Platelet Dysfunction in Homozygous β -Thalassemia

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Summary

This report details the results of studies performed in nine patients with homozygous β -thalassemia evaluating platelet function and prostaglandin formation. Platelet malonyldialdehyde (MDA) formation in the presence of N-ethyl maleimide (NEM 1 mM) or thrombin (0.5 u/ml) was used as an indicator of platelet prostaglandin synthesis. The data on the nine patients revealed two distinct subgroups of patients. Six of nine thalassemics, demonstrated platelet abnormalities. Their mean bleeding time was 7.5 ± 2.5 min (1 SD), significantly prolonged ($P < 0.005$) when compared to a value of 3.5 ± 1.0 min in normal controls. MDA formation in the presence of NEM was significantly decreased ($P < 0.005$) to 2.41 ± 0.49 (1 SD) when compared to a control value of 3.24 ± 0.33 nmoles MDA/ 10^9 platelets. Similarly, the mean value for thrombin induced MDA was 0.98 ± 0.18 nmoles which was decreased ($P < 0.02$) when compared to a value of 1.26 ± 0.2 in the controls. Platelet aggregations with adenosine diphosphate (ADP), epinephrine, and collagen were abnormal in all six patients. However, when platelets from these patients were mixed with platelets from donors who had ingested aspirin 2-8 hr before donation mutual correction and secondary irreversible aggregation of the mixture resulted. No mutual correction was observed when the thalassemic platelets were preincubated with aspirin *in vitro* before mixing with platelets from donors who had recently ingested aspirin. Although the total amount of platelet malonyldialdehyde formed by the thalassemic platelets in response to NEM and thrombin was decreased when compared to normal controls, this reduction was *not* the cause of the platelet aggregation abnormalities. This appears to be so because the amount of MDA, and, thus, prostaglandin endoperoxides synthesized by these platelets in response to external stimuli was sufficient to cause irreversible aggregation of platelets from donors who had recently ingested aspirin, and were, therefore, unable to synthesize their own endogenous platelet endoperoxides. In the remaining three patients, bleeding times, platelet aggregation, and MDA formation was normal. No correlation was observed between the platelet abnormalities noted and the magnitude of iron overload, presence of fibrin degradation products, liver function abnormalities, or the use of iron chelators in the individual patient. Family studies were normal. Although the platelet dysfunction does not appear to be of major significance in the usual patient with thalassemia major under normal circumstances, antiplatelet aggregating agents should be used with caution. Aspirin inhibits platelet endoperoxide and prostaglandin formation and this effect may potentiate the platelet dysfunction present in some patients with thalassemia major.

Speculation

Platelet dysfunction in homozygous β -thalassemia does not appear to be due to the decrease in platelet malonyldialdehyde formation and, thus, platelet endoperoxide formation. The platelet dysfunctional state may be linked with a plasma inhibitory effect.

In homozygous β -thalassemia, studies of red cell membrane components have demonstrated a marked increase in malonyldialdehyde formation (12), together with a significant decrease in the unsaturated fatty acid, arachidonic acid (9). These changes appear to be indicative of red cell membrane lipid peroxidation. Oxidation and precipitation of excess α hemoglobin subunits, and intracellular iron overload resulting in free radical generation are believed to be the major causes for this propensity to peroxidation in the thalassemic red cell. In platelets, free radical formation is an important intermediate step in the conversion of arachidonic acid to the endoperoxides and thromboxanes involved in platelet aggregation (14). This report details the results of studies performed in nine patients with homozygous β -thalassemia evaluating platelet function and platelet prostaglandin formation.

MATERIALS AND METHODS

Blood samples were obtained after informed consent from nine subjects with homozygous β -thalassemia (eight with thalassemia major and one with thalassemia intermedia—Table 1) and from nine control subjects. Ages of the patient and control groups varied between 3-24 and 5-28 yr, respectively. No control or patient had ingested any medication known to affect platelet function for 10 days before blood donation. Six of nine thalassemic subjects were on no medication at the time of study, while three were on continuous subcutaneous infusion of the iron chelator desferrioxamine (Table 1). The studies were performed pretransfusion in all nine thalassemic subjects at hemoglobin concentrations ranging between 9.6-10.5 g/dl. Blood was also drawn contemporaneously from nine controls who had ingested 600 mg of acetyl salicylic acid (ASA) 2-12 hr before blood donation. All blood samples were anticoagulated with 0.1 M buffered citrate anticoagulant in a ratio of 9 parts of blood to 1 part citrate.

