Platelet Arachidonic Acid Metabolism and Platelet Function in Ten Patients With Chronic Myelogenous Leukemia

By Steven J. Jubelirer, Francis Russell, Raymond Vaillancourt, and Daniel Deykin

We have compared the pathways of arachidonic acid (C 20:4) metabolism in platelets from ten patients with Philadelphia chromosome-positive CML with those of seven normal subjects. Platelets were incubated with 3H-arachidonic acid, gel-filtered, and treated with thrombin (5 U/ml). The cyclooxygenase and lipoxygenase-derived products and free arachidonic acid released from the platelets were separated by high pressure liquid chromatography and their radioactivity determined. The total uptake of 3H-C 20:4 by platelets from CML patients did not differ from controls, but the release of radioactivity in response to thrombin was significantly lower (p < 0.01) in CML patients (32.3% ± 4.9% of total radioactivity was released from control platelets; 19.0% ± 7.4% from CML platelets). Both cyclooxygenase and lipoxygenase-derived products were reduced, but there was no specific pattern of abnormality. Although there was no direct correlation between either the WBC or platelet count and impairment of platelet C 20:4 metabolism, the platelets from three patients with accelerated disease released the lowest total amount of 3H-C 20:4 metabolites. In a single patient, studied before and after successful chemotherapy (hydroxyurea), severe abnormalities in platelet arachidonic acid metabolism returned to normal after treatment.

A BNORMALITIES of platelet function have been well described in patients with chronic myelogenous leukemia (CML). Such abnormalities include the following: absent primary or secondary wave aggregation to epinephrine, adenosine diphosphate (ADP), and collagen;1-3 decreased platelet retention to glass-bead columns;4 decreased platelet factor 3 availability;5 and a deficiency of the platelet storage pool.6,8 The underlying nature of these abnormalities, however, is not well understood.

Recent literature has stressed the important physiologic role of arachidonic acid in human platelets, as it is a precursor to thromboxane A2, which induces platelet aggregation.9,10 In this study, we have compared the pathways of arachidonic acid metabolism in platelets from ten patients with Philadelphia chromosome-positive CML with those of seven normal subjects. We focused primarily on the following questions: (1) Is arachidonic acid metabolism normal in CML platelets? (2) Is there a correlation between the clinical state of the patient (as reflected by the WBC, platelet count, and bleeding history) and the degree of impairment of arachidonic acid metabolism? (3) Do abnormalities in platelet aggregation tests directly reflect abnormalities of arachidonic acid metabolism?

MATERIALS AND METHODS

Patients and Controls

Criteria for inclusion of patients into this study were the following: (1) a positive Philadelphia chromosome; (2) low leukocyte alkaline phosphatase; (3) an elevated WBC at some period during their clinical course; (4) a peripheral smear consistent with CML (i.e., myeloid cells at all stages of development). The controls, 4 males and 3 females, were laboratory and hospital personnel and ranged in age from 23 to 43 yr.

None of the patients or controls had ingested aspirin or aspirin-containing medications for at least 7 days prior to testing. All had normal renal function. Seven of ten patients were on chemotherapy (hydroxyurea or myleran) when studied, although all had been on therapy at some time during their course. Informed written consent for the studies was obtained from all patients and controls.

Platelet Function Tests

Bleeding times were performed with the General Diagnostics Simplate II bleeding time device.13 Venous whole blood was obtained from each patient by the two-syringe technique. It was collected into 13 mm x 100 mm plastic tubes containing an acid-citrate dextrose solution in a ratio of one part of anticoagulant to six parts of blood. Platelet-rich plasma (PRP) was obtained by centrifuging the anticoagulated tubes for 15 min at 150 g at room temperature. Platelet-poor plasma (PPP) was obtained by centrifuging the blood for 10 min at 1500 g at 4°C.

Platelet aggregation studies were performed on a Bio-Data Chronolog aggregometer using 0.5 ml of the PRP and 0.05 of the aggregatant at 37°C. When the platelet count in the PRP was greater than 400,000, the aggregation tests were done on PRP diluted with the patient’s PPP to a platelet count of 300,000. The following aggregatants were routinely tested: epinephrine 10−M; ADP, 10−M; collagen, 0.8 μg/ml; and ristocetin, 1.5 mg/ml.

