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Effect of allogeneic blood transfusion on neutrophil functional activity and lymphocyte cytotoxicity in recipient rabbits

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Abstract. The relevance of this paper is that transfusion of allogeneic blood to recipient animals is always associated with immunological risks. In this regard, the purpose of this study was to assess the state of phagocytic activity of blood neutrophils by indicators of phagocytic index, phagocytic number, and oxygen-dependent bactericidal activity, as well as to establish changes in antibody-dependent cytotoxic activity of lymphocytes in recipient rabbits during allogeneic whole blood transfusion. Modelling of blood transfusions was performed on five clinically healthy rabbits by intravenous administration of whole blood at the rate of 5.5 ml/kg of body weight. Blood samples were taken from animals on Days 3, 7, and 23 after blood transfusion. Neutrophil populations were obtained from blood samples by centrifugation on a double density gradient of 1.077 and 1.093 Ficoll-Verografin. The absorption activity of phagocytes was determined in a microscopic test. To investigate the oxygen-dependent bactericidal activity of neutrophils, a spontaneous test with nitroblue tetrazolium was performed. Antibody-dependent cytotoxic activity of lymphocytes was investigated by colorimetric method. It was found that after the transfusion of whole blood, the phagocytic activity of neutrophils increases with a simultaneous decrease in their absorption capacity. On Days 3 and 7, the results of the spontaneous test with nitroblue tetrazolium decreased. This indicates inactivation of the oxygen-dependent bactericidal activity of neutrophil granulocytes during the first phase of post-transfusion immunological reactions. On Day 23, there was an increase in the values of the indicators of the spontaneous test with nitroblue tetrazolium, which indicates the activation of the bactericidal properties of phagocytes. It was found that on Day 3, the antibody-dependent cytotoxic activity of lymphocytes significantly decreased relative to the initial state, and on Days 7 and 23, it increased. An increase in the antibody-dependent cytotoxic activity of lymphocytes should be associated with the active synthesis of antibodies of the late phase of the immune response. Consequently, transfusion of allogeneic blood causes an immune response in recipient rabbits, without causing immediate and long-term transfusion reactions (changes in heart rate, respiratory rate, body temperature). The obtained results are of practical value both for scientists and practising doctors who use transfusion of whole blood and its components to animals with acute anaemia, impaired functional activity of blood coagulation factors, parasitic, and oncological diseases

Keywords: white blood cells, phagocytic index, NBT test, antibodies, granulocytes, immunity

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Introduction

Over the past decade, interest in veterinary transfusiology has increased rapidly. Transfusion of whole blood and its components is vital for parasitic diseases, violation of blood circulation factors, cancer, and polytrauma. That is why whole blood prepared in advance is crucial during intensive veterinary care to sick animals (Lo *et al.*, 2019; Yehorov *et al.*, 2020).

Reactions to blood transfusions in recipient animals are classified as immunological and non-immunological, as well as acute and delayed (Stepura *et al.*, 2020). It is known that acute transfusion reactions are observed in recipient animals (3-8%) after whole blood transfusion (Stepura *et al.*, 2020). In such animals, as in humans, whole blood transfusions can cause severe acute respiratory distress syndrome (Irani *et al.*, 2017; Brugué *et al.*, 2018; Yehorov *et al.*, 2021). This condition is characterized by non-cardiogenic pulmonary oedema. It is arguably related to the response of donor antibodies to recipient leukocytes (Yehorov *et al.*, 2022). It is also known that blood transfusion in dogs causes a simultaneous increase in the number of neutrophil granulocytes and the content of C-reactive protein, which indicates the development of an inflammatory process in the animal's body (Day *et al.*, 2020; Claus *et al.*, 2022).

Blood transfusion is considered an allogeneic tissue transplantation operation, which can have serious complications. Despite strict compliance with the requirements concerning the blood group of the donor and the recipient, as well as conducting an *in vitro* compatibility reaction, an immune conflict may occur in the body of the recipient animal. This is because, apart from transplanted erythrocytes, their body receives many immunogenic corpuscular (leukocyte, platelet) and humoral (plasmatic) antigenic factors that can cause both humoral (activation of class M, G immunoglobulins) and cellular immune response. Notably, a non-cardiogenic pulmonary reaction in recipient animals occurs as a result of transfusion of leukocyte antibodies (Ab). Naturally, a pronounced immune conflict in the body of the recipient animal can occur even with a minimal volume of blood transfusion (Yagi & Holowaychuk, 2016; Poh *et al.*, 2018).

Taylor *et al.* (2021) argue that transfusion responses in cats are unpredictable and vary in severity. Post-transfusion reactions can be both acute and chronic. The most common transfusion reactions are manifested in the form of fever, allergies, as well as circulatory overload associated with transfusion. Transfusion reactions can trigger immune-mediated hemolysis, which causes jaundice and an increase in body temperature, which occurs due to the development of an antigen-antibody complex. Such reactions can also lead to non-hemolytic consequences – fever, itching of the skin, swelling in the facial area of the head (Taylor *et al.*, 2021).