Platelet counts were performed using a Technicon platelet analyzer. Platelet sizing was performed from blood smears prepared from the EDTA-vacutainer tubes by the method of Rivard and Lazerson (10) where platelet diameters were compared to standard-sized polystyrene spheres. Platelet aggregation studies were performed using platelet-rich plasma (PRP) prepared by centrifuging the citrated blood at 150 g (800 rpm) at 22° C for 8 min and adjusting the platelet count to approximately 200×10^9 /liter. The aggregating agents employed included ADP (4 μ M final concentration), epinephrine (6.2 μ M final concentration), and collagen suspensions. Concentrations of aggregating agents were chosen that produced irreversible aggregation of control platelets. Aggregation studies were performed on the nine control and nine thalassemic PRPs, as also on the PRPs obtained from the nine volunteers who had ingested aspirin. After these aggregation studies, mixing experiments were performed in all six experiments where the initial studies revealed abnormalities in the thalassemic patients. For these studies, various proportions of normal or thalassemic PRP (25, 50, and 75%) were mixed with PRP obtained from the aspirin donors (75, 50, and 25%) and the aggregation studies repeated. Finally, aspirin from a stock solution (1 mM at

Table 1. Clinical summary of patients studied

Patient	Sex	Age	Diagnosis	Days since last transfusion	Units of blood transfused to date	Serum ferritin (normal range 7-142)	Chelation Rx
M.S.	F	4	T.M. ¹	24	57	ND ³	-
R.K.	M	9	T.M. ¹	28	104	ND ³	-
R.K.	M	9	T.M. ¹	28	108	1055	-
M.T.	F	3	T.M. ¹	27	39	392	-
E.M.	F	10	T.M. ¹	29	121	971	+
R.W.	F	13	T.I. ²	48	44	448	-
S.M.	M	12	T.M. ¹	29	140	844	+
R.F.	M	22	T.M. ¹	32	212	1848	-
P.M.	F	24	T.M. ¹	30	270	1199	+

¹ T.M. = Homozygous β -thalassemia major.² T.I. = Homozygous β -thalassemia intermedia.³ ND = not done.

Table 2. Thalassaemic patients group B (six of nine) with abnormal platelet function

Measurement	Normal value	M.T.	E.M.	R.W.	S.M.	R.F.	P.M.
Plasma Vitamin E (mg/dl)	1.27 (± 0.40)	0.7	1.0	0.4	1.4	0.8	0.6
FDP (μ g/ml)	<10	0	0	1.25	1.25	2.5	2.5
Platelet count ($1000 \times m^3$)	150 to 350	310	360	420	282	418	405
Ivy Bleeding Time (min) ¹	3.5 (± 1) ¹	12	8	5.5	6.5	5	8
NEM (1 mM) induced MDA (nmoles/ 10^9 platelets)	3.24 (± 0.33)	2.46	2.25	2.21	3.20	2.66	1.71
Thrombin (0.5 u/ml) induced MDA (nmoles/ 10^9 platelets)	1.26 (± 0.2) ¹	0.91	0.87	0.88	1.20	1.24	0.80
Platelet aggregation (ADP, 4 μ M)	Biphasic irreversible	Abn ²	Abn ²	Abn ²	Abn ²	Abn ²	Abn ²
Platelet aggregation (epinephrine, 6.2 μ M)	Biphasic irreversible	Abn ²	Abn ²	Abn ²	Abn ²	Abn ²	Abn ²
Platelet aggregation (collagen)	Lag phase followed by irreversible aggregation	Abn ²	Abn ²	Abn ²	Abn ²	Abn ²	Abn ²

¹ Mean (± 1 SD).² Abn = Minimal single wave aggregation <30% control.

pH 7.4) was added *in vitro* in these six experiments to either control or thalassaemic blood PRP, made up to a final aspirin concentration of 100 μ M, and incubated for 15 min. After this incubation period, mixing experiments were repeated where 25% of these *in vitro* aspirin incubated normal or thalassaemic PRPs were mixed with 75% PRP obtained from the aspirin donors. The PRP was kept in full, tightly-capped plastic test tubes before use, and all aggregating studies were performed within 120 min of platelet harvesting. Platelet lipid peroxide or MDA formation in the presence of NEM 1 mM or thrombin (0.5 units/ml) was performed by a modification (13) of the method of Okuma *et al.* (7) where the products of lipid peroxidation were measured as MDA after its reaction with 2-thiobarbituric acid.

