Intermediate Syndrome of Platelet Dysfunction

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We studied eight patients with intermittent bleeding episodes usually following trauma and associated with the ingestion of medicine known to interfere with platelet function. All patients had a normal or minimally prolonged baseline bleeding time. All had a normal platelet count, glass bead retention test, and platelet serotonin content and a variable pattern of abnormalities in prothrombin consumption and platelet factor 3 availability. However, all showed abnormal platelet aggregation reactions using epinephrine, adenosine diphosphate, and collagen. Following the administration of 975 mg aspirin, our patients' bleeding times became prolonged to a greater extent than the bleeding times of normal controls (range 13 to > 20 min). Review of the literature showed approximately 5% of "normal" controls had findings similar to those we report. We believe we are describing a group of individuals with an intermediate form of platelet dysfunction. Although their bleeding diathesis is not as severe as that of patients with platelet dysfunction syndromes previously described, they do bleed significantly when subjected to trauma following the ingestion of drugs such as aspirin. We propose that this defect is common and should be screened for. The aspirin tolerance test is a simple test for detecting these patients.

BLEEDING TIME has been advocated as a simple test of platelet function sufficiently sensitive to permit diagnosis or exclusion of platelet dysfunction syndromes. When the bleeding time is normal, additional tests of platelet function are usually not performed.

We encountered a group of patients with a qualitative platelet defect manifested by defective platelet function as measured by tests in vitro of platelet aggregation. Their bleeding times, however, were normal or at most only minimally prolonged. Furthermore, in contrast to the lifelong history of bleeding that characterizes most patients with previously described qualitative platelet disorders, our patients experienced only intermittent episodes of bleeding, usually following trauma.

In our first such patient we noted a correlation between the ingestion of drugs known to interfere with platelet function (aspirin, antihistamines) and the severity of postoperative bleeding. This led us to adopt a standard aspirin tolerance test similar to that originally proposed by Quick as part of the evaluation of patients with normal bleeding times but with histories suggestive of a defect in primary hemostasis. In standardizing the aspirin tolerance test we ad-
ministered aspirin to 25 ostensibly normal volunteers. Two were found to have previously undetected von Willebrand disease confirmed by levels of factor VIII procoagulant activity and factor VIII-like antigen, and a third had a von Willebrand-like syndrome. These three subjects were not included in the present study. One volunteer was found to have a qualitative platelet defect with marked prolongation of his bleeding time following aspirin. This latter individual, together with seven patients referred to us for evaluation of easy bruising or untoward bleeding following trauma or surgery, form the basis of this report.

MATERIALS AND METHODS

Blood was drawn with a double-syringe technique through 19- or 21-gauge scalp vein needles. It was promptly mixed with a 0.13 M trisodium citrate anticoagulant (9 parts blood to 1 part anticoagulant). Platelet-rich plasma (PRP) was obtained by centrifugation at 183 g for 10 min at room temperature. Silicone-coated or plastic materials were used throughout. A sample of nonanticoagulated blood was incubated at 37°C for 2 hr to observe qualitative clot retraction.

Platelets. Platelet counts were performed electronically with the Coulter counter Model ZBI and by phase-contrast microscopy. Platelet size determinations were performed with a Hewlett-Packard 1000-channel pulse-height analyzer coupled to the Coulter counter. Platelet size analyses were carried out as described by previous investigators. A “mean window” for each size determination was calculated and a size index for each subject was determined by dividing the observed mean window by the mean window measured in 49 normal subjects.

Bleeding times were performed using a standard template as described by Mielke et al. The mean bleeding time of three incisions was taken as the final value. Platelet retention in glass bead columns was determined by the Salzman method. Platelet aggregation studies were performed using a chronolog aggregometer as described previously. PRP containing 250,000-300,000 platelets/mm³ was harvested as described above. The PRP was kept in full, tightly capped plastic test tubes prior to use. All studies were performed between 45 min and 2 hr after harvesting the platelets, and platelets from a normal control were run concomitantly.

