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 microparticles produced by freezing and thawing of the plasma were highly concentrated in cryoprecipitate and may contribute to its therapeutic effect.

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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. 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, using the radiodinated mouse monoclonal antibody, Tab, 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 0.35% 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 the number of GP IIb molecules is constant in all human plasma.

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Supported by grants from the National Institutes of Health (HL-19996) and the National Aeronautics and Space Administration (NAG 9-5).

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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 mem-
brane 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 cryo-
precipitate. Plasma prepared from PRP contained more plate-
lets 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 mem-
brane microparticles were present in the plasma propor-
tional 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 plasma, obtained by a method to prevent
any platelet contamination or activation, were all normal (normal values = 1.95 ± 1.37 (SD) x 10^{10} 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 signifi-
cantly 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

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Platelet Microparticles (Molecules GP IIb per mL x 10^{10})</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original donor plasma</td>
<td>0.9 ± 0.5</td>
<td></td>
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<tr>
<td>Fresh frozen plasma prepared</td>
<td></td>
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<tr>
<td>from PRP</td>
<td>100 ± 47</td>
<td>157</td>
</tr>
<tr>
<td>Fresh frozen plasma prepared</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from whole blood</td>
<td>25 ± 5</td>
<td>244</td>
</tr>
<tr>
<td>A. Cryoprecipitate</td>
<td>228 ± 30</td>
<td>13</td>
</tr>
<tr>
<td>B. Cryosupernate (cryo-poor</td>
<td>8 ± 2</td>
<td>224</td>
</tr>
<tr>
<td>plasma)</td>
<td></td>
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</tbody>
</table>

The data are the mean values ± 1 SD for ten samples of cell-free
plasma obtained directly from the donors, 13 samples from fresh frozen
plasma bags obtained from platelet-rich plasma (PRP) during the prepara-
tion of platelet concentrates, and five samples from fresh frozen plasma
bags prepared directly from whole blood. The concentration of platelet
membrane microparticles was greater in the plasma prepared from PRP
than in plasma prepared from whole blood (P < .005). The five plasma
bags prepared from whole blood were subsequently fractionated into
cryoprecipitate and the supernatant “cryo-poor plasma” components,
with a significant concentration of platelet membrane microparticles in
the cryoprecipitate fraction compared with the whole plasma (P < .001).
Platelet membrane microparticles were measured using 125I-Tab, a mouse
monoclonal antibody to glycoprotein (GP) IIb.

Fig 1. Platelet membrane microparticles in fresh frozen plasma.
The number of residual platelets in the fresh plasma immedi-
ately after separation from whole blood or from PRP was propor-
tional 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.

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 IIb), ten bags of
cryoprecipitate would contain an amount of platelet mem-
brane GP IIb equivalent to about 4 x 10^9 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 Pro-
eteolytic alteration of fibrinogen and fibronectin due to plate-
let 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 microparti-
cles were highly concentrated by cryoprecipitation. This
process concentrates the large contact-promoting proteins
that interact with the platelet surface,17 fibrinogen, fibronect-
in, and von Willebrand factor, as well as factor VIII and
other proteins,18 and platelet membrane microparticles

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Platelet membrane microparticles 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


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