Bleeding times were performed by a modification of the Ivy Technique (6). In the patient group prothrombin, partial thromboplastin, thrombin times, and liver function studies were performed by standard techniques. Fibrin degradation products (FDP) were evaluated by the technique of Merskey *et al.* (5), and vitamin E levels by the method of Quaife *et al.* (8).

Finally, platelet function studies (bleeding times, aggregations, and malonyldialdehyde formation induced by NEM and thrombin) were also performed on the parents of the two thalassems with the most significant platelet functional abnormalities (M.T. and E.M.).

RESULTS

PLATELET DIAMETER DISTRIBUTION VALUES OF CONTROL VERSUS THALASSEMIC BLOOD

No significant shift in platelet diameter distribution values was observed in the patient population, when compared to a normal control population.

EVALUATION OF PLATELET FUNCTION IN CONTROLS AND IN THALASSEMIC PATIENTS

The data on the nine patients revealed two distinct subgroups of patients. In three of nine patients with thalassaemia (group A), all platelet function studies were normal when compared to the controls. The modified Ivy bleeding time in the nine normal controls was 3.5 ± 1 min (mean ± 1 SD). In patients M.S., R.K., and R.K. bleeding times were within the normal range with values of 4.5, 2, and 2.5 min, respectively. The normal controls demonstrated mean values of 3.24 ± 0.33 (1 SD), and 1.26 ± 0.2 (1 SD) nmoles MDA/ 10^9 platelets after exposure of their platelets to 1 mM NEM or 0.5 u thrombin/ml, respectively. In the three of nine thalassems (group A), values for platelet MDA formation with both NEM and thrombin were within the normal range. In these same three patients, platelet aggregations with ADP (4 μ M) and epinephrine (6.2 μ M) were normal, with biphasic irreversible aggregation similar to that of the control PRPs. Aggregation with collagen was also normal.

However, the remaining six of nine patients with homozygous β -thalassaemia (group B) demonstrated a variety of platelet functional abnormalities as depicted in Table 2. The mean bleeding time in this group of patients was 7.5 ± 2.5 min (1 SD), which was significantly prolonged ($P < 0.005$) when compared to a value of 3.5 ± 1.0 min in the normal controls. These patients also demonstrated a significant decrease in platelet MDA formation in the presence of either NEM or thrombin when compared to the normal controls. Mean value for NEM induced MDA formation was 2.41 ± 0.49 (1 SD) nmoles MDA/ 10^9 platelets, which was significantly decreased ($P < 0.005$) when compared to the value in the control group of 3.24 ± 0.33 nmoles. Similarly, the mean value for thrombin induced MDA was 0.98 ± 0.18 nmoles/ 10^9 platelets which was decreased ($P < 0.02$) when compared to a value of 1.26 ± 0.2 in the controls.

Fig. 1. Aggregation tracings of normal control platelets and those from a patient with homozygous β -thalassemia major. Abnormal aggregations with ADP ($4 \mu\text{M}$), epinephrine ($6.2 \mu\text{M}$), and collagen are demonstrated in the patient tested.

Platelet aggregations were abnormal in all six patients in this group with minimal first wave aggregation with ADP and epinephrine, and minimal aggregation to collagen $<30\%$ of concomitant control PRP. Figure 1 depicts representative platelet aggregation tracings of normal control versus thalassemic PRP using ADP ($4 \mu\text{M}$), epinephrine ($6.2 \mu\text{M}$), and collagen as aggregating agents.

As expected, PRP from the control donors who had ingested aspirin 2–8 hr before donation demonstrated only first wave aggregation with ADP and epinephrine, and minimal aggregation to collagen. These ASA donors had markedly decreased values of 0.27 ± 0.15 (1 SD) and 0.10 ± 0.08 nmoles MDA/ 10^9 platelets after exposure of the platelets to NEM and thrombin, respectively.

