Conference Paper

Assessment of the Impact of Betulin on the Immune Status of Cows with Leukemia-Associated Infection

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Abstract

This study examined the immunity dynamics of bovine brucellosis-complicated leukemia, as well as the efficacy of treatment with betulin. Blood serum samples were taken from cows with specific brucellosis antibodies and bovine leukemia virus antibodies based on the results of complex diagnostic studies. Animals (n = 5) selected based on serological studies were injected subcutaneously with betulin-PEG three times with an interval of 7 days at a dose of 10 mg/kg. Blood was taken for hematological and immunological studies before the injection, and after the first, second and third injections. It was found that leukemia and brucellosis infections were accompanied by a significant increase in the number of leukocytes and lymphoid cells, as well as strong fluctuations in the leukocyte functional and metabolic activity parameters (from insignificant in some individual animals to significantly increased in others). Immunological restructuring of the body after triplicate administration of betulin-PEG was characterized by the restoration of the number of lymphoid cells to the level of clinically healthy animals, as well as a significant increase in the functional and metabolic activity of the neutrophilic granulocytes, especially their oxygen-dependent systems. Based on the results, the product is recommended as an effective means to restrain the leukemia process that is complicated with associated infection, as well as to use for seronegative animals in order to increase nonspecific resistance to BLV infection.

Keywords: leukemia, brucellosis, cattle, immune status, betulin, immunocorrection, associated infection

1. Introduction

Bovine leukemia virus (BLV) infection is widespread in the dairy industry in many parts of the world, especially where most producers do not actively control or reduce the spread of infection in their herds. However, this disease will cost the dairy industry significant financial losses associated primarily with a decrease in milk production and fat content in milk in cases [1-3].

OPEN ACCESS

How to cite this article: Vasily Vlasenko, Valentina Pleshakova, Sayat Bayseitov, and Nadezhda Lescheva, (2021), "Assessment of the Impact of Betulin on the Immune Status of Cows with Leukemia-Associated Infection" in *International research conference on Challenges and Advances in Farming, Food Manufacturing, Agricultural Research and Education*, KnE Life Sciences, pages 693–700. DOI 10.18502/kls.v0i0.9006

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Numerous studies confirm the opinion that the leukemia process is developed against the background of deep defects in the immune mechanisms, while the virus affects the cells of both the innate and adaptive immune systems and disrupts the normal functions of uninfected cells [4-6]. Besides, BLV carriers develop a higher susceptibility to various taxonomic affiliation pathogens (viruses, bacteria, mycoplasmas, endo- and ectoparasites). Associative microparacytocenoses usually have severe outcomes, and it should be noted that researchers do not take into account not only the synergistic effect of individual pathogens but also the antagonistic interaction of the parasitocenosis co-members between themselves resulting ultimately in a significant effect on the macroorganism's natural resistance parameters [7].

The increased immunobiological and protective properties of the animals' body with immune disorders make it possible to restore the immune system activity to the physiological normal state helping to reduce the infection risk, and in cases of serological confirmation of the disease, to prevent the leukemia transition from the asymptomatic stage to the hematological one. On the basis thereof, the search and implementation of effective and accessible biologically active substances increasing the immunological resistance and productive qualities into the leukemia control system is one of the urgent problems [8].

Under the results of various scientists' scientific studies, the animals' increased resistance to BLV infection and productive health is possible with the help of a number of immunomodulating products and biologically active substances, including with the help of their combined use [9, 10].

Lupane triterpenoids, i.e. betulin, betulonic and betulinic acids and their derivatives, are very promising objects to develop products to treat and prevent bovine leukemia. Some researchers proved that betulin has a wide biological activity spectrum (anti-inflammatory, antiseptic, antioxidant, antiviral, and other ones, including anti-HIV and antitumor effects) [11-13]. Besides, the effectiveness and feasibility of betulin products to correct the immune status parameters of the BLV infected cattle are noted [14].

The questions researched by us in this study:

- features of changes in the immune system state in cattle with leukemia-associated infection;

- assessment of the immune system activity state after the use of an immunobiological product to treat the cattle infected with BLV complicated with brucellosis infection.

The research objective is to study the effect of betulin on the natural resistance state in cows with leukemia-associated infection.

2. Methods and Equipment

The research material was the blood and blood serum of red steppe and Holstein-Friesian cows from the agricultural formation of the North Kazakhstan region in the Republic of Kazakhstan.

