

Dynamics of Immunological Parameters of Cattle Infected with Leukemia Virus and its Correction with Betulin–Based Medication

Aleksandr Krasikov

Department of Veterinary
Microbiology, Infectious and
Invasive Diseases
Omsk State Agrarian University
named after P.A. Stolypin
Omsk, Russia
krasikovap1950@mail.ru

Vasilii Vlasenko

Department of Veterinary
Microbiology, Infectious and
Invasive Diseases
Omsk State Agrarian University
named after P.A. Stolypin
Omsk, Russia
vvs-76@list.ru

Valentina Pleshakova

Department of Veterinary
Microbiology, Infectious and
Invasive Diseases
Omsk State Agrarian University
named after P.A. Stolypin
Omsk, Russia
vi.pleshakova@omgau.org

Irina Alekseeva

Department of Veterinary
Microbiology, Infectious and
Invasive Diseases
Omsk State Agrarian University
named after P.A. Stolypin
Omsk, Russia
ia.alekseeva@omgau.org

Abstract— The paper presents the results indicating the possibility of using betulin-based medication for the correction of the immune status of cattle infected with bovine leukemia virus (BLV). To set up the experiment, we designed 3 prototypes of the medication (betulin-B1, B2 and B3), which were tested on 20 cattle units that reacted positively in RID test with the glycoprotein antigen of BLV. 15 experimental animals were formed into 3 groups. Cows of the 1st group (n = 5) were injected subcutaneously with the betulin-B1 preparation three times with an interval of 7 days at a dose of 0.2 ml per 1 kg of live weight, groups 2 and 3 (n = 5) were injected with betulin-B2 and B3, respectively, in the same method. Another five RID-positive cows were used as a control group. After the first and the third administration of medications, hematological and immunological studies were performed. Immunological restructuring of the body after the use of betulin preparations B1 and B3 was characterized by a decrease in the number of lymphocytes due to the population of B-lymphocytes and T-cytotoxic lymphocytes; a decrease in the content of circulating immune complexes, an increase in the functional and metabolic activity of neutrophils, bactericidal and lysozyme activity of blood serum and balance of the protein spectrum. Thus, the revealed changes in the hematological and immunological parameters prove the effectiveness and feasibility of using betulin medication for the correction of the immune status of cattle infected with BLV.

Keywords—cattle, leukemia, immunocorrection, betulin, lymphocytes, NBT test, globulins.

I. INTRODUCTION

Bovine leukemia is a widespread viral pathology among livestock in many countries of the world. [1-4] The bovine leukemia virus (BLV) is able to suppress the general immune resistance of infected animals by destroying the genetic potential of the animals. One of the critical issues is the search and implementation of effective and affordable biologically active substances that increase immunological resistance and productive qualities in the leukemia control system.

Solving the problem of increasing resistance to leukemia, veterinary medicine is developing a scientific direction based on the use of immunomodulating drugs that activate the immune system by stimulating the functional activity of phagocytic cells, generating antibodies, enhancing the

cytotoxic activity of lymphocytes and natural killer cells, inducing the synthesis of interferon and other cytokines. [5]

Therefore, the development and implementation of new, environmentally friendly, biologically active substances of plant origin with antiviral and immunocorrection properties is an important area in veterinary science. Such substances include a drug based on lupane triterpenoids, in particular betulin, which is made from birch bark. A number of researchers have shown that betulin has anti-inflammatory, antiseptic, antioxidant, antiviral, hepatoprotective and other properties. [6-8]

According to a number of researchers [9], the immunomodulatory activity of betulin is associated with its ability to induce the production of endogenous interferon, as well as increase cellular and humoral immunity.

An analysis of the scientific literature showed that so far there are no studies on the effect of betulin and its derivatives on the immune status of cattle infected with leukemia virus.

In connection with the foregoing, the aim of our research was to study the effect of medication based on betulin: betulonic acid, betulinic acid and betulin-CCP (complex chemical preparation) on the state of natural immune resistance in cattle, with a positive RID test for leukemia..

II. METHODS

The work was carried out at the Department of Veterinary Microbiology, Infectious and Invasive Diseases of the Institute at the Institute of Veterinary Medicine and Biotechnologies in the Omsk State Agrarian University; at the Omsk Agrarian Scientific Center, and the farms of Omsk Region. The experimental design was developed by the staff of the Department of Veterinary Microbiology, Infectious and Invasive Diseases of the Institute at the Institute of Veterinary Medicine and Biotechnologies in the Omsk State Agrarian University.

