The use of cryopreserved platelets in the treatment of polytraumatic patients and patients with massive bleeding

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BACKGROUND: The short shelf-life of fresh platelets limits their efficient inventory management and availability during a massive transfusion protocol. Risk of insufficient availability can be mitigated by building an inventory of cryopreserved platelets (CPs).

METHODS: A comparative study of fresh apheresis platelets (FAPs) and CPs was performed. Type-O CPs were processed with DMSO frozen at −80°C and reconstituted in thawed AB plasma. All patients enrolled in the study had the following parameters evaluated on admission: vital signs (body temperature, heart rate, mean arterial pressure), blood count, prothrombin time, activated partial thromboplastin time, fibrinogen level, and, in trauma patients, international severity score. Several outcomes were evaluated: 30-day survival, adverse events, quantity of administered blood products, fibrinogen concentrate and thromboxane (TXA), and laboratory parameters after transfusion (blood count, prothrombin time, activated partial thromboplastin time, fibrinogen level).

RESULTS: Twenty-five (25) patients in the study group received transfusions totaling 81 units of CPs. Twenty-one (21) patients in the control group received a total of 67 units of FAPs. There were no significant differences in patient characteristics ($p > 0.05$) between groups. Both groups were comparable in clinical outcomes (30-day survival, administered blood products, fibrinogen concentrate, TXA, and adverse events). Among posttransfusion laboratory parameters, platelet count was higher in the group transfused with FAPs (97.0×10^9/L) than in the group transfused with CPs (41.5×10^9/L), $p = 0.02025$. Other parameters were comparable in both groups.

CONCLUSION: The study suggests that CPs are tolerable and a feasible alternative to FAPs. However, larger randomized studies are needed to draw definitive conclusions.

Apheresis platelets under normal collection and storage have a 5- to 7-day shelf-life. Due to this short shelf-life platelet inventory is difficult to manage under normal circumstances. When a blood bank is supporting trauma patients with massive bleeding, it can become difficult to supply sufficient platelets to facilitate resuscitation. Uncontrollable bleeding is the second leading cause of death in trauma patients.[1,2] The early intervention with blood products contributes significantly to correction of coagulopathy and to the control of bleeding.[3,4] The rapid administration of the whole spectrum of transfusion products is proven to have a positive impact on patient survival; therefore, most current transfusion protocols and hemostatic resuscitation procedures are based on the coadministration of red blood cells (RBCs) and plasma, supplemented with platelet transfusion.[5–10]

Cryopreserved platelets (CPs) provide a practical solution that has been used operationally by the Dutch Military Health Service over the last 15 years of conflict, as well as in other countries. The logistical challenges associated with

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the need for ultra-low-temperature freezers was outweighed by the easy availability, compatibility, safety, and efficacy of the cryopreserved products and significantly improved survival rates of patients with war injuries treated at Dutch army field hospitals in 2001–2012, when fresh platelets were not available.[9,11–13] In civilian or military hospitals, a CP inventory would assist in managing platelet availability, as well as maintaining an inventory of HLA- /HPA-matched platelets, rare platelets, and autologous platelets.[14–16]

Cryopreservation and storage of frozen platelets significantly prolongs the shelf-life from days to years. Use of CPs improves the logistics of maintaining and providing platelet products where and when fresh products are not available. The production of CPs is not technologically demanding, and they can be easily thawed and reconstituted.[9,13,17–19]

With assistance from the Netherlands Military Blood Bank in Leiden, the Military University Hospital in Prague, Czech Republic (UVN Prague) introduced CPs into clinical practice in September 2014. The introduction was preceded by an in-vitro validation study and was subsequently included in routine production after notification and approval of the Czech State Institute for Drug Control. In the Military University Hospital, CPs are currently indicated for polytrauma and conditions with heavy bleeding. The aim of the study was to determine whether the results of treatment with fresh or frozen platelets were clinically comparable.

MATERIALS AND METHODS

This was a comparative, nonrandomized, observational study. The study groups included patients with massive, life-threatening bleeding following trauma, patients with gastrointestinal bleeding, or patients with other causes of bleeding who required transfusion therapy. The exclusion criterion was clinically significant comorbidities affecting hemostasis (i.e., hemophilia, thrombocytopenia or thrombocytopathy, patients on anticoagulation treatment etc.). Both platelet products, CPs and fresh apheresis platelets (FAPs), are licensed products in the Czech Republic, and there was no need for ethics board approval and patient consent. All patients that were enrolled in the study had the following parameters evaluated on admission and initial assessment: vital signs (body temperature, heart rate, mean arterial pressure), complete blood count (Sysmex XT 2000i, Sysmex corp.), prothrombin time, activated partial thromboplastin time, and fibrinogen level (ACL TOP 500 CT, Instrumentation Laboratory). For all trauma patients, the international severity score (ISS) was also evaluated.

