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Original Article

Preoperative autologous blood donation causes rapid recovery of hematopoietic function of bone marrow in rabbits with acute hemorrhagic shock

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Abstract: *Objective:* Our study aimed to explore the effect of preoperative autologous blood donation (PABD) on hematopoiesis of bone marrow in cases of acute hemorrhagic shock. *Methods:* Rabbits were randomly assigned into a control group (CON), allogeneic blood transfusion group (ABT), autologous blood storage group (ABS), and preoperative autologous blood transfusion group (PAT). The influence of autologous blood storage and transfusion on hematopoietic cells in bone marrow was analyzed. *Results:* Compared with CON group, Hb in stored blood of ABS group decreased significantly ($P<0.05$) and Hb in PAT group also decreased significantly after storage and before shock induction ($P<0.05$). Compared with ABT group, RET% in PAT group and ABS group increased significantly after storage ($P<0.05$). Compared with CON group and ABT group, serum IL-3 and IL-11 in ABS group and PAT group increased significantly ($P<0.05$). Compared with CON group and ABT group, number of nucleated cells in bone marrow increased significantly in ABS and PAT group ($P<0.05$). Compared with CON and ABT group, proportion of bone marrow cells in G_0/G_1 phase decreased significantly in ABS and PAT group ($P<0.05$) but that in S and G_2/M phase increased significantly ($P<0.05$). Compared with CON group, proportion of bone marrow cells in G_0/G_1 phase increased significantly in ABT group ($P<0.05$) but that in S and G_2/M phase decreased significantly ($P<0.05$). *Conclusion:* PABD may reduce Hb and red blood cells in peripheral blood but levels rapidly return to baseline after transfusion. This is better than allogeneic blood transfusion.

Keywords: Preoperative autologous blood donation, shock, hemorrhage, bone marrow cells, nucleated cell counting

Introduction

Preoperative autologous blood donation (PABD) has been an important technique in clinical autologous blood transfusions and has been used in some special patients, such as those receiving major surgery and critically ill patients [1, 2]. Clinical studies have shown that red blood cells (RBCs) could decrease during autologous blood storage, possibly increasing perioperative incidence of iatrogenic anemia [3]. There is evidence showing that risk for perioperative anemia and blood waste increased in patients receiving PABD compared with patients not receiving pre-operative PABD [4, 5]. These significantly limit the clinical application of PABD. However, Delasotta et al. [6] found that use of erythropoietin-α (EPO-α) in patients with mild anemia, to increase preoperative Hb, could reduce the time of postoperative blood transfusions and hospital stays. In addition, some investigators have proposed that Hb reduction might induce increase in EPO as a feedback, leading to rapid recovery of RBC into baseline. Thus, recovery of RBC is more rapid after PABD in anemia patients compared with healthy subjects [7].

Hematopoietic stem cells are a group of special cells in hematopoietic tissues. They have high self-renewal or self-replication capability and can differentiate into precursor cells of other types, which is crucial for homeostasis and tissue repair [8]. In normal bone marrow, most hematopoietic cells are in the quiescent phase. After stimulation, these cells may enter the proliferative and division phase to regulate peripheral cells [9]. In this study, routine blood tests

Table 1. General conditions of rabbits in different groups

Group	n	Body weight (kg)	Dose of anesthetics used (ml)	Volume of blood collected (ml)
CON	6	2.12±0.18	7.3±0.8	0
ABT	6	2.13±0.14	7.5±0.5	0
ABS	6	2.17±0.12	7.7±0.5	40.0±3.2
PAT	6	2.32±0.16	7.8±0.4	41.7±2.7

Data are shown as mean ± SD.

and detection of serum IL-3 and IL-11 were performed in autologous blood storage and blood transfusion. The number of nucleated cells and cell cycle of bone marrow were determined, aiming to explore the influence of autologous blood transfusions on hematopoietic cells in bone marrow. Whether PABD may directly affect the hematopoietic microenvironment requires further study and analysis. In our present study, acute hemorrhagic shock was induced in rabbits followed by resuscitation, aiming to explore the influence of autologous blood storage and transfusion on hematopoietic cells in bone marrow.