The arachidonic acid metabolites released from platelets were separated by high pressure liquid chromatography (HPLC) as described by Russell and Deykin12 and Deykin et al.13 In preparation for the HPLC, 1.8 ml of PRP was labeled with 0.3 ml of 5,6,8,9,11,12,14,15-[3H]-arachidonic acid (72 Ci/m mole) bound to 10% delipidated bovine serum albumin for 20 min at 37°C (pH 6.5). The PRP was then passed over a Sepharose 2B column (1.8 ml PRP/12 ml plastic syringe column) equilibrated with a calcium-free
buffer; and \(^{3}H\)-labeled gel-filtered platelets (GFP) free of unincorporated \(^{3}H\)-arachidonic acid were obtained. The GFP were then stimulated with 5 U/ml of thrombin and incubated for 10 min at 37°C in 13 × 100 mm silicone-coated glass tubes. Each tube contained in 1 ml approximately 1.5 × 10⁶ GFP, 3 mM Ca\(^{2+}\), and 0.5 mg/ml of albumin. After incubation, the reaction was terminated by the addition of 100 μl of 200 mM EDTA (pH 5.0) and 11 μl of DMSO. The platelets were immediately separated by centrifugation at 8000 g for 3 min in a Fisher table-top high-speed centrifuge. The supernatant was then obtained for HPLC analysis.

Epinephrine and adenosine diphosphate (ADP) were obtained from Sigma Laboratories (St. Louis, Mo.). Ristocetin was obtained from New England Nuclear (Boston, Mass.). Highly purified human \(\alpha\)-thrombin (mM = 0.1 U/ml) was kindly provided by Dr. J.W. Fenton III.

All tests were performed within 1 hr of collection of blood.

RESULTS

Patients

The clinical data on the patients are presented in Table 1. The range of the WBC was 6900–567,000/cu mm, while the range of platelet count was 196,000–606,000/cu mm at the time of testing. Of the 10 CML patients studied, only one had a history of bleeding. None of the patients had a prolonged bleeding time.

Platelet Aggregation Studies

The results are shown in Table 2. Six of ten patients had abnormal aggregation to epinephrine (i.e., absent primary or secondary wave aggregation); four of ten had absent secondary wave aggregation to ADP; five of ten had absent aggregation to collagen; and two patients had absent ristocetin aggregation.

Arachidonic Acid Metabolism

The total uptake of \(^{3}H\)-arachidonic acid by platelets from the CML patients did not differ from controls. In contrast, the total release of radioactivity in response to thrombin was significantly lower (<0.01) in the CML patients (32.3% ± 4.9% of the total radioactivity incorporated was released from control platelets; 19.0% ± 7.4% from CML platelets). The data on the release and distribution of arachidonic acid and its metabolites are presented in Table 3. There was no specific pattern of abnormality in the distribution of released metabolites. Six of ten patients had a decrease in the cyclooxygenase-derived products that was more than 2 standard deviations below the mean of the controls; 5 of 10 patients had a similar decrease in released lipoxygenase-derived products; and 3 patients had a similar decrease in the release of both cyclooxygenase and lipoxygenase-derived products. An increase in released free arachidonic acid to levels at least 2 standard deviations above the mean of the controls, reflecting a decrease in its conversion to other metabolites, was found in 8 patients. In only one patient (patient 6) was arachidonic acid metabolism completely normal.

Response to Therapy

One patient was studied before and 4 mo after chemotherapy (2.5 g/day of hydroxyurea) was initiated (Table 4). The platelet aggregation tests and platelet arachidonic acid metabolism normalized after remission was achieved.

DISCUSSION

Our study demonstrates that platelets from patients with CML release less \(^{3}H\)-arachidonic acid and its metabolites in response to thrombin stimulation than do normal platelets. It is possible that the abnormal release reflects preferential release of unlabeled arachidonic acid from CML platelets, but our observations that uptake of \(^{3}H\)-arachidonic acid is similar in both control and patient platelets makes this less likely.

Although the platelets from three patients with the
Table 3. Platelet Arachidonic Acid Metabolism After Stimulation With 5U/ml of Thrombin for 10 min at 37°C

<table>
<thead>
<tr>
<th>Controls (7)</th>
<th>Patients</th>
<th>WBC (x 10⁹)</th>
<th>Platelet Count (x 10⁹)</th>
<th>Total No. of Platelet Aggregation Abnormalities</th>
<th>Percent of Total Radioactivity Released</th>
<th>Percent Distribution of Released Products</th>
<th>Cyclooxygenase Products†</th>
<th>Lipoxygenase Products‡</th>
<th>Arachidonic Acid</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>99</td>
<td>344</td>
<td>0</td>
<td>20</td>
<td>32.2 ± 5*</td>
<td>31.1 ± 1.8</td>
<td>28.5 ± 2.5</td>
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<tr>
<td>2</td>
<td>36</td>
<td>243</td>
<td>0</td>
<td>23</td>
<td>31.1 ± 5.2</td>
<td>30.5 ± 1.6</td>
<td>29.3 ± 2.5</td>
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<tr>
<td>3</td>
<td>7</td>
<td>606</td>
<td>0</td>
<td>26</td>
<td>33.1 ± 5.0</td>
<td>31.9 ± 1.7</td>
<td>29.2 ± 2.3</td>
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<tr>
<td>4</td>
<td>16</td>
<td>280</td>
<td>0</td>
<td>25</td>
<td>34.5 ± 5.5</td>
<td>33.2 ± 1.5</td>
<td>31.3 ± 2.4</td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>345</td>
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<td>40.5 ± 5.9</td>
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<td>38.3 ± 2.7</td>
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<tr>
<td>8</td>
<td>10</td>
<td>269</td>
<td>3</td>
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<td>41.2 ± 1.9</td>
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<td>265</td>
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<tr>
<td>10</td>
<td>567</td>
<td>196</td>
<td>4</td>
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<td>45.2 ± 2.1</td>
<td>44.3 ± 3.0</td>
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</tr>
</tbody>
</table>

