Cent. Eur. J. Med. • 6(2) • 2011 • 181-184
DOI: 10.2478/s11536-010-0062-1

VERSITA

Central European Journal of **Medicine**

The effect of a leukodepletion model on the activation stage of platelets

Research Article

Miodrag Vucic*, Ivan Tijanic, Nenad Govedarevic, Lana Macukanovic, Zoran Pavlovic

Clinic of Hematology, Clinical Centre Nis, University in Nis, 18000 Nis, Serbia

Received 20 April 2010; Accepted 13 October 2010

Abstract: The preparation of thrombocyte concentrates with filtration before storage (in-line) makes it possible to avoid the presence of mononuclear cells in the concentrate and proinflammatory cytokines. Therefore, this filtration may result with decreased activation of thrombocyte receptors in vitro, which may improve therapeutic efficiancy. Methods. We compared two groups, each with 30 therapeutic doses of concentrated thrombocytes. We prepared the first group using the classic model from the buffy coat and the other with concentrated thrombocyte samples filtrated during sampling, so-called in-line, with the WBC filter Imuflex (Terumo). Mononuclear cells (MNC), thrombocyte, and erythrocyte counts in the units of concentrated thrombocytes were obtained on an automatic cell counter, and we used flow cytometry to measure the expression of surface thrombocyte receptors. The results demonstrated that the trombocytes prepared with pre-storage filtration contained a very low level of mononuclear cells and markedly reduced trombocyte receptors. Conclusion. The number of MNC and expression of surface thrombocyte receptors were markedly lower in the concentrated thrombocyte units prepared with in-line filtration. The thrombocytes prepared in this way contain fewer mononuclear cells, are of higher quality, are more functional, and may produce a better therapeutic effect in vivo.

Keywords: *Thrombocytes • Thrombocyte receptors • In-line WBC filtration*

1. Introduction

The presence of mononuclear cells (MNC) in allogenic blood products (concentrated thrombocytes and erythrocytes) can produce a number of adverse effects or reactions. Of these, some of the most important are immunomodulation (mediated by membrane antigens or proinflammatory cytokines), transfusion-induced acute damage to the lungs, graft-against-host disease, and viral transmission [1]. It is also well known that in vitro activation of thrombocytes induce morphologic, functional, and ultrastructural changes. These changes could reduce the viability of thrombcytes, as well as their in vivo functionality and clinical efficacy [2-4].

During harvesting, processing, and storage of blood products, MNC are activated, leading to de novo synthesis of proinflammatory cytokines [5]. During storage, these cytokines can cause thrombocyte activation, with alpha granule and lysosome evacuation from their contents. This means that blood product units with the lowest cytokine levels should be used therapeutically [6,7].

Altered expression of surface antigens such as CD62p, CD42b, CD63, CD36, is notable for the assessment of activation, i.e., functionality of thrombocytes. Reduced expression of these antigens up to 50%, especially of CD62p, has been reported as acceptable (with relatively preserved integrity of thrombocytic alpha granules). A greater reduction of expression to values below 50% substantially improve in vivo thrombocyte recovery, with a greater increase and maintenance in the number of thrombocytes in the host circulation after their therapeutic administration. In contrast, a higher CD62p expression is one of the reasons for more rapid clearance, i.e., the removal of thrombocytes from the host circulation mediated by the reticuloendothelial system [8].

Studies of the expression of CD63 activation marker (its expression being the consequence of externalization of contents of thrombocytic lysosomes) have also indicated less intense changes compared to incrised expression of CD62p [9].

* *E-mail: buca.v@nadlanu.com*

In contrast to the most commonly used leukodepletion model (filtration at the end of storage of blood products – post-storage leukodepletion), filtration during blood sampling or at the beginning of storage (in-line or pre-storage leukodepletion) would markedly reduce cytokine concentrations [10]. It would not only contribute to the reduced incidence of cytokine-mediated transfusion reactions but also create the conditions for reduced activation of thrombocytes in vitro, with improved therapeutic efficacy [11].