The availability of BLV antibodies in blood serum was determined using an immunodiffusion reaction in agar gel - IDR (produced by federal State-Owned Enterprise Kursk Biofabrika - BioK, Russia), enzyme immunoassay - ELISA (produced by ID Vet, France) and an indirect immunofluorescence reaction - IIF. The rose bengal test (RBT), agglutination assay (AA) and complement-fixation test (CFT) with a single antigen (produced by Research and Production Enterprise Antigen, Kazakhstan), as well as ELISA (produced by AniGen, Korea) and IIF were used to detect antibodies to the causative agent of brucellosis. All reactions were performed under the instructions for use of diagnostic kits to detect the specific antibodies in blood serum.

The reaction results were registered and interpreted with the help of an ELISA analyzer Multiscan FX (Thermo Scientific).

10 cows were sampled to test the product; five of them had brucellosis antibodies in the blood serum besides BLV antibodies. The remained 5 clinically healthy cows were used as controls.

The experimental group animals received subcutaneous injection of betulin-PEG, 10 mg/kg three times with an interval of 7 days. Blood samples were taken from individual animals of the experimental and control groups to assess the immune status before the injection, on the 7th, 14th and 21st days after the inoculation of the product.

The cellular link of immunity was studied with the methods of spontaneous, complementary and globulin rosette formation under the guidelines to assess the T- and B-systems of immunity in cattle [15]. The state of the phagocytosis parameters was determined based on the study of the oxygen-producing activity of neutrophilic granulocytes in the nitro blue tetrazolium reduction test (NBT-test) in spontaneous and induced variants photometrically. The functional reserve of neutrophils was calculated as the ratio of the stimulated NBT variant to the spontaneous one to characterize the NBT test further [16]. The oxygen-independent bactericidal system was also assessed with the help of the cationic protein amount using the cytochemical method followed by calculation under the standard method of the average cytochemical coefficient (ACC) [17]. The CIC level in the blood serum was determined under the standard method using polyethylene glycol to assess the humoral link. Leukocytes and leukograms were counted using generally accepted methods.

The results obtained were statistically processed by determining the arithmetic mean (M) and calculating the arithmetic average error (m). The significance of the differences in the results obtained was assessed with the help of the Student's t-test. The results were considered reliable at P≤0.05

3. Results and Discussion

When the immune status was studied before the administration of the product (Table 1), it was found out that the experimental group cows had an increase in the number of leukocytes by 39% and lymphocytes by 61% (p <0.05) compared to the values in the control group animals. The concentration of T-, B- and cytotoxic T-lymphocytes was also increased as a result of these changes but did not reach a significant difference.

TABLE 1: Immunological parameters of the bovine blood before product administration

Index	Group	
	Control	**Experimental**
Leukocytes, thousand / μl	6.88±0.23	9.56±1.11[a]
Lymphocytes, thousand / μl	4.23±0.30	6.83±1.04[a]
T-lymphocytes, thousand / μl	0.66±0.09	0.82±0.14
B-lymphocytes, thousand / μl	1.00±0.02	1.59±0.30
Cytotoxic T-lymphocytes, thousand / μl	0.86±0.04	1.55±0.32
NBT-test, spontaneous, optical density (OD) units	0.24±0.02	0.37±0.09
NBT-test, stimulated, optical density (OD) units	0.24±0.03	0.33±0.09
Functional reserve of neutrophils	0.99±0.06	1.12±0.33
Lysosomalcationicproteins (LCP)	1.50±0.06	2.05±0.30
CIC, C.U.	78.4±4.59	67.4±11.82

ap<0.05.

Changes in the functional and metabolic activity of leukocytes in cows with leukemia-associated infection were accompanied with strong fluctuations, i.e. from insignificant ones in some individual animals to significantly increased parameters in others. It is for this reason that the average group values did not have significant differences as compared with those of the control group despite their increase. For example, the spontaneous NBT-activity level in the experimental group varied from 0.19 to 0.73 units OD, and the average values were 0.37 ± 0.09 versus 0.24 ± 0.02 units OD in control one, i.e. were increased 1.54 times. The same trend was observed when other parameters of the functional activity of neutrophils were analyzed.

The circulating immune complex (CIC) concentration had some tendency to decrease, but the experimental group was characterized with a wide range of individual values of this parameter as it was in the cases described above.