Fresh apheresis platelets

All of the transfused platelets were collected by apheresis devices (Haemonetics MSC+, Haemonetics corp.) from volunteer donors. The collected units were tested for standard infection markers (hepatitis B surface antigen, anti-hepatitis C virus [HCV], anti-human immunodeficiency virus [HIV] 1 and 2, Treponema pallidum antibodies, nucleic acid test hepatitis B virus [NAT HBV], NAT HCV, NAT HIV). The apheresis-collected platelets were resuspended in donor plasma with anticoagulant citrate dextrose solution-A (ACD-A) anticoagulant solution composed of 22 g sodium citrate, 24.5 g glucose monohydrate, 8 g citric acid monohydrate, and 1000 mL water for injection. One unit of FAPs contained $>280 \times 10^9$ platelets. The content of residual leucocytes was reduced to $<1 \times 10^6$/unit by filtration. FAP products were maintained in inventory at room temperature until issued for transfusion or upon expiration of the product.

Platelet cryopreservation—the preparation of cryopreserved platelets

The Valeri method was used, with Dimethyl sulfoxide (DMSO) as a cryoprotectant and removal of the supernatant before freezing.[20,21] FAP products containing $>2.80 \times 10^9$ platelets/unit, blood type O, were used for freezing. Using an infusion set, 75 mL of 27% DMSO was added to the bag with the collected platelets. The final concentration of DMSO in the product was 5–6%, depending on the actual volume. The platelets were transferred to a special cryopreservation bag (Poche de transfert, REF: R4R2074, Fenwal, Inc) using a sterile connection device (CompoDock®, Fresenius Kabi Gmbh). Together with the original bag the platelets were centrifuged for 12 minutes at 1250*g* (Heraeus 6000i centrifuge, Heraeus Instruments GmbH, Labortechnik). A manual extractor was used to transfer the supernatant to the original bag to the final volume of the product of 12–14 mL. The final product bag was labeled, inserted into the carton, placed into a deep freezer, and frozen at −80°C or lower (−80°C to −85°C). The shelf-life of the product is 2 years, when stored at a temperature of −80°C to −65°C.

Thawing and reconstitution of cryopreserved platelets

Prior to clinical use, the CPs were reconstituted in thawed type-AB plasma. Cryopreserved platelets and fresh frozen plasma were initially thawed at a temperature of 30°C (Tool PR 50–300 plasma thawer, Tool s.r.o.). A noncontact thermometer was used to check the temperatures of both products prior to mixing. The CP bag and the thawed plasma bag were connected using a sterile connection device (CompoDock®, Fresenius Kabi Gmbh). The plasma was transferred into the CP and mixed by gentle stirring. Transferring the contents of the bags back and forth (three times) ensures a homogeneous suspension of the product. The final product (in the original platelet bag) was detached from the plasma bag, labeled, and released for transfusion. The thawing and reconstitution did not exceed 30 minutes and the shelf-life of the thawed/reconstituted CP is 6 hours. Each product met the following criteria: platelet count $>200 \times 10^9$/unit, leucocyte count $<1 \times 10^6$/unit, erythrocyte count $<6.8 \times 10^9$/unit,

pH > 6.4, and no aggregates, and the products were processed in a functionally closed system.

Outcomes

The clinical outcomes that were measured included 30-day survival, adverse effects following platelet transfusion, and the total quantity of administered blood products, fibrinogen concentrate and tranexamic acid. The laboratory parameters that were evaluated included complete blood count, prothrombin time, activated partial thromboplastin time and fibrinogen level. The blood samples were collected 1–6 hours after the platelet transfusion.

Statistics

The calculations were carried out using Statistica® software, version 12 (StatSoft s.r.o.) intended for bio-statistical and medical calculations in clinical studies.

The following statistical methods were used: normality test (Shapiro–Wilk test + P–P graphs), descriptive statistics, and analysis of variance, Chi-squared test, and Kolmogorov–Smirnov for the comparison of the two groups.

RESULTS

Patients

Twenty-five patients received transfusion of a total of 81 units of CPs. Twenty-one patients in the control group received transfusion of a total of 67 FAP. The demographics of the patients in both groups are demonstrated in Table 1. No significant differences were observed between the two groups ($p > 0.05$).

