pro-Ala-Gln-Pro-Gly-Val-Tyt) (Galoyan, Aprikyan, 2003, Badalyan, Sargsyan, Galoyan, 2011).

It is noteworthy that PRP has been synthesized by the academician Galoyan and his co-workers in the Laboratory of Biochemistry, Neuroimmunology, Neurohormones and Analytical Chromatography after H. Buniatyan, NAS RA. It is a modulator for both humoral and cellular immunity, also for the differentiation of myelopoiesis and thymocytes as well as for the macrophage activation, as a result of which a great amount of protective antibodies are synthesized preventing the proliferation of the pathogenic bacteria penetrated into the organism (Galoyan, 1997, 2004, 2008, 2011, 2012). So, to increase the immunity in the animals' organism, the amount of immunoglobulins and cytokines, as well as the protective property of the blood serum, different stimulants have been used (Akiyama, Ishida, et. al, 1987, Huang, Nasr, et. al., 1992, Sargsyan, Galoyan, et. al, 2013, Sargsyan, Grigoryan, Galoyan, 2012, Sargsyan, Grigoryan, 2014, Sargsyan, 2015).

The aim of the current research is to study the immuno-stimulating effect of the derivative of PRP galarmin d-15 on the antibody titer of the blood serum in the experimental animals immunized with anti-streptococcal vaccine (Sargsyan, Grigoryan, 2015).

Materials and methods

The experimental works have been implemented at the ANAU research center of "Veterinary Medicine and Sanitary Expert Examination" and at the laboratory of "Biomedical Researches after H. Buniatyan".

For streptococci isolation the internal organs (spleen, liver, cordis, bone marrow), knee intra-articular fluid of two-month-old calves in four animal stocks died from streptococcosis and the milk of the cattle infected with mastitis have been sampled.

To determine the disease reproduction, virulence of the pathogen and the antibody titer of the serum, 36 stocks of 12-15-day-old mice with 15 g-18 g live weight and 18 guinea pigs at the age of 4-5 months with 400 g-500 g live weight have been selected.

To derive the microbial streptococcal (chain of cocci) isolates, glucose, blood, blood serum containing mediums (MPA and MPB) and the Barker mediums (Antonov, Borisova, Volkova, 1986, Nurazyan, 1988, Danielyan, 2002) have been used.

The morphological and antigenic characteristics of the streptococci have been determined in laboratory conditions based on the results of microscopic and micro-biological trials (Antonov, Borisova, Volkova, 1986, Nurazyan, 1988).

The amount of the antigen (streptococcus) has been determined through the bacteriological standard.The chain coccus of anti-streptococcosis vaccine has been inactivated with 0.3 % formaldehyde containing formalin solution. Inactivated glycerine anti-streptococcosis vaccine has been developed from the virulent endemic strains of the disease pathogen.

The vaccine safety has been tested on the experimental animals (mouse), while its purity – in an artificial nutritious medium.

The quantitative and qualitative changes of the formed blood elements have been examined through the generally acknowledged methods (Vasileva, 1982) and by means of immunoassay and hematological analyzers of the series *Star Fax 22400, Elecsys 2010 Roche (German) and Sysmex Xs-100i (Japan).

The lethal doses (LD50) of the streptococci have been identified on the 18 stocks of laboratory white mice with 15 g-18 g live weight, while the poison effect has been enhanced upon the decomposition of erythrocytes (Syurin, Belousova, Fomina, 1984).

The antigen (precipitogen) for the serological concentration determination has been produced from the tested streptococci, while the blood serum of the guinea pig (produced throughout the experimental activities) overimmunized with the appropriate antigen has been used as an antibody (precipitating serum) (Syurin, Belousova, Fomina, 1984, Nurazyan, 1988).

To promote the activity of anti-streptococcus serum the guinea pigs were intramuscularly injected with PRP galarmin d-15 derivative (25 μg per an animal) at the knee part (Figure 1).

Figure 1. Galarmin d-15 derivative.

