Circulating Platelet Aggregates in Sickle Cell Disease Patients with and without Vaso-Occlusion

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With the Technical Assistance of Carol Burger, B.A.

SUMMARY In vivo circulating platelet aggregates (CPA) were evaluated in 18 patients aged 6 to 17 years with sickle cell disease and in 11 age and sex matched normal subjects. Twelve patients with sickle cell disease were in steady state and 6 had vaso-occlusive crises. CPA in patients in steady state were similar to those in normal subjects (mean 6 ± 1 % compared to 5 ± 2 %, respectively), whereas patients with vaso-occlusive crisis in acute state had significantly higher CPA (mean 39 ± 8 %) than patients in steady state or normal control individuals (both \( p < 0.001 \)). CPA decreased in patients with vaso-occlusive crisis (mean 11 ± 4 %) on the tenth day, in association with clinical improvement. This study suggests that in vivo platelet aggregate formation activity, although normal in sickle cell disease patients in steady state, is significantly increased in patients with vaso-occlusive crises.

SICKLE CELL DISEASE (SCD) results in significant morbidity and mortality related to intravascular thromboses. The thrombotic episodes are generally thought to be related to red blood cell sludging in the microvasculature. Recently, coagulation and platelet abnormalities have also been described in SCD. Platelet abnormalities reported in some SCD patients include thrombocytosis, abnormal platelet aggregation, and increased mean platelet volume. Others have, however, reported normal aggregation patterns. Some investigators have proposed that platelet hyperactivity may be a precipitating or contributing factor in the genesis and extension of thrombosis in SCD. However, little information on in vivo platelet aggregate formation activity in SCD and its relationship to the complications of SCD is available. Therefore, the present study was designed to evaluate platelet aggregate formation activity in patients with stable SCD and in vaso-occlusive crisis.

Materials and Methods

Study Populations

Eighteen children, 6 to 17 years-of-age, with SCD documented by hemoglobin electrophoresis, were studied. Twelve of these 18 patients were in steady state with no clinical evidence of complication (Group A). Three of these Group A patients were on hypertransfusion regimens for prior vaso-occlusive episodes. Six patients presented with acute vaso-occlusive crises (Group B). The clinical profile of all these patients is presented in the table. Eleven age and sex matched subjects with no evidence of hematologic disorder were also studied and formed the control group.

Platelet Studies

Circulating platelet aggregates (CPA) were evaluated by a modification of the method of Wu and Hoak. An antecubital vein that had not been previously used was punctured by one of the investigators (P.M.) in all subjects. Free blood flow was obtained and samples of exactly 0.5 ml each were drawn into 2 plastic syringes each containing 2.0 ml of buffered EDTA and into 2 other plastic syringes containing EDTA with formalin. Care was taken in withdrawing blood at the same rate in all subjects. The samples were tilted gently, and allowed to stand for 15 minutes at room temperature. The 4 tubes were then centrifuged simultaneously for 8 minutes at 150 g to obtain platelet-rich plasma (PRP). Platelet counts on both PRP samples were determined under phase microscopy. CPA (%) were calculated as:

\[
\text{CPA} = \left( \frac{\text{Platelet count in EDTA PRP}}{\text{Platelet count in EDTA-formalin PRP}} \right) \times 100
\]

The method is based on the idea that, when present, platelet aggregates will be fixed in the EDTA-formalin mixture and centrifuged down. Therefore, the platelet count of the PRP in EDTA-formalin mixture will be reduced. However, in the syringe with EDTA alone, platelet aggregates are dispersed. Therefore, CPA approaches zero when there are no aggregates, but increases if aggregates are present.

Both EDTA and EDTA-formalin solutions were prepared fresh before use, and were isotonie. All studies were completed within one hour of blood collection. The test for CPA was performed once in all SCD patients in steady state and in control subjects,
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TABLE. Clinical Profile and CPA in Patients With Sickle Cell Disease