It is known that phagocytes are essential in maintaining the constancy of the immune status of the animal body. As non-specific protection factors, they not only capture and digest microorganisms, but also activate specific mechanisms of resistance of the macroorganism. Therefore, it is important to investigate the functional state of phagocytes, specifically neutrophils (Shcherba & Korda, 2018).

The leading characteristic of granulocytes is the assessment of their phagocytic activity. Its decrease can be the result of both a lack of opsonizing factors in the serum, and defects in the cells themselves. It is known that white

blood cells can change their morphological and functional state, taking part in the immune processes of the mammalian body (Yelyseyeva *et al.*, 2020). This ability acts as a criterion for the degree of activity and the nature of the process. Among white blood cells, neutrophils, due to their functional activity, play an important role in the humoral-cellular system of cooperation in the immune response to the antigen. That is why in the mammalian body, neutrophils are both indicators and a universal target for various disorders of homeostasis. Their quantitative and qualitative composition ensures leukocyte activity and is manifested primarily by phagocytic activity (Yelyseyeva *et al.*, 2020).

The hereditary immune system includes large granular lymphocytes – NK cells. Under interleukin-15 (IL-15), they are formed from a lymphocyte stem cell in the bone marrow (Lo *et al.*, 2019). They belong to the system of innate immunity since they develop without rearrangement of the genes of the receptor apparatus. However, unlike other innate immune cells, they recognize molecular structures of antigenic origin that are foreign to the mammalian body. NK cells detect and neutralize altered cells in their own body. At the same time, their targets are cells of the own body that have partially or completely lost the expression of major histocompatibility complex (MHC) class I molecules but express a normal or increased level of surface molecules induced by cellular stress (Lo *et al.*, 2019; Baeva, 2019; Abbas *et al.*, 2020).

Such molecular changes are inherent in cells infected with viruses, cytosolic parasites, as well as for tumour cells. NK cells can neutralize target cells without any prior activation (immunization). This property is called natural cytotoxicity (NK activity) and underlies the term “natural killers”. Furthermore, due to the expression of Fc receptors, NK cells can take part in antibody-dependent cellular cytotoxicity reactions, i.e., neutralize IgG-opsonized antigens. Involvement of Fc receptors in the recognition of antigen epitopes considerably increases the effectiveness of cytotoxicity (Baeva, 2019).

Neutralization (lysis) of infected macroorganism cells and tumour cells is the main function of NK cells. Due to the ability to independently recognize “own-foreign” on the surface of the target cell, cytolysis by NK cells occurs, which distinguishes them from cytotoxic T-lymphocytes. Lysis of target cells is provided by perforin and granzymes – proteins found in the lytic granules of NK cells. During their interaction with target cells, lysis of the latter occurs due to perforins and granzymes – proteins stored in the lytic granules of NK cells, while during their interaction with the target cell, degranulation occurs. Granzymes, entering the cytosol of the target cell with perforin, induce its apoptosis (Lo *et al.*, 2019; Kyivska, 2019).

It is known that phagocytes play an important role in protecting the tissues of the mammalian body and supporting the constancy of the immune status of the body. These cells act as non-specific defence factors that not only phagocytize and process the antigen (Ag), but also mobilize specific resistance mechanisms of the animal body (Legenchuk & Nemyrovych, 2022). Therefore, it is important to understand allogeneic tissue transplantation to investigate the functional state of phagocytes by oxygen-dependent bactericidal activity of blood neutrophils using the nitroblue tetrazolium test (NBT test) (Stasenko, 2019).

The purpose of this study was to investigate the functional properties of neutrophilic granulocytes and antibody-dependent cytotoxic activity of lymphocytes in recipient animals by allogeneic blood transfusion.

Materials and Methods

The study was conducted during 2021-2022 at the Scientific and Educational Laboratory "Animal Blood Bank" of the Acad. I.O. Povazhenko Department of Surgery and Pathophysiology and in the conditions of the Educational and Scientific Production Clinical Centre "Vetmedservice" of the National University of Life and Environmental Sciences of Ukraine.

Experiments on rabbits were conducted per the requirements of the "General Ethical Principles of Conducting Experiments on Animals", approved by the First National Congress on Bioethics (September 20, 2004, Kyiv, Ukraine) (Law of Ukraine..., 2006), the Regulations of the "European Convention on the Protection of Vertebrates Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) (European Convention for..., 1986) and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (2006).

Five clinically healthy rabbits of the White Pannon breed were used in the experiments. The diet of experimental animals corresponded to their need for nutrients and biologically active substances. The animals had free access to water and food.

Blood from donor animals was taken from the jugular vein by a semi-closed method. At the blood sampling site, the animals' fur was shaved, and the skin was treated with a 70% alcohol solution. During the selection of animal blood, the tissues in the place where the jugular vein passes were moderately stretched. The head of the animal was diverted to the side opposite to the side of blood sampling. A jugular vein puncture was performed in the upper and middle third of the neck. At an angle of 45°, a needle was inserted, piercing the skin and vein wall against the blood flow. For the convenience of manipulation, the vein below the puncture site was pressed with the finger of the left hand. Donor blood samples of rabbits were collected in polymer containers with the CPDA anticoagulant (citrate, phosphate, dextrose, and adenine). Recipient rabbits received whole blood transfusions in the amount of 5.5 ml/kg of body weight.

