Application of a microspectral analysis for evaluation of the morphofunctional status of immunocompetent cells in cattle with retroviral diseases

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Abstract. Microspectral analysis is a highly informative way to study the morphofunctional status of cells, including immunocompetent ones. Purpose of the study was an application it for studding of lymphocytes in cattle with enzootic leukemia and viral immunodeficiency. It is important for diagnostics and differential diagnostics of diseases, as well as for analyzing of animal homeostasis and making a prognosis of the disease. Spectral analysis of stained with a Leukodif 200 kit agranulocytes was carried out using the universal color analyzer microspectrophotometer LOMO MSFU-K. The value of the light absorption intensity was measured in the spectrum from 300 to 700 nm. The results of microspectral analysis showed that for cows’ lymphocytes with combined pathology (BLV/BIV), the absorption values in the spectrum of Eosin U and Azure II were 351.2 ± 17.6 and 751.4 ± 37.6 counts, respectively. For animals with BLV and BIV mono-infection, these indicators were 253.3 ± 12.7; 383.3 ± 19.2 and 371.5 ± 18.5; 500.2 ± 24.9 counts. While in intact cows, these indicators were registered at the level of 210.3 ± 10.5 and 173.6 ± 8.6 counts. In intact animals, the acidic and basic components ratio in the cell was uniformly proportional, the ratio coefficient was 0.83 ± 0.04. For BLV, BIV and BLV/BIV-infected animals, this coefficient was 1.34 ± 0.06, 1.51 ± 0.08 and 2.13 ± 0.11, that was in 1.6, 1.8 and 2.6 times higher than in intact animals, which might be an indicator of metabolic acidosis in the cell.

1. Introduction

The problem of enzootic bovine leukemia (EBL) is one of the primary problems for agriculture since the moment of recognizing of the disease as an independent nosological unit. The revelation of another retroviral agent - bovine immunodeficiency virus (BIV), has become an important discovery in the veterinary nosology. Due to the close phylogenetic relationship of cattle retroviruses to each other and to the causative agents of human T-cell leukemia and human immunodeficiency virus, the problem has significant biological and medical value [1].

According to G.C. Buehring et al. data, BLV DNA was detected in the buffy coat cells of blood in 38% of the subjects by PCR and DNA sequencing. IgG antibodies were detected in 32%, IgM in 58%, and IgA in 32% of the subjects [2]. In L. Martinez Cuesta’s scientific report the problem of probability
of malignant neoplasms developing in people with bovine leukemia virus (BLV) is discussed [1]. A number of authors consider this agent as a potentially predisposing to breast cancer in women factor [3, 4, 5, 6].

An important aspect is the fact that EBL and viral immunodeficiency in cattle can be considered as “satellite diseases”. In D. Abdessemed’s opinion, infection of cattle with BIV increases the risk of infection of animals with BLV, that is high probability of mixed infection [7].

According to currently most accepted hypothesis, retroviruses effect on cellular metabolism, using various mechanisms, and activate the proliferation of infected cells, provoking genome instability and somatic changes in carrier cells [8]. This strategy allows pathogens to carry out retroviral expansion in two directions: proliferation results in “cloning” of provirus DNA, in addition, the replication of infectious RNA-containing viral particles occurs more intensively in actively multiplying cells than in dormant ones. Increased mRNA expression in infected lymphocytes is accompanied by significant changes in the production of cytokines and the expression of antigens by immune cells [9]. It was found that animal lymphocytes with a high polyclonal proliferation index have increased viability [10]. Moreover, it is known that infected with retroviruses animal cells, as well as malignantly transformed, acquire different from healthy ones biophysical and morphofunctional properties [11, 12].

The strategy of retroviruses in an infected cell is currently the subject of close researchers’ attention around the world. Some viral proteins demonstrate diffuse cytoplasmic distribution, while others are localized in the nucleoli [12]. The redistribution of biosynthesis products in infected with retroviruses cells is a relevant scientific aspect of veterinary and humane medicine. It is important for the development of modern approaches to diagnostics, differential diagnosis and forecasting in hematopathological processes. Promising to solve this problem is microspectral analysis.

The aim of the study was comparative assessment of the state of agranulocytes BLV, BIV and BLV/BIV - infected and intact animals using microspectral analysis.

2. Materials and methods
The research material were peripheral blood agranulocytes of intact (n = 30), BLV-infected (n = 30), BIV-infected (n = 30) and BLV/BIV infected cattle (n = 30).

Blood was aspirated from the caudal vein (V. caudalis) with a vacuum system into tubes with the K3 EDTA anticoagulant. The tubes were transported to the laboratory at a temperature of + (4-8)° C.

The diagnosis of “BLV and/or BIV infection” was based on the civil service data and laboratory studies using equipment BioRad T100 and Gel Doc XR (USA) by applying PCR kit “LEIKOZ” (InterLabServis, Russia) and our own developments (patent No. 2615465).

Fore isolation of peripheral blood lymphocytes, the Ficoll-Urografin density gradient (1.077 g/ml) was used. Lymphocytes’ fraction dilutions were prepared according to the McFarland Turbidity Standard (0.5) on PBS. One drop of cell suspension was evenly distributed on a defatted glass slide and dried. The preparation was fixed and stained with Leukodif 200 set.

Spectral analysis of the separated agranulocytes was carried out using a universal color analyzer the LOMO MSFU-K microscope-spectrophotometer (Russia). Measurements were made using a standard MSFU-K monochromator at a power of 800A with a measurement step of 0.5 nm and a scan point diameter of 10⁻⁴ mm at 480x magnification (12x40). The intensity of light absorption (Iλ) was recorded in the spectrum range 300 - 700 nm. According to the obtained data, the absorption degree of stained lymphocytes was determined in the spectrum of Eosin U and Azure II.