Outcomes

The 30-day survival rate in patients transfused with CPs was comparable to that of the group transfused with FAPs. In both groups, no adverse events were observed after platelet transfusion. The quantity of administered blood products, fibrinogen concentrate and thromboxane (TXA) in both patient groups were comparable.

There were no differences in patient laboratory results for coagulation (prothrombin time, activated partial thromboplastin time), fibrinogen levels, or hemoglobin/hematocrit for both (CP and FAP) groups (Table 2). However, there was a significant difference in the median platelet count between groups, which was higher after transfusion in patients who received FAPs (97.0×10^9/L) than in patients who received CPs (41.5×10^9/L) (Table 2).

DISCUSSION

The results support that CPs and FAPs are comparable transfusion products when given to massively bleeding patients. However, it is necessary to mention some limitations of the study. This was a retrospective design, with a small number of patients, so definitive conclusions about mortality or safety cannot be drawn. Since there were no institutional transfusion guidelines that direct transfusion practice, the similar number

TABLE 1. Patient characteristics*

Group	Group transfused with CPs (N = 25)	Group transfused with FAPs (N = 21)
Variable		
Age—year (median)	53 (20–80)	50 (27–66)
Sex		
Male—no. (%)	20 (80.0)	12 (57.14)
Female—no. (%)	5 (20.0)	9 (42.86)
Race		
White—no. (%)	25 (100)	21 (100)
Cause of bleeding		
Combined injury/polytrauma (T068) —no. (%)	16 (64)	11 (52.40)
Traumatic subdural hemorrhage (S065) —no. (%)	2 (8)	4 (19.05)
Hemoperitoneum (T810) —no. (%)	2 (8)	4 (19.05)
Other—no. (%)	5 (20)	2 (9.5)
Injury Severity Score—rate (median†)	53.8 (22–75)	59.0 (30–75)
Vital signs on admission		
Body temperature—°C (median)	36.5 (33.1–38.2)	36.7 (33.5–37.8)
Heart rate—beats/min (median)	100 (60–131)	94 (50–130)
Mean arterial pressure—mmHg (median)	85 (53–125)	80 (47–110)
Laboratory results at admission		
Prothrombin time—ratio (median)	1.24 (0.29–2.42)	1.34 (0.8–1.2)
Activated partial thromboplastin time—ratio (median)	1.11 (0.88–2.14)	1.16 (0.80–1.20)
Fibrinogen—g/L (median)	1.48 (0.47–3.81)	1.67 (0.36–4.04)
Platelets count—$\times 10^9$/L (median)	43 (44–206)	53 (16–76)
Hemoglobin—g/L (median)	86 (46–130)	101 (72–145)
Hematocrit—% (median)	26 (13–36)	28 (20–41)

* No significant differences were observed between the two groups ($p > 0.05$).

† Scores range from 0 to 75, with a score of >15 indicating major trauma. Data were unavailable for 7 patients in the cryopreserved platelet (CP)-transfused group and 10 patients in the fresh apheresis platelet (FAP)-transfused group.

TABLE 2. Trial outcomes*

Group	Group transfused with CPs (N = 25)	Group transfused with FAPs (N = 21)
Variable		
30-day survival—no. (%)	19 (76)	17 (81)
Administered blood products, fibrinogen concentrate, and thromboxane		
Red blood cells—no. of units (median)	17.72 (2–41)	16.47 (0–43)
Plasma—no. of units (median)	9.84 (0–21)	9.77 (0–24)
Platelets—no. of units (median)	3.24 (2–8)	3.21 (1–10)
Fibrinogen—no. of g (median)	1.65 (0–10)	1.5 (0–5)
Thromboxane—no. of g (median)	0.825 (0–2)	0.84 (0–2.5)
Adverse events—no. (%†)	0 (0)	0 (0)
Laboratory results after platelet transfusion		
Prothrombin time—ratio (median)	1.23 (1.17–1.57)	1.23 (1.04–1.83)
Activated partial thromboplastin time—ratio (median)	1.11 (0.92–1.69)	1.11 (0.86–1.95)
Fibrinogen—g/L (median)	1.48 (1.10–2.42)	2.04 (1.38–2.28)
Platelet count—$\times 10^9$/L (median‡)	41.5 (20–88)	97 (20–149)
Hemoglobin—g/L (median)	90 (66–120)	98.9 (62–142)
Hematocrit—% (median)	25 (20–36)	28 (19–40)

* No significant differences were observed between the two groups in the listed characteristics except where noted ($p > 0.05$).
† No adverse events that would correspond to post-transfusion reaction were reported to the blood bank.
‡ Platelet counts in the cryopreserved platelet (CP) group after transfusion were significantly lower compared with the fresh apheresis platelet (FAP) group ($p = 0.02025$).

of products transfused in both groups might be due to chance alone. Furthermore, platelet count similarities between FAPs and CPs, taken a maximum 6 hours after the transfusion in a bleeding patient, may be due to consumption alone. Due to the type of patients, who were in serious clinical condition for many reasons, clinicians were not specifically looking for post-transfusion reactions. Thus, although no adverse events were reported, there may have been underreporting of such events.

