CONCISE REPORT

Platelet Membrane Microparticles in Blood Bank Fresh Frozen Plasma and Cryoprecipitate

By James N. George, Elaine B. Pickett, and Robert Heinz

Cryoprecipitate has been demonstrated to correct the bleeding abnormality of patients with some congenital (storage pool disease) and acquired (uremia) platelet abnormalities, but the reason for this effect is unknown. We found significant platelet contamination in plasma harvested to prepare fresh frozen plasma and cryoprecipitate. The platelet membrane microparticles produced by freezing and thawing of the plasma were highly concentrated in cryoprecipitate and may contribute to its therapeutic effect.

THE CRYOPRECIPITATE FRACTION of plasma can correct the bleeding abnormality of some patients with disorders of platelet function. The original observation was made in 1978, by Gerritsen et al,1 who studied eight patients with congenital platelet storage pool deficiency. In each patient, the infusion of 16 bags of cryoprecipitate caused a shortening of the bleeding time, and four of these patients had surgical procedures during cryoprecipitate treatment with no excessive bleeding.1 Infusion of pasteurized human albumin into six of the patients had no effect on the bleeding time.1 This observation was extended by Janson et al2 to the treatment of seven uremic patients. The bleeding time was decreased to normal, or nearly normal, in each patient after the infusion of ten bags of cryoprecipitate, and four of the patients underwent major surgery with no bleeding complications.2 However, there was no control group in this study, and the risks of bleeding with surgery are unknown.

Although the explanation for this effect of cryoprecipitate is unknown, its use is commonly suggested in current textbooks.3-5 The active principal in cryoprecipitate is believed to be a plasma component, and a potential advantage mentioned for its use in these patients is that platelet alloimmunization can be avoided.1,2 However, the appearance of anti-platelet antibodies has been observed in patients with von Willebrand's disease treated only with cryoprecipitate (S.L. Pfueller, Monash University, Victoria, Australia, personal communication, October 1985), as well as in animals immunized with factor VIII-von Willebrand factor prepared from cryoprecipitate.6 To determine if platelet-derived material is present in blood bank plasma products, we measured the platelet concentration in fresh plasma and the resulting platelet membrane microparticles8 after the preparation of fresh frozen plasma and cryoprecipitate.

MATERIALS AND METHODS

Plasma samples were obtained from routine normal donors at the Medical Center Hospital Blood Bank, and plasma fractions were prepared by the Blood Bank personnel using standard methods described in the technical manual of the American Association of Blood Banks.10 An average of 465 mL of blood in our experiments was collected into 63 mL of CPDA-1 anticoagulant in Fenwall PL-146 triple-unit bags. PL-732 bags were used for the platelet concentrates. When platelet concentrates were being made, the blood was centrifuged at 1,800 g for three minutes at room temperature in a RC3-B centrifuge with a horizontal H-6000A rotor (Sorvall Instruments, Wilmington, Del), and the supernatant platelet-rich plasma (PRP) was squeezed off by a plasma expressor (Fenwall Laboratories, Deerfield, Ill). The PRP was then centrifuged at 5,000 g for five minutes at room temperature and the supernatant plasma was expressed off: an average of 157 mL of plasma was removed and 53 mL of plasma left with the platelet concentrate. The supernatant plasma was promptly frozen at −65 °C. Alternatively, the whole blood was centrifuged at 5,000 g for five minutes at room temperature and the plasma was expressed off and promptly frozen. An average of 244 mL of plasma was removed with five donors; therefore, about 70 mL of plasma plus anticoagulant were left with the cells. Samples for platelet counts (Thrombocounter, Coulter, Hialeah, Fla) were obtained from the plasma bags before freezing. Because of the greater volume, cryoprecipitate is prepared in our blood bank only from the plasma that had been separated directly from whole blood. The plasma was frozen at −65 °C and then placed at 4 °C to thaw overnight. It was centrifuged at 5,000 g for eight minutes at 4 °C and the supernatant "cryo-poor plasma" expressed off, leaving an average of 13 mL of plasma with the precipitate to refreeze at −65 °C. Whole blood samples were obtained directly from the phlebotomy tubing after the completion of the donation, anticoagulated with 5 mmol/L EDTA, 1 μg/mL prostaglandin E1, and 0.1 mol/L dibutyryl cyclic adenosine monophosphate, and centrifuged at 12,000 g for ten minutes to obtain plasma for the assay of platelet membrane microparticles.

Platelet membrane microparticles were measured as previously described,8 using the radiodinated mouse monoclonal antibody, Tab,12,13 against glycoprotein (GP) Iib. Plasma samples (0.5 mL) were incubated with 0.5 μg of 125I-Tab for 30 minutes at room temperature. Control samples, subtracted from the plasma samples, contained 0.5 μg of 125I-Tab in buffer. After incubation, the samples were diluted to 7.2 mL with Tyrode's buffer containing 5 mmol/L EDTA and 0.33% bovine serum albumin and centrifuged at 67,000 g for 60 minutes. The buffer was aspirated and the invisible pellet was washed once with 7.2 mL of buffer at 67,000 g for 20 minutes. Then the cpm remaining in the pellet were measured and expressed as molecules of GP Iib per milliliter of plasma, assuming...
that one molecule of Tab binds to one molecule of GP IIb. Because the membrane glycoprotein composition of platelet microparticles in plasma is similar to platelets, the measurement of a single membrane glycoprotein, GP IIb, should provide a reasonable estimate of plasma microparticle concentration.