The reaction of PRP to adenosine diphosphate (ADP), epinephrine, particulate collagen, and ristocetin (final concentration 1.2 mg/ml) were studied. Particulate collagen was prepared from Sigma bovine collagen as described by Hovig. ADP and epinephrine were obtained from Sigma Chemical, St. Louis, Mo. The epinephrine was reconstituted in a semidark room and was stored in a dark compartment at all times. Reactions of platelets to ristocetin were performed according to the method of Lian and Deykin. Platelet factor 3 (PF3) activity was measured as described by Hardisty and Hutton. Three-hour prothrombin consumption was measured by a slight modification of the method of Goldstein et al. Platelet serotonin content was measured by the method of Contractor and platelet nucleotides by the method of Mills and Thomas.

Factor VIII. Factor VIII procoagulant activity was measured by a modification of the one-stage method of Hardisty and MacPherson. Factor VIII-related antigen levels were measured using a modification of the Laurell method as previously described. Aspirin tolerance tests were performed on 21 normal subjects and 8 patients. All were instructed to take no medication for at least 10 days prior to the test. They were allowed clear liquids but not fatty foods for breakfast on the morning of the test, and they were requested not to smoke cigarettes that morning. Prior to the administration of aspirin, blood was drawn for the measurement of platelet adhesiveness, platelet serotonin and nucleotide content, platelet size, aggregation response to ADP, epinephrine, and collagen, activated partial thromboplastin time (APTT) and prothrombin time (PT) tests, factor VIII clotting activity, factor VIII antigen level, prothrombin consumption, and PF3 activity. A three-incision template bleeding time was performed. The subjects were then given 975 mg (three tablets) of aspirin; 2 hr later the bleeding time, prothrombin consumption, PF3, and platelet adhesiveness studies were repeated.
CASE REPORTS

Case 1. P.G. was a 32-yr-old male with a history of intermittent severe bleeding following a tonsillectomy and several dental extractions. He had no family history of bleeding. We had previously evaluated him prior to an elective sigmoid polypectomy. PT, APTT, bleeding time, and platelet count were all normal. At operation major bleeding requiring transfusion occurred. A postoperative wound hematoma developed. In retrospect, it was clear that the patient had ingested aspirin alone or in combination with antihistaminic agents prior to each of his surgical procedures and had ingested both prior to his polypectomy. Subsequent reevaluation revealed abnormal platelet aggregation reactions but a normal bleeding time when he was off all medication. Following aspirin ingestion, his bleeding time became markedly prolonged. He later underwent removal of a nasal polyp. He abstained from any drug known to interfere with platelet function prior to the surgery, and there were no bleeding complications.

Case 2. D.D. was a 25-yr-old male student originally evaluated because of extensive bleeding into an ankle following an athletic injury. He had ingested aspirin to relieve pain. He had a past history of repeated episodes of epistaxis and soft tissue hematomas. His mother bruised easily, and one of her grandfathers was said to have died of hemorrhage, but the details were not known. Prior hemostatic evaluations were reportedly normal.

Case 3. J.R. was a 25-yr-old male evaluated because of an extensive facial hematoma that followed extraction of a wisdom tooth. He had taken aspirin prior to and after the extraction. He had a past history of major soft tissue bleeding requiring 2 units of transfusion following dislocation of a shoulder in an athletic injury. On other occasions, he underwent tonsillectomy and appendectomy without complication. There was no family history of bleeding.

Case 4. M.L. (Mr. L.) was a 45-yr-old male who experienced profuse bleeding following dental extraction at age 8 yr. He had three episodes of upper gastrointestinal tract bleeding following ingestion of large amounts of aspirin. Prior hemostatic evaluations were reportedly normal.

Case 5. R.L. (Mrs. L.) was the 40-yr-old wife of M.L. She had a history of easy bruising following minor trauma. After the delivery of one child, she required 2 units of blood because of excessive postpartum hemorrhage.

Case 6. L.L. was the 13-yr-old daughter of M.L. and R.L. She was evaluated prior to elective surgery because of a past history of prolonged bleeding after tonsillectomy and dental extractions.