There is evidence to suggest that platelets stored frozen are effective in primary hemostasis after thawing. On the other hand, the process of freezing and thawing causes changes in platelet morphology and activates the platelets. Approximately 15% of CP lose their surface Glycoprotein Ib (GPIb). However, there is no observed loss of GPIIb/IIIa. CPs exhibit significantly decreased ristocetin-induced platelet aggregation but do not lose the ability to respond to more potent thrombin-receptor agonists. Although these functional defects are not clinically significant and CPs effectively treat bleeding, one must be cognizant of these changes.[22–24]

In the group of patients transfused with FAPs, a significantly higher platelet count was found in the peripheral blood when compared to the patients transfused with CPs. An increase in the concentration of platelets in the blood count after transfusion might be hard to observe following the transfusion of CPs. This may be due to several causes. First, the broken structure and shape of thawed platelets are associated with a higher amount of phosphatidylserine on their surface which apparently contributes to their lower survival time in circulation and leads to their immediate consumption in hemostasis.[25,26] Another reason may be that CPs are harder for the blood count analyzer to differentiate. Other laboratory parameters were similar in both groups after transfusion, indicating a positive and comparable effect of both platelet products on hemostasis.

According to the European Directive, the shelf-life of CPs is currently 2 years, when stored at ≤−65°C. It is assumed that it will later be extended on the basis of studies. The short post-thaw shelf-life of 6 hours is based on the process of adding the DMSO prior to freezing in an open system and could be improved by use of DMSO in a plastic container and the possibility of using a sterile connection device.

CP are suitable for both civilian and military use, particularly for the treatment of acute conditions associated with massive bleeding when no permanent or sufficient supply of fresh platelets is available. Thawing and reconstitution constitute a simple procedure that takes no more than 30 minutes, and CP production cost is not high. CPs may also find use in other indications, for example as autologous products, as rare or human leukocyte antigen-compatible platelets, or in a wide range of non-transfusion applications. In recent years, there has been a relatively large resurgence of interest in CP as a promising blood product that is being used, tested, and validated in several countries and institutions.[15,22,27–31] The US Army is engaged in a multi-million-dollar development effort for the production, manufacture, and approval (by the US Food and Drug Administration) of a CP product currently undergoing clinical trials.[32] At the Military University Hospital in Prague, Czech Republic, CPs are now used as a standard blood product. From September 2014 to August 2018, a total of 486 units of platelets were administered to 124 patients with severe bleeding. Of these, a total of 305 units of CPs were successfully transfused to 99 patients.