The aim of this study was to assess the number of mononuclear cells and the phenotype of thrombocyte concentrates obtained by various methods of leukodepletion to determine which of the methods are better able to produce thrombocyte concentrates with lower numbers of mononuclear cells and thrombocytes with weaker expression of surface receptors, thus preserving viability and function. Studies have so far failed to produce any clear answers to these questions, perhaps because of different study methodologies, varying sensitivity of the assays used, or biologic variations of the donors. Results of our study showed that in-line trombocyte preparation significantly reduced the trombocyte-receptor expression. Thrombocytes prepared in this way contain fewer mononuclear cells, are of higher quality, are more functional, and may produce an improved therapeutic effect in vivo.

2. Material and Methods

Preparation of thrombocyte concentrates. We studied two groups, each having 30 therapeutic doses of concentrated thrombocytes obtained from 360 units of whole blood from volunteer blood donors.

1) Thrombocyte preparation using the classic model with fourfold bags. Thrombocytes were prepared using fourfold bags from the units of whole blood (450 mL) from vollunteer blood donors (Macopharma, CPD/SAGM, France). The blood was processed within 6 hours of sampling; the first centrifugation (Heraus 8500i) on an automatic extractor TACE-II (Terumo, Japan) produced the buffy coat (leukocyte-thrombocyte layer), erythrocytes, and plasma; the second centrifugation was performed after 2 hours of buffy-coat storage, and the thrombocyte concentrate was obtained.

2) Thrombocyte preparation using the bags with an in-line filter. Thrombocytes were prepared using fourfold bags with an in-line filter (Imuflex-WB-SP CPD/SAGM, Terumo, Japan) from the whole-blood units (450 mL) from volunteer blood donors. Investigation of the mechanisms of leukocyte removal with polyurethane has shown that the majority of leukocytes are trapped mechanically in small pores or dimples in the material. Very limited interaction with cell material and the absence of cellular or protein activation result in blood products of superior purity. The blood was processed within 6 hours of sampling; whole blood was filtrated with an in-line thrombocyte preserving filter (WB-SP filter), and then the first centrifugation (Heraus 8500i) on an automatic extractor TACE-II (Terumo, Japan) produced the leukoreduced buffy coat (leukocyte-thrombocyte layer), leukoreduced erythrocytes, and leukoreduced plasma; the second centrifugation was performed after 2 hours of buffy-coat storage, and the thrombocyte concentrate was obtained.

The thrombocytes prepared in both ways were then placed in the horizontal stirrer (Teknolabo Instruments, Italy) with controlled temperature (22±2°C), and the samples were taken 12 hours after the preparation for assessment.

Determination of mononuclear cell count. The number of MNCs was measured in the samples obtained from concentrated thrombocyte units with an automated cell counter (Beckman-Coulter).

Flow cytometry analysis.

Platelet expression of CD62p and CD63, as indicators of platelet activation, were measured by flow cytometry as described by Marquardt et al. [12], with minor modifications. Briefly, platelet samples ($1x10^6$/tube) were incubated with fluorescein isothiocyanate conjugated (FITC) CD62p and CD63 antibodies (Beckman Coulter), according to the manufacturer's recommendations, in darkness for 15 minutes at 4^oC. Nonspecific binding was detected by the control cells, which were incubated with phosphate-buffered saline (PBS) alone. After incubation, cells were washed twice with PBS and analyzed (10,000 analyzed cells per sample) with the Epics XL (Coulter) flow cytometer. Forward and side scatter properties, as well as settings to discriminate platelets from other contaminating cells in the sample, were as specified by Harrison et al. [13]. Results were expressed as the percentage of antibody-positive platelets.

Statistical analysis: Results were presented as mean±standard deviation(SD). Significant difference between the groups were analyzed with Student`s t test.

3. Results

Taking into account the significance of contaminating cells in prepared platelet samples, we first evaluated the number of monocytes in platelet samples prepared with two different methods of leukodepletion (classic method

Table 1. Volume and number of blood cells of final filtered components compared to nonfiltered units.