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Sex</th>
<th>Past History</th>
<th>Day 1</th>
<th>Day 10</th>
<th>Treatment</th>
<th>C.P.A. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. I.P.</td>
<td>10</td>
<td>F</td>
<td>Cerebrovascular accident, recurrent pneumonia</td>
<td>Stable</td>
<td>—</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>2. M.M.</td>
<td>14</td>
<td>F</td>
<td>Chronic leg ulcers, psoriasis</td>
<td>Stable</td>
<td>—</td>
<td>Hypertransfusion</td>
<td>6</td>
</tr>
<tr>
<td>3. S.M.</td>
<td>12</td>
<td>F</td>
<td>Arteriovenous fistula, cholelithiasis</td>
<td>Stable</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>4. A.S.</td>
<td>9</td>
<td>M</td>
<td>Unremarkable</td>
<td>Stable</td>
<td>—</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>5. J.R.</td>
<td>13</td>
<td>M</td>
<td>Cerebrovascular accident</td>
<td>Stable</td>
<td>—</td>
<td>Hypertransfusion</td>
<td>0</td>
</tr>
<tr>
<td>6. N.N.</td>
<td>14</td>
<td>F</td>
<td>Unremarkable</td>
<td>Stable</td>
<td>—</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>7. A.H.</td>
<td>5</td>
<td>F</td>
<td>Aplastic crises</td>
<td>Stable</td>
<td>—</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>8. Y.D.</td>
<td>10</td>
<td>F</td>
<td>Gangrene lower extremity</td>
<td>Stable</td>
<td>—</td>
<td>Hypertransfusion</td>
<td>8</td>
</tr>
<tr>
<td>10. D.E.</td>
<td>10</td>
<td>F</td>
<td>Unremarkable</td>
<td>Stable</td>
<td>—</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>11. B.E.</td>
<td>7</td>
<td>M</td>
<td>Unremarkable</td>
<td>Stable</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>12. L.W.</td>
<td>6</td>
<td>M</td>
<td>Unremarkable</td>
<td>Stable</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. A.S.</td>
<td>13</td>
<td>M</td>
<td>Unremarkable</td>
<td>Cerebrovascular accident</td>
<td>Partial recovery</td>
<td>Exchange transfusion</td>
<td>60</td>
</tr>
<tr>
<td>14. D.P.</td>
<td>15</td>
<td>M</td>
<td>Unremarkable</td>
<td>Generalized bone pain</td>
<td>Resolved</td>
<td>Analgesics and hydration</td>
<td>47</td>
</tr>
<tr>
<td>15. G.G.</td>
<td>17</td>
<td>M</td>
<td>Unremarkable</td>
<td>Localized bone pain</td>
<td>Unchanged</td>
<td>Analgesics and hydration</td>
<td>20</td>
</tr>
<tr>
<td>16. M.M.</td>
<td>14</td>
<td>F</td>
<td>Chronic leg ulcers</td>
<td>Localized bone pain</td>
<td>Resolved</td>
<td>Analgesics and hydration</td>
<td>12</td>
</tr>
<tr>
<td>17. K.G.</td>
<td>17</td>
<td>M</td>
<td>Frequent episodes bone pain</td>
<td>Cerebrovascular accident</td>
<td>Partial recovery</td>
<td>Exchange transfusion</td>
<td>50</td>
</tr>
<tr>
<td>18. H.J.</td>
<td>7</td>
<td>M</td>
<td>Unremarkable</td>
<td>Bone pain &amp; aplastic crisis</td>
<td>Resolved</td>
<td>Transfusion</td>
<td>46</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39 ± 8</td>
</tr>
</tbody>
</table>

and twice in SCD patients with complications, on the first and tenth days of presentation.

Calculations

The mean and standard error of CPA values in each group were calculated. Student's t-test was used for paired and unpaired statistical computation. A p value of less than 0.05 was considered significant.

Results

Control Subjects

The duplicate values in each subject were similar (mean variation, 2%). CPA in normal control subjects varied from 0 to 10 %, mean 5 ± 2 %.

Sickle Cell Disease Patients

The results of platelet studies in individual patients are presented in the table. The duplicate values obtained in each patient agreed closely (mean variation, 5 %).

Group A. CPA in patients with SCD in steady state ranged from 0 to 11 %, mean 6 ± 1 %. The 3 patients on hypertransfusion regimen, Nos. 2, 5 and 8, had CPA within normal range, 6, 0 and 8 % respectively. Mean CPA in Group A patients was similar (p-NS) to that in the control subjects.