Case 7. C.L. was the 11-yr-old daughter of M.L. and R.L. She also bled severely after tonsillectomy and after dental extraction.

Case 8. R.B. was a 31-yr-old male who initially volunteered as one of our normal subjects for the aspirin tolerance test. His history, however, showed that he bruised easily and bled for a prolonged period of time following extraction of a wisdom tooth. His father had been called a “bleeder,” but the patient was unaware of the details.

RESULTS

All patients were tested on at least three separate occasions. The defects found were present on each examination.

The platelet count and the platelet size index were normal in all patients (Table I). The initial bleeding time was minimally prolonged in two patients, the prothrombin consumption was abnormal in four, and PF3 availability was decreased in three (Table I). Two patients (P.G. and R.B.) had no detectable abnormality. One patient (D.D.) showed an abnormality in all three tests.

All patients had a marked impairment in tests in vitro of platelet aggregation in response to ADP, epinephrine, and collagen. Typical aggregation curves for patient D.D. are shown in Fig. 1; those for the “L.” family are shown in Fig. 2. The curves for patients P.G., J.R., and R.B. were similar and are not shown. At a final concentration of ADP that induced complete aggregation in all normals (2 × 10^-6 M), all patients showed only minimal aggregation and rapid
disaggregation. At higher concentrations, patients showed, at best, aggregation followed by rapid disaggregation. All patients showed impaired response to epinephrine; at a concentration that produced typical biphasic aggregation in all of our 21 normal subjects (2 x 10^{-6} \text{M}) all patients showed either no response at all or a pattern of primary aggregation only. At a higher dose (10 x 10^{-6} \text{M}) Mr. and Mrs. L. showed delayed aggregation; the other patients showed only primary aggregation. The response to a standard preparation of collagen was variable. At a concentration that produced aggregation in all normal subjects, all patients failed to respond. At higher concentrations, the platelets of some patients showed relatively normal aggregation patterns. Platelet aggregation studies in response to ristocetin were also performed. Complete aggregation was induced in 35 normal subjects and in all 8 patients at a final concentration of 1.2 mg ristocetin/ml PRP.

Platelet glass bead retention, factor VIII–related antigen, and factor VIII procoagulant activity were normal in all patients, although studies on C.L. were at the lower limits of normal (Table 2).

The platelet serotonin content was normal in four patients and slightly ele-
Fig. 2. Typical baseline platelet aggregation reactions of the "L." family as compared to a normal control.

vated in the four members of the "L." family (Table 3). Platelet ADP levels were normal in seven of eight subjects studied; platelet ATP levels were normal in six of seven patients studied (Table 3). D.D., the one individual with low platelet ADP, also had low platelet ATP and a normal ADP/ATP ratio.

Aspirin Tolerance Test

The effect of the ingestion of 975 mg aspirin on the template bleeding time in 21 normal volunteers is shown in Fig. 3. The mean preaspirin bleeding time (±2 SD) was 4.0 ± 2.8 min. Two hours after aspirin ingestion the mean bleeding time increased to 7.3 ± 4.0 min. The longest bleeding time actually obtained was 12.5 min; this "control subject" had no history of bleeding, and all other tests performed were normal. Following aspirin ingestion, there was no change in platelet adhesiveness, prothrombin consumption, or PF3 activity. In all patients the bleeding time following aspirin ingestion was prolonged beyond the mean ± 2 SD of the controls (Fig. 4). The shortest bleeding time observed in our patients 2 hr following aspirin ingestion was 13 min; the longest exceeded 20 min. There was no correlation between the bleeding time before and the length-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Factor VIII Procoagulant Activity (U/ml)</th>
<th>Factor VIII-related Antigen (U/ml)</th>
<th>Glass Bead Retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.G.</td>
<td>0.72</td>
<td>0.59</td>
<td>45</td>
</tr>
<tr>
<td>D.D.</td>
<td>0.67</td>
<td>0.67</td>
<td>40</td>
</tr>
<tr>
<td>J.R.</td>
<td>0.75</td>
<td>0.53</td>
<td>25</td>
</tr>
<tr>
<td>R.B.</td>
<td>1.20</td>
<td>1.20</td>
<td>54</td>
</tr>
<tr>
<td>M.L. (Mr. L)</td>
<td>0.91</td>
<td>1.08</td>
<td>30</td>
</tr>
<tr>
<td>R.L. (Mrs. L)</td>
<td>0.82</td>
<td>1.04</td>
<td>58</td>
</tr>
<tr>
<td>L.L.</td>
<td>0.67</td>
<td>0.69</td>
<td>23</td>
</tr>
<tr>
<td>C.L.</td>
<td>0.56</td>
<td>0.52</td>
<td>16</td>
</tr>
<tr>
<td>Normal Range</td>
<td>0.50–1.56</td>
<td>0.46–1.52</td>
<td>16–68</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.03 ± 0.53</td>
<td>0.99 ± 0.53</td>
<td>42 ± 26</td>
</tr>
</tbody>
</table>
enning of the bleeding time following aspirin ingestion. In our patients, as with the normal subjects, there was no consistent change in platelet glass bead retention, PF3 availability, or prothrombin consumption tests.

