Effects of Platelet Transfusions: Plug Formation and Maintenance of Vascular Integrity (36096)

ALBERT J. ROY AND ISAAC DJERASSI
(Introduced by Saul Malkiel)
The Children's Cancer Research Foundation, and the Department of Pathology, the Children's Hospital Medical Center, Boston, Massachusetts 02112

The hemostatic functions of platelets include promotion of blood clotting (1–3), clot retraction (4, 5), vascular support (6–8), and platelet plug formation (9). Although much information of a qualitative nature is presently available concerning the relationship of platelets to these various functions, there is a dearth of quantitative knowledge concerning platelet requirements to correct the sequelae of thrombocytopenia. Knowledge of this kind is essential if a rational use of platelets is to be made in the clinic. As more and more information about platelet transfusion has accrued over the past several years, the use of platelets for transfusion in thrombocytopenic patients has increased tremendously. In the presence of this increased use, the need for guidance in terms of platelet dosage on the one hand, and more specific knowledge of patient response to such dosages on the other, becomes more acute.

Prolonged bleeding in thrombocytopenic patients from traumatized vessels results, at least in part from inability to form hemostatic platelet plugs (10). It is generally agreed that the bleeding time is a measure of platelet plug formation (10, 11). The occurrence of spontaneous hemorrhages in thrombocytopenic individuals suggests that blood platelets are also involved in maintaining the integrity or continuous reparative processes of blood vessel structures. Bigelow *et al.* (12) in agreement with previous work by Drinker and Yoffey (13), have shown that in severe thrombocytopenia, red blood cells or extrinsic particulate material may reach the lymph channels without gross morphologic or microscopic changes of the blood vessel walls. The abnormally high concentration of red blood cells in the lymph of thrombocytopenic animals results, therefore, from an increase in the permeability of blood and lymph vessels to formed blood elements and is considered to be a measure of the integrity of blood vessel structure. This represents an entirely different aspect of the hemorrhagic tendency in thrombocytopenia from the inability to form hemostatic platelet plugs. Although Freireich *et al.* (14) demonstrated, in 1963, that there is a relationship between platelet count and the frequency of hemorrhage and that this frequency increases precipitously as the platelet count falls below 50,000/mm^3 the requirements in terms of platelet transfusion to correct the various defects in hemostasis are not really known. The studies reported here were intended to investigate the relationship of platelet transfusion to hemostatic plug formation and to the endothelial supporting function of platelets. Information of this nature could be of value in clinical studies on platelet replacement in thrombocytopenic patients.

Materials and Methods. Seventeen mongrel dogs, weighing from 9 to 11 kg were rendered thrombocytopenic by total body X-irradiation.[1] Seven to 10 days later, the dogs were anesthetized with 45 mg/kg iv Nembutal; and the thoracic duct was cannulated according to the method of Brown and Hardenburgh (15). Lymph samples were collected in 2 ml of 4% sodium citrate for 5 min every 0.5 hr. The total red cell output in the lymph was determined by multiplying the

[1] Irradiation factors: 470 R, given in 2 doses of 235 R delivered to each side of the body; 250 kV, 15 mA X-ray source with 0.25 mm Cu and 1.0 mm Al filters; source–target distance of 110 cm; rate of 5.2 R/min at the skin surface as determined with a Victoreen dosimeter.

TABLE I. Effect of Small Amounts of Fresh Platelets on the Platelet Count, Lymph RBC Output, and Bleeding Time of Thrombocytopenic Dogs.

Platelets (amt infused; units)		Platelet (count/mm³)		RBC (output/mm³ × 10⁶)		Reduction (%)	Bleeding time		
							Initial	Minimum (after infusion)	
		Initial	Maximum	Initial	Lowest		(min)	(min)	(sec)
1[a]		5000	119,000	2288	216	91	>30	8	00
		14,000	64,000	9635	468	95	>30	7	30
		24,000	59,000	5404	1226	80	>30	8	45
		9000	44,000	1987	270	86	>30	>30	
	Av	13,000	71,500			88			
0.5		26,000	70,000	10,660	950	91	>30	7	30
		20,000	59,000	3457	345	90	>30	5	45
		13,000	57,000	1581	171	89	>30	4	20
		20,000	27,000	1728	164	91	>30	20	35
	Av	20,000	53,500			90			
0.25		3000	22,000	3224	328	89	>30	24	45
0.125		2000	9000	7081	3135	56	>30	>30	
		32,000	32,000	5330	2040	62	>30	13	00
		3000	13,000	1474	570	61	>30	>30	
		7000	16,000	6930	5328	23	>30	>30	
	Av	11,000	17,000			51			
0.05		22,000	22,000	463	463	0	>30	>30	
		4000	5000	545	94	83	>30	>30	
		2000	13,000	2730	2240	11	>30	>30	
		8000	8000	8270	3147	65	>30	>30	
	Av	9000	12,000			40			
	PPP[b]	17,000	17,000	1455	1455	0	>30	>30	

[a] One unit of PRP contains 10^{11} platelets.

