Platelet function in beta-thalassaemia major

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SUMMARY Abnormal platelet aggregation was found in eight (44%) of 18 patients with beta-thalassaemia major and transfusional iron overload. The aggregation defect bore no correlation with the degree of hepatic fibrosis, liver function tests, whether or not splenectomy had been performed, the degree of iron overload, haematocrit, platelet count, serum vitamin E level, or leucocyte ascorbate concentration. Only three of the 18 patients showed prolonged bleeding times as well as abnormal platelet aggregation, and only one of these suffered clinically significant haemorrhage.

The results show that a proportion of patients with beta-thalassaemia major have abnormal platelet function. It is possible, however, that the in vitro abnormality might be due partly to artefacts induced by manipulations required to remove the abnormal thalassaemic red cells, and this may explain the much lower incidence of significant haemorrhage.

A mild bleeding tendency characterised by easy bruising and frequent epistaxes has been observed in a significant minority of patients with beta-thalassaemia major (Hilgarter et al., 1963; Hilgarter and Smith, 1964). There does not, however, appear to be a close correlation between the severity of the clinical haemorrhagic tendency and the degree of deficiency of plasma coagulation factors which arises in this disorder largely as a result of parenchymal liver disease.

Eldor (1978) found that 14 of 15 patients with beta-thalassaemia major and four of five with beta-thalassaemia minor had in vitro defects of platelet function, the extent of which was directly related to the severity of haemorrhagic symptoms. The purpose of the present study was further to investigate the frequency and cause of platelet qualitative defects in beta-thalassaemia major and to determine whether these in vitro defects have any relation to clinical bleeding or to prolongation of the bleeding time.

Patients and methods

Eighteen patients (8 females, 10 males, 11 splenectomised, aged 8 to 26 years) with beta-thalassaemia major were included in this study. The total amount of blood received ranged from 64 to 429 units (mean 192 units) and serum ferritin from 1560 to 22 000 μg/l (mean 10 600 μg/l). Two patients had frequent epistaxes. All patients denied having ingested any drug known to influence platelet function for at least 20 days before being tested.

Leucocyte ascorbate was measured by the method of Denson and Bowers (1961), serum vitamin E by the method of Hashim and Schuttringer (1966), and serum ferritin by the method of Addison et al. (1972). All other chemical and haematological tests were done by standard methods.

Platelet function tests

Blood was collected into 1/10th volume of 3·8% tri-sodium citrate in polystyrene tubes and centrifuged at 180 g for 10 minutes at room temperature to obtain platelet-rich plasma (PRP). In 12 patients (Table 1) this was not adequate to remove all of the red cells and so an aliquot of red cell contaminated PRP was harvested, and the remaining blood was recentrifuged as described above. An aliquot of the PRP, now almost free of red cells was collected, and the remaining blood was recentrifuged at 2000 g for 20 minutes at 4° to obtain platelet-poor plasma. Platelet and red cell counts were carried out on both PRP samples.

In order to demonstrate the effect of red cells on the platelet aggregation pattern, in six cases a sample PRP obtained after the first spin (sample A) was compared with PRP obtained after a repeat spin (sample B).

Platelet aggregation was measured by a modification of the turbidimetric method of Born (1962). PRP (0·45 ml) was incubated at 37°C in a Payton dual-channel aggregometer (Payton Associates, Ontario, Canada) with 0·05 ml of aggregating agent.
The stirring speed was 1000 rpm. The aggregating agents used and their final concentrations were adenosine diphosphate (ADP) 2 μM, adrenaline 2 μM, and ristocetin 1-5 mg/ml. Collagen (bovine, Achilles tendon) was suspended in buffered saline (pH 7.35) to a concentration of approximately 1 mg/ml. With all reagents, platelet aggregation was quantitated by measuring the percentage fall in the optical density of the PRP 3 minutes after the addition of the aggregating agent.

Platelet adenine nucleotides were assayed by a modification (Hardisty et al., 1970) of the method of Holmsen et al. (1966). von Willebrand factor in plasma was determined by the method of Weiss et al. (1973) using fresh, unfixed suspensions of washed human platelets.

### Results

A mild prolongation of the prothrombin time and kaolin partial thromboplastin time was observed in 14 (88%) and 11 (69%), respectively, of the 16 patients tested (Table 1). The platelet count was reduced (<150 × 10⁹/l) in one, normal in seven, and elevated (>400 × 10⁹/l) in 10 (59%).

Three patients showed a prolonged bleeding time (cases 1, 2, and 3), and all three showed reduced platelet aggregation with two or more of the aggregating agents used (Table 1). The defect was greatest when ADP or adrenaline were used, less consistent results being obtained with collagen and ristocetin. A further three patients (cases 4, 5, and 6) exhibited a milder degree of abnormality. In all six cases the platelet defect was characterised by the absence of, or a reduction in, the secondary wave of aggregation. Two examples of this (cases 1 and 4) are shown in Figure 1. Two additional patients (cases 7 and 8) had reduced aggregation with ristocetin only. One of the patients who had a history of epistaxis (case 3) had abnormal platelet function, but the other (case 15) had normal function. von Willebrand factor assays were normal in all the patients except case 10, who showed an increased level (300%). The platelet adenine nucleotide concentration and the ATP:ADP ratio were also normal in all cases. The aggregation defect was not correlated with the degree of hepatic fibrosis, splenectomy, units of blood transfused, serum ferritin, liver function tests, haematocrit, platelet count, serum vitamin E level, or leucocyte ascorbate concentration.