RESULTS

Significant numbers of platelets were present in the plasma used for fresh frozen plasma and to prepare cryoprecipitate. Plasma prepared from PRP contained more platelets than plasma prepared by a single centrifugation of whole blood (33,420 ± 5,615 (SD, n = 5) v 13,796 ± 5,204 (n = 5) platelets per microliter, P < .001). The centrifugation force is the same for both preparations and sufficient to sediment all platelets. More platelets may be present in the plasma separated from the platelet concentrates because less plasma is left with the platelet pellet than is left above the platelet–white cell layer when the whole blood is separated by the single fast centrifugation. Also, the presence of red cells may affect platelet sedimentation. After frozen storage, as for clinical use, intact platelets were lysed and platelet membrane microparticles were present in the plasma proportional to the original platelet content (Fig 1). The correlation was significant (r = .89, P < .001). Therefore, the microparticle concentration in fresh frozen plasma prepared from PRP was greater than in fresh frozen plasma prepared directly from whole blood (Table 1). The microparticle concentrations of the donors’ original plasmas, obtained by a method to prevent any platelet contamination or activation, were all normal (normal values = 1.95 ± 1.37 (SD) × 1010 molecules of GP IIb per milliliter plasma, n = 21, ref 10) and therefore were a negligible contribution to these blood bank plasma samples. The platelet membrane microparticles were significantly concentrated within the cryoprecipitate. In these five samples, the microparticle concentration of cryoprecipitate was 29-fold that of the cryosupernatant plasma, with a range of 20- to 34-fold. The cryoprecipitate contained only 5% of the volume of the original plasma but 48% of the total microparticles. The microparticle concentration was 265-fold that of the donors’ original plasma. Using an assumption that the platelet content of GP IIb equals 72,358 molecules per cell (the value we measured by 125I-Tab binding to intact platelets after thrombin-induced secretion, assuming that platelets have no intracellular pool of GP IIb11), ten bags of cryoprecipitate would contain an amount of platelet membrane GP IIb equivalent to about 4 × 109 platelets, or about 20 mL of blood.

DISCUSSION

Plasma separated to prepare the standard blood bank products, fresh frozen plasma and cryoprecipitate, contained a significant number of platelets. When platelet concentrates were prepared, the resulting supernatant plasma contained more platelets than if the plasma was harvested directly from the whole blood. Platelet counts as high as 39,000 per microliter were observed. Platelet contamination of bank plasma products may have clinically relevant effects, such as cleavage of von Willebrand factor by the platelet calcium-activated protease with loss of the largest multimers.14 Proteolytic alteration of fibrinogen and fibronectin due to platelet protease activity may also occur.15,16

The concentration of platelet membrane microparticles in fresh frozen plasma after thawing was proportional to the number of platelets originally present, and these microparticles were highly concentrated by cryoprecipitation. This process concentrates the large contact-promoting proteins that interact with the platelet surface, fibrinogen, fibronectin, and von Willebrand factor, as well as factor VIII and other proteins,16,18 and platelet membrane microparticles

![Fig 1. Platelet membrane microparticles in fresh frozen plasma. The number of residual platelets in the fresh plasma immediately after separation from whole blood or from PRP was proportional to the concentration of membrane microparticles present after freezing and thawing (correlation coefficient, r = .89, P < .001). The data are from five bags obtained from centrifugation of whole blood and five bags obtained from centrifugation of PRP during the preparation of platelet concentrates.](https://www.bloodjournal.org/content/bloodjournal/103/10/1843/F1.large.jpg)
may be concentrated by an interaction with these proteins. We have found that platelet microparticles in plasma contain the membrane GP Ib and GP IIb-IIIa that can interact with these proteins. The ability of platelet microparticles to bind to fibrinogen, fibronectin, or von Willebrand factor may be enhanced by cryoprecipitation, similar to the enhanced ability of platelets to interact with fibrinogen and von Willebrand factor that are bound onto a solid support. Red cell fragments may also be concentrated in cryoprecipitate, as the cryoprecipitate from Rh(D) donors can immunize Rh(D)− patients.

A hemostatic function for platelet microparticles remains speculative. The amount of microparticles in ten to 16 bags of cryoprecipitate is only equivalent to the platelets in about 20 to 30 mL of blood, but the dispersion of the platelets’ contact-promoting membrane glycoproteins into small particles may amplify their effectiveness. An analogous phenomenon is the shedding into media by tissue culture cells of membrane microparticles, termed adherons, that promote both cell adhesion to the substratum and cell–cell aggregation. This function can be blocked by a monoclonal antibody to a 170,000-molecular weight membrane protein shared by the intact cells and adherons. The mechanism of adheron function is unknown, but it is assumed to relate to the concentration of cell adhesion molecules in small particles that can facilitate contact between cells and surfaces. A similar property may be postulated for platelet membrane microparticles.

Because our data were obtained by standard procedures in a general hospital blood bank, these observations should be relevant to plasma components prepared for routine clinical use. Therefore, the presence of platelet membrane microparticles must be considered in interpreting the effects of transfused plasma products.

ACKNOWLEDGMENT
We thank Drs Rodger P. McEver, Geoffrey Johnston, Jean-Philippe Rosa, and G. David Roodman for advice and criticism, and Judi Skinner for the preparation of this manuscript.

REFERENCES

Platelet membrane microparticles in blood bank fresh frozen plasma and cryoprecipitate

JN George, EB Pickett and R Heinz