Materials and methods

Animals and grouping

Male New Zealand rabbits (n=24; specific pathogen free), weighing 1.9-2.4 kg, were purchased from Songlian Experimental Animal Center in Shanghai (Lot number: SCXK[Hu]-2012-0011). Animals were randomly assigned into four groups with a random number table (n=6 per group): control group (CON), allogeneic blood transfusion group (ABT), autologous blood storage group (ABS), and preoperative autologous blood transfusion group (PAT). Animals were given ad libitum access to water and food. All procedures were performed according to Animal Welfare and Use in Research.

Autologous blood collection and storage

Blood (about 12% of total blood volume) was collected from the ear central artery at a speed of 3-5 ml/min under an aseptic condition. Arterial blood was preserved in a bag containing sodium citrate solution. The bag was sealed, the number of the rabbit and date of blood collection were marked on the bag. The bag was stored at 4-6°C. The above procedures were repeated three times in ABT group and PAT group with an interval of 1 week. Last blood collection was performed 72 hours before shock induction.

Shock induction and resuscitation

In ABS group and PAT group, bloodletting was done at a rate of 3 ml/min/kg until mean arterial pressure (MAP) reached 40 mmHg and maintained for 30 minutes, suggesting successful establishment of an acute hemorrhagic shock (AHS) model [10]. In ABT group, stored allogeneic blood of ABS group was transfused via the right femoral vein. In PAT group, autologous blood was transfused via the right femoral vein. Blood transfusion was performed at a rate of 3 ml/min until MAP returned to baseline levels. In CON group and ABS group, only the femoral artery and vein were separated without induction of shock.

Collection of bone marrow

At 24 hours after resuscitation, rabbits were anesthetized and bone marrow biopsy was performed at 1 cm below the tibial plateau after sterilization. About 1 mL of bone marrow was collected into a tube containing 125 U of heparin. Bone marrow was filtered through a filter for blood transfusion and the resultant bone marrow was anticoagulated for further use.

Detection of serum IL-3 and IL-11

At five time points [before blood storage (T_1), after blood storage (T_2), before hemorrhagic shock (T_3), resuscitation 0 min (T_4), resuscitation 24 h (T_5)], peripheral blood (2.0 mL) was collected. Centrifugation was done 2 hours later at 3000 rpm/min for 5 minutes. Serum was collected and stored at -20°C. Serum contents of IL-3 and IL-11 were determined by enzyme-linked immunosorbent assay.

Counting of nucleated cells and cell cycle of bone marrow

The above bone marrow (20 μL) was mixed with 380 μL of 1% HCl and then 20 μL of resultant solution was used for cell counting. Nucleated cells were counted within 4 grids, followed by averaging. According to results from cell counting, 5×10^5 of bone marrow cells were lysed with

Table 2. Hb and RET at different time points in four groups

Parameters	Group	n	T_1	T_2	T_3	T_4	T_5
Hb (g/L)	CON	6	118.5±7.8	117.5±7.7	117.2±5.9	116.8±7.7	117.3±6.3
	ABT	6	119.0±7.5	118.5±9.1	117.5±7.0	106.3±9.0[a,c]	108.5±9.7
	ABS	6	117.3±7.8	106.7±6.9[a,b,c]	104.0±7.3[a,b,c]	105.7±6.2[a,c]	105.8±7.3[a,c]
	PAT	6	116.5±8.2	106.0±5.2[a,b,c]	104.0±5.0[a,b,c]	111.5±8.5	113.8±7.9
RET (%)	CON	6	2.99±0.63	3.19±0.67	3.10±0.55	3.03±0.56	3.13±0.64
	ABT	6	2.97±0.55	2.98±0.48	3.04±0.41	2.94±0.50	2.95±0.58
	ABS	6	3.11±0.59	5.32±1.05[a,b,c]	5.32±0.90[a,b,c]	5.26±1.01[a,b,c]	4.98±0.90[a,b,c]
	PAT	6	3.00±0.57	5.10±1.03[a,b,c]	5.12±0.97[a,b,c]	5.12±0.80[a,b,c]	4.98±0.83[a,b,c]