In an attempt to normalize the aggregation patterns of the six thalassemic PRPs (group B) and that obtained from the aspirin donors, mixing studies were performed. Mixing of 75% aspirin donor PRP with 25% thalassemic PRP caused complete normalization of the resultant mixture in all six experiments using ADP ($4 \mu\text{M}$), epinephrine ($6.2 \mu\text{M}$) and collagen. Similar normalization of aggregation tracings was obtained when mixing studies were performed with 50% aspirin donor PRP and 50% thalassemic PRP. However, correction of aggregation tracings was not obtained when the mixture contained 25% aspirin donor PRP and 75% thalassemic PRP. No correction was obtained with ADP, epinephrine, or collagen in all six experiments when 25% PRP from the six abnormal thalassemic patients was incubated *in vitro* with aspirin before mixing with 75% PRP obtained from aspirin donors. When control PRP was similarly incubated *in vitro* with aspirin before mixing with PRP obtained from aspirin donors no correction was also observed. Figure 2 is a composite of superimposed platelet aggregation tracings demonstrating abnormal aggregation in response to $4 \mu\text{M}$ ADP in both the thalassemic and ASA control. Mutual correction is observed in mixing experiments where 75% ASA control PRP (0.3 ml) is mixed with 25% thalassemic PRP (0.1 ml) before addition of the aggregating agent. It will also be noted in this graph that no mutually corrective effect was observed when the ratios were reversed, i.e., 25% ASA control PRP (0.1 ml) was mixed with 75% thalassemic PRP (0.3 ml) before the addition of the aggregating agent.

COAGULATION SCREENING TESTS, LIVER FUNCTION STUDIES, AND PLASMA VITAMIN E LEVELS IN CONTROLS VERSUS THALASSEMIA BLOOD

No abnormalities were noted in the prothrombin, partial thromboplastin, or thrombin times in the patients with homozygous β -thalassemia. FDP were also normal in all nine patients. A signifi-

Fig. 2. Aggregating tracings of aspirin donor platelets and those from a patient with homozygous β -thalassemia demonstrating abnormal aggregation with ADP ($4 \mu\text{M}$) in both cases. Normalization of the tracing is seen in the mixing experiment where 0.3 ml (75%) aspirin control PRP is mixed with 0.1 ml (25%) thalassemic PRP. No correction of the tracing is seen in the mixing experiment where 0.1 ml (25%) aspirin control PRP is mixed with 0.3 ml (75%) thalassemic PRP.

cant decrease was noted in plasma vitamin E levels in the patient group as a whole ($P < 0.01$) with a mean level of 0.77 ± 0.29 (1 SD) mg/dl when compared to the control value of 1.27 ± 0.4 mg/dl. Liver function abnormalities were found in all thalassemic

patients and included elevations of SGOT (nine of nine) and total bilirubin (three of nine).

PLATELET FUNCTION STUDIES IN PARENTS OF GROUP B THALASSEMICS

Studies in all four parents evaluated were normal. Mean bleeding time was 4.1 ± 1.1 min (1 SD). Platelet MDA formation in the presence of both NEM and thrombin was normal at 3.21 ± 0.09 and 1.22 ± 0.18 nmoles/ 10^9 platelets, respectively. Platelet aggregations with ADP ($4 \mu\text{M}$), epinephrine ($6.2 \mu\text{M}$), and collagen were also normal.

DISCUSSION

The results of the present study show that out of nine patients with homozygous β -thalassemia evaluated, six demonstrated platelet functional abnormalities. The abnormalities included prolongation of bleeding times, abnormal aggregations with ADP, epinephrine, and collagen, and decreased formation of platelet MDA, in the presence of both NEM and thrombin. These laboratory findings were not associated with a clinically significant bleeding tendency in these six patients. However, a history of epistaxis and petechial rashes in the presence of normal platelet counts have been occasionally observed in patients with homozygous β -thalassemia (4).