Group B. CPA in patients with vaso-occlusion varied from 12 to 60 %, mean 39 ± 8 %, which was significantly higher (p < 0.001) than that in Group A SCD patients or control subjects. CPA in 2 patients with cerebrovascular accidents, Nos. 13 and 17, were 60 % and 50 % respectively. In contrast, CPA in patients with localized bone pain, Nos. 15 and 16, were 20 and 12 % respectively and in one with generalized bone pain, No. 14, 47 %. The only patient with aplastic crisis, No. 18, with no clinical or laboratory evidence of infection or a history of drug ingestion, had 46 % CPA.
The 2 patients with cerebrovascular accidents recovered with mild residual weakness. Bone pain alone in 2 of 3 patients resolved with hydration and analgesics and persisted in one. The patient with bone pain and aplastic anemia improved with blood transfusion. Repeat CPA evaluation 10 days later showed CPA values to decline significantly \((p < 0.01)\) to \(11 \pm 4\%\).

**Discussion**

The present study shows that CPA in patients with SCD in stable state are within normal range. However, vaso-occlusive states in SCD patients are associated with a significant increase in CPA at the onset of crisis. This increase in CPA declines to normal levels as the clinical state improves. In addition, the magnitude of increase in CPA correlates with the clinical severity of vaso-occlusive phenomena.

The technique for quantitating CPA employed in this study has been found to be useful in assessing in vivo platelet aggregate formation in several other studies.\(^8\) CPA does not appear to be affected by the intake of food or use of drugs such as anticoagulants.\(^11\) The test is simple and rapidly performed. The duplicate values in individual patients are very similar, if tested within one hour of obtaining blood.

Several investigators have observed abnormalities in platelet function in SCD patients\(^3^\) and postulated that platelet dysfunction may play a role in the genesis of thrombotic events in SCD patients. Our studies clearly show that in vivo platelet aggregate formation activity is normal in SCD patients in steady state, but abnormally high in each SCD patient with vaso-occlusive crisis. Whether increased platelet aggregate formation during acute thrombotic event is primarily responsible for the initiation of the event or is a secondary phenomenon in response to tissue injury\(^6\) is not clear from these studies. As the platelets come in contact with collagen in the blood vessels damaged by sludging of sickle cells, release of thromboxane \(A_2\) occurs leading to vasoconstriction and formation of platelet aggregates,\(^12^\)\(^13\) thus compromising blood flow. Release of catecholamines in response to tissue ischemia and the metabolites of ischemia per se, i.e. ADP, could cause further platelet activation. Whatever the precise mechanisms involved, the increase in platelet aggregate formation in the microvasculature could limit blood flow\(^14\) and augment tissue ischemia produced by sludging of sickled cells in the blood vessels. Normal CPA values in SCD patients in steady state suggest that the increase in CPA is unique to SCD patients with acute vascular insufficiency and tissue injury.

Our study also shows that the magnitude of increase in CPA corresponds to the severity of vaso-occlusive crises. The two patients with cerebrovascular accidents had highest CPA values (60 and 50 \%). One patient with generalized bone pain had higher CPAs (47 \%) than the other 2 with only localized bone pain (20 and 12 \%). CPAs were also high (46 \%) in one patient with aplastic crisis possibly the result of generalized vaso-occlusion in the bone marrow.

Reduced in vivo aggregate formation activity also correlated with improvement in the clinical status (table). In one patient, No. 15, no decline in CPA was observed, while his clinical status was unchanged. In all other patients with vaso-occlusive phenomena, CPA returned to normal levels in association with clinical improvement. The observation of normal CPA in 3 SCD patients on hypertransfusion regimen for previous vaso-occlusive episodes, but presently in steady state, further suggests that increased in vivo platelet aggregate formation activity indicates only acute tissue injury.

We and other investigators\(^8\)\(^-\)\(^10^\)\(^,\)\(^18\) have implicated increase in CPA in vaso-occlusive phenomena such as myocardial infarction and cerebrovascular accidents (adult patients without SCD). It is possible that similar mechanisms are operative in children with SCD presenting with vaso-occlusive complications. Our study further suggests that the magnitude of increase in CPA may also indicate extent of tissue injury. Since the test for quantitating CPA can be done easily, it may be used as a diagnostic and therapeutic guide. To assess the possible usefulness of this test, further studies in a large number of SCD patients with and without complications are warranted.

**References**

Circulating platelet aggregates in sickle cell disease patients with and without vaso-occlusion.
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