Review Article

Cryopreserved platelets: a narrative review of its current role in transfusion therapy

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Objective: To review the current role of cryopreserved platelets (CPP) in transfusion medicine.

Background: The short shelf life of platelet concentrates (PCs) provokes logistic problems in providing timely PC transfusions during long holiday periods when long or major transportation barriers exists (severe weather, military operations…) or for patients highly alloimmunized by human leukocyte antigens (HLA)/human platelet antigens (HPA) antibodies. In the European Union the only approved strategy authorized to circumvent the limited storage time of PCs is the cryopreservation using 6% dimethyl sulfoxide (DMSO), at temperature of –80 ℃ or below.

Methods: A review of articles referenced in PubMed published in English before 2020 studying the effect of cryopreservation on platelets *in vivo* and *in vitro* was performed.

Conclusions: Using *in vitro* techniques available to characterize platelets, significant changes in the structure and function of CPP after thawing are detected. The changes detected suggest that cryopreservation provokes an increase in the procoagulant activity of platelets. However, *in vivo* studies in healthy volunteers and in patients, have shown the efficacy and safety of the transfusion of CPP in different clinical scenarios. Nevertheless, its current use is limited to situations where liquid stored platelets is not available mainly in military operations, for patients with complex HLA/HPA alloimmunization or for patients with massive bleeding.

Keywords: Platelet storage; platelet cryopreservation; platelet transfusion

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Introduction

Platelets, the smallest cellular fragment (since they do not have nucleus, strictly speaking, we can not name them, cells) in blood circulation, play an essential role in hemostasis, tissue restoration and inflammation (1,2). Their capacity to adhere and aggregate on subendothelial structures exposed after any injury in the blood vessel wall guarantees the formation of a hemostatic plug that, later, will be stabilized by the fibrin mesh, resulted from the activation of the coagulation cascade.

This intrinsic capacity of platelets to react to many sorts of stimuli, which is essential to their hemostatic function, combined to their short life span in circulation (around 10 days) (3) make them very difficult to handle and store under standard blood bank conditions. In contrast to the other blood components for transfusion that we can store them for weeks (red blood cells concentrates up to 7 weeks when additive solutions are used) (4) or even years (fresh frozen plasma at temperatures below –25 ℃ up

to 3 years) (4), in the case of platelets we can store them just days, specifically 5 days if they are stored at 22 ℃ under continuous agitation in gas permeable plastic bags or up to 7 days if measures to prevent or detect bacterial contamination are applied (4).

In order to extend the storage of platelet concentrates (PCs) beyond the 7 days, the only approved alternative in the European Union is to add a cryoprotectant and freeze them at temperatures of –80 ℃. This paper reviews the current methods available for freezing platelets, the impact that such methods have on platelet structure and function and what the main uses of this form of platelet preservation are in the light of the most recent publications We searched in PubMed the papers published until 2020 in English where cryopreserved platelets (CPP) where studied. We present this article in accordance with the Narrative Review reporting checklist (available at https://aob.amegroups.com/article/view/10.21037/aob-21-31/rc).

Current methods for freezing platelets

As many other advances occurred in the history of Transfusion Medicine, the cryopreservation of platelets was developed to allow the availability of PC for transfusion in the battlefield. And in this sense, the role of retired Navy Capt. C. Robert Valeri, MD, of the Naval Blood Research Laboratory at Boston University School of Medicine has been paramount. During almost 50 years, as a Director of the Naval Blood Research Laboratory he studied and developed methods in the area of frozen blood components, some with undisputable practical effects such as providing evidence for a two-week post-thaw shelf life of deglycerolized red blood cells or the use of dimethyl sulfoxide (DMSO) as platelet cryoprotectant. In 2009 Dr. Valeri received the Lifetime Achievement Award from the United States of America Armed Services Blood Program because "Today, the entire Department of Defense frozen blood program a vital part of contingency operations all over the world is a direct result of his work and the transfusion of deglycerolized red cells has saved many lives" (5).

Dr. Valeri published the first method for cryopreserving platelets in 1972 (6). However, the method required a controlled rate of temperature decrease which was cumbersome and time consuming and storage in the gas phase of liquid nitrogen at –150 ℃. Two years later his group reported a simplified method where 6% DMSO was used and the freezing was performed placing the PC after adding DMSO, in a mechanical freezer at –80 ℃. However, after thawing, this method required the washing of the PC before transfusion (7).