[b] These values are the average for 10 animals which received platelet-poor plasma.

red cell count by the volume of lymph in the sample.

Blood samples for platelet counts were obtained in duplicate from a standardized incision in a shaved and washed portion of the inner surface of the ear. The method of Brecher and Cronkite (16) employing phase-contrast microscopy was used for the actual counting of platelets. The bleeding time was determined by observing the duration of bleeding from the above incision when the blood was absorbed every 30 sec using Whitman filter paper No. 2.

Platelet counts, bleeding time, and lymph RBC output were determined before and at 30 min intervals for 4 hr following infusion of dog platelet concentrates or platelet-poor plasma (PPP). An average unit of platelets (obtained from 1 pint of blood) contained approximately 10^{11} platelets. The dosage of platelets administered was subsequently expressed in fractions of a unit of concentrate. The infusions were given through a cannula placed in the jugular vein during the cannulation of the thoracic duct.

The relationship of bleeding time to the level of circulating platelets in thrombocytopenic animals was studied separately in 12 irradiated dogs without cannulation of the thoracic duct.

Platelet-rich dog plasma (PRP) was prepared by bleeding normal donor dogs in standard ACD-containing plastic bags. Five hundred milliliters of blood were collected on

each occasion in 75 ml of ACD.[2] The PRP was prepared by centrifugation at 4° using a refrigerated centrifuge (International Model PR2). The blood was centrifuged at 2300 rpm for 5 min and the PRP was transferred to an empty satellite bag. The PRP was centrifuged at 3500 rpm for 6 min to concentrate the platelets. The platelet-poor plasma was removed; and 20 ml was returned to resuspend the platelets. Volumes of the concentrate containing 5×10^9 (0.05 unit) to 4×10^{11} (4 units) platelets were infused over a 10 min period. The control dogs received 20 ml of platelet-poor plasma.

Results. Bleeding time in 20 normal dogs in this laboratory averaged 4 min 29 sec, with a standard deviation of 2 min 08 sec and a range of 2 min 30 sec to 9 min 45 sec.

Platelet counts, bleeding times, and bleeding into the lymph were studied simultaneously in 17 thrombocytopenic dogs (Table I). The infusion of 1.25×10^{10} platelets (0.125 unit) in 4 dogs decreased the output of red blood cells in the thoracic duct lymph by an average of more than 50%. In 2 of 4 dogs which received only 5×10^9 platelets (0.05 unit), the red cell output in the lymph was also reduced by more than one half (83 and 65%, respectively). The average decrease of the lymph RBC output in all 4 dogs in this group was 40%. Minimal or no increase in the number of circulating platelets was observed in these animals.

Bleeding times, determined simultaneously with lymph studies, remained prolonged (more than 30 min in 7 of the 8 animals). Nine additional dogs received more than 1.25×10^{10} (0.125 unit) platelets. The platelet counts increased from very low levels to 50,000/mm^3 or more in 6 of these animals. A substantial (more than 60%) reduction of lymph red cell output was seen in all 9 dogs regardless of the posttransfusion platelet count. The bleeding time, on the other hand, remained prolonged in the animals with platelet counts of less than 50,000/mm^3.

In the 10 dogs which received only platelet-poor plasma, there was no effect on the platelet count nor was there any reduction of the bleeding time during the 4 hr period of observation. Concomitantly, the output of red blood cells in the lymph increased from an average of 1455×10^9 cells to an average of 1620×10^9 cells/5 min period, an increase of 11%.

The relationship of the levels of platelets to bleeding time was studied in a separate group of 12 dogs without cannulation of the thoracic duct. Platelet-rich plasma in excess of 0.125 unit (1.25×10^{10} platelets) was given as shown in Table II. The platelet counts increased to more than 50,000/mm^3 in 8 animals. The prolonged bleeding time in 7 of these 8 was reduced to normal. In 3 of 4 dogs in which the platelet count failed to reach 50,000/mm^3, the bleeding time remained markedly abnormal. The combined data for pre- and postinfusion bleeding times (Table III) show prolonged values in 40 of 42 occasions in which the platelet counts were below 50,000/mm^3. Conversely, in 15 of 17 determinations, the bleeding time was within normal limits when the platelet count exceeded 50,000/mm^3.