The study was based on blood plasma samples taken from five rabbits on Days 3, 8, and 23 after transfusion. The population of blood neutrophils was obtained by centrifugation (Hettich EVA laboratory centrifuge, Germany) on a double density gradient of 1.077 and 1.093 Ficoll-Verografin (Yamashita *et al.*, 2019).

The phagocytic activity of blood granulocytes (absorbing activity of phagocytes) was determined in a microscopic test. When investigating the phagocytic activity of neutrophils, standard latex particles for phagocytosis (10% polystyrene suspension) with a diameter of 1.0-1.3 µm were

used as test systems. The functional activity of phagocytes was assessed by determining phagocytic number (PN), % is the number of phagocytizing cells per 100 counted; phagocytic index (FI), c.u. is the average number of latex captured by one phagocyte.

A suspension of neutrophil cells was introduced into a test tube in the amount of 0.1 ml and incubated at 37°C for 60 min with 0.1 ml of latex suspension. Smears were prepared on glass slides, dried, fixed, and stained according to Romanowsky-Giemsa. 100 neutrophils were counted in each smear (Crawford *et al.*, 2022).

The oxygen-dependent bactericidal activity of blood neutrophils was determined by a spontaneous nitroblue tetrazolium test (HBT test). When setting up a spontaneous HBT test, phagocytes were cultured in the presence of nitroblue tetrazolium without prior cell activation (Grasso *et al.*, 2019). The oxygen-dependent bactericidal activity of neutrophils in the HBT test was investigated using the spectrophotometric method (Grasso *et al.*, 2019). A suspension of phagocytes in culture medium 199 (1×10⁶ cells/ml) was introduced into centrifuge tubes and centrifuged at 1,000 rpm for 5 minutes. Subsequently, the supernatant fraction was removed, and 0.2 ml of HBT solution in a phosphate-salt buffer with a mass fraction of 0.2% was added to the phagocytes. Cells were cultured for 60 min at 37°C in a water-saturated atmosphere with a constant CO₂ level (5%). After incubation, the tubes were centrifuged at 1,000 rpm for 1-2 minutes. Then the supernatant fluid was removed, and 0.2 ml of HCl was added to the phagocytes. The test tubes were thoroughly shaken and centrifuged again under the same conditions. Subsequently, the supernatant fraction was removed from the test tubes and 0.2 ml of dimethyl sulphoxide was added for phagocyte lysis. After cell lysis, 3 ml of phosphate-salt buffer was added to the test tubes. The optical density of diformazan in the samples was measured on a Ulab 102 UV spectrophotometer (China) at an optical wavelength of 492 nm in cuvettes (1×1 cm) against a solution of 2.8 ml of phosphate buffer with 0.2 ml of dimethyl sulfoxide (Grasso *et al.*, 2019).

Antibody-dependent cytotoxic activity of lymphocytes was determined by the colorimetric method (Grasso *et al.*, 2019).

Statistical processing of the research results was performed using the "Statistica 5.0" software (StatSoft Inc., USA). Differences between two indicators of the compared samples were considered statistically significant at P<0.05, P<0.01, P<0.001 (Filimonova *et al.*, 2005).

During the studies, the main clinical indicators (body temperature, heart rate, respiratory rate) were monitored in experimental animals.

Results and Discussion

Changes in the phagocytic activity of neutrophil granulocytes in the blood of recipient rabbits during allogeneic whole blood transfusion are presented in Table 1.

Table 1. Dynamics of changes in the phagocytic activity of neutrophilic granulocytes in rabbit blood during allogeneic whole blood transfusion (M ± m, n = 5)

Seq. No.	Cells	Initial state	Day 3	Day 7	Day 23
1	Phagocytic index, c.u.	26 ± 2.50	30 ± 1.50	37 ± 2.80**	39 ± 3.40**
2	Phagocytic number, %	7.86 ± 0.18	7.12 ± 0.13**	6.20 ± 0.18***	6.87 ± 0.24**

Note: * P<0.05; ** P<0.01; *** P<0.001, significant relative to the initial state

As a result of determining the phagocytic activity of neutrophil granulocytes in rabbits after whole blood transfusion, the phenomenon of “phagocytosis dysfunction” was identified. Thus, during whole blood transfusion, the phagocytic index increased in recipient rabbits on Days 7 and 23 of the experiment by 42.3% and 50.0% ($P < 0.01$), respectively (Table 1).

Therewith, the phagocytic number significantly decreased relative to the initial state on Day 3, Day 7, and Day 23 of the experiment by 9.4% ($P < 0.01$), 22.0% ($P < 0.001$), and 13.0% ($P < 0.01$), respectively.