The object of the study was cattle of holsteinized Black Pied breed, three to five years of age, weighing 550-600 kg, belonging to the manufacturing agricultural enterprise of the Omsk district of the Omsk region in the amount of 20 units, with a serological study which established a positive RID reaction to BLV.

In the research and production laboratory of the Department of Veterinary Microbiology, Infectious and Invasive Diseases, prototypes of preparations for parenteral administration were made: betulin-B1 (betulinic acid); betulin-B2 (betulonic acid) and Betulin-CCP (B3).

Experimental studies on the effect of Betulin preparations on the state of natural immune resistance in the cattle organisms that were RID positive were carried out on 4 groups of animals, with 5 animals in each group.

The cows of the experimental groups were injected subcutaneously with betulin medication three times, with an interval of 7 days (the 1st experimental group – B1; the 2nd experimental group – B2; the 3rd experimental group – B3) at a dose of 0.2 ml per 1 kg of live weight. The animals of the control group were not injected with drugs. Blood sampling in all groups of animals was performed on the 7th and 21st days from the start of the experiment.

The functional activity of neutrophilic granulocytes in experimental animals was determined by the presence of lysosomal cationic proteins (LCP) according to the method of M.G. Shubich with bromophenol blue. [10] When analyzing samples, the percentage of positively reacted cells was calculated and the average cytochemical coefficient (ACC) was calculated in accordance with standard methods. We also evaluated the oxygen-producing activity of neutrophils in the reduction reaction of nitro-blue tetrazolium (NBT test) in spontaneous (NBTsp) and stimulated (NBTst) variants with a photometric assessment of the result. Additionally, the functional reserve of neutrophils was calculated ($FRN = NBTst / NBTsp$). The concentration of circulating immune complexes (CIC) in blood serum was determined by precipitation with polyethylene glycol with a molecular weight of 6000.

The population of T-lymphocytes (E) was determined in the reaction of spontaneous rosetting with sheep erythrocytes, the population of B-lymphocytes (EAC) was determined in the reaction of complementary rosetting with bull erythrocytes, which formed immune complexes with heterophilic antibodies and complement, and the population of cytotoxic T lymphocytes (EA) in the reaction of indirect globulin rosette formation with bovine erythrocytes, which formed immune complexes only with heterophilic antibodies.

The functional activity of neutrophils in the NBT test, the number of immunocompetent cells and the content of circulating immune complexes were determined in accordance with the guidelines for assessing the immune status in bovine leukemia. [11]

Leukocyte counts and leukograms were performed according to generally accepted methods.

The bactericidal activity of blood serum was determined according to the method of O.V. Smirnova and T.A. Kuzmina [12], lysozyme activity of blood serum - according to V.G.

Dorofeychuk. [13] Albumin and globulin fractions of the protein were determined by the method of capillary electrophoresis on agar gel plates using the Paragon electrophoresis system (Vital Diagnostic kits, Russia).

Statistical processing of the obtained data was carried out using methods of variation statistics. The arithmetic mean value (M) and the mean square error of the mean value (m) were calculated. In assessing the significance of differences (p) between the two mean values of Mx and Mu, the Student t-test was used. Differences in results were considered statistically significant at a significance level of $P > 0.05$.

III. RESULTS

After a single injection of the betulin-based medication that we designed (Table 1), a significant increase in the number of leukocytes was observed in the cows of the experimental groups: by 1.3–1.6 times in comparison to the control group. Also, an increase of lymphocytes concentration was found: in the 2nd group – up to 6.52 ± 0.29 thousand / μl and in the 3rd group – up to 6.05 ± 0.28 thousand / μl , against the control group – 4.32 ± 0.46 thousand / μl . The increase in the number of lymphoid cells was due to all their subpopulations, especially the growth of the number of T-lymphocytes in the 2nd group by 74% ($p < 0.05$), as well as cytotoxic T-lymphocytes in the 2nd and 3rd groups – 69 and 93% ($p < 0.05$), respectively, compared with the same indicators of the control group.

The introduction of betulin-based medication to the virus carrier positively affected the oxygen-dependent neutrophil metabolism.

Thus, according to the results of studies in the NBT test, the cows of the experimental groups showed a decrease in the spontaneous tetrazolium activity of neutrophils, especially animals of the 3rd group, in which the changes reached a significant difference relative to the control group. Indices of stimulated NBT test, on the contrary, tended to increase insignificantly in the 1st and 3rd groups. In addition, another parameter, defined as the ratio of the stimulated NBT test to the spontaneous (functional reserve of neutrophils), significantly increased to 1.14 ± 0.01 in the 1st experimental group and to 1.17 ± 0.03 in the 3rd group, compared with the control group – 0.91 ± 0.08 . Such changes indicated a higher bactericidal activity of neutrophils.