Basic mechanisms of platelet function include adhesion, contraction, and secretion of platelet constituents contained in platelet dense bodies and α granules. An impairment in secretion can be due to a reduction of serotonin and the nonmetabolic pool of platelet adenine nucleotides, *i.e.*, a storage pool defect, or due to an impairment of prostaglandin synthetic ability, although platelets in this latter situation usually contain a normal complement of serotonin and storage pool nucleotides. In both types of abnormalities the second wave of platelet aggregation is absent such that the performance of platelet aggregation studies will not elucidate the type of defect present.

Smith *et al.* (11) has demonstrated that platelet MDA (a by-product of the platelet endoperoxides) may be used as an indicator of platelet prostaglandin synthesis. Prostaglandin biosynthesis appears to play a key role in platelet secretion and the second wave of platelet aggregation. After various aggregating stimuli, phospholipase is activated in the platelet membrane, which leads to the splitting of arachidonic acid from platelet membrane phospholipid. Prostaglandin cyclooxygenase catalyzes the synthesis from arachidonic acid of the labile endoperoxides PGG_2 and PGH_2 . These endoperoxides in the presence of thromboxane synthetase (an enzyme present in platelet microsomes) causes the synthesis of thromboxane A_2 , the most potent inducer of platelet aggregation and release yet identified. In these studies, determination of platelet MDA formation with NEM and thrombin revealed that the donors who had recently ingested aspirin manifested a near complete absence of platelet malonyldialdehyde formation, concomitant with abnormal single wave reversible platelet aggregation. Platelet aggregation tracings in the six of nine group B thalassemic patients were similar to the aspirin donors. However, although the total amount of platelet MDA formed by these thalassemic platelets in response to NEM and thrombin was decreased when compared to normal controls, this reduction was *not* the cause of the platelet aggregation abnormalities. This appears to be so because the amount of MDA, and, thus, prostaglandin endoperoxides synthesized by these platelets in response to external stimuli was sufficient to cause irreversible aggregation of platelets from donors who had recently ingested aspirin, and were, therefore, unable to synthesize their own endogenous platelet endoperoxides. The absence of complete and irreversible aggregation when a mixture of platelets from aspirin donors was combined with thalassemic platelets preincubated with aspirin *in vitro* also serves to demonstrate that the thalassemic platelet dysfunction cannot be attributed to the abnormality in prostaglandin synthesis. The mixing studies are based on those of Gerrard *et al.* (2) who have elegantly demonstrated that platelets from

patients with the Hermansky-Pudlak syndrome (adenine nucleotide storage pool deficient) correct the defective aggregation and release of aspirin-treated normal platelets by releasing sufficient amounts of platelet endoperoxides to cause aggregation of the aspirin platelets.

In the only previous abstract in the literature, Markenson and Hilgartner (4) demonstrated abnormal bleeding times in 3 of 30, and abnormalities in platelet aggregation in 13 of 30 patients with β -thalassemia major. They showed that the dysfunction appeared to be due to a plasma factor by demonstrating that normal donor gel filtered platelets were made functionally abnormal when incubated in platelet poor plasma from the thalassemic patients who had manifested platelet dysfunction. In this study, no correlation was observed between the platelet dysfunctional state and liver function studies, the presence of FDP, the use of an iron chelator, or the magnitude of iron overload in the individual patient. From Tables 1 and 2, it will be seen that the most severe platelet dysfunctional state was seen in M.T., the youngest patient, who has received the least number of red cell transfusions, who is not receiving an iron chelator, and who has the lowest ferritin level of the group as a whole. That the platelet dysfunctional state is not directly related to iron deposition in platelets was also shown by Markenson and Hilgartner (4) who analyzed the thalassemic platelet spectrophotometrically for iron with negative results. Hereditary causes for the platelet functional abnormalities were also ruled out by our family studies.

Low serum levels of vitamin E have been previously demonstrated in thalassemia major (9), with the suggestion that the low levels are due to a secondary consumption of the antioxidant due to membrane oxidation. However, serum α -tocopherol levels do not correspond with red cell MDA values after peroxide exposure (12), and thalassemic patients with the most significant membrane lipid abnormalities were those with the longest intertransfusion interval (9). Because the E deficient state has been associated with platelet hyperaggregability (3), the platelet hypofunction in these thalassemic patients cannot be attributed to the concomitantly present E deficiency.