DISCUSSION

During the past decade, an increasing number of tests of platelet function have been developed. Although the advances in methodology have increased our knowledge of platelet biochemistry, it has been difficult to relate abnormalities observed with empirical tests in vitro of platelet function to a basic understanding of how platelets function or fail to function in response to vascular injury. Not surprisingly, there is no universally accepted classification of qualitative platelet disorders, although four general groups are now relatively well defined.15,16

Thrombasthenia is a severe familial disorder, originally characterized by a long bleeding time and impaired clot retraction. The primary phase of platelet

Table 3. Patient Platelet Serotonin, ADP, and ATP Studies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serotonin (nmol/10^9 Platelets)</th>
<th>ADP (nmol/10^9 Platelets)</th>
<th>ATP (nmol/10^9 Platelets)</th>
<th>ATP/ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.G.</td>
<td>3.9</td>
<td>47</td>
<td>88</td>
<td>1.87</td>
</tr>
<tr>
<td>D.D.</td>
<td>4.1</td>
<td>25</td>
<td>46</td>
<td>1.84</td>
</tr>
<tr>
<td>J.R.</td>
<td>3.4</td>
<td>60</td>
<td>82</td>
<td>1.37</td>
</tr>
<tr>
<td>R.B.</td>
<td>3.4</td>
<td>45</td>
<td>88</td>
<td>1.96</td>
</tr>
<tr>
<td>M.L. (Mr. L)</td>
<td>6.5</td>
<td>57</td>
<td>81</td>
<td>1.42</td>
</tr>
<tr>
<td>R.L. (Mrs. L)</td>
<td>6.2</td>
<td>48</td>
<td>70</td>
<td>1.46</td>
</tr>
<tr>
<td>L.L.</td>
<td>6.4</td>
<td>56</td>
<td>74</td>
<td>1.32</td>
</tr>
<tr>
<td>C.L.</td>
<td>6.5</td>
<td>53</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal Range</td>
<td>3.4–5.8</td>
<td>34.4–73.6</td>
<td>55–107</td>
<td>1.26–1.84</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.6 ± 1.15</td>
<td>54 ± 19.6</td>
<td>81 ± 26</td>
<td>1.55 ± 0.29</td>
</tr>
</tbody>
</table>

Fig. 3. Template bleeding times in 21 normal volunteers prior to and 2 hr following ingestion of 975 mg aspirin. Extended line, mean bleeding time.
aggregation in response to ADP, epinephrine, and collagen is absent or impaired. Levels of platelet fibrinogen and certain glycolytic enzymes may be diminished, and a decrease in glutathione peroxidase in these patients has been found.17

Patients affected with a second disorder, Bernard-Soulier syndrome, also experience severe bleeding.18,19 The patients are frequently moderately thrombocytopenic, and their platelets, which are larger than normal, have a shortened survival time. The platelets respond normally to ADP and collagen but are uniquely resistant to the aggregating action of ristocetin. In contrast to effects in patients with von Willebrand disease, the addition of factor VIII does not restore sensitivity to ristocetin.18

