Inflamm. res. 50 (2001) 107–111
1023-3830/01/020107-5 $ 1.50+0.20/0

Inflammation Research

Accumulation of the neutrophil-derived protein YKL-40 during storage of various blood components

C. Cintin[1], J.S. Johansen[2], F. Skov[3], P.A. Price[4] and H.J. Nielsen[1,5]

[1] Surgical Immunology Laboratory, Hvidovre University Hospital, University of Copenhagen, 2650 Hvidovre, Denmark
[2] Department of Rheumatology, Hvidovre University Hospital, University of Copenhagen, 2650 Hvidovre, Denmark
[3] Blood Transfusion Unit, Nykøbing Falster County Hospital, Denmark
[4] Department of Biology, University of California San Diego, La Jolla, CA, USA
[5] Department of Surgical Gastroenterology 435, Hvidovre University Hospital, University of Copenhagen, 2650 Hvidovre, Denmark, Fax: 0045 3632 3760, e-mail: h.j.nielsen@ofir.dk

Received 29 May 2000; returned for revision 10 October 2000; accepted by I. Ahnfelt-Rønne 20 October 2000

Abstract. *Objective and Design:* Post transfusion infectious complications associated with allogeneic blood components may depend on storage time and may be related to extracellular accumulation of bioactive substances during storage. YKL-40 is a glycoprotein located in the specific granules of the neutrophils. While exocytosed it may play a role in inflammation and remodelling of the extracellular matrix. We studied the potential accumulation of YKL-40 in blood components during storage.
Methods: Using a RIA method extracellular accumulation of YKL-40 was determined in supernatants from whole blood, plasma-reduced whole blood, buffy-coat-depleted SAGM (saline-adenine-glucose-mannitol) blood, whole blood leukocyte depleted by prestorage filtration, and whole blood leukocyte depleted by bedside filtration. The blood was donated by volunteer, healthy blood donors, and stored under standard blood bank conditions for 35 days.
Results: Extracellular accumulation of YKL-40 increased significantly in a time-dependent manner during storage for 35 days of non-filtered whole blood, plasma-reduced whole blood, and SAGM blood, respectively. Prestorage leukocyte depletion of whole blood prevented extracellular YKL-40 accumulation, while YKL-40 accumulation was not reduced by bedside leukocyte depletion.
Conclusion: YKL-40 appears to accumulate extracellularly in a time-dependent manner in standard erythrocyte components. Prestorage leukocyte depletion by filtration of whole blood may be an effective procedure to prevent extracellular YKL-40 accumulation during storage of erythrocyte components.

Key words: Blood transfusion – YKL-40 – Leuko-depletion

Correspondence to: H.J. Nielsen

Introduction

The frequency of post trauma infectious complications appears to be amplified by transfusion with allogeneic blood components [1]. The mechanisms leading to development of infectious complications in relation to blood transfusion are still not known in details, but transfusion-induced impaired immune competence, donor leukocyte surface antigens and various soluble bioactive substances may play a role [2–4]. Presumably due to disintegration of granulocytes and platelets during storage of blood components [5, 6] various granule associated bioactive substances are accumulated extracellularly in a time-dependent manner [4, 7]. Some of these substances, such as myeloperoxidase (MPO), eosinophil cationic protein (ECP), eosinophil protein X (EPX), histamine, neutrophil elastase, activated complement 3 (C3a), and vascular endothelial growth factor (VEGF), are well known to play significant roles in inflammation and infectious complications [4, 7, 8]. Thus, results from clinical studies have suggested that post trauma morbidity and mortality in relation to blood transfusion may be related to storage time of the transfused components [9–12].

Pulmonary complications, including pneumonia [11–14] are frequently observed after blood transfusion, and particularly, the rare but severe lung complication adult respiratory distress syndrome may be related to transfusion with blood components [15, 16]. Thus, transfusion-induced impaired immune competence to bacterial contamination [17], and substances from the liquid portion of blood components may be involved in development of lung complications [3, 18, 19]. Impaired immune competence [20] and post trauma pneumonia [10] in relation to transfusion may also be dependent on storage time of the blood.

