Effects of allogeneic blood transfusion on the immunity parameters in recipient rabbits

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Article info
Received 10.04.2023
Received in revised form 12.05.2023
Accepted 24.05.2023

Introduction

Transfusion of blood or plasma can cause serious and potentially life-threatening reactions in the recipients. Over the recent decade, transfusion medicine has advanced, and currently many canine patients receive blood or its components. Plasma components such as fresh-frozen plasma (FFP) are mostly used for treatment of coagulopathies (Santo-Domingo & Le-...
those of researches on critical patients. In 76% of such patients, there
developed post-transfusion neutrophilic leukocytosis. Furthermore, there
was seen a positive correlation between leukocytosis and concentration of
IL-8 in the erythrocyte mass. Moreover, the patients that had received
allogenic blood transfusion (Izbicki et al., 2004) had no clinical signs
characteristic of acute allergic reactions.

Insignificant blood-transfusion-caused immunological reactions were
also observed in the clinically healthy dogs after autologous-blood transfu-
sion. The researchers (McMichael et al., 2010) noted increase in seg-
mented-nucleus neutrophils, fibrogen, and C-reactive protein in the recip-
ient animals after transfusion of 21-day erythrocytes.

Studies also revealed that similar immune reaction was observed in
healthy dogs that had received transfusion of 28-day erythrocytes. Those
animals were seen to have neutrophilic leukocytosis and increase in the
level of proinflammatory cytokine monocyte chemoattractant protein-1
(MCP-1). Results of the both studies demonstrate that transfusion of eryth-
rocytes can cause immunological reactions in healthy dogs, while causing
no clinical signs of the transfusion reaction (changes in frequency of car-
diac contractions, respiration rate, and body temperature). Also, there is a
need for studies and scientific substantiations of immunological reactions
in clinically healthy animals, and also feline and canine patients after blood
transfusion (Callan et al., 2013).

According to the results of the study (Egorov et al., 2022), after allo-
geneic transfusion of whole blood to the recipient rabbits, the content of
M-class immunoglobulins in blood serum increased on the 3rd day of the
experiment, and decreased on the 23rd day.

After allogeneic transfusion of whole blood to the recipient rabbits,
the contents of G and A-class immunoglobulins in blood serum decreased
over a 23-day experiment. Allogeneic transfusion of whole blood to the recip-
et rabbits activated the formation of circulating immune complexes in
blood serum of the animals, which settle in the perivascular space and
cortex of the kidney, and activate complement and inflammation (Egorov
et al., 2022).

The literature data (Drannik, 2010) suggest that humoral specific im-
munity is realized by B-lymphocytes that synthesize five classes of im-
munoglobulins. Cell-mediated immunity is represented by population of
T-lymphocytes, including helpers, killers, and suppressors. A peculiarity
of specific immunity is that T- and B-lymphocytes have antigen-identifying
receptors on their surface, with the help of which there occurs the process of identification of antigen and differentiation of self- and non-self
antigens. If necessary, with time, there activate mechanisms of synthesis
of antibodies, or T-lymphocyte-killers, which have a clear specificity to
antigens. Once an immune reaction dies out, there remains a specific im-
munological memory that allows the immune system of animals to quick-
ly react if an antigen invades the organism again (Rabson et al., 2006; Drannik, 2010).

The objective of the study was determining the influence of allogenic
transfusion of whole blood on changes in the immune reaction of the recip-
ient animals at cellular level of the recipients’ blood.

Materials and methods

The experiments on the animals were carried adhering to the re-
quirements of the General Ethical Principles of Performing Experiments
on Animals, approved by the 1st National Congress of Bioethics and the
Positions of the European Convention for the Protection of Vertebate
Animals used for Experimental and other Scientific Purposes, and the
Law of Ukraine on Protection of Animals from Abuse. A permit for using
animals in the experiments according to the scheme was received from the
local commission of Bioethics of the National University of Life and
Environmental Sciences of Ukraine (as of 10/27/2020), Protocol No. 31-1.