Thirty-three years later Dr. Valeri's group published a modification of the second method (8). The modification consisted in concentrating the platelets and removing the supernatant before freezing. So the final method can be summarized as follows: to leukoreduced [the same group had established earlier that leukoreduction increased the recovery after transfusion from 64% to 74% (9)], PC collected by apheresis or prepared from whole blood donations, DMSO is added under agitation over a 5-minute period, to reach a final concentration of 6%. After, the unit, in a 300 mL PVC bag, is centrifuged at 1,250 ×g for 10 minutes and all the supernatant solution is removed, leaving only about 10–15 mL with the platelet pellet in the bag. Following, the bag is placed at –80 ℃ in a mechanical freezer for up to 2 years (9). The frozen bag is thawed in a water bath maintained at 37 ℃ in approximately 5 minutes then the platelets are diluted with 0.9% saline solution and stored at room temperature for as long as 6 hours without agitation (8). However, other groups have shown the feasibility of resuspending the thawed platelet in platelet additive solution (PAS), a mixture of plasma and PAS (10) or fresh frozen plasma (11).

Impact of freezing and thawing on platelets

In spite of adding the cryoprotectant the process of freezing and thawing has profound effects on platelets that can be characterized using different techniques that will be reviewed following.

Platelet count

Freezing and thawing using the no-wash Valeri's method provokes a decrease in the platelet count between 20% to 30%. Interestingly, Johnson *et al.* reported that, when measured 1-hour after thawing the decrease was of 31%, however when the thawed platelet, resuspended in a mixture of 50% plasma and 50% PAS G (SSP+, MacoPharma, Tourcoing, France) were stored for 24 h in a platelet plastic bag under continuous agitation, there was an increase in the platelet count so the final decrease was 23% (10). Slichter *et al.*, looked at the impact of freezing and thawing in the platelet content of 42 units transfused to patients. They reported a 20% to 25% decrease in the platelet content of

the units after thawing (12).

Platelet structure

One easy way to study the structure of platelets during platelet preparation and storage is flow cytometry combined to fluorescein labeled monoclonal antibodies and proteins that bind to different molecules present at the membrane of platelets (13). Several studies have looked at the impact of freezing-thawing on platelet structure using flow cytometry. Our group reported changes associated with PC cryopreservation using 6% DMSO (14). They reported statistically significant increases in the percentage of platelets that were positive for P-selectin (CD62P, 24%), lysosomal integral membrane protein of 53 kDa (LIMP, CD63, 10.6%), factor Va (FVa 29.1%) and von Willebrand factor 12.3%). They also reported a significant decrease in the expression of glycoprotein (GP) IV (27%) and GPIbα (70%) with no significant changes measured in GPIIb-IIIa expression. For reference, the changes observed after 10 days of liquid storage at 22 ℃ under continuous agitation, in the binding of FVa was significantly lower (18.9%) (13).

Johnson *et al.* instead of using FVa to measure the exposure of phosphatidylserine in the outer layer of platelet membrane, used the binding of annexin A5 (10). They also reported a significant increase in the binding of annexin A5 to platelets 1 hour after thawing, (61%) however that percentage decreased to 30% after 24 hours of storage.

An important aspect to highlight of the effect of platelet freezing and thawing is the formation of microparticles. Microparticles are small fragments (between 100 and 1,000 nm in diameter) originating from the cytoplasmic membrane that are shed by platelets upon activation by thrombin, collagen and complement (15). It has been shown that the freezing and thawing of the platelets causes a significant increase in the release of microparticles. Johnson *et al.* reported that 6 hours after thawing the number of microparticles in the bag increased from just a few millions to 60,000 million. Interestingly after 24 hours of storage at 22 ℃ under agitation, the number of microparticles decreased about 60% when the platelet had been resuspended in plasma while in PAS G the reduction was only around 30% (16). Raynel *et al.* characterized the microparticles generated during platelet cryopreservation; interestingly they found that in comparison to microparticles found in fresh platelets, microparticles generated during freeze and thawing had more expression of GPIV, GPIIb and the GPIb-V-IX complex, and contained more cytoskeletal proteins such as actin or filamin A (17).

Platelet function

Several approaches have been used to study the impact of the freezing-thawing process in platelet function. Lozano *et al.* reported the changes in the aggregatory response to arachidonic acid, collagen, collagen plus epinephrine, adenosine diphosphate (ADP) and ristocetin before and after freezing and thawing. They found a significant decrease in the aggregatory response to arachidonic acid (69%), collagen (80%), collagen plus epinephrine (66%), ADP (73%) and ristocetin (51%) (14).

The adhesive and aggregatory capacities of platelets after freezing and thawing have been also tested under flow conditions (18). Our group reported the results of one study where the adhesive and aggregatory capacity of thawed platelets under flow conditions in an *in vitro* model was tested (19). In comparison to fresh whole blood and blood reconstituted with platelets stored up to 5 days under standard conditions, that showed a similar capacity of adhering to the exposed subendothelium, thawed platelets showed a 50% reduction of the surface covered by platelets (19).