Data are shown as mean ± SD. [a]*P*<0.05 *vs*. CON group; [b]*P*<0.05 *vs*. ABT group; [c]*P*<0.05 *vs*. T_1.

Table 3. WBC, RBC, and PLT at different time points in four groups (n=6)

Parameters	Group	n	T_1	T_2	T_3	T_4	T_5
WBC ($\times10^9$/L)	CON	6	9.3±2.1	9.1±2.3	9.5±3.0	9.7±3.5	9.7±3.8
	ABT	6	9.6±2.6	9.5±2.8	10.1±3.3	11.7±4.0	13.5±3.7
	ABS	6	9.2±2.2	8.8±3.0	9.4±3.1	10.0±3.3	10.2±3.4
	PAT	6	8.9±1.8	9.1±2.4	9.6±3.4	11.7±3.5	12.2±3.6
RBC ($\times10^{12}$/L)	CON	6	5.5±0.6	5.6±0.5	5.4±0.6	5.5±0.7	5.3±0.8
	ABT	6	5.4±0.8	5.5±0.8	5.3±0.7	5.0±0.6[a,c]	5.1±0.6[a,c]
	ABS	6	5.5±0.8	4.7±0.7[a,b,c]	4.7±0.7[a,b,c]	4.7±0.8[a,b,c]	4.8±0.7[a,b,c]
	PAT	6	5.6±0.6	4.7±0.5[a,b,c]	4.8±0.5[a,b,c]	5.4±0.8	5.4±0.8
PLT ($\times10^9$/L)	CON	6	406.3±92.3	415.6±103.6	411.1±107.8	408.7±104.3	413.0±98.1
	ABT	6	403.0±133.8	422.1±126.5	423.5±117.2	414.3±122.5	407.7±130.8
	ABS	6	418.2±121.8	413.4±116.4	409.4±115.4	405.7±118.0	411.6±120.5
	PAT	6	429.0±146.0	419.8±136.1	421.6±127.8	417.4±126.9	430.4±136.9

Data are shown as mean ± SD. [a]*P*<0.05 *vs*. CON group; [b]*P*<0.05 *vs*. ABT group; [c]*P*<0.05 *vs*. T_1.

1 mL of RBC lysis buffer for 5 minutes, followed by centrifugation at 1000 rpm/min for 5 minutes. The supernatant was collected and mixed with 1 mL of pre-cold PBS for re-suspension. The solution was transferred into 1.5 mL centrifuge tube. Cells in this solution were washed twice with PBS and then fixed in 1 mL of pre-cold 70% ethanol over night. After washing in PBS twice, centrifugation was done and 0.5 mL of propidium iodide solution was added. Cells were resuspended and incubated at 37°C for 30 minutes in the dark. Cell cycle was detected by flow cytometry and data were analyzed with Cell Quest software.

Statistical analysis

Statistical analysis was performed with SPSS version 13.0. Quantitative data are expressed as mean ± standard deviation. After homogeneity of variance test, one way analysis of variance (ANOVA) and LSD tests were employed for comparison of data among groups.

Results

General conditions of rabbits in different groups

There were no significant differences in body weight and dose of anesthetics used. The volume of blood collected was also comparable between ABS group and ABT group (**Table 1**).