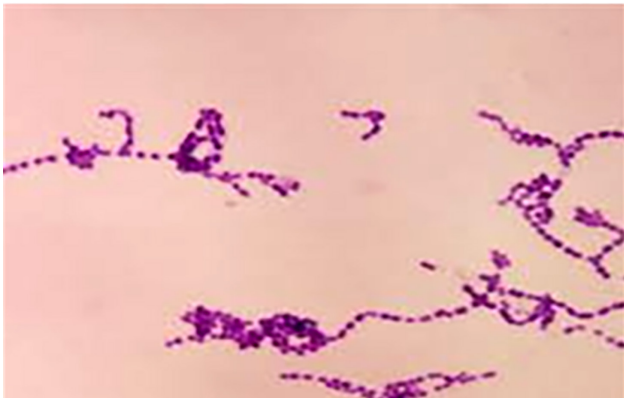

Figure 3. Streptococcus.

The antibody titer of anti-streptococcal serum was determined on the 7th -14th days of post infection period (Nurazyan, 1988). The investigated blood sera were diluted through physiological solution with the ratio of 1:5-1:40.

The effect of galarmin d-15 derivative and antibiotics on the streptococci was determined in vitro (Durgaryan, Matevosyan, Seferyan, Sargsyan, Grigoryan, Galoyan, 2011).

Results and discussions

As a result of microscopic investigations in the tissue smears received from the pathological materials and bacterial isolates, chain cocci with the size of 0.5-1.0 µm have been detected (Vorobyova, Bykova, 2003), while on the blood agar hemolytic zone round the bacterial colonies has been observed and in Barker's medium black colonies have been marked (Figures 2, 3).

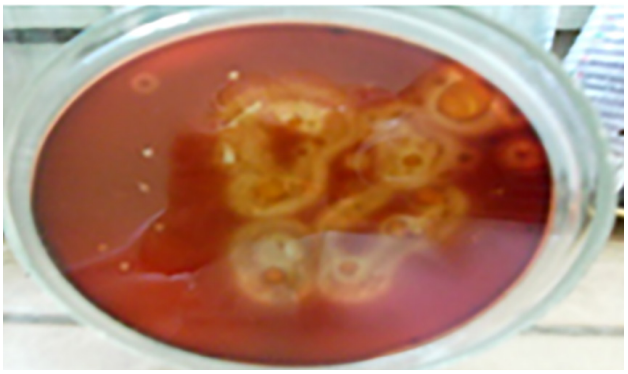

Figure 2. Blood agar (haemolysis).

Streptococci peculiar to the classical form have been detected in the smears produced from the mentioned colonies. The pathogenicity of the mentioned pathogen and the immune-stimulating activation of the galarmin d-15 derivative have been tested and determined in the white mice with 15 g-18 g live weight. The experimented mice were proportionally divided into three groups with 6 mice per a group.

The mice of the first group were injected with streptococcus (20-30 mln/mL), those of the second group were injected with galarmin d-15 derivative (10 µg /animal) combined with streptococcus (20-30 mln/mL) and the mice of the third group (control) were injected with physiological solution (1mL/animal) intramuscularly (Durgaryan, Matevosyan, Sferyan, Sargsyan, Grigoryan, Galoyan, 2011).

During the five-day post infectious period the mortality of the mice infected with 24 h. bacterial isolate made 83.3-91.6 %, while when treated with galarmin d-15 derivative combined with streptococci it made only 15-25 % (Figures 4, 5).

Taking into account the research results it should be mentioned that no morphological changes (size, concentration, colour) have been observed in the internal organs of the mice treated with the derivative of galarmin d-15 and streptococci. While the internal organs of the mice of the first and third groups have been subjected to modifications, and in the tissue smears produced from these organs, recruitments of the chain cocci have been recorded.

The results obtained during the research period testify that the galarmin d-15 derivative is endowed with immunoreactivity, which has inhibited the bacterial growth in the body of the mice infected with streptococci.

