Study of Hematological Blood Parameters Depending on the Mode of Administration of the Adjuvant - Antigen-Carrier

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Abstract. The article presents experimental materials on the effect of a synthetic adjuvant on the hematological parameters of the blood of laboratory animals, depending on the method of administration. Currently, the search continues for new effective vaccine preparations based on polymeric compounds with immunostimulating properties. An important condition for development of certain carriers (adjuvants) is creation of a drug that can stay in the body for a long time and not cause development of inflammatory reactions, and also does not affect the biochemical and hematological parameters of the blood of animals.

1 Introduction

The study of adjuvants dates back to the 20s of the XIX century, when Gaston Ramon showed the possibility of enhancing the humoral immune response by introducing additional substances – adjuvants.

Adjuvants are used to increase the immunogenicity of highly purified bacterial and viral antigens, toxoids, recombinant and synthetic antigens, and etc. Inclusion of adjuvants in the composition of vaccines provides a faster formation of pronounced and long-term specific immunity. The expediency of using adjuvants in vaccines is to increase the immunogenicity of vaccines, change the nature of the immune response, reduce the amount of antigen required for successful immunization, reduce the frequency of vaccine administration and increase the intensity of the immune response in animals with reduced immunological activity [1,5].

We, together with scientists from SSU named after N.I. Chernyshevsky developed a new adjuvant based on a chemical polyelectrolyte substance. It is planned to use it in the composition of vaccines.

Purpose of the paper: to study its effect on the animal organism in order to ensure its safety.

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2 Methods

The work was carried out in 2020 on the basis of the Testing Center for Veterinary Drugs at the Saratov State Agrarian University. 3 groups of white rats weighing 200-220 g were formed. Control group 1, group 2 administered the tested adjuvant orally using a gastric tube in a volume of 0.5 ml 1%, group 2 intramuscularly and group 3 subcutaneously 1 % 0.1 ml. After 24 hours, blood was taken to obtain hematological and biochemical parameters [8]. The blood was examined on a MINDREY hematological automatic analyzer. The obtained digital study material is biometrically processed.

3 Results

Blood, together with lymph and intercellular fluid, is the body's fluid, i.e. the environment in which cells, tissues and organs function and which contains powerful defense components - cellular and humoral, i.e. the animal organism has a natural resistance to diseases [4].

In the blood of mammals, the main function of erythrocytes is the transport of respiratory gases, since with this form the diffusion surface increases and the diffusion distance decreases. In addition, due to their shape, erythrocytes have a great ability to reversibly deform when passing through narrow curved capillaries. The content of erythrocytes and hemoglobin is subject to significant fluctuations, which can be caused by various physiological and pathological reasons. [2]

The number of red blood cells and the level of hemoglobin are provided in Table 1.

Table 1. Parameters of peripheral blood of rats (biochemical and hematological) 24 hours after the administration of the adjuvant.

No.	Indicator	Control	Oral administration	Intramuscular injection	Subcutaneous administration
1	Erythrocytes, 10^{12}/l	6.8±0.2	6.69±0.24*	6.59±0.21	7.2±0.09
2	Hemoglobin concentration, g/l	153±0.32	151.6±0.64	151.4±0.64	152.6±0.83
3	Hematocrit, %	38.44±1.62	39.9±0.68	38.66±0.95	39.8±0.53
4	Average erythrocyte volume (MCV), fl	63.08±0.39	63.9±0.52	63.5±0.41	64.0±0.46
5	Average hemoglobin content in erythrocyte (MCH), pg	21.62±0.20	21.66±0.29	21.42±0.19	21.64±0.04
6	Erythrocyte hemoglobin concentration (MCHC), %	35.06±0.26	34.54±0.23*	34.52±0.11*	34.8±0.22
7	Platelet count, 10 ^{9}/l	1,408±20.87	1,406±24.81	1,425±21.72	1,418±19.31
8	Number of leukocytes, 10^{9}/l	9.78±0.46	10.44±0.05	10.3±0.2	9.9±0.34

No.	Indicator	Control	Oral administration	Intramuscular injection	Subcutaneous administration
9	Lymphocytes, %	73.6±0.98	72.8±0.42	74.4±0.87	73.0±0.84
10	Monocytes, %	4.2±0.19	3.8±0.26*	2.8±0.28*	4.6±0.69
11	Granulocytes, %	22.2±1.14	23.0±0.67	22.8±0.66	22.4±1.4
P> 0.95; (**) - P> 0.99; (***) - P> 0.999					

4 Discussion

Our studies have shown that the number of erythrocytes in animals of the control group is (6.8 ± 0.2) 1,012/l, and in the experimental groups with oral administration (6.69 ± 0.29) 1,012/l, with intramuscular (6, 59 ± 0.21) 1,012/l, with subcutaneous administration (6.59 ± 0.21) 1,012/l. The hemoglobin concentration in all groups ranged from (153±0.32) g/L to (152.6±0.83) g/L.