The third and fourth disorders, sometimes loosely grouped together under the broad term "thrombopathia," are characterized by the inability of the patient's platelets to release nonmetabolic ADP and serotonin. The third disorder, termed "storage-pool disease," is characterized by diminished to absent storage pools of adenine nucleotides and serotonin;20 the absence of these storage organelles, the platelet-dense bodies, may be the underlying cause of the disorder. Bleeding is moderate to severe and the bleeding time is usually prolonged. The patient's platelets respond to ADP, epinephrine, and collagen by forming primary aggregates only; the second wave of aggregation, thought to be dependent at least in part on the release of adenine nucleotides, does not occur. In contrast, the fourth functional platelet disorder is characterized by normal amounts of storage pool contents, and normal numbers of dense bodies are present. Presumably the defect in these patients reflects an inability to release platelet constituents on exposure of platelets to aggregating agents.

Patients with von Willebrand disease may on occasion be confused with patients having qualitative platelet defects. The bleeding time is prolonged in
both, and decreased retention of platelets in glass bead columns is associated with both disorders. However, in von Willebrand disease levels of factor VIII procoagulant activity and factor VIII-related antigen are decreased, while platelet aggregation reactions to ADP, epinephrine, and collagen are characteristically normal. Although abnormal reactions have been described in patients with low levels of factor VIII procoagulant activity and factor VIII-related antigen, such patients do not fulfill the criteria of classical von Willebrand disease and to date do not clearly fit into any single category of coagulopathy.

Our data confirm the prior observations that aspirin ingestion does not significantly alter platelet adhesiveness, prothrombin consumption, or PF3 activity. The prolongation of the bleeding time following aspirin ingestion in normal volunteers was similar to that reported previously. The data presented in Table 1 and Figs. 1 and 2 distinguish the patients we present in this report from most of those previously described. The data in Table 2 exclude von Willebrand disease. The normal bleeding time, the normal clot retraction, and the ability of the platelets to respond to ADP, collagen and epinephrine by forming primary aggregates differed from the typical features of thrombasthenia. The absence of thrombocytopenia, the normal platelet size, and the normal response to ristocetin separate our patients from those with Bernard-Soulier syndrome. On the basis of normal serotonin content, normal nucleotide content, and ADP/ATP ratio, our patients did not have storage-pool disease. The defect in our patients most closely resembled that described in those patients with normal storage-pool contents but an impaired release mechanism, except that our patients had a normal to only minimally prolonged bleeding time.

Karpatkin and Lackner recently postulated that in some patients, especially those with recent onset and a negative family history, the “thrombopathia” syndrome may be an autoimmune disorder. Our patients differed from theirs in several ways. We did not have a preponderance of female patients, and a positive family history was frequently present. None of our patients ever had evidence of an underlying immunologic disorder. Finally, mixing experiments employing patient plasma and normal platelets gave normal aggregation reactions and PF3 values. Therefore we consider it unlikely that the defect seen in our patients had an autoimmune basis.

The features that characterized our patients therefore are threefold: (1) a history of intermittent, excessive bleeding in response to trauma in association with a normal platelet count and a normal to minimally prolonged bleeding time, (2) exaggerated prolongation of the bleeding time in response to aspirin, and (3) an abnormal pattern of platelet aggregation, suggestive of a defect in the platelet release mechanism. None of these features by itself is specific. However, taken together, these three findings appear to characterize a group of patients with a mild defect of platelet function that would not be detected by routine coagulation screening tests.