Specific substances such as MPO and elastase derived from the neutrophilic granulocytes are suspected to be involved in pneumonia [21]. However, other neutrophil derived proteins may as well participate. YKL-40, a matrix protein of

specific granules (22), is elevated in serum of patients with pneumonia [23]. YKL-40 is a member of the 18-glycosylhydrolase family [24, 25], which also includes bacterial chitinases and chitinase related proteins. Although the exact physiological function of YKL-40 is unknown at present, the pattern of its expression in normal and disease states suggest a role in inflammation, degradation and/or remodelling of the extracellular matrix [22–27].

Due to the possible association between blood transfusion and storage time-dependent increased risk of pneumonia, we studied the potential extracellular accumulation of YKL-40 during storage of various blood components.

Material and methods

Study 1

Peripheral venous blood (2 × 4.5 ml) was drawn from 18 unpaid, healthy, volunteer blood donors into ice-chilled endotoxin-free tubes (Becton-Dickinson, Mountain View, CA, USA) containing 0.5 ml sodium citrate (0.129 mol/l) and aprotinin (12.5 KIU/ml). One set of blood samples was centrifuged at 2,500g for 10 mins at 4 °C, and the supernatants were carefully separated from the cells and kept at –80 °C until analysis of plasma YKL-40. From the other set of samples 2 ml of blood were mixed with 8 ml of sterile water (1-in-four dilution) in endotoxin-free tubes and frozen-thawed five times to ensure complete disintegration of all cellular elements. Subsequently, the diluted blood samples were centrifuged at 2,500 g for 10 mins at 4 °C, and the supernatants were separated from the cellular debris and kept at –80 °C until determination of the total (intra- plus extracellular) content of YKL-40.

Simultaneously, one unit of whole blood (450 ml) was donated by each of the 18 blood donors under standard conditions into blood bags (Baxter Optipac SA, La Chatre, France) containing 63 ml citrate, phosphate, dextrose and adenine (CPDA) solution as anticoagulant. The 18 units were prepared as follows: 6 units were left as whole blood (WB), 6 units of plasma-reduced WB were prepared by removing 200 ml plasma from each of the 6 units of WB, and 6 units of SAGM (saline-adenine-glucose-mannitol) blood were prepared by centrifugation of 6 units of WB at 5,000 g for 8 mins. By using the top and bottom system (Baxter SA) the red cells were by the bottom outlet subsequently transferred to a separate bag including 100 ml SAGM solution. All units were stored in the blood bank under standard conditions for 35 days at 4 °C. One ml of stored blood was drawn from all units after initiation of storage (day 0), and on storage days 2, 5, 9, 14, 21, 28, and 35. To ensure a homogeneous distribution of cells and plasma/SAGM solution the blood bags were gently mixed before the samples were drawn. The samples were centrifuged at 4 °C at 2,500 g for 10 mins, and the supernatants were carefully separated from the cells and kept frozen at –80 °C until determination of extracellular YKL-40 levels.

Study 2

Blood (450 ml) was donated by each of 43 unpaid, healthy, volunteer blood donors as previously described. The 43 units of WB were prepared as follows: 8 units were stored under standard conditions for 35 days at 4 °C, and 8 units were leukocyte depleted by filtration (leuko-depletion) at room temperature within 4 h using inline leukofilters (WB5, Pall Medsep Ltd, Portsmouth, UK) and then stored for 35 days. One ml of blood was drawn from these 16 units after initiation of storage (day 0) and on storage days 21 and 35. These samples were prepared and stored frozen at –80 °C as previously described. Furthermore, 9 units were stored for 7 days before leuko-depletion using the bedside filter RC100 (Pall Ltd), 9 units were stored for 21 days and 9 units were stored for 35 days before similar leuko-depletion. One ml of blood was drawn from these 27 units after initiation of storage and just before and after leuko-depletion on the respective days. Supernatants from all the samples were prepared and stored frozen at –80 °C as described.

