Effect of Agitation on Platelet Aggregation and Microaggregate Formation in Banked Blood

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Blood stored at 4 C in ACD or CPD solution develops microaggregates composed primarily of fibrin and platelets. This debris has been implicated in the pathogenesis of post-traumatic pulmonary insufficiency in man. Recent work indicates that gentle agitation of the blood during storage appears to decrease debris formation. These studies were undertaken to establish more clearly the effect of agitation on debris formation. Blood was drawn in CPD from healthy young males, non-aspirin ingesting donors and stored at 4 C. One-half of the bags were gently and continuously agitated for 21 days and the other half remained stationary. At the end of the storage period, platelet counts and screen filtration pressures were measured. Agitated blood showed significantly less debris formation and significantly higher platelet counts. Gentle agitation was shown to be an effective method for preventing debris formation in banked blood.

BLOOD stored at 4 C in ACD or CPD solution develops microaggregates composed primarily of platelets and fibrin.[1,2,8,12] This debris in transfused blood has been shown to produce changes in pulmonary hemodynamics in animals[4,7] and has been implicated in the pathogenesis of post-traumatic pulmonary insufficiency in man.[5,9,10] Recent studies in this laboratory suggested that agitation of blood during storage appears to decrease the formation of debris.[1,2,11] The present studies were undertaken to more clearly define the effects of agitation upon debris formation in banked blood.

Materials and Methods

Ten units of fresh human blood were collected in the customary fashion in plastic bags containing CPD solution and stored at 4 C. Blood was drawn from healthy male donors between the ages of 24 and 30, with no history of aspirin ingestion for a 14-day period prior to donation.

Five units of the blood were placed in a wire basket mounted on an electrical rotating platform agitator with an 11-inch diameter platform, a velocity of 26 rpm and rotating at a 30° angle from horizontal. The entire apparatus was placed in the refrigeration unit and allowed to gently agitate, continuously for the 21-day storage period.

The remaining five units of blood were stored in the refrigeration unit for 21 days with no form of agitation.

On day 21, the following studies were performed: Platelet Counts (Model 21, Coulter Electronics); and Screen Filtration Pressure (SFP) and debris weight determinations as previously described.[6]

Results

Platelet counts of the agitated samples were considerably higher than the platelet counts for the non-agitated samples (Table 1).

Debris weights and SFP values of agitated samples were considerably less than corresponding values for non-agitated samples (Table 2).

Discussion

From analysis of Tables 1 and 2, there appears to be an inverse relationship between platelet counts and debris weights. This inverse relationship has been previously demonstrated[1,2] using serial platelet counts and debris measurements for the 21-day storage period. This is not surprising since it appears that platelet aggregation is a necessary precursor to significant debris formation.[1,2,8] Platelet aggregation may account for the decrease in platelet count observed in non-agitated stored blood.

In blood left unagitated for 21 days, separation of the red cells and the plasma occurs into approximately equal volumes. If one assumes that the platelets are entirely in the plasma fraction, then their concentration in the separated

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TABLE 1.

	Mean Day 21 Platelet Counts	p
Agitated samples	154,000/mm³ ± 25,000	<0.001
Non-agitated samples	68,000/mm³ ± 20,000	

TABLE 2.

	Mean Day 21 Debris Weight	p
Agitated	0.1 mg/cc ± 0.1	<0.001
Non-agitated	0.8 mg/cc ± 0.2	

fractions will be twice their concentration when dispersed in whole blood. Since aggregation can result only from platelet collisions, their initial aggregation can be closely approximated by the kinetics equation for a second order reaction: $r = k(\text{platelets})^2$, where 4 = the rate of aggregation, k = a constant determined by the number of platelet collisions per aggregation, and $(\text{platelets})^2$ = the square of the platelet concentration. On the basis of the concentration factor alone, one would expect that since the concentration of platelets in the serum equal two times the concentration in whole blood, the rate of platelet aggregation in non-agitated blood would be four times faster than in agitated blood. Carrying this one step further then, debris formation in gently agitated blood would be expected to form debris at a rate one-fourth that of non-agitated blood.

From Table 2, we see that agitated blood formed one-eighth the debris of non-agitated. Based on the crude assumptions made in setting up the kinetics model, this would appear to be a rather good correlation between the theoretical model and the observed results. It is likely that the platelets in non-agitated blood may be found in a narrow layer within the serum fraction, thus further increasing the effective platelet concentration. If this were taken into account, our theoretical model would approximate the observed results even more closely. Another possible explanation for the discrepancy between predicted and observed values could be that platelet red cell interactions slow platelet aggregation and thus slow debris formation.

Severe agitation has been shown to induce self-aggregation of platelets,[14] possibly by rupturing the platelet membrane with subsequent ADP release. Agitation in this experiment, however, was gentle and actually reduced platelet aggregation and debris formation.

Several reports indicate that platelets are no longer viable after 3 to 5 days.[3,13] On this basis, it seems likely that agitation would be necessary for only 5 days. If, however, platelets continue to aggregate throughout the entire storage period as recent work has indicated,[1,2] then agitation would be necessary during the entire storage period.

References

1. Arrington, P. J. and McNamara, J. J.: Mechanism of Microaggregate Formation in Stored Blood. Ann. Surg., 179:146, 1974.
2. Arrington, P. J. and McNamara, J. J.: Microaggregate Formation in Stored Blood From Aspirin Ingesting Donors. (Submitted for publication)
3. Cohen, P., Gardner, F, and Barnett, O.: Reclassification of the Thrombocytopenias by the ^{51}Cr Labeling Method for Measuring Platelet Life Span. N. Engl. J. Med.: 264:1350, 1961.
4. Hissen, W. and Swank, R. L.: Screen Filtration Pressure and Pulmonary Hypertension. Am. J. Physiol., 209:715, 1965.
5. Jenevein, E. P. and Weiss, D. L.: Platelet Microemboli Associated with Massive Blood Transfusion. Am. J. Pathol. 45:313, 1964.
6. McNamara, J. J.: Effective Filtration of Banked Blood. Surgery, 71:594, 1972.
7. McNamara, J. J., Burran, E. L., Larson, E. J., *et al.*: Effect of Debris in Stored Blood on Pulmonary Microvasculature. Ann. Thorac. Surg., 14:133, 1972.
8. McNamara, J. J., Molot, M. D. and Stremple, J. F.: Changes in Some Physical Properties of Stored Blood. Ann. Surg., 174:58, 1971.
9. McNamara, J. J., Molot, M. D. and Stremple, J. F.: Screen Filtration Pressure in Combat Casualties. Ann. Surg. 172:334, 1970.
10. Moore, F. D., *et al.*: Post-Traumatic Pulmonary Insufficiency. Philadelphia, W. B. Saunders, 1969.
11. Suehiro, G. T., Soeter, J. R., Nakagawa P, *et al.*: Comparison of Filtering Efficiency of Three New In-line Blood Transfusion Filters. (Submitted for publication)
12. Swank, R. L.: Alterations of Blood on Storage: Measurement of Aging Platelets and Leukocytes and Their Removal by Filtration. N. Engl. J. Med., 265:728, 1961.
13. Tranum, B. L. and Hunt, A.: In-vivo Survival of Platelet Prepared in CPD Anticoagulants. Transfusion, 12:168, 1972.
14. Unpublished data from this laboratory.