The average erythrocyte volume (MCV) in the group that received an oral adjuvant was 1 % higher than in the control and other groups and amounted to (64.0±0.46) fl.

The average content of hemoglobin in the erythrocyte (MCH) in all groups was at the same level within the range (21.42±0.19) pg to (21.66±0.29) pg.

The next day, in the groups in which the administration of the adjuvant was carried out orally and intramuscularly, the concentration of hemoglobin in the erythrocyte (MCHC) decreased by 1 % compared with the indicators of the control group – (35.06±0.26)%.

Analyzing the parameters of erythrocytes and the level of hemoglobin in animals of the control and experimental groups, we note their insignificant changes of about 1 % and were within the physiological norm [12-14].

The hematocrit level in the test groups varied within 1 % and corresponded to the physiological norm for the given animal species.

White blood cells play not only an essential role in natural immunity, but also a significant role in specific acquired immunity [6].

When comparing the total number of leukocytes between groups, we found that the highest total leukocyte count was observed in the group of oral administration of polyelectrolyte and amounted to (10.44±0.05) 109/l. With intramuscular administration by 5.3 % and subcutaneous administration by 1.2 % higher compared with the control group. We believe that the increase in the number of leukocytes in the experimental groups is associated with activation of cellular defense factors when exposed to an adjuvant.

Therefore, when analyzing the results in the animals of the studied groups, it shall be emphasized that the hematopoietic function allows for the most active leukopoiesis in the body of rats that were injected with the adjuvant.

The state of the leukocyte formula, represented by the level of granulocytes and agranulocytes, is reflected in Table 1.

Granulocytes include the following types of leukocytes – basophils, eosinophils and neutrophils.

Basophils are analogs of mast cells that are found in the blood. Basophils bind the immunoglobulin E-multivalent allergen complex and are thus effector cells immunoglobulin E-mediated hypersensitivity of the immediate type.

In addition to Fc receptors for immunoglobulins E and G, basophils carry membrane receptors for complement and its components [7].

Eosinophils express receptors for immunoglobulin E and can bind the antigen: immunoglobulin E-antibody complex. Eosinophils are especially effective in destroying

infectious agents that stimulate the synthesis of immunoglobulins E, such as helminths. In addition, they contain the cellular Fc receptor for immunoglobulins G and complement receptors. Like neutrophils, eosinophils are phagocytes [8-11, 15].

Neutrophils, respond quickly to chemotactic stimuli, phagocytose and destroy foreign particles (for example, microbes), can be activated by cytokines, and are the main cell population during acute inflammation. Neutrophils contain receptors for immunoglobulin G and proteins of the complement system, they migrate and concentrate in three places of its activation. Therefore, they actively phagocytose opsonized particles and function as effector cells of humoral immunity [15].

5 Conclusion

The results of the study show that in the blood of the experimental groups of rats, the process of formation and maturation of nonspecific defense cells - granulocytes - proceeds most intensively. This state indicates the activation of cellular defense factors and, as a consequence, the high resistance of the animal organism to infections.

Lymphocytes are key cells in the immune system that provide basic immune responses. The structural organization of lymphocytes and their ability to circulate and exchange between blood, lymph and tissues is very important for the formation and specificity of the immune response. Lymphocytes are the only cells in the body that are able to specifically recognize and differentiate various determinants, which has been confirmed by a number of pieces of evidence [15].

Comparison of the results between the groups showed that the lymphocyte count varied slightly between the groups. We observed the highest rate in rats that were injected intramuscularly with the adjuvant. Their number was - (74.4±0.87) %.

Monocytes are one of the main cell populations of the immunobiological system, the primary function of which is phagocytosis. They are the dominant cells of the mononuclear phagocytic system, perform several functions in immunity, are the first to encounter foreign particles, and most often are the first cells to interact with microorganisms at the time of their introduction into the body. The data obtained indicate that the level of monocytes in the blood of the studied animals was within the physiological norm, however, after oral administration, their number decreased by 10 %, and in the group after intramuscular administration by 33.3 % [12-14].

The obtained indicators of peripheral blood indicate that, regardless of the method of administration of the antigen-carrier adjuvant, the hematological indicators corresponded to the physiological norm for this type of animal. Our data are consistent with the hematological parameters of rat blood, obtained by scientists I.N. Kravchenko, O.N. Khokhlovoy, 2008, which determined the hematological parameters of CD rats (sprague dawley) and normal CD 1 mice free from pathogenic flora [3]. In addition, an inflammatory or allergic process does not develop at the injection area.

The preclinical studies carried out prove the possibility of using an antigen-carrier adjuvant (polyelectrolyte) in the form of an adjuvant in the design of vaccines.

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