The studies were carried out at the basis of the Bank of Animal Blood
NNL of the Department of Surgery and Pathophysiology named after
academician I. O. Povazhanko of the National University of Life and
Environmental Sciences of Ukraine and in the conditions of the Vetmed-
servis Education-Research Center. In the experiments, we used five cli-
nically healthy White Pannon rabbits. The diet of the experimental animals
were given in the nutrition and biologically active compounds. The animals had free access to water and fodder. Blood of the donor ani-
mals was drawn from the jugular vein using semi-closed method. In the
place from blood was to be drawn, the hair of the animals was shaved, and
the skin was treated with 70% alcohol solution. During blood collection,
the animals were fixated so the tissues were moderately stretched in the
region of the jugular vein. The head of an animal was pulled away from
the blood draw. In the area of the upper and middle thirds of the neck, we
performed puncture of the jugular vein. At the angle of 45°, we injected
the needle, puncturing the skin and the vein wall against the blood flow.

For convenience of the procedure, the vein below the puncture region was
pressed by the finger of left hand. The samples of donor blood from the
rabbits were collected into polymer containers with anticoagulant CPDA
(citrate, phosphate, dextrose, and adenine) (Malyuk et al., 2022). The recip-
ient rabbits received transfused allogeneic whole blood in the amount of
5.5 mL/kg of body weight. The materials for the study were the blood
samples taken from five rabbits on the 3rd, 7th, and 23rd days after trans-
fusion.

The morphological studies of blood (number of leukocytes, lympho-
cytes, monocytes, and granulocytes) of the animals were performed using a
Mindray BC-2800 Vet hemotological analyzer (China).

Amount of T-lymphocytes in blood of the rabbits was examined with
the use of ram erythrocytes by the method of spontaneous rosette forma-
tion according to Jondal et al. (1972). The method of identification of T-
lymphocyte number is based on the interaction between membrane
receptors of lymphocytes and indicator cells (ram erythrocytes). Accord-
ing to the number of erythrocytes, adsorbed by one lymphocyte, we
determined extent of the activity of T-cells, because the phenomenon of
rosette formation is conditioned by amount of receptors on their surface
(Mazurkevich et al., 2014).

Amount of B-lymphocytes in blood of the rabbits was measured ac-
cording to Mazurkevich et al. (2014). B-lymphocytes on their surface
have receptors to C3 component of the complement, though have no re-
ceptors to ram erythrocytes, and therefore a direct interaction of such
erythrocytes with B-lymphocytes is impossible. Combining erythrocytes
and B-lymphocytes requires mediation by the complement and anti-
erythrocyte antibodies. For this purpose, erythrocytes were processed
using hemolytic serum containing anti-erythrocyte antibodies. There
formed an antigen-antibody complex, with which the complement
bonded. Because receptors to C3 component of the complement have B-
lymphocytes, during a rosette formation (by number of lymphocytes that
linked three and more erythrocyte-antibody-complement complexes), one
can conclude on the number of B-lymphocytes (Fig. 1) (Mazurkevich
et al., 2014). Number of O-cells was counted as follows: from 100% overall
lymphocytes, we subtracted the total numbers of T-lymphocytes and
B-lymphocytes (Duda & Prus, 2019).

The data were statistically analyzed using ANOVA and the Tukey’s
test. The difference between the parameters was considered significant at
P < 0.05.

Results

Results of studying quantitative value and percentage parameter of leukocytes and their subpopulations in blood of rabbits on days 3, 7, 23 of the experiment after allogeneic transfusion are presented in Table 1 and Figure 1. As Table 1 demonstrates, after allogeneic transfusion, the number of leukocytes in blood of the recipient rabbits increased by 35.3%, 74.9%, and 43.1% on the 3rd, 7th, and 23rd days of the experiment, respectively. That is, in the recipient animals, post-transfusion leukocytosis took place. We should note that increase in the number of leukocytes in the experimental animals was within the physiological parameters.