Thus, in case of whole blood transfusion in the body of recipient rabbits, the absorption capacity of blood neutrophils decreases against the background of an increase in their phagocytic activity, which indicates a dysfunction

of phagocytosis. The obtained data indicate considerable compensatory capabilities of neutrophils, which can maintain the overall balance in the phagocytic system under conditions of intense load on specific and non-specific links of immunity. Notably, under these conditions, the body temperature of the experimental animals was within the normal range. The established regularities coincide with the results of studies described by other authors (Callan *et al.*, 2013; Suddock & Crookston, 2022).

Oxygen-dependent phagocyte metabolism was determined using the NBT test. This test reflects the degree of functional irritation of phagocytic cells and is an indirect indicator of the functional activity of cells. The NBT test was used to characterize the ability of phagocytes to destroy microbial cells, i.e., their antibacterial activity (Fig. 1).

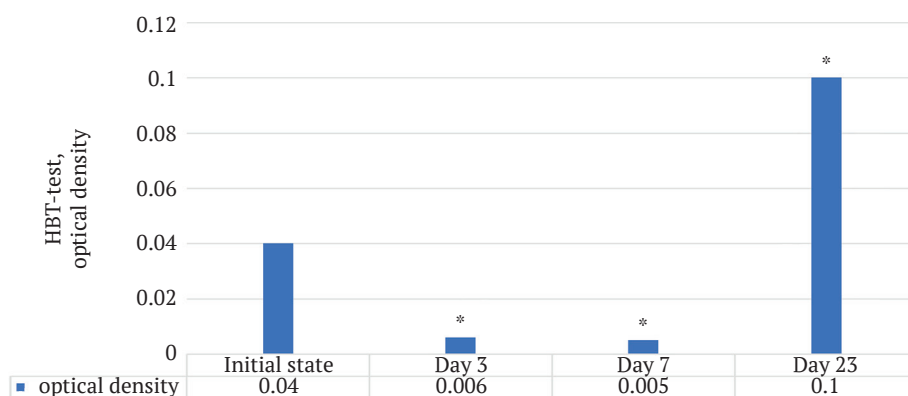


Figure 1. Dynamics of functional activity of neutrophils in the reaction of reduction of nitroblue tetrazolium in rabbit blood during allogeneic whole blood transfusion

Note: * $P < 0.05$, values are significant relative to the initial state

Indicator of the NBT test (Fig. 1) in rabbits, whole blood transfusions experienced a decrease of 15% ($P < 0.05$) and 12.5% ($P < 0.05$), respectively, compared to the initial state. At the same time, on Day 23 of the experiment, the level of this indicator increased by 16.6% ($P < 0.05$) relative to the initial state. The established increase in the value of this indicator during whole blood transfusion correlates with the dynamics of the phagocytic index (PI), which was also marked by an increase, but already by 50.0% ($P < 0.01$) relative to the initial level (Table 1). Activation of antibacterial oxygen-dependent systems within phagocyte cells on Day 23 after whole blood transfusion probably occurs due to the destruction of transplanted cells in the recipient animal body during this period (Yagi & Holowaychuk, 2016; Day *et al.*, 2020).

Portnov (2021) notes that the HBT test scores may also increase at the initial stage of acute bacterial infections. While in the subacute and chronic course of the infectious process, their decrease is noted. Rehabilitation of the body from pathogens is accompanied by the restoration of the values of this indicator. A sharp decrease in its level indicates decompensation against infectious defences and is considered a prognostically unfavourable sign.

A complex of reactions aimed at recognizing, absorbing, and eliminating microscopic particles from the body is called phagocytosis. Kling *et al.* (2020) indicate that many cell types are more or less capable of phagocytosis, but the phagocytic activity of neutrophils is most important for the immune system.

Krüger (1995) noted that the storage of blood affects all its components. Preliminary removal of polymorphonuclear neutrophils from the blood improves the quality of its components. Therewith, a decrease in their number can lead to the disappearance of important mechanisms of antibacterial protection.

Antibody-dependent cytotoxic activity of lymphocytes in the blood of recipient rabbits during allogeneic whole blood transfusion was investigated. The dynamics of antibody-dependent cytotoxic activity of lymphocytes in the blood of recipient rabbits during whole blood transfusion is presented in Figure 2.

On Day 3 of experimental studies, in the case of allogeneic whole blood transfusion to recipient rabbits, the antibody-dependent cytotoxicity of leukocytes significantly decreased by 16% relative to the initial state ($P < 0.01$) (Fig. 2). The described pattern is similar to the results of other researchers (Yagi & Holowaychuk, 2016; Day *et al.*, 2020). It is probable that the decrease in the antibody-dependent cytotoxic activity of lymphocytes in the peripheral blood of recipient rabbits on Day 3 of experimental studies relative to the initial state is due to the activation of the activity of the immune system as a whole and the synthesis of class M immunoglobulins (pentamers), to which natural killer cells (NK cells) have no specific receptors. Notably, it is Class M antibodies that are synthesized by plasma cells during the acute period of the immune response, i.e., in the case of first contact with certain epitopes of antigens (Keir *et al.*, 2012).