Whole blood preparations contain the full amount of cellular elements and plasma donated by the donor. The cell-free content is approximately 340 ml. Plasma-reduced WB (packed erythrocytes) contains the full amount of cellular elements donated by the donor, but the plasma content is reduced with 200 ml. Therefore, the cell-free content is approximately 140 ml. SAGM blood contains the full amount of erythrocytes, but the leukocyte amount is reduced with 75%, the plasma volume with 90% and more than 99% of the platelets are removed. The cell-free content is approximately 110 ml. Prestorage leuko-depletion reduces the leukocyte count to less than 0.5×10^6/unit, while bedside leukofiltration is less effective, dependent on storage time [28]. In addition, previous results have shown that bioactive substances accumulated during storage may be slightly and insignificantly reduced by bedside leukofiltration [28].

YKL-40 analysis

A RIA method was used for determination of YKL-40 [25]. The antiserum was raised in rabbits immunised with purified intact human YKL-40, and purified human YKL-40 was used for standard and tracer. The intra-assay and inter-assay variations were less than 6.5% and 12%, respectively, and the sensitivity was 20 µg/l.

Statistical analysis

The statistical analysis was performed with Sigma Stat (SPSS Inc. Chicago, IL). Results are expressed as median and range. Comparison between groups was calculated by the non-parametric Mann-Whitney-U-test and within groups by Wilcoxonís rank sum test. P values less than 0.05 were considered significant.

Results

Study 1

At the day of blood donation the plasma YKL-40 levels of the donors ranged from 12 to 95 µg/l, and their total extra- and intracellular content ranged from 200 to 1,200 (g/l (Table 1). There were no significant differences of plasma concentrations or total contents between the donors for the three different blood components.

During storage the extracellular concentration of YKL-40 increased significantly in a time-dependent manner (Table 2). By using the cell-free content of the specific blood preparations the amount of extracellularly accumulated YKL-40 was calculated. Thereby, the exact amount of YKL-40 per

Table 1. YKL-40 concentrations in plasma, and total extra- and intracellular content in blood from healthy blood donors.

	YKL-40 concentration (µg/l)	
Donors of blood prepared as	Citrated plasma	Total content
Whole blood	53 (12 – 95)	750 (400 – 1,200)
Plasma-reduced whole blood	32 (26 – 87)	520 (420 – 1,080)
SAGM blood	32 (20 – 34)	560 (200 – 840)

Values are given as median and range. No significant differences were found in the YKL-40 levels in citrated plasma and in the total content of YKL-40 of donors of blood for the 3 different blood components. N = 6 for each blood component.

Table 2. Extracellular YKL-40 concentrations (µg/l) during storage of erythrocyte components at 4 °C.

Storage day	Whole blood	Plasma reduced whole blood	SAGM blood
0	53 (10–98)	32 (27–99)	17 (12–40)
2	59 (10–96) ns	32 (25–109) ns	17 (12–38) ns
5	70 (45–100) ns	61 (33–177) p = 0.031	30 (13–42) ns
9	86 (53–108) ns	102 (41–215) p = 0.031	44 (19–65) p = 0.031
14	98 (61–143) p = 0.031	149 (68–387) p = 0.031	55 (23–68) p = 0.031
21	108 (80–161) p = 0.031	142 (94–271) p = 0.031	59 (22–67) p = 0.031
28	105 (71–179) p = 0.031	201 (104–336) p = 0.031	58 (24–71) p = 0.031
35	99 (80–143) p = 0.031	151 (84–319) p = 0.031	57 (24–72) p = 0.031

Values are median and range. P-values represent differences compared to initial values (Wilcoxonís test). N = 6 for each blood component. ns = not significant.

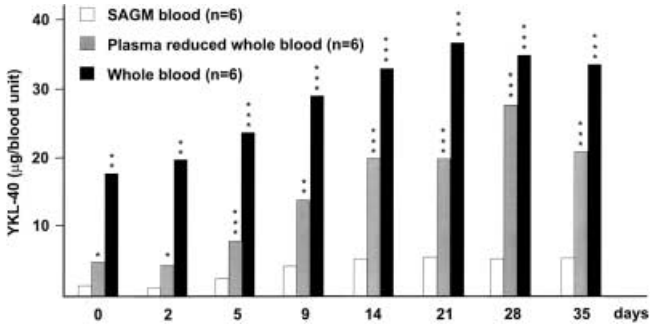

Fig. 1. Time-dependent accumulation of extracellular YKL-40 per unit SAGM blood, plasma reduced whole blood and whole blood during storage at 4oC for 35 days. Values are given as median. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.002$ (Mann-Whitney's test: the extracellular YKL-40 level in the plasma-reduced whole blood or whole blood preparations versus the level in SAGM blood at the different days).