Method of leukodepletion	Volume (ml)	WBC (x10^6/unit)
Classic method	59.8±9.9	53.2±58.7
In-line filtered	60.9±10.6	0.03±0.04*

*The total cell number was detected by automated cell sorter as described in the section on material and methods. Results are presented as mean±SD. * p<0.0001 compared to corresponding control.*

Table 2. The effect of various methods of leukodepletion on CD62p and CD63 expression in human platelets.

Surface marker	Leukodepletion method	
	Classic model	In-line filter
CD62p	59.6±15.6	29.8±9.4*
CD63	32.2±10.3	12.9±5.3*

*Expression of CD62p and CD63 in platelets was evaluated by flow cytometry, as described in the section on material and methods. Results are expresed as mean±SD of positive cells. * p<0.05 compared to the coresonding control.*

and so-called in-line filter preparation). Table 1 shows that use of the WBC filter during platelet sampling significantly decreased the number of contaminating cells (monocytes) in platelet samples (p<0.001), as determined by an automated cell counter.

Table 1 Volume and number of blood cells of final filtered components compared to nonfiltered units.

Since our previous results showed that platelet samples filtrated via the WBC filter contained a significantly decreased number of monocytes, which cold be responsible for platelet activation, in our next experiments we tried to evaluate the expession of CD62p and CD63 molecules as activators of platelet activation. The results (Table 2) show significantly decreased expression of CD62p (p<0.05) and CD63 (p<0.05) molecules in platelet samples filtrated during sampling, compared with the platelet samples prepared using the classic model from the buffy coat. These results correlated with a decreased number of monocytes in platelet samples, indicating the important role of WBC filter application in the platelet-activation process.

4. Discussion

In our study, we tried to assess the impact of various leukodepletion models on the presence of MNCs in thrombocyte concentrates and the expression of thrombocyte activation receptors in various preparations of thrombocyte concentrates.

The number of MNCs was significantly higher in the thrombocyte concentrates prepared from the buffy coat compared with those prepared with use of filtration during blood sampling or at the beginning of storage (in-line or pre-storage leukodepletion). This could be explained by an effective in-line leukodepletion, where leukocyte removal with polyurethane shows that the majority of leukocytes are trapped mechanically in small pores or dimples in the material. Very limited interaction with cell material and the absence of cellular or protein activation result in blood products of superior purity with only one filtration step [14].

In addition to our study, others have also investigated platelet activation in several pathophysiological states by measuring specific markers of activated platelets with the use of flow cytometric analysis. P-selectin (CD62p) is a commonly used marker of activated platelets. P-selectin exsists on the membrane of alpha granules in resting (nonacivated) platelets. A level below 50% indicates the preservation of thrombocytic alpha granules, i.e., a reduced in vitro thrombocyte activation [15].

Those thrombocytes prepared using the classic method from the buffy coat had a significantly higher expression of P-selectin as an activation marker compared to thrombocyte concentrates filtrated in-line, indicating that the thrombocytes with high P-selectin expression are activated, i.e. functionally, morphologically, and ultrastructurally altered, and are less effective therapeutically. This could be explained as the absence or reduced presence of MNCs and inflammatory cytokines in in-line filtrated thrombocyte concentrates, neutralizing the phenomenon of cytokine-induced thrombocyte activation [16].

Assessment of the expression of CD63 activation marker also showed that the marker was significantly reduced in in-line filtrated thrombocytes. The recorded difference was less intese than P-selectin, wich was the consequence of externalization of the contents of thrombocytic lysosomes. It was assumed that a more potent stimulus was needed to empty the lysosomes of their contents than to empty the thrombocytic alpha granules [12].

Our results are of clinical relevance since they show that the preparation of thrombocyte concentrates with in-line filtration is more efficient than the preparation from the buffy coat, reducing the possibility of host sensitization with isoantigens and producing improved therapeutic outcomes.