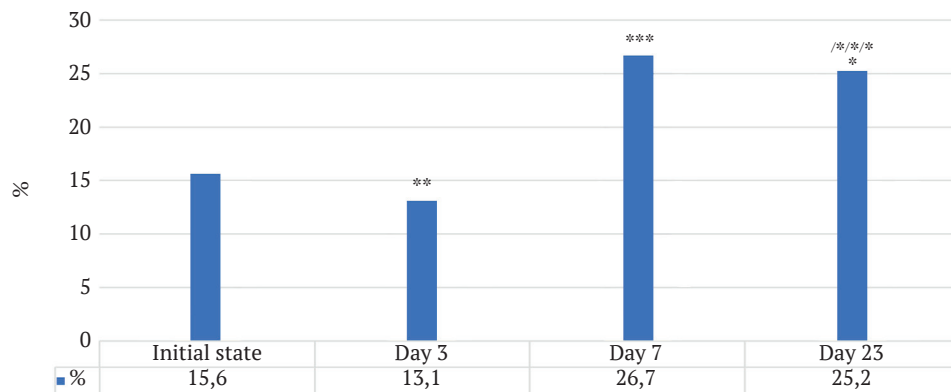


Figure 1. Dynamics of antibody-dependent cytotoxic activity of lymphocytes in the blood of recipient rabbits during allogeneic whole blood transfusion

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, values are significant relative to the initial state, and /*** $P < 0.001$, significant compared to the previous stage of the experiment

On Day 7 of experimental studies (Fig. 2) when whole blood was transfused to recipient animals, the antibody-dependent cytotoxic activity of blood lymphocytes increased by 71.3% ($P < 0.001$) relative to the initial state. Notably, at this time of the experiment, antibody-dependent cellular cytotoxicity increased by 2 times compared to 3 days of the experiment. An increase in antibody-dependent cell cytotoxicity on Day 7 of the experiment relative to the initial state and 3 of its days should be associated with the active synthesis of late-phase immune response antibodies (IgG) that interact with Fc domains with surface receptors of NK cells. Class G immunoglobulins have only 2 antigen binding centres and a lower molecular weight, thereby providing greater specificity with epitopes of corpuscular and humoral antigens (red blood cells, white blood cells, platelets, plasma proteins), thus providing stable avidity (antibody-antigen relationship) (Abbas *et al.*, 2020).

On Day 23 of the experiment, the cytotoxic activity of blood lymphocytes significantly increased relative to the initial state by 61.5% ($P < 0.05$), and relative to Day 3 of the experiment by 1.9 times (/*** $P < 0.001$). The increase in antibody-dependent cell cytotoxicity on Day 23 of the experiment on allogeneic whole blood transfusion in recipient rabbits is probably associated with the active synthesis of Class G immunoglobulins by plasma cells, which actively opsonize transplanted antigens, forming an Ag-Ab complex, and thereby activate antibody-dependent cell cytotoxicity reactions (Australian Red Cross..., 2020). At the same time, antibody-dependent cell cytotoxicity tended to decrease by 5.6% compared to Day 7 of the experiment (26.7%). Such changes occur due to apoptosis of transplanted platelets and leukocytes on Days 7-9 days and 14-16 after transfusion, which coincides with the results of research by Day *et al.* (2020).

The decrease in antibody-dependent cellular cytotoxicity in experimental animals is caused by a decrease in the antigenic load, especially corpuscular antigens, on the immune system of recipient animals on Day 23 relative to Day 7 of the experiment. The results obtained are consistent with those of Dani *et al.* (2017), Poh (2018) and Yagi & Holowaychuk (2016).

As noted by Ueta *et al.* (2018), when recipient lymphocytes and donor stimulating cells are co-cultured, cytotoxic

T lymphocytes appear among the cells. They can destroy target cells that carry antigens that are also present on stimulating cells. The study of cellular cytotoxicity in mixed lymphocyte culture in some cases allows predicting whether the graft will stimulate the formation of cytotoxic T-lymphocytes or not.

Crawford *et al.* (2019) demonstrated that transfusion of donor-specific blood induces alloresponses and leads to immunosuppression. Using fully allogeneic combinations of rat blood cells in scientific experiments, scientists have found that red blood cell products can directly modulate the function of immune cells. This is called transfusion-associated immunomodulation (TRIM), which provides an appropriate adaptive mechanism. A single blood transfusion effectively induces a T-cell-dependent helper response of antibody-forming cells of the main histocompatibility complex (MHC) Class I with a change in the class of immunoglobulins and a donor-specific response of regulatory lymphocyte cells (Treg). This occurs mainly in the spleen, probably by indirect allorecognition via resident dendritic cells (DCs).

Notably, during the studies in experimental animals, the main clinical indicators (body temperature, heart rate, respiratory rate) stayed within the physiological parameters and corresponded to the range of values of 38.5-39.5°C, 180-250 heartbeats/min, 50-60 respiratory movements/min, respectively.

Thus, the results of the conducted studies indicate that whole blood transfusion causes complex immunological changes in the body of recipient animals in the form of a two-phase course of post-transfusion immunological reactions. The first phase is characterized by inhibition of phagocytic activity of neutrophilic leukocytes and the HBT test index. The second one can be considered a stimulation phase. It is characterized by reverse immunological processes relative to the first phase. By stimulating the phagocytic and, specifically, oxygen-dependent bactericidal activity of neutrophil granulocytes in recipient rabbits.