Routine blood test

When compared with CON group, Hb reduced significantly after storage in ABS group and still remained at a low level at T_5 (P<0.05). Hb reduced significantly after storage and before shock induction in PAT group (P<0.05) but increased significantly immediately and at 24 hours after resuscitation (P<0.05). When compared with ABT group, Reticulocyte % increased significantly after storage in PAT group and ABS group (P<0.05), still remaining at a high level 24 hours after resuscitation (P<0.05). In

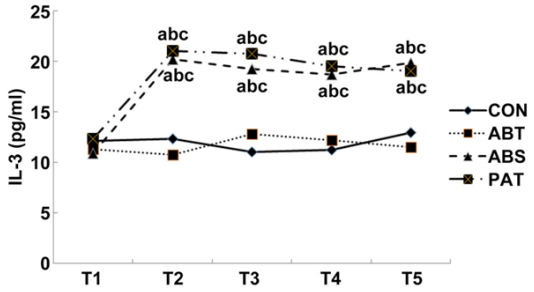

Figure 1. Serum IL-3 content at different time points in four groups ($\bar{x} \pm s$, n=6). [a]*P*<0.05 *vs.* CON group; [b]*P*<0.05 *vs.* ABT group; [c]*P*<0.05 *vs.* T_1.

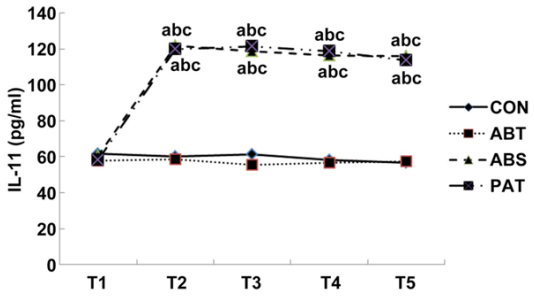

Figure 2. Serum IL-11 content at different time points in four groups ($\bar{x} \pm s$, n=6). [a]*P*<0.05 *vs.* CON group; [b]*P*<0.05 *vs.* ABT group; [c]*P*<0.05 *vs.* T_1.

Table 4. Number of nucleated cells in the bone marrow of rabbits in 4 groups

Group	n	Number of nucleated cells ($\times 10^7$/ml)
CON	6	3.16±1.09
ABT	6	2.28±0.92
ABS	6	6.30±1.75[a,b]
PAT	6	5.64±2.42[a,b]

[a]*P*<0.05 *vs.* CON group; [b]*P*<0.05 *vs.* ABT group.

Table 5. Cell cycle of bone marrow at 24 hours after resuscitation in 4 groups

Group	n	G_0/G_1	S	G_2/M
CON	6	83.66±2.55[b]	10.71±2.13[b]	5.46±1.51[b]
ABT	6	94.21±3.57[a]	4.40±2.26[a]	1.40±1.66[a]
ABS	6	75.42±3.67[a,b]	16.27±1.56[a,b]	8.14±2.58[b]
PAT	6	75.75±4.44[a,b]	17.54±2.72[a,b]	6.71±2.39[b]

Data are shown as mean ± SD. [a]*P*<0.05 *vs.* CON group; [b]*P*<0.05 *vs.* ABT group.

the four groups, WBC count and PLT count were comparable at different time points (P>0.05). When compared with T_1, the RBC in PAT group reduced significantly at T_2 and T_3 (P<0.05) but returned to baseline level at T_4 (P>0.05). In ABT group, RBC reduced significantly (P<0.05) and still remained at a low level at T_5 (P<0.05 vs. baseline) (**Tables 2** and **3**).

Serum IL-3 and IL-11 in peripheral blood

When compared with CON group and ABT group, serum contents of IL-3 and IL-11 increased significantly in ABS group and PAT group after storage (P<0.05). When compared with T_1, serum contents of IL-3 and IL-11 increased significantly in ABS group and PAT group after storage (P<0.05) and remained at a high level 24 hours after resuscitation (P< 0.05) (**Figures 1** and **2**).

