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Possible red blood cell damage due to iatrogenic transfusion filter-related mistakes during blood transfusion

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Abstract

Background: Red cell concentrates are usually transfused through a transfusion filter. Iatrogenic accidents can occur in which the red blood cell concentrate is transfused through the infusion filter by mistake. Therefore, we examined the extent of red blood cell damage in red cell concentrates transfused through transfusion and infusion filters *ex vivo*.

Methods: We evaluated red blood cell damage using lactate dehydrogenase, free hemoglobin, and erythrocyte membrane phosphatidylserine expression in red cell concentrates transfused through transfusion and infusion filters (transfusion rate: 100mL/h). In addition, we performed hemolytic experiments using a 50% glucose solution in red cell concentrates transfused through both filters.

Results: Red cell concentrates transfused through infusion filters revealed higher lactate dehydrogenase, free hemoglobin, and phosphatidylserine expression levels than those transfused through transfusion filters. The 50% glucose solution induced hemolysis of the red cell concentrates from the infusion filters more strongly than those from the transfusion filters.

Conclusion: An iatrogenic mistake from the transfusion filter to the infusion filter might induce red blood cell damage.

Keywords: Hemolysis, phosphatidylserine, lactate dehydrogenase, free hemoglobin, transfusion filters

Introduction

Transfusion-associated hemolysis is caused by either immune-mediated or non-immune hemolysis. Antibodies and/or complement proteins induce a hemolytic reaction during the acute (acute hemolytic transfusion reaction) [1] and delayed (delayed hemolytic transfusion reaction) phases [2]. Non-immune mediated causes of transfusion-associated hemolysis include thermal injury [3], osmotic injury [4], mechanical injury [5], infection [6], drugs [7] and others. In mechanical injuries, defective blood administration causes induced transfusion-associated hemolysis [8]. Blood components such as red cell concentrates (RCC), fresh frozen plasma, platelet concentrates, and others, are usually transfused through a transfusion filter with a pore size of 170–200μm to remove clots, aggregates, and other particles [9]. On the other hand, the therapeutic solution is usually infused intravenously through an infusion filter (intravenous fluid set) with a pore size of 15μm to remove small-sized crystals and glass. As transfusion incident reports are dependent on the practitioner's knowledge of transfusion practice, new nurses sometimes mistakenly set the transfusion route from the transfusion filter to the infusion filter [10]. There are no recent reports regarding iatrogenic administration setting mistakes in transfused patients. Therefore, we examined the effects of infusion filters on red cell damage compared with a routinely used transfusion filter *ex vivo*. As a result, blood transfused through the infusion filter might be more fragile and easily lysed by the hyperosmolar solution. We concluded that adequate transfusion filters should be used in transfusion practice.

Materials and methods

Materials

Transfusion filters were purchased from Termo Co. Ltd. (Osaka, Japan). Infusion filters were purchased from Nipro Co. (Osaka, Japan). A 50% glucose solution (Otsuka Pharmaceutical, Tokyo, Japan) was used for the hemolytic experiments. The non-irradiated RCC (Japanese Red Cross Society, Tokyo, Japan) used in the experiments were out of date. The RCC is leukocyte-depleted before storage and must be transfused within 21 days after phlebotomy in Japan; we used RCC within 1 week after the expiration date for the experiments.

Evaluation of red blood cell damage in dynamic and static tests using blood passed through the transfusion and infusion filters

In the dynamic test, RCC was transfused through the transfusion or infusion filter at a flow rate of 100mL/hour. Lactate dehydrogenase (LD) and free hemoglobin (fHb) in the RCC plasma were used as hemolytic markers and measured by BML (Tokyo, Japan). In addition, we measured phosphatidylserine (PS) exposure on the erythrocyte membrane. In the static test, RCC from the transfusion and infusion filters were co-infused with the 50% glucose solution at a 1:1 ratio (final concentration: 25% glucose solution). Thirty minutes after mixing at room

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Table 1. Red blood cell damage in blood transfused through infusion filters.

	Transfusion filter N=4	Infusion filter N=4
Lactate dehydrogenase (IU/L)	50.5 (0.5)	58.3(0.9)*
Free hemoglobin (mg/dL)	15.2 (0.3)	18.5 (0.6)*
% phosphatidylserine	0.39 (0.09)	1.04 (0.01)*

Data represent the means with standard errors in parentheses. The results are representative of 3 independent experiments.
*p<0.05 vs. transfusion filter.

Table 2. Effects of a 50% glucose solution on blood transfused through infusion filters.