Conclusions

It was found that allogeneic whole blood transfusion to recipient rabbits increases the phagocytic activity of blood neutrophils, with a simultaneous decrease in their absorption capacity.

On Days 3 and 7 after allogeneic whole blood transfusion to recipient rabbits, there is a significant decrease in the value of the spontaneous HBT test in the animal body, respectively, by 15% and 12.5%, which indicates inactivation of oxygen-dependent bactericidal activity of neutrophilic granulocytes during the first phase of post-transfusion immunological reactions.

On Day 23 after whole blood transfusion, a significant increase in the value of the spontaneous HBT test by 16.6% occurs in the body of rabbits, which indicates the activation of oxygen-dependent mechanisms of phagocyte killing and proves their bactericidal property.

It was found that on Day 3 after allogeneic whole blood transfusion, antibody-dependent cellular cytotoxicity decreases by 16% in the body of recipient rabbits. The authors of this study believe that the established pattern may be related to the synthesis of Class M immunoglobulins by plasma cells in the first days of the experiment, to which natural killers do not have specific Fc receptors.

On Day 7 of the experiment, 71.3% of antibody-dependent cellular cytotoxicity is significantly activated in recipient rabbits relative to the initial state. A probable increase in the value of this indicator indicates the activation of the synthesis of immunoglobulins of the secondary immune response (Ig G), to which natural killers (NK) have a complementary Fc receptor.

Antibody-dependent cytotoxic activity of lymphocytes on Day 23 after whole blood transfusion significantly increased by 61.5% in the body of recipient rabbits. The value of this indicator on Day 23 of the experiment in experimental animals was less by 5.6% relative to its Day 7, which occurs due to simultaneous natural apoptosis of allogeneic platelets and white blood cells within 7-16 days after whole blood transfusion.

Further studies will concern the investigation of the response of the cellular link of immunity (T, B, and O lymphocytes), in dynamics, in recipient animals by allogeneic transfusion.

References

- [1] Abbas, A.K., Lichtman, A.G., & Pillai, S. (2020). *Fundamentals of immunology: Functions and disorders of the immune system*. Kyiv: VSV "Medicine".
- [2] Australian Red Cross Lifeblood. (2020). *Blood component information: An extension of blood component labels*. Retrieved from <https://transfusion.com.au/BCI>.
- [3] Baeva, O.V. (2019). Cytotoxic lymphocytes: Expression of adhesion molecules and activation markers. In *Current issues of biology and medicine: Materials of the All-Ukrainian scientific conference with international participation* (pp. 8-10). Cherkasy: FOP Belinska O. B.
- [4] Brugué, C.B., Ferreira, R.R.F., Sanchez, I.M., Graça, R.M.C., Cardoso, I.M., de Matos, A.J.F., & de Gopegui, R.R. (2018). *In vitro* quality control analysis after processing and during storage of feline packed red blood cells units. *BMC Veterinary Research*, 14(1), article number 141. doi: 10.1186/s12917-018-1458-4.
- [5] Callan, M.B., Patel, R.T., & Rux, A.H. (2013). Transfusion of 28-day-old leucoreduced or non-leucoreduced stored red blood cells induces an inflammatory response in healthy dogs. *Vox Sanguinis*, 105(4), 319-327. doi: 10.1111/vox.12058.
- [6] Claus, M.A., Poh, D., Smart, L., Purcell, S.L., Boyd, C.J., & Sharp, C.R. (2022). Effect of leukoreduction on inflammation in critically ill dogs receiving red blood cell transfusions: A randomized blinded controlled clinical trial. *Journal of Veterinary Internal Medicine*, 36(4), 1248-1257. doi: 10.1111/jvim.16487.
- [7] Crawford, T.M., Andersen, C.C., Hody, N.A., Robertson, S.A., & Stark, M.J. (2022). Effect of washed versus unwashed red blood cells on transfusion-related immune responses in preterm newborns. *Clinical & Translational Immunology*, 11, article number 1377. doi: 10.1002/cti2.1377.
- [8] Crawford, T.M., Andersen, C.C., Hody, N.A., Robertson, S.A., & Stark, M.J. (2019). The contribution of red blood cell transfusion to neonatal morbidity and mortality. *Journal of Paediatrics and Child Health*, 55(4), 387-392. doi: 10.1111/jpc.14402.
- [9] Dani, C., Poggi, C., & Gozzini, E. (2017). Red blood cell transfusions can induce proinflammatory cytokines in preterm infants. *Transfusion*, 57(5), 1304-1310. doi: 10.1111/trf.14080.
- [10] Day, M.J., Mackin, A., & Littlewood, J.D. (2000). Manual of canine and feline haematology and transfusion medicine. *Canadian Veterinary Journal*, 44(8), article number 663.
- [11] European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. (1986, March). Retrieved from http://zakon4.rada.gov.ua/laws/show/994_137.
- [12] Filimonova, N.B., Fil, I.O., & Mykhailova, T.S. (2005). Statistical analysis of data according to the principles of science-based medicine. Comparison of groups in quantitative terms. *Medicine of Ukrainian Transport*, 4, 86-93.
- [13] Grasso, M., Pacella, G., Sangiuliano, N., De Palma, M., Puzziello, A., & Grasso, M. (2019). Gastric cancer surgery: Clinical outcomes and prognosis are influenced by perioperative blood transfusions. *Updates Surgery Journal*, 71(3), 439-443. doi: 10.1007/s13304-019-00622-7.
- [14] Irani, M.S., Karafin, M.S., & Ernster, L. (2017). Red cell exchange to mitigate a delayed hemolytic transfusion reaction in a patient transfused with incompatible red blood cells. *Journal of Clinical Apheresis*, 32(1), 59-61. doi: 10.1002/jca.21461.
- [15] Keir, A.K., McPhee, A.J., Andersen, C.C., & Stark, M.J. (2012). Plasma cytokines and markers of endothelial activation increase after packed red blood cell transfusion in the preterm infant. *Pediatric Research*, 73(1), 75-79. doi: 10.1038/pr.2012.144.
- [16] Kling, L., Schreiber, A., Eckardt, K.-U., & Kettritz, R. (2020). Hypoxia-inducible factors not only regulate but also are myeloid-cell treatment targets. *Journal of Leukocyte Biology*, 110(1), 61-75. doi: 10.1002/JLB.4RI0820-535R.
- [17] Krüger, J. (1995). The impact of polymorphonuclear neutrophils on the quality of stored cellular blood products. *Infusionsther Transfusionsmed*, 22(3), 159-163. doi: 10.1159/000223116.