The results of aggregation of PRP prepared by both standard (A) and prolonged (B) centrifugation (see methods section) are shown in Table 2. Aggregation measured as percentage fall in optical density was below normal in all six PRP samples prepared by the standard method. However, in most cases a biphasic aggregation curve was observed (see curves A in Fig. 2). There was considerable loss of platelets after recentrifugation, but nevertheless in nearly every instance removal of the red cells resulted in a marked increase in the percentage aggregation (Table 2). The exceptions were case 4, who did not show any...
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Fig. 1 Platelet aggregation in beta-thalassaemia. Arrows indicate the point of addition of the aggregating agent.

improvement, and cases 4, 7, and 8, in whom ristocetin-induced aggregation worsened after the second centrifugation.

Discussion

The results of these studies have shown an in vitro defect of platelet aggregation with one or more aggregating agents in eight of 18 patients with beta-thalassaemia major. One of these eight (case 3) had a mild bleeding tendency, and this patient, together with two others with a platelet defect (cases 1 and 2),

Table 2 Effect of red cells on platelet aggregation

<table>
<thead>
<tr>
<th>Patient</th>
<th>PRP platelet count (10⁹/l)</th>
<th>PRP red cell count (10⁹/l)</th>
<th>Platelet aggregation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>1020</td>
<td>350</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>814</td>
<td>393</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
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</tr>
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<td>18</td>
<td>748</td>
<td>532</td>
<td>32</td>
</tr>
<tr>
<td>Normal range</td>
<td>50 - 90</td>
<td>45 - 90</td>
<td>50 - 90</td>
</tr>
</tbody>
</table>

Results from case 17. PRP samples labelled A contained 12 × 10⁹ red cells per litre and 389 × 10⁹ platelets per litre. PRP samples labelled B contained < 1 × 10⁹ red cells per litre and 300 × 10⁹ platelets per litre. Arrows indicate the point of addition of the aggregating agent.
had a prolonged bleeding time. However, seven of the patients with a platelet defect had undergone a liver biopsy within a week of these studies, and six had had a splenectomy within the previous four years with no specific haemostatic cover and with no untoward bleeding. In no case was the platelet aggregation pattern considered to be grossly abnormal, and this is in contrast to the 'profound' changes in a similar group of patients reported by Eldor (1978). The incidence of a mild bleeding tendency in the latter patients was also apparently much higher than in our group although most had undergone splenectomy without bleeding complications.

The aetiology of the platelet defect appears to be quite variable. In some cases the aggregation abnormality was restricted to one agent only, particularly ristocetin (cases 7 and 8), and was more or less absolute. In others, the platelet release reaction was impaired, although in such cases a normal adenine nucleotide profile excluded storage pool deficiency. The association between a functional platelet defect and scurvy is well established (Wilson et al., 1967). Vitamin C deficiency is a recognised feature of iron overload (Lynch et al., 1967; Wapnick et al., 1970) and, although reduced levels of leucocyte ascorbate were observed in the majority of our patients, there was no correlation with the platelet defect. Vitamin E deficiency has also been well documented in patients with beta-thalassaemia major (Hyman et al., 1974). Little is known about the role of vitamin E in thrombopoiesis or platelet function. However, high platelet counts have been reported with vitamin E deficiency (Ritchie et al., 1968; Keeton, 1976), and Khurshid et al. (1975) reported impaired platelet aggregation with ristocetin in an infant with vitamin E deficiency in whom the platelet function returned to normal after vitamin E treatment. All our patients were deficient in vitamin E, but the severity of this did not correlate with the platelet aggregation results, although it may have contributed, together with splenectomy, to the thrombocytosis observed in some of our patients.

In view of the low incidence of bleeding manifestations in the patients with abnormal platelet aggregation, we have considered alternative explanations for the in vitro defect. Because of the reduced size and volume of the red cells in beta-thalassaemia major it is difficult to prepare PRP sufficiently free of red cells to monitor satisfactorily platelet aggregation by nephelometry. In many instances, prolonged centrifugation was needed, and this resulted in PRP platelet counts which, as shown in Table 1, were lower than those in the whole blood in most cases. It is possible that prolonged centrifugation selectively removes the young metabolically active platelets which are larger and denser than older cells. The latter might be expected to show somewhat reduced aggregability. On the other hand, it is clear from the results in Table 2 that removal of red cells from PRP by prolonged centrifugation improves overall platelet aggregability, particularly to ADP, adrenaline, and collagen. Thus any deleterious effect of prolonged centrifugation on the function of the platelet population is masked. Another factor that may have influenced the aggregation results is the 'platelet refractoriness' induced by a variety of manoeuvres, including centrifugation. Ristocetin-induced aggregation has been shown to be particularly influenced by this phenomenon (Hutton, unpublished observation), and it may explain the abnormality seen in cases 4, 7, and 8 with this reagent after prolonged spinning.

Thus it is possible that the high incidence of abnormal platelet aggregation in thalassaemic patients (44% in our study and 93% in Eldor's group) might be due partly to artefacts induced by in vitro manipulations. This suggestion is to some extent supported by the low incidence of clinically significant haemorrhagic symptoms in such patients. Nevertheless, excessive bleeding can occur in association with platelet disorders of the type we describe (Lusher and Barnhart, 1977).

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References


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