



Letter to the Editor

Response to the Article on Dimethyl Sulfoxide–Cryopreserved Platelets Published in Transfusion Medicine Reviews Volume 28(4)



We were interested in the article “Review of in vivo studies of dimethyl sulfoxide cryopreserved platelets” by Slichter et al [1] and would like to draw attention to our work in this field, which is described in the monograph “Non-surgical bleeding diathesis in anemic thrombocytopenic patients: role of temperature, RBC, platelets, and plasma clotting proteins” [2]. This monograph reports the role of red blood cell function to exert a hemostatic effect together with the platelet hemostatic function of fresh, liquid-preserved, and dimethyl sulfoxide (DMSO)–cryopreserved platelets to reduce the bleeding time and nonsurgical blood loss in healthy volunteers, patients, and baboons. The monograph provides the data on 45 peer-reviewed articles published by the Naval Blood Research Laboratory (NBRL) related to hemostatic function of viable red blood cells and the hemostatic function of fresh, liquid-preserved, and DMSO–cryopreserved platelets.

The article “A simple method for freezing human platelets using 6% DMSO and storage at -80 degrees C” by Valeri et al [3] reports the use of aspirin-treated healthy volunteers to assess hemostatic function of the thawed and washed platelets resuspended in plasma to correct the aspirin-induced prolonged bleeding time 2 hours and 24 hours after transfusion of autologous platelets in male volunteers. The article reports that, in the 12 volunteers, the bleeding time was reduced by about 50% 2 hours after infusion in 9 of the 12 subjects, and 24 hours after the transfusion of the autologous washed platelets, the bleeding time in the 12 volunteers was significantly reduced ($t = 4.130$, $P < .01$). Our laboratory over the past 40 years has used aspirin-treated healthy volunteers to assess the hemostatic function of allogeneic compatible fresh non–aspirin-treated platelets and autologous non–aspirin-treated liquid-preserved and cryopreserved platelets to assess both their in vivo survival and hemostatic function. Slichter et al have identified 5 problems using the aspirinated prolonged bleeding time to evaluate platelet hemostatic function. Our numerous publications do not agree with Slichter et al objections to the use of the aspirin-treated healthy volunteers to assess the in vivo survival and hemostatic function of fresh, liquid-preserved, and cryopreserved platelets in human volunteers and healthy baboons.

In 1968, the NBRL developed a method in healthy volunteers to produce a platelet dysfunction by aspirin treatment of the healthy volunteer to assess the survival and hemostatic function of fresh, liquid-preserved, and DMSO–cryopreserved platelets. The monograph on “Non-surgical bleeding diathesis in anemic thrombocytopenic patients: role of temperature, red blood cells, platelets, and plasma clotting proteins” demonstrated that the bleeding time is affected by temperature, red blood cell count, platelet count, platelet size, platelet function, and plasma clotting proteins factor VIII, fibrinogen, and the von Willebrand

factor [2]. The study of healthy aspirin-treated volunteers controls these factors that affect the bleeding time. Like anemia, aspirin treatment inhibits platelet function [4,5].

The aspirin-induced prolonged bleeding time to evaluate hemostatic effect of platelets has been used and published to assess the effect of washing autologous non–aspirin-treated platelets by Pineda et al [6] and the effect of filtering autologous non–aspirin-treated platelets by Brecher et al [7].

The hemostatic effect of liquid-preserved platelets stored at room temperature for 3.4 days was compared with platelets frozen with 6% DMSO stored at -80°C for as long as 2 years, thawed, washed, and stored in plasma for 5 hours before transfusion to patients with anemia and thrombocytopenia after cardiopulmonary bypass surgery by Khuri et al [8]. The platelets frozen with 6% DMSO and stored at -80°C for 2 years, thawed, washed, and stored in plasma at room temperature for 5 hours reduced nonsurgical blood loss and the need for allogeneic red blood cells, and allogeneic fresh frozen plasma had reduced in vivo survival 2 hours after transfusion of 24% compared with the in vivo survival of 37% for liquid-preserved platelets stored at room temperature for 3.4 days.

There is a current discussion regarding the hemostatic effectiveness of platelets stored at 4°C and platelets stored at room temperature [9].

Clinicians have suggested that platelets stored at 4°C provide better hemostasis in actively bleeding patients than platelets stored at room temperature. There is a current recommendation that platelets stored at 4°C are indicated for actively bleeding patients and that platelets stored at room temperature are indicated to increase the platelet count in patients with thrombocytopenia to prevent bleeding.

