

Dynamics of Blood Aminoacids in the Wounds' Treatment of Cows with Hydrocele Ointment

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Abstract—This article introduces the actual problem that is a study of proposed by the authors Hydrocele ointment in amino acids' metabolism of cows' blood in inflammation of traumatic origin. Hydrocele ointment has shown a positive effect on inflammatory process and amino acids' metabolism of animals treated with the drug. Amino acid levels reached physiological parameters on the 10th day after treatment; in the control group this parameter was higher than normal.

Keywords—Amino acids, blood protein, Hydrocele ointment, inflammation, repair.

I. INTRODUCTION

DESPITE the long history of inflammation study and much information devoted to the problem of inflammation, attention to this matter is still active. Such interest in the problem of inflammation is due to the fact that many diseases often seemingly different in their manifestations and causative factors based on it.

The most common causes of inflammation are a mechanical damage of animal organism. A severe mechanical damages lead to the development of traumatic disease. Traumatic disease is characterized by the formation and development of complex local and systemic pathological processes.

Local pathological processes caused by primary and secondary alteration. Inflammation accumulates toxic products and biologically active substances. Soaked into the blood they cause multiple organ failure. This syndrome includes post-traumatic encephalopathy, acute cardiovascular, respiratory, gastrointestinal, liver failure, immune dysfunction.

To reduce the influence of the source of inflammation body develops complex physiological reactions to localize the source and restore of disturbed functions. Plasma proteins play a major role in this body reaction. [1].

Amino acid levels of blood are indicators of protein metabolism. Changing of amino acid fund, accumulation of certain amino acids, or their metabolites, and vice versa, reducing of other's concentration can serve as an indicator of the direction of protein metabolism, severity of disease progression, mobilizing the body's defenses [2].

For the treatment of wounds there are suggested a large number of drugs where leading role belongs to the ointments

and creams [3],[4]. However, they do not give the desired result. In this regard, we have proposed Hydrocele ointment for the treatment of wounds which includes hydrogen peroxide, lanolin, petrolatum and methyl cellulose as the components.

In connection with the above to determine the effectiveness of Hydrocele ointments that was proposed to regulate inflammation process and stimulate regenerative processes, we studied the amino blood acid composition of cows with injuries.

Purpose. The purpose of this study was to investigate the dynamics of blood amino acids in the treatment of cows' wounds with Hydrocele ointment.

II. MATERIALS AND METHODS OF RESEARCH

Experiments were carried out at the farm «Kremlyovskaya» of Kochenevskiy district of Novosibirsk region and in the laboratory «Leukosis», of the Institute of Experimental Veterinary of Siberia and the Far East of the RAA (Russia Agrarian Academy).

Study included cows selected on the basis of analogies with the wounds of distal extremities, totally in amount of 27. Experimental animals were divided into 3 groups. First control group included 7 animals, the second study group – 10, and third study group – 10.

Before treatment it was carried out conventional wounds toilet in surgery techniques. For this purpose it was used 0.5% solution of ammonia, 2% solution of chloramine, solution of Furacilinum 1:5000, 5% alcoholic solution of iodine.

After blood collection and wound toilet animals of the second group were treated with Vishnevsky ointment on the inflicted wounds. After blood collection and wound toilet animals of the third group were treated with Hydrocele ointment. Amount of applied ointment depended on the wound area in accordance with condition of uniform coverage of the wounds. The first group of animals was control.

Blood for biochemical analysis was sampled in the morning before feeding from the jugular vein into the Florina testtubes with Trilon-B (disodium dihydrogen ethylenediaminetetraacetate) in amount of 3-4 ml, and for serum preparation – into the bacteriological test tubes in amount of 10-15 ml.

To get the serum the blood sample was placed into an incubator for 3 hours at a temperature of 37°C, and after retraction of a clot it was precipitated by centrifugation (1000 rpm for 15 min.) at room temperature. To determine the

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biochemical composition it was used fresh stabilized blood and fresh serum.

Blood sampling was performed before treatment and in 10 days after treatment starting. It was determined total protein and amount of 13 amino acids including 7 essential ones: threonine, valine, methionine, isoleucine, leucine, histidine, lysine; 6 interchangeable ones: asparagine, glutamine, proline, glycine, alanine, tyrosine in the blood.

Definition of total protein and blood amino acids indexes has been carried out using comparative spectrum analyzer of approximated infrared (IR) region Infrapid-61 fixing full diffusely-reflected spectrum of approximated IR region on the wavelengths from 1300 to 2400 nm with the minimum interval of 1 nm.

Comparative IR spectrometry is based on regularity according to which there is a good correlation between diffuse reflection of multi-component homogeneous samples measured at definite values of a wave length close to IR region and number of separate components.