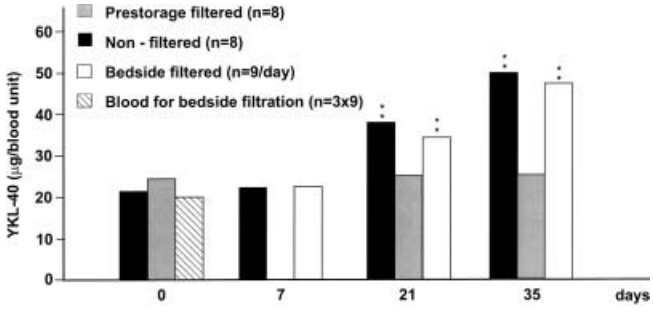

Fig. 2. Supernatant content of YKL-40 in non-filtered and prestorage filtered whole blood, and in whole blood stored for 7, 21 and 35 days, respectively, after bedside leucofiltration. All values given on day 0 are before filtration. Values are given as median. *$p < 0.05$; and **$p < 0.01$ (Wilcoxonís test: the extracellular YKL-40 level in whole blood at the different times after storage and leukofiltration versus the level in prestorage filtered whole blood).

unit is presented in Fig.1, which shows a storage time-dependent significant accumulation of extracellular YKL-40 in all three different blood preparations.

Study 2

The extracellular contents of YKL-40 in the various blood preparations are presented in Fig. 2, which confirms, that the protein accumulates during storage in a time-dependent manner in whole blood. Prestorage leuko-depletion prevents accumulation of YKL-40 during storage for 35 days. Furthermore, YKL-40 did not accumulate from day 0 to day 7 in the blood for bedside leuko-depletion. The accumulated amount of the protein during 21 days or 35 days of storage, respectively, was not reduced by bedside leukofiltration. The final amount of YKL-40 in these particular units was similar to the amount accumulated before leuko-depletion.

Discussion

Recently, efforts have been made to further characterise the mechanisms leading to blood transfusion-induced adverse effects by focusing on presynthesised intracellular granule-associated leukocyte- and platelet-derived bioactive substances. A great variety of bioactive substances stored intracellularly may participate in a concerted action leading to post transfusion infectious complications, including pneumonia and other lung complications (4). Neutrophilic granulocytes examined after storage for 24 h have initiated disintegration [5] and already present dysfunction, such as alterations in bactericidal activity, chemotaxis, aggregation, and superoxide synthesis [29]. In addition, loss of neutrophil mobility has been demonstrated as early as 5 h after storage [30]. While the defective oxidative burst is one of the earliest indicators of granulocyte damage and occurs within hours after blood is donated into storage bags, granular release and cell membrane disintegration follow shortly and continue throughout the period of storage. In Denmark the SAGM erythrocyte components are stored for 35 days and at that time approximately 80–90% of the granulocytes have disintegrated [31]. Thereby, granule-associated bioactive substances are released and accumulate extracellularly in various blood components, such as erythrocyte, platelet and plasma components, which have not been leuko-depleted by filtration [4–7, 32]. Some of these substances, such as histamine, MPO, ECP, EPX, elastase, and C3a, are well known as being involved in development of inflammatory processes and of postoperative infectious complications [4].

The neutrophil-derived matrix protein YKL-40, located in the specific granules [23] is one of the putative bioactive substances, which may be involved in development of post transfusion adverse effects. In the present study we showed that YKL-40 accumulates in erythrocyte components during storage in a time-dependent manner. The highest content per unit of erythrocytes was found in whole blood, whereas the lowest content was shown in SAGM blood. Whole blood contains the full amount of leukocytes donated by the donor, while the content is reduced with approximately 75% in SAGM blood. Moreover, the low amount of residual plasma

in SAGM blood is diluted with the SAGM solution, and the cell-free amount is only approximately 110 ml in SAGM blood compared to approximately 340 ml in whole blood. This may explain why the content of YKL-40 in SAGM blood units were even lower than the content in prestorage leuko-depleted whole blood.