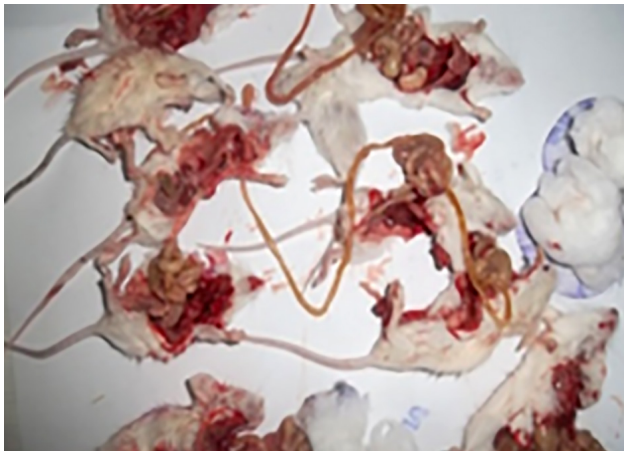

Figure 4. Mice infected with streptococcosis.

Figure 5. Galarmin d-15+inactivated streptococci.

Thus, the main objective of our research is to promote the activity of immunocompetent cells of the experimental animals treated with anti-streptococcal vaccine through the galarmin d-15 derivative which predetermines the amount of the synthesized antibodies and their immunogen activities.

So, 18 guinea pigs with 400 g-500 g live weight have been selected for our investigations. The guinea pigs have been similarly divided into three groups with 6 animals per a group. To enhance the vaccine activation the galarmin d-15 has been administered twice intramuscularly with 48 hours' interval.

The guinea pigs of the first group were injected with galarmin d-15 (25 µg /animal) combined with inactivated streptococci (2 bln/animal), the animals of the second group were treated with inactivated streptococci (2 bln/animal) and the pigs of the third group were injected with 0.9 % physiological solution (2 mL/animal) intramuscularly.

The immunity of the animals of the first and second groups was determined through the reinfection of the bacterial isolate with viable streptococci. Hence, the viability of the experimental animals treated with the vaccine and galarmin made 83.3 %, while that of the animals treated only with vaccine was 66.6 %.

The activity of the anti-bacterial serum, as well as the quantitative and qualitative changes of the formed blood elements have been evaluated on the 7th and 14th post-infection days (p. i.).

During the investigation considerable quantitative increase in IgG, lymphocytes, polymorphonuclear leukocytes and monocytes has been observed, which have exceeded the same index data in the animals of second (vaccinated with inactivated streptococci) and third (intact) groups in 2.2 times. From the diluted (1:5-1:40) blood serum of the animals treated with galarmin derivative and anti-streptococcal vaccine 0.3-0.5 ml mass was poured out into test-tubes and the same amount of hydrochloric acid antigen produced from the streptococci has been added in the tube.

As a result, the precipitate (grey-white cloud) of the blood serum in the animals of the first group was marked in case of 1:5, 1:10, 1:20 dilution, that of in the second group was fixed in case of 1:5 and 1:10 dilution, while in the third group it was excluded at all (Syurin, Belousova, et.al, 1984, Nurazyan, 1988, Sargsyan, 2015). It is noteworthy that the antibody titer of the blood serum in the animals treated with galarmin d-15 derivative was higher than the blood serum indices in the animals of other groups.

Conclusion

In the result of investigations it has been found out that galarmin d-15 derivative has increased the reactivity of the organisms in the experimental animals and a great amount of anti-streptococcal specific antibodies have been synthesized, which have been evaluated by means of serological reaction.

The timely detection of the disease pathogen and prevention of the streptococcosis propagation is a primary objective in the livestock and veterinary medicine sector.

The galarmin d-15 derivative has demonstrated bacteriostatic and the antibiotics have shown bactericidal characteristics In vitro.

Galarmin d-15 can be applied parallel to vaccination, which will increase the reactivity of the organism. The synthesized specific antibodies endowed with high antigenic properties will be used for diagnostic, therapeutic and preventive purposes.

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