This study, as well as our 4 years of clinical experience using CPs in practice, suggests that cryopreserved platelets are a well-tolerated and viable alternative to FAPs in practice. Although large, randomized studies are needed to draw definitive conclusions, we are confident that continued clinical use will support the use of CPs as effective and safe.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. World Health Organisation: cause-specific mortality and morbidity. 2009. http://www.who.int/whosis/whostat/EN_WHS09_Table2.pdf.
2. Cothren CC, Moore EE, Hedegaard HB, et al. Epidemiology of urban trauma deaths: a comprehensive reassessment 10 years later. World J Surg 2007;31:1507-11.
3. Hess JR, Brohi K, Dutton RP, et al. The coagulopathy of trauma: a review of mechanisms. J Trauma 2008;65:748-54.
4. Brohi K, Cohen MJ, Ganter MT, et al. Acute coagulopathy of trauma: hypoperfusion induces systemic anticoagulation and hyperfibrinolysis. J Trauma 2008;64:1211-7.
5. Perkins JG, Cap AP, Spinella PC, et al. An evaluation of the impact of apheresis platelets used in the setting of massively transfused trauma patients. J Trauma 2009;66(Suppl 4):S77-84 [discussion: S84–5].
6. Simmons JW, White CE, Eastridge BJ, et al. Impact of policy change on US Army combat transfusion practices. J Trauma 2010;69(Suppl 1):S75-80.
7. Holcomb JB, Zarzabal LA, Michalek JE, et al. Increased platelet: RBC ratios are associated with improved survival after massive transfusion. J Trauma 2011;71(2 Suppl 3):S318-28.
8. Perkins JG, Cap AP, Spinella PC, et al. Comparison of platelet transfusion as fresh whole blood versus apheresis platelets for massively transfused combat trauma patients (CME). Transfusion 2011;51:242-52.
9. Noorman F et al. Transfusion: −80° C frozen blood products are safe and effective in military casualty care. PLoS One 2016; 11:1-18.
10. Hess JR, Thomas MJ. Blood use in war and disaster: lessons from the past century. Transfusion 2003;43:1622-33.
11. Lelkens CC, Koning JG, de Kort B, et al. Experiences with frozen blood products in The Netherlands military. Transfus Apher Sci 2006;34:289-98.
12. Badloe J, Noorman J. The Netherlands experience with frozen −80°C red cells, plasma and platelets in combat casualty care. Transfusion 2011;51(Suppl):24A.
13. Noorman F, Strelitski R, Badloe JF. −80°C Frozen platelets are activated compared to 24-hour liquid platelets and quality of frozen platelets is unaffected by a quick preparation method (15 min) which can be used to prepare platelets for the early treatment of trauma patients for the early treatment of trauma patients in military theatre. Transfusion 2012;52(Suppl S3):33A.
14. Holley A, Marks DC, Johnson L, et al. Frozen blood products: clinically effective and potentially ideal for remote Australia. Anaesth Intensive Care 2013;41:10-9.
15. Daly PA, Schiffer CA, Aisner J, et al. Successful transfusion of platelets cryopreserved for more than 3 years. Blood 1979;54: 1023-7.
16. Schiffer CA, Aisner J, Wiernik PH. Frozen autologous platelet transfusion for patients with leukemia. N Engl J Med 1978;299:7-12.
17. Amer KA, Proper AS, Prose CH. Effect of different resuspension media on the post-thaw characteristics of frozen blood. Br J Haematol 1980;44:635-44.
18. Isbister J. Frozen Blood: time for outcomes. Anaesth Intensive Care 2013;41:5-7.
19. Neuhaus S, Wishaw K, Lelkens C. Australian experience with frozen blood products on military operations. Med J Aust 2010; 192:203-5.
20. Handin RI, Valeri CR. Improved Viability of Previously Frozen Platelets. Blood 1972;40:509-13.
21. Valeri CR, Ragno G, Khuri S. Freezing human platelets with 6 percent dimethyl sulfoxide with removal of the supernatant solution before freezing and storage at −80 degrees C without postthaw processing. Transfusion 2005;45:1890-8.
22. Royce A, Reade MC, Johnson L, et al. CLIP (cryopreserved vs. liquid platelets for surgical bleeding): protocol for a randomized controlled trial. Heart Lung Circ 2015;24:e57-8.
23. Owens M, Werner E, Holme S, et al. Membrane glycoproteins in cryopreserved platelets. Vox Sang 1994;67:28-13.
24. Raynel S, Padula MP, Marks DC, et al. Cryopreservation alters the membrane and cytoskeletal protein profile of platelet microparticles. Transfusion 2015;55:2422-32.
25. Nagata S, Suzuki J, Segawa K, et al. Exposure of phosphatidylserine on the cell surface. Cell Death Differ 2016;23:952-61.
26. Lentz BR. Exposure of platelet membrane phosphatidylserine regulates blood coagulation. Prog Lipid Res 2003;42:423-38.
27. Johnson L, Reade MC, Hyland RA, et al. In vitro comparison of cryopreserved and liquid platelets: potential clinical implications. Transfusion 2015;55:838-47.
28. Yılmaz S, Çetinkaya RA, Eker İ, et al. Freezing of apheresis platelet concentrates in 6% dimethyl sulfoxide: the first preliminary study in Turkey. Turk J Haematol 2016;33:28-33.
29. Ding GL, Qin WS, Zhao LY, et al. Key preparation technique and clinical application of frozen platelets. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2016;24:1226-31.
30. Cohn CS, Dumont LJ, Lozano M, et al. Vox Sanguinis International Forum on platelet cryopreservation. Vox Sang 2017;112:669-84.
31. Cohn CS, Dumont LJ, Lozano M, et al. Vox Sanguinis International Forum on platelet cryopreservation: summary. Vox Sang 2017;112:684-8.
32. Solicitation Number: MTEC-18-06-CPP "Development and Evaluation of a Cryopreserved Platelet (CPP) Product for the US Military," Amendment 1, Medical Technology Enterprise Consortium, Amended Issue Date July 25, 2018. https://mtec-sc.org/active-solicitations