The amount of leukocytes and thereby their granule-associated bioactive substances are reduced by prestorage leuko-depletion [7, 32]. In contrast, recent results have indicated that bedside leuko-depletion at the time of transfusion is less effective to retain leukocytes compared with prestorage leuko-depletion [28], and that bioactive substances accumulated during storage are not retained in the filters [28]. These observations were confirmed by the present results, which show that the amount of YKL-40 accumulated during storage could not be significantly reduced by bedside leuko-depletion.

Although it is still debated by few authors (33–35) it is well established that perioperative transfusion of allogeneic blood components increases the risk of postoperative infectious complications [2, 4, 10–14, 36, 37]. The mechanisms are not finally disclosed, but bioactive substances from the blood preparations are suspected to play a certain role [3, 4]. Recent results may support this notion, as the frequency of blood transfusion-related postoperative and post trauma infectious complications seem to depend on blood storage time [9–12]. It has previously been speculated that leukocyte surface antigens might be associated with post transfusion adverse effects, such as impaired immune responses and postoperative infectious complications [38]. Therefore, it was anticipated that approaches such as predeposit autologous blood donation and subsequent reinfusion when indicated would reduce the frequency of post transfusion adverse effects, including postoperative infectious complications [39]. However, this assumption was not confirmed by subsequent reports, which suggest that the frequency of adverse effects after autologous transfusion may be similar to the frequency after allogeneic transfusion [40, 41]. These latter results may support the assumption, that bioactive substances accumulated during storage may play a significant role in the mechanism leading to adverse effects by transfusion with blood preparations. YKL-40 may as such participate in the concerted action with various other bioactive substances derived from transfused blood components [4], but its possible specific relation to pneumonia has to be confirmed in future studies [23].

Although the present and other previous studies have shown that prestorage leuko-depletion may be advantageous to bedside leuko-depletion [4, 7, 28], the clinical results of the value of transfusion with leuko-depleted blood components may seem conflicting; some studies show beneficial effects and others show that the frequency of adverse effects are unchanged compared with transfusion with components, which have not been leuko-depleted [4, 36]. However, with the present knowledge of adverse effects to various blood components, and the influence of storage time and preparation methods, these studies [33–35] need to be re-evaluated. In addition, evaluation and comparison of studies where different patient populations have been included need specific consideration. Results obtained among patients undergoing hip or knee replacement surgery or cardiac by-pass surgery are difficult to compare with results from patients undergoing surgery for colorectal cancer or trauma, as the bacterial contamination in the latter patients may play a dominating role [42].

In conclusion, it was shown that the neutrophilic granulocyte derived matrix protein YKL-40 accumulated extracellularly during storage of standard erythrocyte components in a time-dependent manner. While prestorage leuko-depletion could prevent this accumulation, bedside leuko-depletion was ineffective in reducing the substance already accumulated before the actual filtration procedure. The exact biological function of YKL-40 is still not known, but it is supposed to participate in inflammation and remodelling of the extracellular matrix. Therefore, large amounts of YKL-40 transfused with non-filtered blood preparations may be detrimental, at least for trauma and surgical patients [43]. Prestorage leuko-depleted blood components should be considered at least to specific patient populations at risk of developing postoperative infectious complications.

Acknowledgements. The expert technical assistance by Inger Aakaard and Susanne Munch, Department of Rheumatology, Kirsten Vangsgaard, Department of Surgical Gastroenterology, Hvidovre Hospital, and the staff of the Blood Transfusion Unit, Nykøbing Falster County Hospital is gratefully acknowledged.

The study received financial support from The Michaelsen Foundation, The Johan and Lise Boserup Fund, The Kornerup Foundation, The Danish Pharmacy Foundation of 1991, The Aage and Johanne Louis-Hansen Fund, and The Danish Cancer Society (grant # 99 100 21).

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