- [18] Kyivska, Yu.O. (2019). *Prooxidant-antioxidant and immune mechanisms in experimental rhinitis of various genesis and pathogenetic justification of local application of natural antioxidants* (Doctoral thesis, National University of Pharmacy, Kharkiv, Ukraine).
- [19] Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment". (2006, February). Retrieved from <https://zakon.rada.gov.ua/laws/show/3447-15>.
- [20] Legenchuk, O.V., & Nemyrovych, Yu.P. (2022). Phagocytic activity of neutrophilic granulocytes of oral fluid in children with acute forms of leukemia. *Medical Science of Ukraine*, 18(1), 30-34. doi: 10.32345/2664-4738.1.2022.05.
- [21] Lo, N.C., Macagno, M., Sangiolo, D., Bertolaccini, L., Aglietta, M., & Merlano, M.C. (2019). NK-mediated antibody-dependent cell-mediated cytotoxicity in solid tumors: Biological evidence and clinical perspectives. *Annals of Translational Medicine*, 7(5), article number 105. doi: 10.21037/atm.2019.01.42.
- [22] Poh, D. (2018). *Blood banking in small animal medicine: A survey of current practices and assessment of a technique to prevent transfusion-related complications*. Murdoch: Comparative Health Research Group.
- [23] Portnov, A. (2021). *Spontaneous test with NST*. Retrieved from https://m.ilive.com.ua/health/spontanny-test-s-nst_105353i15972.html.
- [24] Shcherba, V.V., & Korda, M.M. (2018). Phagocytic and metabolic activity of blood neutrophils in rats with periodontitis against the background of hyper- and hypothyroidism. *Herald of Problems of Biology and Medicine*, 1:2(143), 373-377. doi: 10.29254/2077-4214-2018-1-2-143-373-378.
- [25] Stasenko, A.A. (2019). Functional properties of neutrophil granulocytes of blood and peritoneal exudate in patients with peritonitis. *Medical Case*, 7-8, 22-29.
- [26] Stepura, N.N., Zamotayeva, G.A., Terekhova, G.N., & Volynets, I.P. (2020). The content of circulating immune complexes in patients with diffuse toxic goiter complicated by autoimmune ophthalmopathy. *Endocrinology*, 25(4), 305-309. doi: 10.31793/1680-1466.2020.25-4.305.
- [27] Suddock, J.T., & Crookston, K.P. (2022). *Transfusion reactions*. Tampa: StatPearls Publishing.
- [28] Taylor, S.E., Spada, E., Callan, M.B., Korman, R., Leister, E., Steagall, P., Lobetti, R., Seth, M., & Tasker, S. (2021). 2021 ISFM consensus guidelines on the collection and administration of blood and blood products in cats. *Journal of Feline Medicine and Surgery*, 23(5), 410-432. doi: 10.1177/1098612X21100707.
- [29] Ueta, H., Kitazawa, Y., Sawanobori, Y., Ueno, T., Ueha, S., Matsushima, K., & Matsuno, K. (2018). Single blood transfusion induces the production of donor-specific alloantibodies and regulatory T cells mainly in the spleen. *International Immunology*, 30(2), 53-67. doi: 10.1093/intimm/dxx078.
- [30] Yagi, K., & Holowaychuk, M.K. (2016). Evolution of veterinary transfusion medicine and blood banking. In *Manual of veterinary transfusion medicine and blood banking* (pp. 3-12). Hoboken: John Wiley & Sons. doi: 10.1002/9781118933053.
- [31] Yamashita, Y.I., Hayashi, H., Imai, K., Okabe, H., Nakagawa, S., Kitamura, F., Uemura, N., Nakao, Y., Yusa, T., Itoyama, R., Yamao, T., Umesaki, N., Miyata, T., Chikamoto, A., Shimokawa, M., Baba, H., & Yamashita, Y.I. (2019). Perioperative allogeneic blood transfusion does not influence patient survival after hepatectomy for hepatocellular carcinoma: A propensity score matching analysis. *World Journal of Surgery*, 43(1), 2894-2901. doi: 10.1007/s00268-019-05085-w.
- [32] Yehorov, O.V., Malyuk, M.O., & Kozlovskaya, G.V. (2020). Sterility monitoring of cat stored donor blood. *Ukrainian Journal of Veterinary Sciences*, 11(1), 143-151. doi: 10.31548/ujvs2020.01.015.
- [33] Yehorov, O.V., Malyuk, M.O., & Kozlovskaya, G.V. (2021). Sterility monitoring of canine packed red blood cells. *Ukrainian Journal of Veterinary Sciences*, 12(2), 67-73. doi: 10.31548/ujvs2021.02.007.
- [34] Yehorov, O.V., Malyuk, M.O., & Savchuk, M.V. (2022). Changes in the content of immunoglobulins and circulating immune complexes in the blood serum of recipient rabbits after allogeneic whole blood transfusion. *Scientific Bulletin of NULES of Ukraine*, 5(99). doi: 10.31548/dopovidi2022.05.010.
- [35] Yelyseyeva, I.V., Zhdamarova, L.A., Bilozersky, V.I., Kolpak, S.A., & Balak, O.A. (2020). Study of the dynamics of phagocytic activity of neutrophils after repeated vaccinations of laboratory animals with an experimental diphtheria bacterial antigen preparation. *Ukrainian Journal of Medicine, Biology and Sports*, 5(1), 50-55. doi: 10.26693/jmbs05.01.050.