Table 1

<table>
<thead>
<tr>
<th>Cells</th>
<th>Initial condition</th>
<th>3rd day</th>
<th>7th day</th>
<th>23rd day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, 10^9/L</td>
<td>5.06 ± 0.34</td>
<td>6.82 ± 0.89</td>
<td>8.78 ± 1.74</td>
<td>7.16 ± 1.20</td>
</tr>
<tr>
<td>Lymphocytes, 10^9/L</td>
<td>2.96 ± 1.37</td>
<td>3.94 ± 0.78</td>
<td>5.88 ± 1.12</td>
<td>4.70 ± 0.83</td>
</tr>
<tr>
<td>Monocytes, 10^9/L</td>
<td>0.40 ± 0.16</td>
<td>0.22 ± 0.08</td>
<td>0.30 ± 0.16</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>Granulocytes, 10^9/L</td>
<td>1.70 ± 0.62</td>
<td>2.51 ± 0.38</td>
<td>2.58 ± 0.33</td>
<td>2.15 ± 0.22</td>
</tr>
</tbody>
</table>

Note: different letters indicate significant difference between each other within one line of the table according to the Tukey test (P < 0.05) with the Bonferroni correction.

Compared with the initial condition, number of lymphocytes in the rabbits after transfusion of whole blood increased by 44.8%, 112.2%, and 69.0% on the 3rd, 7th, and 23rd days of the experiment, respectively. Quantity of monocytes in blood of the experimental animals decreased compared with the initial condition on days 3 and 23 of the experimental studies by two times, and by 25% on the 7th day of the experiment. Number of granulocytes in blood of the experimental animals was also greater than initially on days 3, 7, and 23 of the experiment, by 47.1%, 52.9%, and 52.9%, respectively. Therefore, allogeneic transfusion of blood to the recipient rabbits caused leukocytosis throughout the study period by increasing lymphocytes and granulocytes.

Percentage of lymphocytes (Fig. 2) in the rabbits after transfusion of whole blood had not significantly changed by the 3rd, 7th, and 23rd days of the experiment.

Fig. 2. Dynamics of changes in the ratio of lymphocytes in blood of the rabbits following whole-blood transfusion (x ± SD, n = 5): ordinate indicates the share of lymphocytes (%); no significant (P < 0.05) differences between the groups were

Percentage of monocytes (Fig. 3) in the rabbits after transfusion of whole blood significantly dropped 2.7, 2.4, and 2.8 times on days 3, 7, and 23 of the experiment, respectively.

Granulocyte percentage (Fig. 4) in the rabbits following whole-blood transfusion did not change significantly.

We should note that we observed no rise in body temperature and no clinical signs of allergic reactions in the recipient rabbits. Percentage of T-lymphocytes in the recipient rabbits (Table 2) after transfusion of whole blood significantly increased compared with the initial condition on the 3rd day of the experiment (by 35.3%). On the 23rd day, percentage of T-lymphocytes significantly decreased (by 21.2%). Such changes in T-lymphocytes in the recipient animals resulted from antigen (humoral and cellular) irritation during allogeneic transfusion. Percentage of B-lymphocytes in the recipient rabbits (Table 2) did not change after allogeneic-blood transfusion.

Fig. 3. Dynamics of changes in monocytes in blood of the rabbits following allogeneic whole-blood transfusion (x ± SD, n = 5): ordinate indicates the share of monocytes (%); different letters indicate samplings that significantly differ one from another according to the Tukey test with the Bonferroni correction.

Fig. 4. Dynamics of content of granulocytes in blood of the rabbits following allogeneic-whole-blood transfusion (x ± SD, n = 5): ordinate indicates the share of granulocytes (%); no significant differences (P < 0.05) between the groups were found according to the Tukey test.