Measuring technique is of a comparative nature and, consequently requires calibration testing using conventional chemical measurements. This means that it is necessary to compose a calibration series of 30-50 samples and carry out its chemical analysis for a component, which at the later stage will be measured using the device. The same series of samples should be measured on the whole range of the device. Data obtained in such a way (spectral and chemical analysis data) should be included into a special program-application, which using regression analysis method will select effective wavelengths, changes of diffusely-reflected spectrum, where will correlate with concentration of the desired component and calculate linear relationship coefficients.

Biochemical composition of blood and blood serum have been determined by recording data of spectrum diffusive absorption/reflection from 1300 to 2400 nm against the reference, modulation of obtained data into pre-selected coefficients of linear relationship individual for each component.

Before serial changes the device has been heated at least 10 minutes (according to the instruction). In the program-application the mode 'calculation of concentration' was selected, where there were determined coefficients for the substrate's desired components content calculation. A cuvet was filled with a sample of biological fluid thereat avoiding formation of air bubbles; glasses of the cuvet were closely rubbed until appearing of colored splotches and after preliminary scanning of the reference sample (40 s), the investigated sample was scanned (45 s). After each sample the cuvet was washed with running water, then with distilled water and de-oiled by wiping with a sponge treated with 90% rectified ethyl alcohol. Data received by the device were transferred via communications ports system to the buffer of the program-application, which calculated desired values and allowed conveying measurement results on a printer immediately and/or saving them in a file of the 'text DOS' format. Approximate time required for study of one sample was 3-4 minutes.

III. STUDY RESULTS

It was shown that the locally developing pathological process has overall effect to the body, which was reflected in our case as change of serum protein amount and blood amino acids.

The results of determined blood total protein and amino acids are shown in Table I. It shows that the amount of total serum protein of cows with traumatic injuries (wounds) compared with healthy cows is reduced by 17.0%.

TABLE I
 DYNAMICS OF BLOOD AMINO ACIDS IN WOUNDS TREATMENT OF COWS WITH HYDROCELE OINTMENT

Indicators, %	Rate	Before treatment	In 10 days after treatment	
			Vishnevsky ointment	Hydrocele ointment
Asparagine	0,80±0,26	1,27±0,18	1,05±0,35	0,78±0,31
Threonine	0,25±0,17	0,41±0,16	0,33±0,26	0,27±0,13
Glutamine	1,60±0,28	2,31±0,26	1,93±0,27	1,80±0,41
Proline	0,55±0,21	0,83±0,18	0,66±0,24	0,59±0,16
Glycine	0,25±0,18	0,48±0,15	0,30±0,18	0,28±0,17
Alanine	0,35±0,19	0,62±0,16	0,39±0,16	0,37±0,18
Valine	0,51±0,22	0,87±0,19	0,67±0,25	0,49±0,19
Isoleucine	0,22±0,11	0,40±0,23	0,29±0,14	0,24±0,10
Leucine	0,7±0,166	1,19±0,23	0,84±0,26	0,79±0,18
Tyrosine	0,14±0,09	0,25±0,19	0,20±0,13	0,16±0,10
Histidine	0,31±0,20	0,52±0,25	0,46±0,13	0,33±0,11
Lysine	0,42±0,15	0,76±0,28	0,59±0,22	0,48±0,12
Methionine	0,28±0,12	0,48±0,18	0,32±0,16	0,30±0,13
Replaceable	3,69±0,31	5,76±0,38	4,53±0,32	3,98±0,31
Essential	2,75±0,28	4,56±0,45	3,5±0,38	2,90±0,21
Total	6,44±0,42	10,32±1,11	8,03±0,47	6,88±0,39
Total protein g / l	72,5±2,15	60,2±1,17	82,5±1,15	87,2±1,04

With the reduction of total protein it is an increasing of individual amino acids level. Thus, if compare with normal animals with wounds it was observed increasing of total studied amino acids by 60.2%, including non-essential amino acids by 56.1%, essential amino acids by 65.8%. In terms of individual amino acids it was observed not same increasing in the degree of change. Number of separate nonessential amino acids increased by 50,9% for proline, by 92,0% for glycine, by 77,1% for alanine, by 78,6% for tyrosine, by 58,8% for asparagine, by 44,4% for glutamine.

The inflammatory process was characterized by the increase of all essential amino acids, in particular: by 70,6% for valine, by 81,8% for isoleucine, by 56,6% for leucine, by 71,4% for

methionine, by 64,0% for threonine, by 80,9% for lysine and by 67,7% for histidine.

In healthy cows the share from the amount of amino acids was 57,3% and 42,7% for nonessential and essential respectively. At inflammation caused by a trauma this figure amounted to 55,8% and 44,2% for nonessential and essential respectively.

Application of ointments with curative purpose exerts attenuated influence on the dynamics of total protein and amino acids, however this influence depends on the type of an ointment.

In 10 days after the start of treatment with Vishnevsky ointment the level of total protein in blood increased by 37,0% as compared to the indexes before treatment.

The amount of nonessential amino acids and essential amino acids decreased by 21,3% and 23,2% respectively as compared to the data obtained before treatment.