Вплив алогенної трансфузії крові на показники функціональної активності нейтрофілів і цитотоксичності лімфоцитів в організмі кролів-реципієнтів

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Анотація. Актуальність наукового дослідження зумовлена тим, що переливання алогенної крові тваринам-реципієнтам завжди пов'язане з імунологічними ризиками. У зв'язку з цим, метою роботи було оцінити стан фагоцитарної активності нейтрофілів крові за показниками фагоцитарного індексу, фагоцитарного числа та кисень-залежної бактерицидної активності, а також визначити зміни антитілозалежної цитотоксичної активності лімфоцитів у кролів-реципієнтів за алогенної трансфузії цільної крові. Моделювання переливання крові здійснювали на п'яти клінічно здорових кролях, шляхом внутрішньовенного введення їм цільної крові з розрахунку 5,5 мл/кг маси тіла. На 3, 7 та 23 добу після гемотрансфузії у тварин відбирали зразки крові. Зі зразків крові отримували популяцію нейтрофілів шляхом центрифугування на подвійному градієнті щільності 1,077 і 1,093 фіколу-верографіну. Поглинальну активність фагоцитів визначали у мікроскопічному тесті. Для вивчення кисень-залежної бактерицидної активності нейтрофілів проводили спонтанний тест з нітросинім тетразолієм. Колориметричним методом досліджували антитілозалежну цитотоксичну активність лімфоцитів. Встановлено, що після трансфузії цільної крові відбувається підвищення фагоцитарної активності нейтрофілів з одночасним зменшенням їх поглинаючої здатності. На 3 і 7 добу показники спонтанного тесту з нітросинім тетразолієм зазнавали зниження. Це свідчить про інактивацію кисень-залежної бактерицидної активності нейтрофільних гранулоцитів за першої фази посттрансфузійних імунологічних реакцій. На 23 добу відзначалося підвищення значень показників спонтанного тесту з нітросинім тетразолієм, що вказує на активацію бактерицидної властивості фагоцитів. З'ясовано, що на 3 добу антитілозалежна цитотоксична активність лімфоцитів вірогідно зменшувалася відносно вихідного стану, а на 7 і 23 добу – збільшувалася. Підвищення антитілозалежної цитотоксичної активності лімфоцитів слід пов'язувати з активним синтезом антитіл пізньої фази імунної відповіді. Отже, трансфузія алогенної крові спричиняє імунну відповідь у кролів-реципієнтів, не викликаючи при цьому негайних і віддалених трансфузійних реакцій (зміни частоти серцевих скорочень, частоти дихання, температури тіла). Отримані результати становлять практичну цінність як для науковців, так і для практикуючих лікарів, які застосовують переливання цільної крові та її компонентів тваринам за гострої анемії, порушенні функціональної активності факторів згортання крові, паразитарних і онкологічних захворюваннях

Ключові слова: лейкоцити, фагоцитарний індекс, НСТ-тест, антитіла, гранулоцити, імунітет