Table 2

<table>
<thead>
<tr>
<th>Cells</th>
<th>Initial condition</th>
<th>3rd day</th>
<th>7th day</th>
<th>23rd day</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-lymphocytes</td>
<td>52.9 ± 2.4a</td>
<td>71.6 ± 4.9b</td>
<td>57.6 ± 9.2c</td>
<td>41.7 ± 19.9a</td>
</tr>
<tr>
<td>B-lymphocytes</td>
<td>19.0 ± 2.5c</td>
<td>19.4 ± 1.8b</td>
<td>21.4 ± 2.1a</td>
<td>24.5 ± 17.7ab</td>
</tr>
<tr>
<td>O-lymphocytes</td>
<td>28.1 ± 1.3a</td>
<td>9.0 ± 1.0c</td>
<td>21.4 ± 1.3b</td>
<td>33.8 ± 13.3c</td>
</tr>
</tbody>
</table>

Note: see Table 1.

Number of T-lymphocytes (Fig. 5) in the experimental animals increased by 92.3%, 2.2 times and 28.2% on days 3, 7, and 23 of the experiment, respectively, compared with the initial number.

Compared with the initial number, B-lymphocytes (Fig. 6) in the experimental animals increased by 2.3 and 2.1 times on the 7th and 23rd days of the experiment, respectively. Therefore, after allogeneic blood transfusion, the number of B-lymphocytes in the recipient animals had been increasing throughout the experiment, indicating activation of specific link of immunity. Number of O-lymphocytes (Fig. 7) in the experimental animals decreased compared with the initial number by 2.2 times on the 3rd day of the experiment and increased by 56.6% and 2.0 times on the 7th and 23rd days, respectively. The increase in O-lymphocytes on the 7th and 23rd days of the experiment clearly indicates the activation of the immune response not only by specific link of immunity but by non-specific as well.
nary specialists. Use of whole blood and blood components for treating anemia in animals draws the attention of practicing veterinary doctors. However, reaction of the immune system in recipient animals to transfusion is concerning (Callan et al., 2013; Suddock et al., 2019).

Errors in blood transfusion in veterinary clinics occur during practice of drawing, processing, storing, and transfusing blood. One of the complications associated with blood transfusion is immune reaction that varies by severity from light signs to potentially lethal reactions. Most often, a recipient animal has an immune response not only to transplanted erythrocytes but also products of cell life that formed during storage. While blood components are being stored, pro-inflammatory cytokines form, which can lead to acute immune reactions in animals receiving a transfusion. Concentration of pro-inflammatory cytokines depends on duration of blood storage. Also, immune reactions in recipient animals after whole-blood and blood-component transfusion occur because of neglect by veterinary-clinic personnel regarding identification of blood group and performing a crossmatching (major and minor) (Poh, 2018).

Analysis of the results we obtained suggest that whole-blood transfusion to the recipient rabbits can be positive because allogeneic transplantation of blood to the recipient animals caused no clinical picture of immune response: rectal temperature, pulse rate, and respiratory rate remained within the physiological parameters, and also we observed no TRALI and DIC syndrome. Our results are consistent with the study by Brand (2002).

It has to be noted that after transfusion of whole blood to the recipient animals, we saw leukocytosis in the rabbits on the 3rd, 7th, and 23rd days of the experiment. Our results correlate with the studies by Claus et al. (2022). We also observed that the number of granulocytes in the blood of the experimental animals was higher than the initial amount throughout the study. Our studies are coherent with the studies by a number of authors (Izbicki et al., 2004; McMichael et al., 2010; Callan et al., 2013).

After the transfusion of whole blood, percentages of T, B, and O-lymphocytes in the recipient rabbits also significantly increased compared with the initial number, which is consistent with the experimental data from a number of authors (Izbicki et al., 2004; McMichael et al., 2010; Callan et al., 2013). Therefore, the studies we performed regarding allogeneic transplantation of whole blood suggest that transfusion of whole blood causes a cascade of complex immunological reaction in the recipient animals. We should note that after allogeneic transfusion of whole blood to the recipient rabbits, we observed no pronounced post-transfusion clinical symptoms (tachycardia, tachypnea, edema of the eyelids, auricles, body-temperature rise). Results of those studies are consistent with the data from a number of authors (Izbicki et al., 2004; McMichael et al., 2010).