Procoagulant activity

The combination of an increased expression of phosphatidylserine on the outer layer of the platelets and the generation of a great number of microparticles both with known procoagulant capacity led to the idea that frozen-thawed platelet might have a potential thrombotic capacity in the recipient of a transfusion. To explore this hypothesis several techniques have been applied.

Johnson *et al.* studied the procoagulant capacity of thawed platelets compared to before freezing using thromboelastography (TEG 5000, Haemoscope Co, Niles, IL, USA) (16). They found that freezing and thawing was associated to a significant reduction in the R-time, almost a 50%. R-time measures the time taken until de appearance of the first sign of thrombus formation. The maximum amplitude (MA), parameter that measures the strength of the clot formed was just slightly decreased after thawing (16). Cid *et al.* performed a similar study but using thromboelastometry (TEM, Pentapharm GmbH, Munich, Germany) employing two different tests EXTEM (tissue factor and phospholipid are used as activators or the coagulation)

and FIBTEM (cytochalasin is added to inhibit platelet cytoskeleton and contractibility) (19). TEG and TEM use a similar technology, i.e., blood is incubated at 37 ℃ in a cup where a pin with a detector system is placed to measure the formation of the clot. In the case of TEG the cup is in movement while in the TEM is the pin which is in movement (20). In EXTEM compared to control whole blood, thrombocytopenic blood to which frozen-thawed platelets were added, provoked a 40% shortening of the clotting time and also a 48% reduction in the maximum clot firmness (MCT), i.e., an increased procoagulant capacity and a reduced capacity of platelets to keep the strength of the clot. That was confirmed with the measurement in the FIBTEM test, reduction in the clotting time of 47% without significant changes in MCT due to the inhibition of platelet contractibility (19).

The procoagulant capacity of the platelets generated during the freezing and thawing can be also studied measuring the thrombin generation. Johnson *et al.*, found that the supernatant from CPP was capable to increase around 10 times the peak of thrombin generation when compared to values observed before freezing (16).

In vivo studies of CPP in healthy volunteers

Slichter *et al.* reviewed the studies published looking at the effect of platelet cryopreservation on post-transfusion platelet recovery and survival using radiolabeling in healthy volunteers (21). If only the studies performed using the second method described by Dr. Valeri (no-wash) were considered, 3 studies in 32 healthy volunteers have been reported. In those studies, the mean recovery of CPP after transfusion was 33%±10% (mean ± standard deviation) in comparison to fresh platelet that was 63%±9%, i.e., a reduction of 48%. Regarding platelet survival, fresh platelets were found to have a mean survival of 8.6±1.1 days in the circulation, while CPP had 7.5±1.2 days, i.e., the survival was an 89%±15% of that of the fresh. Those studies suggest that the cryopreservation of platelets provokes a loss of about 50% of the CPP and that the other 50% can circulate *in vivo* during a time similar to that of the fresh.

In 4 of the studies, involving 32 healthy volunteers who received CPP, no adverse effects were reported (21).

In vivo studies of CPP in patients

Since the 70s of the last century 27 papers have reported the clinical efficacy and safety of platelet transfusions cryopreserved in DMSO (12,21-24).

In the already mentioned review that Slichter *et al.*, published in 2014 (21), they reported that in 18 studies, where the platelet loss associated with the freezing and thawing in DMSO was analyzed, the mean of platelet loss was 28%±12% with a range of 13% to 55%. About the post-transfusion response in general the recovery of CPP was about 48% of fresh platelet while the 1 h corrected count increment (CCI) was 52% of fresh. The reported 24 h-CCI of the CPP varied from 27% to 64% of fresh. Slichter concluded that the responses to CPP, both autologous and allogeneic were similar to that observed to platelets stored from 5 to 7 days at 22 ℃ under continuous agitation transfused to thrombocytopenic patients (21).

Regarding adverse events, 6 studies reported 101 patients who received 181 cryopreserved units, and no adverse effects were reported. In 4 studies including 72 patients, receiving 181 cryopreserved PCs bad odor or bad taste (metallic) was noted that was likely related to residual DMSO, however the frequency of these events was not reported (21).

Slichter *et al.* reported a study where CPP using no-wash Valeri's method were transfused to bleeding hemato-oncology patients with thrombocytopenia (12). Patients with a World Health Organization (WHO) bleeding score of 2 or more were randomized to receive 0.5 units, 1 unit, 3 units of cryopreserved PCs or 1 apheresis unit stored under standard conditions. WHO grade 2 bleeding is any gross organ system bleeding, WHO grade 3 bleeding is severe enough to require a red blood cell transfusion(s) and WHO grade 4 is a life/organ function-threatening bleeding.

Twenty-four patients were included in the study. Fifty-eight percent of the patients transfused with a CPP product showed an improvement in the bleeding in comparison to a 50% in the patients who received the standard product. There were no thrombotic events considered related to any of the study platelet transfusions. There were 11 serious adverse events reported in five patients who received CPPs but all of them were considered related to their underlying clinical condition (12).