When assessing the oxygen-independent mechanisms of phagocytosis, a slightly different picture was established. So, as a result of the determination of cationic proteins of blood neutrophils, a decrease in their number in all groups by 24–61% was noted compared with the control group.

The concentration of CIC in serum (despite the fact that in the 1st group it was increased by 24%, and in the 3rd group, on the contrary, decreased by 37%, in comparison to the control group) did not reach significant differences.

TABLE I. IMMUNOLOGICAL PARAMETERS OF BLOOD OF EXPERIMENTAL CATTLE GROUPS AFTER SINGLE INTRODUCTION OF BETULIN-BASED MEDICATION

INDEX	ANIMAL GROUP			
	<i>control</i>	<i>1st experimental (B1)</i>	<i>2nd experimental (B2)</i>	<i>3rd experimental (B3)</i>
LEUKOCYTES, THOUSANDS/MCL	6.32±0.69	9.20±0.42**	10.20±0.20**	8.30±0.25*
LYMPHOCYTES, THOUSANDS/MCL	4.32±0.46	4.43±0.27	6.52±0.29**	6.05±0.28*
T LYMPHOCYTES, THOUSANDS/MCL	0.65±0.07	0.77±0.06	1.13±0.14*	0.86±0.07
B LYMPHOCYTES, THOUSANDS/MCL	1.14±0.23	0.94±0.11	1.80±0.22	1.82±0.25
CYTOTOXIC T LYMPHOCYTES, THOUSANDS/MCL	0.96±0.17	1.08±0.11	1.62±0.12*	1.85±0.23*
SPONTANEOUS NBT TEST, OPTICAL DENSITY UNITS	0.29±0.02	0.26±0.02	0.23±0.02	0.23±0.01*
STIMULATED NBT TEST., OPTICAL DENSITY UNITS	0.25±0.02	0.30±0.02	0.24±0.02	0.27±0.01
FUNCTIONAL RESERVE OF NEUTROPHILS	0.91±0.08	1.14±0.01*	1.08±0.03	1.17±0.03*
LCP	1.46±0.22	1.11±0.12	0.64±0.13*	0.57±0.09**
CIC, C.U.	72.4±12.57	90.2±7.64	66.6±8.55	45.6±8.56

* $p < 0.05$; ** $p < 0.01$

When studying the parameters of the immune system after the triple administration of betulin-based medication, a tendency to an increase in the number of leukocytes was also observed, but only in the 1st and 2nd groups. This increase was most significant in the 2nd group (9.26 ± 0.66 ; 6.60 ± 0.91 thousand / μl , $p < 0.05$).

The concentration of lymphocytes in the experimental animals was significantly reduced, with the exception of the 2nd group, in which the absolute number of lymphocytes increased due to an increase in the number of leukocytes. It should be noted that the proliferation of lymphoid cells in this group occurred mainly due to B-lymphocytes and cytotoxic T-lymphocytes, the concentration of which increased by 29% and 40%, respectively, compared with the control group.

The opposite trajectory of indicators was observed in the 1st and 3rd groups, in which the content of B-lymphocytes and cytotoxic T-lymphocytes decreased, it is especially possible to

distinguish the 1st group, where the number of B-lymphocytes decreased to 0.61 ± 0.06 thousand / μl , against 1.03 ± 0.15 thousand / μl ($p < 0.05$) in the control group, and cytotoxic T-lymphocytes – up to 0.61 ± 0.04 thousand / μl , against 0.88 ± 0.11 thousand / μl ($p < 0.05$).

The administration of medication contributed to an increase in the functional and metabolic activity of neutrophils in all experimental groups, which was characterized by a decrease in spontaneous tetrazolium activity, an increase in the induced activity and an increase in the functional reserve, as a consequence. The most distinct changes of this kind were observed in the 1st and 3rd groups.

The results of the lysosomal-cationic test showed the absence of significant differences in the experimental groups relative to the control, although there was a slight tendency to a decrease in cationic proteins of neutrophils.