5. Conclusion

Our results show that reduced presence of MNCs and reduced expression of thrombocyte activation markers can be obtained by leukodeopletion of thrombocyte concentrates at the beginning of storage (in-line) with use of an Imuflex filter and that these thrombocytes are more viable and therapeutically more successful. Therefore, this method can be recommended for therapeutic preparation of thrombocytes, since the presence of cytokines within them has been reduced to the lowest possible level.

References

[1] Bubel S., Wilhelm D., Entelmann N., Kirchner H., Kutler., Chemokines in stored platelet concentrates, Transfusion., 1996; 36,445-449

[2] Bordin JO., Hedlle NM., Bjachman MA, Biological effects of leukocytes presented in transfused cellularr blood products., Blood., 1994, 84, 1703-21.

[3] Maly MA, Hadacova I, Hajek P, Zemanek D, Veselka J. High-residual platelet activity despite dual antiplatelet treatment associated with subacute stent trombosis. Cent Eur J Med, 2009; 4, 119-124.

[4] Salehiomran A, Ahmadi H, Karimi A, Tazik M, Dowlatshahi S, Fathollahi S, Abbasi S. Transfusion associated in-hospital mortality and morbidity in isolated coronary artery bypass surgery. Cent Eur J Med, 2009; 4. 286-292.

[5] Metcalfe P., Williamson LM., Reutelingsperger CP, Activation during preparation of therapeutic platelets afects deterioration during storage, Comparative Flow cytometric study of different product methods, B J Haematology., 1997, 98,86-95

[6] British Commitee for Standards in Haematology, Blood Transfusion Taskforce. Guidlines on the clinical use of leukocyte-depleted blood components, Transfusion-Medicine., 1998,8,59-71

[7] Wadhawa M., Kraildashiri P., Dilger P., Gaines R, Cytokine levels as perfomance indicators for white blood cell reduction of platelet concentrates, Vox Sanguinis., 2002,83,125-136

[8] Schuerholz T., Losche W., Keil O., Friedrich L., Simon T., Marx G, Acute short-term hyperglycemia impairs platelet receptor expression even in healty adults in vitro, Med Sci Monit., 2008, 14,12,BR,294-298

[9] Murasaki K., Kawana M., Murasaki S., Tsurumi Y., Tonoue K., Hagiwara N., Kasanuki H, High P-selectin expression and low CD36 occupancy on circulating platelets are strong predictors of restenosis after coronary stenting in patients with coronary artery disease, Heart Vessels., 2007,22,229-236

[10] Paunovic D., Van Der Meer P., Kjeldsen-Kragh R., Larson S et al, Multicenter evaluation of whole-blood filter that saves platelets, Transfusion.,2004,44,8,1197-203

[11] Lozano M., Perez-Ceballos E., Rivera J., Paunovic D., et al, Evaluation of a new wholw-blood filter that allows preparation of platelet concentrates by platelet –rich plasma method, Transfusion., 2003,43,1723-1728

[12] Marquardt L., Ruf A., Mansmann U., Winter R., Schuler M., Buggle F., Mayer H., Grau AJ, Course of platelet activation markers after ischemic stroke, Stroke., 2002, 33, 2570-2574

[13] Harrison P., Ault KA., Chapman S., Charie L., Davis B., Fuj Marquardt L., Ruf A., Mansmann U., Winter R., Schuler M., Buggle F., Mayer H., Grau AJ, Course of platelet activation markers after ischemic stroke, Stroke., 2002, 33, 2570-2574

[14] Krailadsiri P., Seghachian J., Lorna M, Platelet storage lesion of WBC-reduced pooled buffy coat-derived platelet concentrates prepared in three in-process filter-storage bag combinations, Transfusion., 2001,41,243-250

[15] Balint B., Vucetic D., Trajkovic-Lakic Z., Petakov M et al, Quantitative, functional, morfological and ultrastructural recovery of platelets as predictor for criopreservation, Haematolog(Budap)., 2002,32,4,363-75

[16] Metzelaar M .J., Wijngaard PL., Peters PJ., Sixma J.J., Niuwenhius HK., & Clevers HC, CD 63 antigen. A novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in in eucaryotic cells, B Biol Chem .,1991, 266, 3239-3245