Our data obtained at the NBRL, Boston, MA, demonstrate that storage of human platelets at 4°C for 24 hours produces platelets with reduced in vivo survival of 38% and reduced lifespan of 3 days but improved hemostatic function to reduce the bleeding time in aspirin-treated human volunteers, whereas platelets stored at room temperature for 24 hours have in vivo survival of 50% after transfusion and normal lifespan but do not correct the aspirin-induced prolonged bleeding time in healthy volunteers [10].

Studies in aspirinated baboons transfused with fresh, liquid-preserved, and DMSO–cryopreserved autologous platelets show that autologous baboon platelets do not correct the aspirin-induced prolonged bleeding time when the autologous platelets are stored at room temperature for 3 days and 5 days [11]. Cryopreserved baboon platelets frozen with 6% DMSO and stored at -80°C , thawed, washed, and resuspended in plasma reduce the bleeding time in aspirin-treated baboons and have better in vivo survival 2 hours after transfusion compared with autologous baboon platelets stored at room temperature for 5 days. The liquid-preserved autologous baboon platelets stored at 22°C for 5 days do not correct the prolonged bleeding time in aspirin-treated baboons, whereas the cryopreserved autologous platelets correct the aspirin-induced increased bleeding time in the baboons.

Conflict of Interest: The authors have no conflicts to report.

The studies performed at the NBRL have demonstrated that human and baboon platelets frozen with 6% DMSO and stored at -80°C thawed, washed, and stored in plasma and human platelets frozen with 6% DMSO and the supernatant DMSO removed before freezing by storage in mechanical freezers at -80°C after thawing and dilution with 0.9% NaCl have a bimodal population of platelets [12]. One population behaves like platelets stored at 4°C with reduced *in vivo* survival and reduced lifespan and improved hemostatic function. The other populations behave like platelets stored at room temperature with 48% *in vivo* survival and normal lifespan [13]. Clinicians are now requesting that bleeding patients receive platelets stored at 4°C and patients with thrombocytopenia receive platelets stored at room temperature to increase the platelet count to prevent bleeding. Autologous baboon platelets stored at room temperature for 3 and 5 days do not correct the aspirin prolonged bleeding time [11].

The Food and Drug Administration has licensed the storage of human platelets at room temperature for 5 days. The numerous publications on liquid-preserved platelets stored at room temperature for 5 days have reported the *in vivo* recovery 1 to 2 hours after transfusion and the lifespan of the liquid-preserved platelets. Do the 5-day liquid-preserved platelets exert a hemostatic function to reduce the bleeding time and the nonsurgical blood loss in patients with thrombocytopenia? The study of fresh, liquid-preserved, and cryopreserved platelets in aspirin-treated volunteers with increase in bleeding time has provided extremely important data on the hemostatic function of preserved platelets. The survival and hemostatic effectiveness of liquid-preserved and previously frozen platelets published by Valeri [10] provided the data to show that platelets stored at 4°C are more hemostatically effective than platelets stored at room temperature to treat bleeding patients. The survival and hemostatic function of human platelets frozen with 6% DMSO and stored at -80°C have a bimodal population of platelets after thawing, washing, and storage in plasma like human platelets treated with 6% DMSO, removal of the supernatant DMSO before freezing followed by thawing, and dilution in the 0.9% NaCl before transfusion [12]. One population of GPIb normal and reduced annexin V binding platelets and the other population of GPIb reduced and increased annexin V binding are produced in human platelets frozen with 6% DMSO and stored at -80°C . The platelets frozen with DMSO are washed before transfusion, whereas the platelets that are concentrated to remove the DMSO before freezing are thawed and diluted with 0.9% NaCl before transfusion. The population of GPIb reduced and increased annexin V binding platelets have increased hemostatic function to reduce the nonsurgical blood loss observed in patients with trauma treated by the Netherlands military over the past 10 years. The experience with the use of frozen platelets by the Netherlands military has demonstrated that DMSO-cryopreserved platelets provided excellent hemostatic function to reduce nonsurgical blood loss in patients subjected to traumatic injuries without any untoward effects.

The aspirin-treated human volunteers and the aspirin-treated baboons provided important data on the survival and hemostatic function of fresh, liquid-preserved platelets, and cryopreserved platelets. The experience over the past 10 years by the Netherlands military has demonstrated the safety and the hemostatic effectiveness of platelets frozen with 5% DMSO, removal of the supernatant DMSO before freezing and

storage at -80°C for at least 2 years, thawing, and dilution with AB plasma to treat patients with traumatic injuries [14,15]. The NBRL has documented over the past 40 years that aspirin-treated healthy volunteers provide important *in vivo* data on the hemostatic function of autologous and allogeneic compatible preserved platelets to correct the prolonged bleeding time, which has correlated with the hemostatic function of preserved platelets to reduce the bleeding time and nonsurgical blood loss in patients with anemia and thrombocytopenia.

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