Nucleated cell counting

Compared with CON group and ABT group, the number of nucleated cells in bone marrow increased significantly in ABS group and PAT group (P<0.05) (**Table 4**).

Cell cycle of bone marrow (flow cytometry)

Compared with CON group and ABT group, the proportion of cells reduced significantly in G_0/G_1 phase and increased significantly in S phase and G_2/M phase in ABS group and PAT group (P<0.05). Compared with CON group, the proportion of cells in G_0/G_1 phase increased significantly in ABT group but that in S phase and G_2/M phase reduced significantly (P<0.05) (**Table 5** and **Figure 3**).

Discussion

PABD entails the collection and storage of patient blood in the preoperative period (typically, days to weeks before surgery), to be later used in an emergency or surgery. However, several studies have shown that autologous blood storage may reduce RBC to different extents and even increase perioperative incidence of iatrogenic anemia, significantly limiting wide application of PABD [3, 11]. In this study, our results confirmed that autologous blood storage could reduce peripheral Hb and RBC, leading to the failure of preoperative RBC recovery. There is evidence showing that the median time to recovery of RBC in healthy volunteers is 36 days, after donation of 550 mL of blood in the absence of iron supplement [12]. Thus, some clinicians [13] have proposed that autologous blood collection should be done four

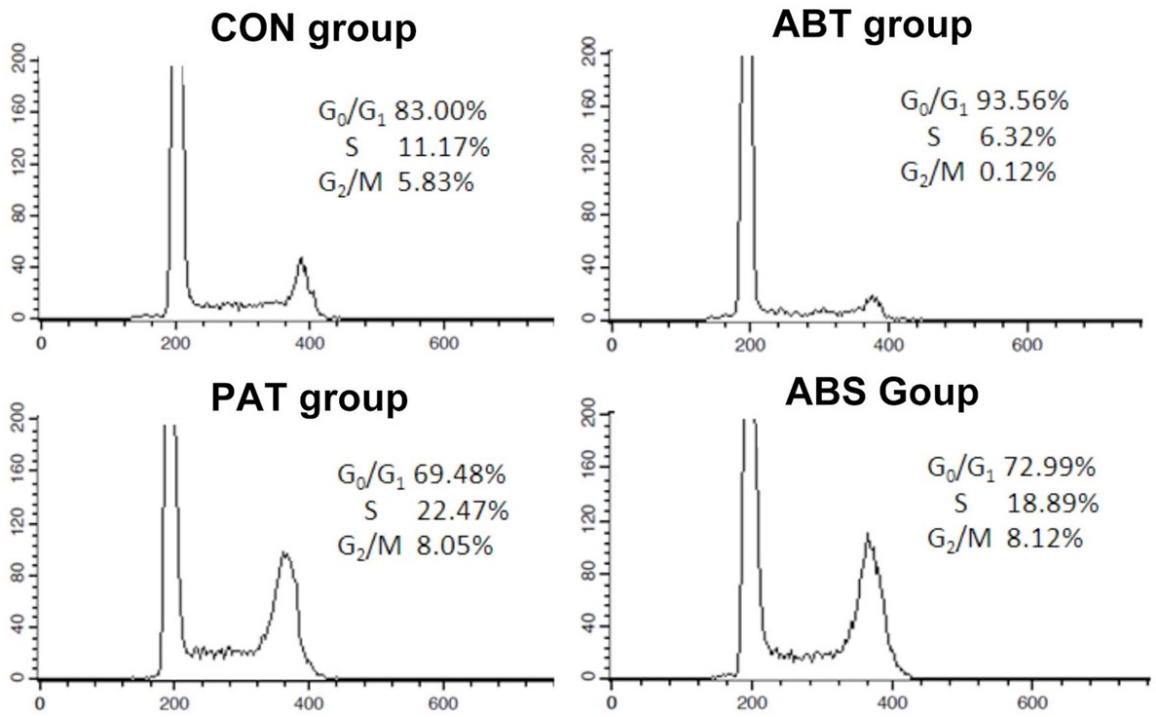

Figure 3. Cell cycle of bone marrow at 24 hours after resuscitation in 4 groups.