In 1999 a randomized controlled clinical trial, where the effect of CPP was compared to liquid preserved platelets after cardiopulmonary bypass was published. Seventy-three patients undergoing cardiopulmonary bypass were randomized to receive transfusions of cryopreserved (stored up to 2 years) or liquid preserved platelets, although finally, data of only 53 patients (24 receiving CPP and 29 receiving liquid preserved platelets) was analyzed. Platelet

cryopreservation was performed using the "old" Valeri method, i.e., washing the platelet after thawing. In both groups no adverse effects of the transfusion were observed. The patients in the group of CPP received lower dose of platelets per patient $(4.5\pm2.1)\times10^{11}$ compared to the group receiving standard platelets, $(6.9\pm3.9)\times10^{11}$, P=0.008. Also, the median postoperative blood loss per patient was lower: 1,721 mL in the cryopreserved group *vs.* 2,299 mL in the standard platelets group, P=0.007. The volume of blood products transfused to the patients receiving CPP was significantly lower, 1,933±1,042 mL compared to the group of patients receiving standard platelets, 3,426±1,963, P=0.012 (25).

In 2019, another randomized controlled trial was published that had investigated the role of CPP in cardiac surgery, but this time cryopreservation was performed using the Valeri "no-wash" method. The study was a double-blind, pilot, multicenter randomized controlled trial involving high-risk cardiothoracic surgical patients. The primary outcome was feasibility and safety of the protocol. In the study 23 patients received CPP and 18 received standard platelets. Although the blood loss was similar in both groups, significant postoperative hemorrhage composite bleeding endpoint occurred in nearly twice as many patients in the standard group compared to the group receiving CPP [55.6% *vs.* 30.4, P=0.10). The group receiving CPP received more platelet units (median 2, P=0.012)] and less red blood cell transfusion (median 3, P=0.23) compared to the group receiving standard platelets (platelets median 1, red cells median 4). There were no differences in adverse effects in both groups. The authors concluded that the transfusion of CPP was associated with no evidence of harm and that a study testing safety and hemostatic effectiveness was warranted (23).

Bohonek *et al.* reported also in 2019, an observational study performed in the Military University Hospital of Prague where CPP are indicated for polytrauma and conditions with heavy bleeding. The aim of the study was to determine whether the results of treatment with fresh of frozen platelets were clinically comparable in a group of patients with massive, life threatening bleeding (of trauma, gastrointestinal, and other origins) (24) Twenty-five patients received a total of 81 units of CPP while 21 patients in the control group, received al total of 67 liquid stored platelets. The 30-day survival rate in both groups of patients was similar (76% in the group receiving CPP and 81% in the group receiving liquid stored platelets). There were no statistically significant differences in the number of blood components transfused between the two groups. Only the median platelet count after transfusion was statistically significant higher in the groups of patients receiving liquid stored platelets in comparison to the group receiving CPP (97.0×10^9/L *vs.* 41.5×10^9/L, P=0.02) among the laboratory parameters measured (24).

Current uses of CPP in routine

In 2017, a Vox Sanguinis International Forum investigated the current use of CPP in routine in 12 different countries (26,27). Only in 7 of them, the product was being used (Australia, Belgium, Czech Republic, the Netherlands, Poland, Spain, Switzerland) in a variety of settings. Among those settings, highly alloimmunized patients in need of human leukocyte antigens (HLA)/Human platelet antigens (HPA) matched units (autologous or allogeneic units), armed forces and heavily bleeding patients. The Netherlands Armed Forces, during the past 16 years, have frozen 2,554 apheresis platelets units of which 1,152 were transfused to 350 patients in several conflicts (26). In Poland cryopreserved units are used for neonatal and intrauterine transfusions, immune refractory patients and the stem cell transplant population but only when liquid-stored units are not available (26). A publication reviewing recent mass shootings events, identified some trauma centers transfusing up to 42 therapeutical platelet units in the day of the event (28) suggesting the potential role of CPP to assure that the needs are covered in those consumption peaks.

Summary

Since the development of the cryopreservation method by Valeri, CPP have been available for almost 50 years. However, its use in routine has been limited probably by the technical complications associated with its freezing and thawing and for the impact that the procedure provokes in the platelets. For these reasons currently its use is limited to some scenarios. One is armed forces in order to assure platelet transfusion therapy to combat casualties in locations where liquid platelets are not easily available. Another use is to assure that highly HLA and/or HPA alloimmunized patients with hematology-oncology disorders will have products available for transfusion during the aplasia period associated to chemotherapy treatment. Other potential uses of CPP is for covering supply shortages in remote areas or in situations of increased demand such as mass casualties events.

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