Discussion. The above observations indicate that transfusion of small numbers of blood platelets well below the numbers required to restore the bleeding time to normal, can reduce the abnormal red cell content of the lymph in thrombocytopenic animals. These findings are interpreted as evidence that the platelets may sustain vascular integrity or repair blood vessel structures by an unknown mechanism, presumably discrete from that of hemostatic plug formation, which determines the duration of bleeding from a mechanically injured blood vessel. The possibility that small numbers of platelets may suffice to plug hypothetical minute holes in the blood vessel wall through which red cells escape into the lymph spaces is possible, but unlikely, since discontinuity of endothelial structures in bleeding thrombocytopenic animals is not generally observed (17, 18) and diapedesis of red cells across blood vessels has been shown to occur in the presence of normal platelet counts (19), as well as in thrombocytopenic animals. Increased diapedesis of red cells in irradiated

[2] Anticoagulant solution containing: 0.8% citric acid (hydrous); 2.2% sodium citrate; and 2.45% dextrose (hydrous).

TABLE II. Effect of Transfusion of Fresh Platelets on the Platelet Count and Bleeding Time of Thrombocytopenic Dogs.

Platelets (amt infused; units)		Platelet (count/mm³)			Bleeding time: Initial (min)	Bleeding time: Initial (sec)	Bleeding time: Minimum (after infusion) (min)	Bleeding time: Minimum (after infusion) (sec)
		Initial	Increase	Maximum				
4		138,000	101,000	239,000	14	20	4	05
		69,000	64,000	133,000	5	00	4	30
		38,000	103,000	141,000	>30		7	20
		3000	153,000	156,000	>30		7	10
	Av	62,000	105,000	167,000				
3		11,000	86,000	97,000	>30		5	30
		11,000	87,000	98,000	16	10	4	20
	Av	11,000	86,500	97,500				
1		134,000	46,000	180,000	6	35	5	05
0.25		8000	17,000	25,000	>30		>30	
		30,000	21,000	59,000	18	00	18	00
		11,000	22,000	33,000	16	00	16	00
		25,000	12,000	37,000	>30		8	45
		11,000	26,000	37,000	>30		11	75
	Av	17,000	20,000	38,000				

thrombocytopenic animals has been attributed to endothelial damage other than formation of discrete holes (20); and has also been shown to be reduced or eliminated by the injection of platelet factor 3 (8, 9), or soybean phosphatides (21), neither of which is capable of forming hemostatic plugs.

As shown in the above study, bleeding time and increased RBC diapedesis can be differentiated with regard to the amount of transfused platelets needed for their correction. To obtain adequate hemostatic plug formation (as measured by the bleeding time) a certain minimal concentration of platelets is apparently required. Under the conditions of the present experiments, this minimum appears to be of the order of 50,000/mm³. When adequate numbers of platelets were transfused, a markedly prolonged bleeding time was restored to normal. Smaller platelet increments were not effective in this respect. On the other hand, a gradation of effects on bleeding into the lymph was observed with various doses of transfused platelets. With large numbers of platelets in the circulation, a marked reduction in the lymph red blood cell output usually occurred. When smaller numbers were given, lesser but definite effect on this aspect of hemostasis was seen.

It is not clear why a minimum number of circulating platelets is needed to normalize the prolonged bleeding time. Possibly, sufficient platelets must be available per unit of time at the site of injury to quickly form a

TABLE III. Effect of the Level of the Platelet Count on the Bleeding Time of Thrombocytopenic (X-irradiated) Dogs.

No. (%) of occasions when bleeding time was:	Platelet (count/mm³) (× 10³): 0–25	26–50	51–75	>75
>30 min	28 (90)	4 (40)	0 (—)	0 (—)
10–30 min	3 (10)	5 (50)	1 (14)	1 (10)
Within normal limits	0 (—)	1 (10)	6 (86)	9 (90)

plug of sufficient strength, before it can be dislodged by the blood flowing under pressure. When the platelet counts are low, adequate numbers of platelets may not reach the injured site quickly enough to form a stable "plug."

While it is true that there is some variation in the initial platelet counts as well as in the initial output of red blood cells in the lymph in the present studies, it should be pointed out that in all but 3 animals the initial platelet count was below 40,000/mm^3, which represents a reduction of 85 to 90% from normal. With regard to the bleeding into the lymph, previous studies (22) demonstrated that the output of red blood cells in the lymph of normal dogs averaged 16 $\times$ 10^9 cells per 5 min period with a range from 0 to 75. The bleeding observed, therefore, in all of the experimental dogs in the studies presented here, although varying considerably from one to another, demonstrate marked abnormalities in the lymph red blood cell composition.

In view of the known difficulties in closely correlating platelet counts, and bleeding, either in the clinic or experimentally, we feel that the model used in these studies is the closest possible quantitative evaluation of thrombocytopenic bleeding, as Jackson *et al.* (23) and Woods *et al.* (24) had previously demonstrated.