weeks before surgery, possibly allowing recovery of 90% of stored RBC. However, the detrimental influence of blood storage and exceeding time limits of blood storage will be unavoidable in some environments. In this study, we used the recommended method for autologous blood storage, according to the American Association of Blood Banks (AABB) [14]. Under this condition, the body is in a state of subacute hemodilution which is clinically important for reduction in intra-operative loss of RBC [15].

In addition, our results also indicate that RET of peripheral blood increased significantly after autologous blood collection and remained at a high level 24 hours after resuscitation. Reticulocytes refer to immature RBCs and mainly reflect the hematopoietic state of erythrocyte lineage in bone marrow. Thus, they have been used as an important marker of bone marrow hematopoiesis. In blood, Hb reduces which induces elevation of EPO as a feedback. Then, EPO is able to stimulate production of reticulocytes to promote recovery of RBC. However, the number of RBC regenerated is closely related to the time from last blood collection to surgery [7, 16]. This suggests that PABD may induce production of EPO to stimulate the generation of reticulocytes, leading to rapid recovery of RBC into baseline levels.

IL-3 and IL-11 have been proven to be positive hematopoietic regulators in the hematopoietic microenvironment. IL-3 and IL-11 may act synergistically. They may stimulate the production of megakaryocyte progenitor cells and increase generation of erythrocyte, granulocyte, and megakaryocyte lineages in peripheral blood. Some studies [17, 18] have confirmed that IL-11 was effective in preventing and treating thrombocytopenia after chemotherapy and aplastic anemia and might reduce the requirement for platelet transfusion. In this present study, serum IL-3 and IL-11 increased significantly after autologous blood storage in PAT group and ABS group, lasting to 24 hours after resuscitation. In ABT group, serum IL-3 and IL-11 failed to increase. In the presence of loss of peripheral blood, a feedback may be initiated to increase hematopoietic growth factors in the blood and stimulate growth and differentiation of hematopoietic cells in bone marrow.

Nucleated cells in the bone marrow are important indicators reflecting the extent of bone marrow cell proliferation. Bone marrow hematopoietic cells experience periodic DNA replica-

tion and continuous proliferation and apoptosis, which maintains the balance between bone marrow cells and peripheral blood cells. However, cell cycle progression is regulated by multiple signaling pathways and multiple factors. Studies have revealed that induced proliferation or apoptosis of hematopoietic cells is crucial for the therapy of cancers [19]. In addition, studies have reported the clinical mobilization of bone marrow cells from quiescent (G_0/G_1) phase to proliferation phase (S and G_2/M) which is able to treat related hematological diseases and partially relieve tissue ischemia [20]. Results of our study showed that when compared with ABT group, the proportion of bone marrow cells in S phase and G_2/M phase increased significantly and number of nucleated cells elevated significantly in ABS group, suggesting that PABD promotes DNA synthesis and mitosis, induces hematopoietic cells progressing from quiescent phase to proliferation phase, and facilitates hematopoietic cells proliferation. All of these are helpful in promoting the recovery of hematopoietic function. Moreover, the proportion of bone marrow cells in G_0/G_1 phase increased significantly but that in proliferation phase reduced significantly in ABT group, suggesting that allogeneic blood transfusion may inhibit proliferation of bone marrow hematopoietic cells. The specific mechanism, however, requires further study.

These findings suggest that peri-operative mild anemia and reduction in Hb are not contradictions to PABD. On the contrary, PABD may promote division of bone marrow hematopoietic cells and maintain the proportion of bone marrow reticulocyte in a high level, which are beneficial for post-operative recovery of Hb and RBC.

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Disclosure of conflict of interest

None.

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