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Hemostatic Function of Liquid Preserved and Cryopreserved Platelets

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Abstract

The hemostatic function of liquid preserved and cryopreserved platelets has been studied at the NBRL, Inc., Boston, MA for the past 50 years. Since 1968, aspirin treated male volunteers have been studied to measure the reduction in the increased bleeding time by allogeneic and autologous platelets. Studies performed in aspirin treated male volunteers have shown that frozen platelets are superior to fresh platelets and liquid preserved platelets to reduce the aspirin induced increased bleeding time. A study performed in patients following Cardio Pulmonary Bypass (CPB) has reported that frozen platelets were superior to liquid preserved platelets stored at room temperature (22°C) for 3.4 days to reduce the non-surgical blood loss and reduce the need for red blood cells and fresh frozen plasma transfusions.

Keywords: Hemostatic function; Liquid preserved; Cryopreserved platelets

Introduction

For the past 18 years, the Netherlands military has reported the therapeutic effectiveness and safety of frozen platelets together with frozen RBC and frozen plasma to treat casualties in war zones in Afghanistan and Iraq.

These studies in aspirin treated male volunteers, in patients following Cardio Pulmonary Bypass (CPB), and in patients with traumatic injuries now support the freezing of platelets which can be stored at -80°C for at least 2 years, thawed and diluted with 10-20 mL of 0.9% NaCl to eliminate the need for platelets in fresh whole blood and platelets stored at 22°C.

Studies in normal volunteers

The data obtained in aspirin treated male volunteers show that a single unit of autologous platelets treated with 5% or 6% DMSO, frozen at 2 to 3°C per minute, stored at -80°C in mechanical freezers for at least 8 months, thawed, washed, and resuspended in autologous plasma provided a significant reduction in the increased bleeding time 24 hour after transfusion [1-3] similar to 4 units of fresh allogeneic platelets stored at room temperature (22°C) for 4 hours, 8 units of liquid preserved autologous platelets stored at room temperature (22°C) for 24 hours, and 12 units of liquid preserved autologous platelets stored at room temperature (22°C) for 48 hours [4].

Studies in patients undergoing cardiopulmonary bypass

In the study of patients following Cardio Pulmonary Bypass (CPB), the hemostatic function of allogeneic group O leukoreduced apheresed frozen ($4.5 \pm 2.1 \times 10^{11}$) platelets per patient was superior to liquid preserved ($6.9 \pm 3.9 \times 10^{11}$) platelets per patient stored at 22°C for 3.4 days. The leukoreduced group O apheresed platelets were treated with 6% DMSO, frozen at 2 to 3°C per minute in a -80°C mechanical freezer for at least 2 years, thawed, washed and suspended in autologous plasma stored at room temperature without agitation for 5 hours prior to transfusion. The superior hemostatic function of the frozen allogeneic platelets compared to the liquid preserved allogeneic platelets stored at 22°C for 3.4 days in patients following CPB was documented by the reduction in nonsurgical blood loss and the reduction in red blood cell and Fresh Frozen Plasma (FFP) transfusions in 24 patients who received the frozen platelets with *in vivo* survival of 24% one to two hours following transfusion compared to the 29 patients who received the liquid preserved platelets with *in vivo* survival of 37% one to two hours following transfusion [5]. In aspirated male volunteers the hemostatic function of the frozen platelets was superior to that of the fresh platelets and the liquid preserved platelets stored at room temperature (22°C) for 24 to 48 hours reported by [1-4]. Allogeneic frozen platelets stored at -80°C for at least 2 years and allogeneic liquid preserved platelets stored at 22°C for 3.4 days were transfused to patients following CPB. Significant reduction in nonsurgical blood loss and reduction in the need for red blood cells and Fresh Frozen Plasma (FFP) transfusions were observed for the patients transfused with the frozen platelets [5] support the use of frozen platelets to treat patients subjected to traumatic injuries to reduce nonsurgical blood loss and reduce the need for red blood cells and Fresh Frozen Plasma (FFP) transfusions.

Simplification of the Freezing Method to Eliminate the Need for Post-Thaw Washing

A simple method to freeze human platelets treated with 6% DMSO and frozen at 2 to 3°C per minute by storage in a -80°C mechanical freezer, thawed, washed, and resuspended in autologous plasma [2] was modified in March 2000 to eliminate the need to wash the platelets following thawing and resuspension in plasma. In the modified procedure to freeze platelets treated with 6% DMSO, the DMSO treated platelets are centrifuged at 1250×g for 10 minutes to remove the supernatant DMSO prior to freezing at 2 to 3°C per minute by storage in a -80°C mechanical freezer for at least 2 years. The thawed, previously frozen platelets are diluted with 10 to 20 mL 0.9% NaCl and stored at room temperature for six hours prior to transfusion [6].

For the past 18 years, Lelkens CC and co-authors in the

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Netherlands military have frozen platelets with the modified method which eliminates the need for post-thaw washing by removal of the supernatant DMSO prior to freezing. Lelkens CC and co-authors [7] have utilized the modified method to freeze platelets by the removal of the supernatant DMSO following centrifugation at 1250×g for 10 minutes, freezing at 2 to 3°C per minute by storage at -80°C for at least 2 years, thawed, and resuspended in a unit of AB plasma.