	Transfusion filter N=4	Infusion filter N=4
Lactate dehydrogenase (IU/L)	198.8 (61.8)	555.8(30.9)*
Free hemoglobin (mg/dL)	94.8 (17.2)	216.3 (15.6)*
% phosphatidylserine	30.1 (1.6)	40.5 (1.0)*

Data represent the means with standard errors in parentheses. The results are representative of 3 independent experiments.
*p<0.05 vs. transfusion filter.

temperature (20°C), the co-infused blood was analyzed (LD, fHb, and PS expression).

Flow cytometric analysis of PS exposure

PS expression of the erythrocytes in the co-incubated blood was measured using a modified flow cytometric assay, as previously described [11-12]. Annexin V-fluoresceinisothiocyanate (FITC) was used as a marker for PS positivity, while anti-glycophorin A-phycoerythrin (PE) was used as an erythrocyte identifier. Sample blood was diluted 10-fold with a binding buffer (Beckman Coulter, Inc.). We added 1 μL of diluted blood to a container with 1 μL of anti-glycophorin A-PE (anti-CD235a; Beckman Coulter, Inc.) and 1 μL of annexin V-FITC (Annexin V-FITC Apoptosis Detection Kit; Beckman Coulter, Inc.). This mixture was diluted with 100 μL of binding buffer and then spatulated. Under cool temperatures and protection from light, the mixture was allowed to react for 15 min, and further diluted with 400 μL of binding buffer and analyzed following agitation. The forward scatter of the cells stained with glycophorin A was determined, and the annexin V fluorescence intensity was measured in FL-1 by fluorescence-activated cell sorting (FC500; Beckman Coulter, Inc.) with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. PS exposure was expressed as the percentage of annexin-V-binding erythrocytes. The study design was approved by the Ethics Review Board of our institution.

Statistical analysis

We compared differences between groups using Wilcoxon analysis. The data is represented as the mean ± standard error (SE). All statistical tests were conducted using JMP version 8.0 (SAS Institute, Inc., Cary, NC, USA). The level of statistical significance was set at $p < 0.05$.

Results

Ex vivo dynamic red blood cell damage in blood transfused through infusion filters

Gross hemolysis was absent in the plasma transfused through the infusion and transfusion filters at a flow rate of 100mL/h. However, the LD and free Hb levels in the blood transfused through infusion filters were significantly higher than those transfused through transfusion filters, as shown in (Table 1). In addition, erythrocyte membrane PS expression in the blood transfused through the infusion filters was significantly higher than that transfused through the transfusion filters.

In vitro hemolytic effects of a 50% glucose solution on blood transfused through an infusion filter

Gross hemolysis was present in the plasma transfused through the infusion and transfusion filters, at 30 min after mixing the co-infused blood and 50% glucose solution at room temperature (20°C).The LD and free Hb levels of co-infused blood transfused through the infusion filters with the 50% glucose solution were significantly higher than those transfused through the transfusion filters, as shown in (Table 2). In addition, PS expression on the erythrocyte membrane from the co-infused blood transfused through the infusion filter with the 50% glucose solution was significantly higher than that transfused through the transfusion filter.

Discussion

Blood transfused through an infusion filter with a pore size of 15μm was more fragile than that through a routinely used transfusion filter, as shown in (Table 1). Although no strong hemolysis was observed, these data suggested the possibility that blood transfused through an infusion filter might be lysed in transfused patients. The erythrocyte membrane PS expression in the blood transfused through the infusion filters was significantly higher than that transfused through the transfusion filters (infusion filter: 1.04±0.01%, transfusion filter: 0.39±0.09%). Since erythrocyte membrane PS expression is a marker of aging erythrocytes [13], macrophages present in the liver and spleen may easily phagocytose erythrocytes with high PS expression [14]. Therefore, erythrocytes transfused through an infusion filter might have shortened intravascular life compared with those transfused through a transfusion filter. High-percentage glucose solutions have been shown to induce hemolysis and enhance the expression of PS on erythrocyte membranes [15]. The high-percentage glucose solution (50% glucose) induced hemolysis in the co-infused blood transfused through the infusion filters to a greater extent that than that transfused through the routinely used transfusion filters. These data suggested that the erythrocytes from the blood transfused through the infusion filters were

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damaged. Erythrocyte membrane PS expression also increases in hemolytic anemia [**16**].

These data suggested that iatrogenic mistakes from the transfusion filter to the infusion filter could induce transfusion-associated hemolysis. Therefore, adequate education regarding administration settings is critical for nurses who perform blood transfusion.

Conclusions

An iatrogenic mistake from the transfusion filter to the infusion filter might induce red blood cell damage.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HF designed and planned the experiments, performed the research, and wrote the manuscript. RS performed the PS expression experiments. SN performed the experiments and analyzed the data.

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