It is of interest also, that when massive bleeding into the lymph is present, some platelets are still found in the circulation. Since the bleeding can be affected by minimal transfusions of platelets which do not increase the platelet count, the question of the functional adequacy of the "residual" platelets can be raised.

Although platelet transfusion increasing the platelet count to above 50,000/mm^3 consistently reversed sequelae of X-ray-induced thrombocytopenia, at least one of the defects, the increased endothelial permeability to red cells can be improved significantly by fewer platelets than are required to normalize the bleeding time. Platelet transfusions, which result in little or no increase in the platelet count may therefore be of some hemostatic value. Understanding of the specific platelet function in need of correction in an individual thrombocytopenic patient may provide some guidance for the dosage and schedules of administration of platelet transfusions.

Summary. Separate mechanisms of action account for the effects of platelets on maintenance of vascular endothelium and on bleeding from mechanically injured vessels. Circulating platelets in excess of 50,000/mm^3 are needed in thrombocytopenic dogs to consistently reduce their bleeding time to normal levels. Significant reduction of red cell diapedesis into the lymph, an index of endothelial damage, was observed following transfusions of small amounts of platelets in the same animal model whether or not the platelet count was increased. The known dependence of the bleeding time on "hemostatic plug" formation suggests that higher levels of circulating platelets are needed for its adequate formation, while small numbers of functional platelets may be needed to support the integrity of vascular endothelium. Since endothelial changes appear to account for initiating spontaneous bleeding and lack of hemostatic plug formation for its continuation, various programs of platelet replacement may be needed by thrombocytopenic individuals requiring prophylactic or therapeutic platelet transfusions.

1. Seegers, W., Thomas, R., Landeburu, R., and Mannen, E., Prothrombin, Recent Chem. Progr. **21**, 1 (1960).

2. Seegers, W., Proc. Conf. Platelets, 3rd, Nat. Acad. Sci., Nat. Res. Counc., Washington, DC **1959**, 57.

3. Seegers, W., "Blood Platelets" (Henry Ford Hosp. Int. Symp.), p. 241. Little, Brown, Boston (1961).

4. Conley, G., Proc. Conf. Platelets, 3rd, Nat. Acad. Sci., Nat. Res. Counc., Washington, DC **1959**, 40.

5. Conley, G., "Blood Platelets" (Henry Ford Hosp., Int. Symp.), p. 437. Little, Brown, Boston (1961).

6. Roy, A., Diss. Abstr. B **28**, (1967).

7. Johnson, S., Blood Clotting Enzymol. **1967**, 379. 79.

8. Johnson, S., VanHorn, D., Pederson, H., and Marr, J., Transfusion **6**, 11 (1966).

9. Roskam, J., Hughes, J., Bounameaux, Y., and Salmon, J., Proc. Conf. Platelets, 3rd, Nat. Acad. Sci., Nat. Res. Counc., Washington, DC **1959**, 32.

10. Marcus, A., and Zucker, M., "The Physiology

of Blood Platelets," p. 65. Grune & Stratton, New York (1965).

11. Roskam, J., Arrest of Bleeding: Physiology, Pharmacology, Pathology, Thomas, Springfield, ILL (1954).

12. Bigelow, R., Furth, J., Woods, M., and Storey, R., Proc. Soc. Exp. Biol. Med. **76**, 734 (1951).

13. Drinker, C., and Yoffey, J., Lymphatics, Lymph and Lymphoid Tissue, Harvard Univ. Press, Cambridge, MA (1941).

14. Freireich, E., Kliman, A., Gaydos, L., Mantel, N., and Frei, E., Ann. Intern. Med. **59**, 277 (1963).

15. Brown, C., and Hardenburg, H., Surgery **29**, 502 (1959).

16. Brecher, G., and Cronkite, E., J. Appl. Physiol. **3**, 365 (1950).

17. Bedson, S., J. Pathol. Bacteriol. **25**, 94 (1922).

18. Spaet, T., Blood **7**, 641 (1952).

19. Quick, A., Ann. N.Y. Acad. Sci. **115**, 252 (1964).

20. Kahn, J., and Furth, J., Blood **7**, 404 (1952).

21. Djerassi, I., Farber, S., Roy, A., and Yoshimura, H., J. Clin. Invest. **41**, 770 (1962).

22. Djerassi, I., and Roy, A., Circ. Res. **10**, 758 (1962).

23. Jackson, D., Sorenson, D., Cronkite, E., Bond, W., and Fliedner, T., J. Clin. Invest. **38**, 1689 (1959).

24. Woods, M., Gamble, F., Furth, J., and Bigelow, R., Blood **8**, 545 (1953).

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