Under the influence of Vishnevsky ointment the level of separate amino acids decreased as compared to indexes before treatment. Separately for each certain group of amino acids the decrease was: a) by 20,5% for neutral non-polar amino acids of proline, by 37,5% for glycine, by 37,1% for alanine, by 20,0 for tyrosine, by 17,3% for asparagine, by 16,4% for glutamine; essential amino acid: by 30,0% for valine, by 27,5% for isoleucine, by 29,9% for leucine, by 33,3% for methionine, by 19,5% for threonine, by 11,5% for histidine, by 22,4% for lysine.

Under the influence of Vishnevsky ointment the share of nonessential and essential amino acids form the total amount changed and made 56,4% and 43,6% respectively.

The undertaken studies have shown that Hydrocoel ointment has a more favorable effect on inflammatory process as confirmed by the results of amino acids dynamics study.

Thus, at application of Hydrocoel ointment for curative purposes the level of total protein increased by 44,8% as compared to the data obtained before treatment.

Amino acid levels compared with pretreatment decreases and approaches the level of standards. Thus, the level of essential amino acids compared to the norm increased by 7.8%, and the data pre-treatment decreased by 30.9%. The number of amino acids was higher than normal by 5.9%, and the data pre-treatment decreased by 36.4%.

Research evidence that the dynamics of the content of each amino acid has its own characteristics. Thus, as compared to the data obtained before treatment the level of nonessential amino acids decreased: by 28,9% for proline, by 41,7% glycine, by 40,3% for alanine, by 36,0% for tyrosine, by 38,6% for asparagine, by 22,1% for glutamine; essential amino acids: by 43,7% for valine, by 40,0% for isoleucine, by 33,6% for leucine, by 34,1% for threonine, by 37,5% for methionine, by 36,5% for histidine, by 36,8% for lysine.

IV. DISCUSSION OF RESULTS

Analysis of our data and research can be noted that the shifts of the studied parameters are dependent on the stage of the inflammatory process. It is known in the complex inflammatory process there are three main stages: alteration,

exudation of emigration and proliferation. These stages are in close relationship with each state of the organism and environmental conditions. Given this biological feature, we experimentally obtained data are considered in this aspect.

In the discussion of the studied biochemical indices of inflammation the first two stages were combined in a single phase, and the stage of proliferation - in a different phase of inflammation.

In the first phase of inflammation in the blood of cows decreases the total amount of protein. Reducing the total amount of protein associated with the fact that in this phase is dominated by the processes of alteration. On the increased breakdown of proteins in inflammation and accumulation in the focus of the decay products of proteins albumoz, peptones and amino acids (tyrosine, leucine, histidine, etc.) reported in the works D.E.Alperna [5].

Further reduction of the total protein is apparently connected with the fact that albumin pass into the inflamed tissue where involved in the neutralization of toxic substances [6].

According to Merezhinsky M.F. and Isakova V.K. the increase in the level of amino acids in blood is explained not only by degradation of lesional tissues but reflectory protein breakdown in tissues located symmetrically to traumatized region as well as in internals (liver, spleen, kidney etc.) [7], [8].

Reduction of these changes and their rapid normalization is a priority doctor. For this purpose, we proposed to use an ointment hydrocele.

Cows that have used the ointment for the treatment of hydrocele in 10 days after the start of treatment the level of amino acids close to the physiological parameters. Less sharp quantitative changes of amino acids are apparently related to the influence of Hydrocoel ointment. Comprising methylcellulose Hydrocoel ointment firmly coats a wound surface and so reduces alternating influence of endogenous and exogenous products. Besides, under the influence of this ointment the conditions predisposing to secondary alteration are eliminated. Hydrogen dioxide contained in the ointment helps to decrease contamination and the degree of wound abscess. As a result the damaging effect of the area of inflammation on the body remits and regenerative processes activate.

The acceleration of regenerative processes is accompanied by increased synthetic processes which are involved not only amino acids, but peptides and polypeptides. Using the latter leads to incomplete cleavage of protein and lower receipt of the final product in the blood of their cleavage of amino acids. The works of S. Rivera et al. mention that amino acids play an important role in proliferation of not only normal but malignant cells in particular [9].

In the period of plentiful growth of granulation tissues in the blood of cows treated with Hydrocoel ointment the amount of amino acids was less than in control animals. It should be noted that dynamics of separate amino acids has its own peculiarities. The most limited amino acids are alanine, asparagine, histidine and tyrosine. All essential amino acids:

valine, isoleucine, methionine, threonine, lysine were in high demand. Minimal changes in the dynamics were marked in such amino acids as glycine, leucine and methionine. Such dynamics is apparently connected with the function performed by each amino acid.

Wound healing in cows which used the ointment hydrocele occurred on the 10th day after starting treatment.

V. CONCLUSION

Thus, studies have shown that the ointment hydrocele has a positive effect on the inflammatory process, which showed earlier normalization of the amino acid composition of the blood of cows with the alignment with the performance of cows treated with Vishnevsky ointment.

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