Lelkens CC and co-authors reported that in 2001 two patients were treated with frozen platelets. One elderly woman with gunshot wounds in the pelvic region and one young soldier with acute ITP (viral). Both patients experienced unstoppable bleeding due to a low platelet concentration. Although the platelet count barely rose after transfusion, the bleeding of each patient stopped within 20 minutes after transfusion of one thawed platelet concentrate in AB plasma. The Netherlands military has reported that frozen platelets are life-saving and that the use of a walking blood bank to provide fresh whole blood and liquid preserved platelets was abolished when frozen platelets were available in theatre. From 2001, frozen platelets and frozen blood bank facilities always have been part of the standard equipment of Dutch deployed military hospitals [7].

The protocol reported by Valeri et al [6] to freeze platelets has been evaluated by Dumont LJ and co-authors [8]. The *in vitro* recovery of the frozen platelets and the *in vivo* recovery 1 to 2 hours following transfusion and the lifespan of the frozen platelets in healthy volunteers are reported [8].

Use of the Bleeding Time to Evaluate the Function of Preserved Platelets

The hemostatic function of fresh, liquid preserved, and cryopreserved platelets evaluated in aspirin treated male volunteers has been reported by Handin and Valeri [4], Valeri, [1,2], and Spector JI et al [3]; and in patients subjected to CPB surgery by Khuri et al [5]. These studies show that the hemostatic function of the frozen platelets was superior to the hemostatic function of platelets in fresh whole blood and liquid preserved platelets stored at 22°C for 24 hours, 48 hours, and 3.4 days.

The NBRL has reported that liquid preserved platelets stored at 22°C (room temperature) for 2 days reduced significantly the increased bleeding time in aspirin treated male volunteers 24 hours after transfusion reported by Handin and Valeri [4]. In aspirin treated baboons, autologous platelets stored at 22°C for 2 days and autologous cryopreserved baboon platelets reduced the aspirin induced increase in the bleeding time in the baboons whereas in aspirin treated baboons, autologous baboon platelets stored at 22°C for 3 days and 5 days with agitation did not reduce the aspirin increased bleeding time reported by Valeri et al. [9].

Current Studies of "Chilled" And Lyophilized Platelets

The recent publications by the U.S. Army and Mayo Clinic investigators have reported that "chilled platelets" stored in the liquid state at 4°C in platelet additive solutions for at least 3 days are being evaluated to treat bleeding patients with nonsurgical blood loss [11,12]. The survival and function of autologous chilled platelets stored in Platelet Additive Solutions (PAS) at 4°C for 3 days [11,12] should be measured in aspirin treated male volunteers and compared to autologous platelets treated with 6% DMSO, the supernatant DMSO removed prior to freezing at 2 to 3°C in a -80°C mechanical freezer, thawed, and resuspended in 0.9% NaCl [6].

The survival and function of autologous lyophilized reconstituted platelets [13] should be measured in aspirin treated male volunteers and compared to autologous frozen platelets [6]. The allogeneic chilled platelets [11,12] and allogeneic lyophilized platelets [13] should be studied in patients following CPB as reported by Khuri and co-authors [5] to document the hemostatic function of the "chilled platelets" stored at 4°C in Platelet Additive Solutions (PAS) for 3 days [11,12] and the lyophilized, reconstituted platelets [13] compared to liquid preserved platelets stored at 22°C and the frozen platelets [6].

Conclusion

Universal donor group O leukoreduced apheresed 2.5 to 3.0×1011 platelets stored at 22°C for 48 hours to allow for the mandated testing for infectious disease markers treated with 6% DMSO, the supernatant DMSO removed by centrifugation at 1250×g for 10 minutes prior to freezing at 2 to 3°C per minute with storage at -80°C for at least 2 years, thawed and resuspended in 10 to 20 mL of 0.9% NaCl and stored at room temperature for 6 hours contain a bimodal population of platelets: one population of GPIb normal and annexin V reduced platelets which circulate like platelets stored at 22°C for 24 hours and a population of GPIb reduced and increased annexin V binding platelets which exert an immediate hemostatic effect like platelets stored at 4°C for 24 hours [6,7,10].

The clinical experience reported by Khuri and co-authors in J Thorac Cardiovasc Surg [5] in patients following CPB surgery transfused with frozen platelets and the clinical experience by the Netherlands military in the treatment of wounded casualties in war zones in Afghanistan and Iraq with universal donor frozen group O RBC, frozen group O platelets, and frozen AB plasma have demonstrated the therapeutic effectiveness and safety of the frozen platelets for the past 18 years [6,7,10].

The NBRL modified procedure to freeze platelets was provided to the Netherlands military in March 2000 and this procedure was published by Valeri and co-authors [6] to freeze universal donor group O Rh-positive and group O Rh-negative leukoreduced apheresed 2.5 to 3. 0×1011 platelets treated with 6% DMSO, centrifuged at 1250×g for 10 minutes to remove the supernatant DMSO prior to freezing at 2 to 3°C per minute by storage at -80°C in mechanical freezers for at least 2 years, thawed, and diluted with 10 to 20 mL of 0.9% NaCl and stored at 22°C for 6 hours prior to transfusion provide platelets that function to immediately reduce nonsurgical blood loss. This method to freeze platelets is simple and a routine blood bank needs a -80°C mechanical freezer with a dual-cascade air-cooled compressor and a tank of liquid carbon dioxide to maintain the temperature of the frozen platelets at -80°C for at least 2 years. The platelets are thawed and resuspended in 10 to 20 mL of 0.9% NaCl and stored at room temperature (22°C) for 6 hours without agitation prior to transfusion.

The universal donor leukoreduced apheresed group O, 2.5 to 3.0×1011 frozen platelets should replace platelets in fresh whole blood stored at room temperature (22°C) for 4 hours and liquid preserved platelets stored at room temperature (22°C) with agitation for 5 days.

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