Discussion

Transfusion of blood and blood components has been extremely relevant in veterinary medicine over the recent years. At the same time, after allogeneic blood transfusion to the recipient animals, there occurs an immune response (Malinak et al., 2022). Active and mostly efficient transfusion of blood and its components in veterinary medicine is performed in case of various-genesis anemia (post-hemorrhagic, hemolytic, aplastic) in animals (Yagi et al., 2016; Yehorov et al., 2020). As is known, anemia in animals occurs quite often and requires immediate interference of veterinary specialists. Use of whole blood and blood components for treating anemia in animals draws the attention of practicing veterinary doctors. However, reaction of the immune system in recipient animals to transfusion is concerning (Callan et al., 2013; Suddock et al., 2019).

Analysis of the results we obtained suggest that whole-blood transfusion to the recipient rabbits can be positive because allogeneic transplantation of blood to the recipient animals caused no clinical picture of immune response: rectal temperature, pulse rate, and respiratory rate remained within the physiological parameters, and also we observed no TRALI and DIC syndrome. Our results are consistent with the study by Brand (2002).

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After the transfusion of whole blood, percentages of T, B, and O-lymphocytes in the recipient rabbits also significantly increased compared with the initial number, which is consistent with the experimental data from a number of authors (Izbicki et al., 2004; McMichael et al., 2010; Callan et al., 2013). Therefore, the studies we performed regarding allogeneic transplantation of whole blood suggest that transfusion of whole blood causes a cascade of complex immunological reaction in the recipient animals. We should note that after allogeneic transfusion of whole blood to the recipient rabbits, we observed no pronounced post-transfusion clinical symptoms (tachycardia, tachypnea, edema of the eyelids, auricles, body-temperature rise). Results of those studies are consistent with the data from a number of authors (Izbicki et al., 2004; McMichael et al., 2010).

Conclusion

We determined that the recipient animals underwent post-transfusion leukocytosis. We should note that increase in the number of leukocytes in the experimental animals was within the physiological parameters. According to the results of the study, the amount of lymphocytes in the recipient rabbits increased after whole-blood transfusion by 41.2%, 2.1 times, and 65.5% on the 3rd, 7th, and 23rd days of the experiment, respectively. Percentage of T-lymphocytes in the recipient rabbits after transfusion of blood increased by 35.3 % and 8.9% on the 3rd and 7th days of the experiment. Our results correlate with the studies by Claus et al. (2022). We also observed that the number of granulocytes in the blood of the experimental animals increased compared with the initial condition by 48.8%, 51.8%, and 26.5% on the 3rd, 7th, and 23rd days of the experiment, respectively. Number of granulocytes in the blood of the experimental animals increased compared with the initial amount. Number of granulocytes in the blood of the experimental animals increased compared with the initial condition by 48.8%, 51.8%, and 26.5% on the 3rd, 7th, and 23rd days of the experiment, respectively. Percentage of T-lymphocytes in the recipient rabbits after transfusion of blood increased by 35.3 % and 8.9% on the 3rd and 7th days of the experimental studies, respectively. We should also note a significant 21.2% decrease in the percentage of T-lymphocytes on the 23rd day. Percentage of B-lymphocytes in the recipient rabbits after transfusion of allogeneic blood increased by 2.1%, 12.6%, and 28.9% on days 3, 7, and 23, respectively, indicating the activation of specific link of immunity. Following the transfusion of whole blood, percentage of O-lymphocytes in the recipient rabbits dropped 3.1 times and by 25.3% on days 3 and 7 of the experiment, respectively. On the 23rd day of the experiment, this parameter increased by 20.3%. The perspectives for further research are study of allogeneic transfusion of blood components and parameters of specific and non-specific links of immunity in the recipient animals.

The authors declare no conflict of interest.

References


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