Unlike the red cell, the platelet in thalassemia major does not demonstrate an increase but rather a decrease in MDA formation. This is interesting in light of the previous red cell studies of Rachmilewitz *et al.* (9) who demonstrated a decrease in arachidonic acid in the red cell membrane in thalassemia, suggesting that peroxidation of membrane may have occurred. In the platelet, because arachidonic acid is the main substrate for platelet endoperoxide and prostaglandin synthesis, it is possible that decreased levels of this polyunsaturated fatty acid, if present in the platelet membrane in thalassemia, will ultimately lead to decreased endoperoxide and prostaglandin formation.

The bleeding time is the most important *in vivo* assay of platelet adhesion and aggregation available at the present time. It is suggested that bleeding times be performed on all patients with thalassemia before surgical procedures. The platelet dysfunction does not appear to be of much clinical significance in the usual patient with thalassemia major under normal circumstances. It is well documented, however, that the ingestion of aspirin can markedly prolong the bleeding time in subjects with underlying abnormalities either in platelet function, or in the fluid phase of coagulation (1). Aspirin inhibits platelet endoperoxide and prostaglandin formation. Because the inhibition of platelet endoperoxide formation may potentiate the platelet dysfunction already present in some patients with thalassemia major, this drug should be used with caution in this disorder.

REFERENCES AND NOTES

1. Czapek, E. E., Deykin, D., Salzman, E., Lian, E. C., Hellerstein, L. J., and Rosoff, C. B.: Intermediate syndrome of platelet dysfunction. *Blood*, 52: 103 (1978).
2. Gerrard, J. M., White, J. G., Rao, G. H. R., Krivit, W., and Witkop, C. J.: Labile aggregation stimulating substances (LASS): the factor from storage pool deficient platelets correcting defective aggregation and release of aspirin treated normal platelets. *Br. J. Haematol.*, 29: 657 (1975).
3. Lake, A. M., Stuart, M. J., and Oski, F. A.: Vitamin E deficiency and enhanced

- platelet function: reversal following E supplementation. *J. Pediatr.*, 90: 722 (1977).
4. Markenson, A. L., and Hilgartner, M. W.: Plasma inhibitor of platelet function in β -thalassemia. (Abstract) *Pediatr. Res.*, 11: 626 (1977).
 5. Merskey, C., Kleiner, G. J., and Johnson, A. J.: Quantitative estimation of split products of fibrinogen in human serum relation to diagnoses and treatment. *Blood*, 28: 1 (1966).
 6. Mielke, C. H., Kaneshiro, M. M., Maher, I. A., Weiner, J. M., and Rapaport, S. I.: The standardized normal bleeding time and its prolongation by aspirin. *Blood*, 34: 204 (1969).
 7. Okuma, M., Steiner, M., and Baldini, M.: Studies on lipid peroxides in platelets. I. Method of assay and effect of storage. *J. Lab. Clin. Med.*, 75: 283 (1970).
 8. Quaife, M. L., Scrimshaw, N. S., and Lowry, O. H.: A micromethod for assay of total tocopherols in blood serum. *J. Biol. Chem.*, 180: 1229 (1949).
 9. Rachmilewitz, E. A., Lubin, B., and Shohet, S.: Lipid membrane peroxidation in beta thalassemia major. *Blood*, 47: 495 (1976).
 10. Rivard, G. E., and Lazerson, J.: A simple method for platelet sizing. *J. Lab. Clin. Med.*, 86: 547 (1975).
 11. Smith, J. B., Ingberman, C. M., and Silver, M. J.: Malondialdehyde formation as an indicator of prostaglandin production by human platelets. *J. Lab. Clin. Med.*, 88: 167 (1976).
 12. Stocks, J., Offerman, E. L., and Modell, C. B.: The susceptibility to auto oxidation of human red cell lipids in health and disease. *Br. J. Haematol.*, 23: 713 (1972).
 13. Stuart, M. J.: Malonaldehyde formation as a measure of platelet survival. In: M. J. Silver, J. B. Smith, and J. J. Kocis: *Prostaglandins in Hematology*. pp. 57-68. (Spectrum Publications, New York, 1977).
 14. White, J. G., Rao, G. H. R., and Gerrard, J. M.: Effects of Nitro blue tetrazolium and vitamin E on platelet ultra structure, aggregation and secretion. *Am. J. Pathol.*, 88: 387 (1977).
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