A review of the literature shows that in several other series in which the platelet function of ostensibly normal volunteers was studied individuals similar to our patients can be identified. ten Cate et al. studied 80 allegedly normal volunteers and found 8 subjects with repeatedly abnormal platelet aggregation.
PLATELET DYSFUNCTION SYNDROME

Aspirin was administered to 7 of those 8 subjects: in 3 the bleeding time, measured by a technique essentially similar to the standard template test, was prolonged from 2-4 to over 15 min; in the other 4 the bleeding time was measured by the Ivy technique and remained within normal limits. Zucker and Peterson found 2 of 10 subjects studied whose platelets did not produce a normal secondary wave of aggregation in response to epinephrine, and Smith et al. found 1 normal volunteer in a group of 11 whose platelets responded to epinephrine only by primary aggregation. In contrast, in a later study, Hardisty et al. described 60 individuals all of whom had normal aggregation reactions. Weiss and co-workers in two small series found normal aggregation reactions in 16 subjects when receiving a placebo as compared to aspirin.

Therefore a thorough literature review shows that (including our data) in a total of 199 volunteers, all of whom were known not to have ingested aspirin before testing, 12 were found to have impaired aggregation responses, an incidence of approximately 6%. In view of the relatively small numbers and the short interval of time this study encompasses, this figure of 6% may well be off by several fold. However, even if this is so, this syndrome is still sufficiently common to warrant inclusion in the differential diagnosis of bleeding disorders.

We believe that our data, in conjunction with the previously cited studies, indicate that there exists a syndrome of mild platelet dysfunction characterized by intermittent bruising and bleeding, normal to minimally prolonged bleeding in response to a standardized hemostatic challenge, and enhanced sensitivity to the deleterious effects of aspirin on hemostatic function. The platelets from such patients do not respond normally to aggregating agents in tests in vitro of platelet function and appear to have a defect in the release of serotonin.

To date we have not been able to further investigate the exact nature of the platelet defect in these patients. Their platelet aggregation patterns are similar to those of the patient recently described as having mild platelet cyclooxygenase deficiency by Malmsten et al. and some of our patients may indeed have such a defect. More likely, however, they will eventually prove to consist of a heterogeneous group of individuals with a variety of mild defects of platelet function.

Without as yet knowing the exact mechanism(s) for the platelet disorder(s) we encountered, we think the relatively frequent occurrence of this syndrome has several important implications. First, it may offer an explanation for easy bruising and/or postoperative bleeding in some individuals with a normal bleeding time. Since postoperative bleeding can apparently be avoided in such individuals by abstinence from drugs known to interfere with platelet function, identification of this syndrome is clinically important. Second, the concomitant occurrence of platelet dysfunction and a variety of disorders of the soluble clotting mechanism, such as factor IX and factor XI deficiency, has been reported. If mild platelet dysfunction occurs relatively commonly, its association with other diseases is bound to occur to some degree on the basis of chance alone. Such an association might then represent the occurrence of two disease entities in an individual rather than linkage of platelet dysfunction syndromes with disorders of the soluble coagulation mechanism.

Finally, aspirin has been advocated for several years as a potential anti-
thrombotic agent. Aspirin has been suggested as therapy in multiple diseases known to involve formation of platelet aggregates, such as myocardial infarction and transient ischemic attacks, and for prevention of postoperative venous thrombosis.\(^{37}\) Several large clinical trials have been approved to study the advisability of the widespread use of aspirin both prophylactically and therapeutically in such diseases.\(^{38-40}\) If the administration of aspirin increases greatly, and if the incidence of the minimal platelet dysfunction syndrome is as low as 5%, one can expect that on a random basis a relatively large number of individuals with undiagnosed minimal platelet dysfunction will undergo some type of surgical procedure while on aspirin. Such surgery is associated with an increased risk of excessive bleeding and hence possibly with increased morbidity. Because both aspirin ingestion and the syndrome of minimal platelet dysfunction we describe are relatively common, we consider minimal platelet dysfunction to be clinically important. Although it can be diagnosed by repeated platelet aggregation studies, the aspirin tolerance test appears to be a simple, reliable method of detecting mild platelet dysfunction syndromes. We therefore suggest that the aspirin challenge test become an integral component in the diagnosis of disorders of platelet function.

REFERENCES

30. Deleted
Intermediate syndrome of platelet dysfunction
EE Czapek, D Deykin, E Salzman, EC Lian, LJ Hellerstein and CB Rosoff