TABLE II. IMMUNOLOGICAL PARAMETERS OF BLOOD OF EXPERIMENTAL CATTLE GROUPS AFTER TRIPLE INTRODUCTION OF BETULIN-BASED MEDICATION

INDEX	ANIMAL GROUP			
	<i>CONTROL</i>	<i>1ST EXPERIMENTAL (B1)</i>	<i>2ND EXPERIMENTAL (B2)</i>	<i>3RD EXPERIMENTAL (B3)</i>
LEUKOCYTES, THOUSANDS/MCL	6.60±0.91	7.60±0.53	9.26±0.66*	6.26±0.59
LYMPHOCYTES, THOUSANDS/MCL	4.22±0.66	3.91±0.25	5.26±0.35	3.63±0.34
T LYMPHOCYTES, THOUSANDS/MCL	0.61±0.07	0.45±0.04	0.78±0.07	0.51±0.07
B LYMPHOCYTES, THOUSANDS/MCL	1.03±0.15	0.61±0.06*	1.33±0.17	0.84±0.12
CYTOTOXIC T LYMPHOCYTES, THOUSANDS/MCL	0.88±0.11	0.61±0.04*	1.23±0.14	0.66±0.06
SPONTANEOUS NBT TEST, OPTICAL DENSITY UNITS	0.23±0.02	0.17±0.01*	0.41±0.02***	0.20±0.02
STIMULATED NBT TEST., OPTICAL DENSITY UNITS	0.18±0.01	0.30±0.01***	0.43±0.02***	0.34±0.03**
FUNCTIONAL RESERVE OF NEUTROPHILS	0.82±0.06	1.82±0.17**	1.04±0.01*	1.80±0.28**
LCP	1.68±0.18	1.40±0.14	1.36±0.11	1.57±0.15
CIC, C.U.	87.2±17.65	21.8±5.12**	41.4±8.27*	23.2±3.88**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

The use of betulin-based preparations also had a normalizing effect on the content of immune complexes in blood serum. Thus, the concentration of CIC was reduced in

the 1st group by 75% ($p < 0.01$), in the 2nd – by 53% ($p < 0.05$) and in the 3rd – by 73% ($p < 0.01$), compared with the control group.

TABLE III. DYNAMICS OF CHANGES IN PROTEIN PROFILE AND INDICATORS OF NON-SPECIFIC IMMUNE RESISTANCE IN CATTLE AFTER INTRODUCTION OF BETULIN-BASED MEDICATION

INDEX	ANIMAL GROUP			
	CONTROL	1 ST EXPERIMENTAL (B1)	2 ND EXPERIMENTAL (B2)	3 RD EXPERIMENTAL (B3)
ALBUMINS	30.2±1.03	39.2±1.42	38.6±1.28	38.1±1.22
ALPHA GLOBULINS	18.0±1.44	18.4±2.12	19.2±1.48	18.6±0.92
BETA GLOBULINS	21.3±1.34	16.8±1.01	17.4±0.91	17.8±0.88
GAMMA GLOBULINS	20.4±1.13	26.1±1.34*	23.4±1.16*	28.6±1.42*
SERUM BACTERICIDAL ACTIVITY	38.3±2.12	46.4±2.83	48.6±2.91	48.9±2.61
SERUM LYSOZYME ACTIVITY	12.4±0.9	16.7±1.12	18.9±1.18	17.3±1.22

* $p < 0.05$.

Along with the described changes in cattle of the 1st and 3rd experimental groups, a tendency to restore the disturbed balance of the protein spectrum of blood serum by the time of the third injection of drugs was noted.

A significant increase in gamma globulins in animals of the 1st, 2nd and 3rd experimental groups was established up to 26.1 ± 1.34 ; 23.4 ± 1.16 and 28.6 ± 1.42 , respectively (Table 3).

At the same time, a decrease in beta globulins was observed in the all three experimental groups. Also, in animals that were injected with betulin-based preparations, an increase in albumin was recorded in the 1st, 2nd and 3rd experimental groups up to 39.2 ± 1.42 ; 38.6 ± 1.28 and 38.1 ± 1.22 , respectively.

Confirmation of the immunomodulatory effect of betulin based medication is the significant increase of bactericidal and lysozyme activity of blood serum in the animals of the 1st, 2nd and 3rd groups.

IV. CONCLUSION

The use of betulin based medication restrains the development of pathological process in cattle infected with BLV, optimizes immunological homeostasis indicators, characterized by a decrease in the number of lymphocytes within the physiological norm, a significant decrease in the CIC content and an increase in the functional and metabolic activity of neutrophils.

In addition, the research has shown that the studied medication restores the ratio of serum proteins and stabilizes a number of indicators of the immune response – it increases serum bactericidal and lysozyme activity, in particular.

Based on the foregoing, the proposed betulin based medication can be recommended for non-specific preventive measures for cattle with the RID positive profile of BLV.

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