Analysis of the immune system parameters after the first injection of the product also showed significant differences in the number of leukocytes and lymphocytes but the concentration of lymphocytes, on the contrary, decreased to 3.23 ± 0.12 versus 4.27 ± 0.38 thous/µl ($p < 0,05$) in the control group as opposed to the previous study. As a result of the transformation of lymphocytes observed, there was also a decrease in the number of B-lymphocytes and especially T-lymphocytes with the concentration in the experimental group 0.36 ± 0.06 versus 0.65 ± 0.06 thous/µl in the control animals.

The changes also affected the functional activity of leukocytes. In particular, a significant decrease in spontaneous tetrazolium activity from 0.30 ± 0.02 to 0.24 ± 0.01 units OD was found as compared to the control group on the 7th day after product administration. Such restructuring contributed to the increased functional reserve of neutrophils (respectively, 0.90 ± 0.08, 1.11 ± 0.01; $p < 0.05$).

Besides a decrease in reactive oxygen species detected by the NBT-test, the activity of cationic proteins responsible for oxygen-independent metabolism also decreases. Thus, the average cytochemical coefficient (ACC) of LCP in animals treated with betulin decreased by 35% as compared with the control group but these differences were not statistically significant.

The CIC concentration insignificantly increased in the blood serum of the experimental group animals.

No significant differences between the parameters of the experimental and control groups were found by us after the second injection of the product. The animals treated with an immunostimulating agent retained the tendency towards a slight increase in the number of leukocytes, the functional reserve of neutrophils and, at the same time, a decrease in the concentration of lymphocytes and their subpopulations, as well as spontaneous NBT activity of neutrophils. A distinctive feature was a decreased CIC value with a simultaneous increase in the activity of cationic proteins to the level of values in the control group during this period of studies compared with the previous one (respectively, 1.45 ± 0.30, 1.48 ± 0.13).

A follow-up study performed in 7 days after the third administration of betulin (Table 02) showed a tendency to the increased leukocytes within the physiological normal state in the experimental group animals as compared with the control one (respectively, 6.66 ± 0.79, 8.30 ± 0.35; $p > 0.05$).

TABLE 2: Bovine blood immunological parameters after the third injection of the product

Index	Group	
	Control	Experimental
Leukocytes, thousand / μl	6.66±0.79	8.30±0.35
Lymphocytes, thousand / μl	4.25±0.57	4.19±0.29
T-lymphocytes, thousand / μl	0.63±0.08	0.57±0.04
B-lymphocytes, thousand / μl	1.04±0.15	0.98±0.12
Cytotoxic T-lymphocytes, thousand / μl	0.89±0.10	0.62±0.08
NBT-test, spontaneous, optical density (OD) units	0.25±0.02	0.26±0.02
NBT-test, stimulated, optical density (OD) units	0.21±0.02	0.33±0.02b
Functional reserve of neutrophils	0.84±0.06	1.27±0.05b
Lysosomalcationicproteins (LCP)	1.57±0.17	1.74±0.18
CIC, C.U.	85.2±1.13	63.2±11.62

bp<0.01.

The absolute content of lymphocytes, as well as their subpopulations (T- and B-lymphocytes) in the blood of cows treated with the product corresponded to the level characteristic for the control group animals.

A distinctive feature of the immune status during this study period was a significant increase in the induced tetrazolium activity to 0.33 ± 0.02 versus 0.21 ± 0.02 units OD in the control group and, as a consequence, the functional reserve of neutrophils by 1.55 times (p <0.01) suggesting a higher oxygen-dependent microbicide activity of neutrophils in the experimental group animals. It should be pointed out that the level of lysosomal cationic proteins responsible for oxygen-independent mechanisms of bactericidal activity of leukocytes did not change significantly.

A decrease in the blood serum CIC concentration of the experimental animals to 63.2 ± 11.62 versus 85.2 ± 11.13 CU was observed besides the changes noted; however, it did not achieve a meaningful difference.

4. Conclusion

The use of betulin for leukemia-associated infection in cattle provides optimization of the immune system parameters characterized with the restoration of the lymphoid cells number to the level of clinically healthy animals, as well as a significant increase in the functional and metabolic activity of neutrophilic granulocytes, especially their oxygen-dependent systems.

The results obtained make it possible to recommend the product as an effective means to restrain the leukemia process complicated with associated infection, as well as to use it for seronegative animals to increase nonspecific resistance against BLV infection

Conflict of Interest

The authors declare that there is no conflict of interest.

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