Statistical processing of the data included the arithmetic mean (M) and the arithmetic mean error (m) determination. The difference between the control and the experimental groups was statistically significant considered at P≤0.05.

3. Results and discussions
Eosin U, which is part of the Leukodif 200 staining kit, is classified as acid dye, with the help of which substances and structures of a basic (protein) nature are revealed. The absorption spectrum of
the pure organic dye of eosin is 470 nm. Another component of the staining kit, Azur II, is the basic dye used to stain rich in nucleic acids structures (nuclei, nucleoli, ribosomes), as well as the amorphous component of the intercellular substance. Azur II is a two-component complex dye, its absorption spectrum is in the range of 620-665 nm. When staining biological objects, the phenomenon of metachromasia is often observed, which can be associated with the interaction of dyes with each other and depends on the kinetics of biological processes in the cell, so the absorption range can somewhat vary. So the absorption region for Eosin Y in biological objects begins already with 360 nm, and for Azure II - with 580 nm [14].

The main absorption peaks were precisely recorded in the range of characteristic wavelengths for these dyes (figure 1, table 1).

| λ, nm | Intact | BIV infected | BLV/BIV infected | BLV infected |
|-------|--------|--------------|------------------|--------------|
| 350   | 31.3±1.5| 50.1±2.6*#   | 35.5±1.7         | 33.3±1.6     |
| 400   | 97.5±4.9| 110.3±5.6    | 110.7±5.5        | 73.3±3.7*#   |

Figure 1. The absorption strength of stained lymphocytes at different wavelengths (a - intact; b - BLV-infected; c - BIV-infected; d - BLV/BIV-infected).

Table 1. Indicators of absorption strength of lymphocytes from intact and retrovirus-infected cattle.
| Value | 450 | 500 | 550 | 600 | 650 | 700 | 750 |
|-------|-----|-----|-----|-----|-----|-----|-----|
| 195.4±9.8 | 210.3±10.5 | 98.7±4.9 | 93.5±4.6 | 173.6±8.6 | 156.2±7.8 | 34.4±1.7 |
| 310.4±15.5* | 371.5±18.5* | 146.6±7.3* | 189.1±9.5* | 500.2±24.9* | 461.6±23.1* | 50.3±2.6 |
| 286.9±14.3*# | 351.2±17.2* | 175.5±8.8*# | 188.3±9.4* | 751.4±37.6*# | 725.2±36.3* | 99.6±4.9*# |
| 115.2±5.8*# | 253.3±12.7*# | 150.7±7.5* | 148.3±4.7*# | 383.3±19.2*# | 345.4±17.3*# | 148.3±4.7*# |

Note:
* - statistically significant differences between the control and experimental groups (p <0.05);
# - statistically significant differences between the experimental groups (p <0.05).

As it follows from the illustrated in Figure 1 data, when studying the agranulocytes’ absorption strength, significant differences were recorded depending on their status. For cows’ lymphocytes with combined pathology (BLV BIV), the absorption values (Iλ) in the spectrum of Eosin U and Azur II were 351.2 ± 17.6 and 751.4 ± 37.6 counts, respectively. For animals with BLV* and BIV mono-infection, these indicators were 253.3 ± 12.7; 383.3 ± 19.2 and 371.5 ± 18.5; 500.2 ± 24.9 counts respectively. While in intact cows, these indicators were recorded at the level of 210.3 ± 10.5 and 173.6 ± 8.6 counts.

Based on the fact that the absorption intensity is higher, then higher the substance concentration, it is possible to calculate the ratio of basic and acidic components in the cells. According to our data, in intact animals, the ratio of basophilic and oxyphilic components in the cell was uniformly proportional, that is, the ratio was 0.83 ± 0.04. For BIV, BLV and BLV/BIV-infected animals, this coefficient has averaged been 1.34 ± 0.06; 1.51 ± 0.08 and 2.13 ± 0.11, that is, it was 1.6; 1.8 and 2.6 times higher than that in intact ones.

Blood mononuclear cells are characterized by a high nuclear-cytoplasmic ratio. However, this indicator is not identical to the ratio of basophilic and oxyphilic cellular components, which in our opinion is more informative, since it reflects not so much structural features as the functional state of the cell. Thus, we cannot confirm that the content of nucleic acids has sharply increased in the cell, since Azure II stains all basophilic structures, including the amorphous component of the intercellular substance. In addition, AFM scanning of animals’ lymphocytes with retroviral infection did not reveal such significant morpho-topographic changes compared to intact animals [12, 15]. While inflammation and intoxication markers were detected by hematological studies of cattle with enzootic leukemia and viral immunodeficiency [16].

That is, we can assume that in this case metabolic acidosis develops in the cell. The cause of metabolic acidosis is the accumulation of ketone bodies and other under-oxidized intermediate metabolites. Increased metabolic cells activity can cause acidosis. In this case, this can equally be due to the production of viral particles and the lymphocytes polyclonal proliferation. It is likely that the toxic effect of viral proteins in infected lymphocytes is a predisposing factor.

4. Conclusion
Thus, BLV-BIV mixed infection is accompanied by more profound changes in animal homeostasis than mono-infection. We consider that the registered aberrances were caused by structural and metabolic changes in the infected cells. The data allow us to assess the infectious status and the state of animal homeostasis.

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