*Mean ± 1 SD.
†TXB₂, HHT, and PGD₂.
‡HETE.

most accelerated disease (patients 5, 9 [blast crisis], and 10) had the most impaired arachidonic acid metabolism, we found no direct correlation between the level of WBC, platelet count, and degree of impairment of arachidonic acid metabolism. This observation is well illustrated by patient 1, with a WBC of 99,000 and 20% of total incorporated radioactivity released, in comparison to patient 7, with a WBC of 17,700 and 18% of incorporated radioactivity released. In another example, patient 3 had a platelet count of 606,000, 26% of total incorporated radioactivity released, and a slight decrease in release of cyclooxygenase-derived products (27%), whereas patient 8 had a platelet count of 269,000, 22% of the radioactivity released, and a more marked decrease in released cyclooxygenase-derived products.

We also found that abnormalities in platelet aggregation in these patients did not necessarily reflect abnormalities in arachidonic acid metabolism. For example, the four patients with normal platelet aggregation studies (patients 1–4) had a mild decrease in release of radioactivity in response to thrombin and variable abnormalities in the distribution of metabolites (e.g., patient 3, with reduced amounts of released lipoxygenase-derived products, and patient 4, with a reduced amount of released cyclooxygenase-derived products). The one patient (patient 6) with normal arachidonic acid metabolism had absent secondary wave aggregation to both epinephrine and collagen.

The results of previous studies on platelet arachidonic acid metabolism and CML have been variable. Keenan et al.14 reported a reduction in NEM-stimulated malonyldialdehyde (MDA) production in 10 patients with various myeloproliferative disorders, including 1 of 4 patients with CML. Gerrard et al.6 studied 8 patients with CML, 7 of whom had absent secondary wave aggregation to epinephrine. These 7 patients had normal MDA production, and in two of these patients, the conversion of arachidonic acid to HETE, HHT, and thromboxane B₂, as measured by mass spectroscopy, was normal. Mannucci et al.7 in a study of platelet function in 40 patients with a myeloproliferative disease, found normal levels of thromboxane B₂ (measured by radioimmunoassay) in these patients, including 9 patients with CML. Okuma and Uchino15 reported a deficiency of platelet lipoxygenase activity in 4 of 7 patients with CML. These same investigators,16 in a subsequent study of 33 patients with various myeloproliferative disorders, reported markedly decreased lipoxygenase activity in 8 of 14 patients with CML and significantly decreased cyclooxygenase activity in only 1 of these patients.

The findings of Okuma and Uchino contrast with ours. This discrepancy can be explained in part by the following differences in their methodology compared to ours: (1) by using exogenous ¹⁴C-arachidonic acid,
in contrast to stimulating prelabeled platelets, they by-passed the controls exerted by phospholipases, the primary determinants of arachidonic acid metabolism within the platelet; (2) by omitting calcium from the medium, they employed conditions that preferentially emphasized the cyclooxygenase pathway, since the lipoxygenase pathway in general is calcium stimulated; (3) by using very short incubation intervals (30 sec), they selected conditions under which cyclooxygenase activity was at or near maximum but under which lipoxygenase activity was not at maximal activity. In a study by Deykin et al., total platelet cyclooxygenase production was approximately 70% of maximum at 1 min and reached peak values at 3 min, whereas lipoxygenase production reached approximately 30% of maximum at 1 min, 80% at 3 min, and maximum by 10 min following thrombin stimulation in the presence of calcium.

It is pertinent to speculate on the basis of the variability and nonspecific nature of abnormalities in arachidonic acid metabolism and platelet function in these patients. Penington and Streatfield suggested that heterogeneity of platelet production in the bone marrow occurs in myeloproliferative disorders, with both normal and abnormal populations of platelets serving different functions. In patients with normal arachidonic acid metabolism and platelet function, a normal clone of megakaryocytes and platelets would predominate. In the one patient studied before and after chemotherapy, the marked improvement in arachidonic acid metabolism could well be explained by suppression of production of the abnormal megakaryocyte clone and increased production of normal megakaryocytes and platelets. The different platelet populations in CML may each have different sensitivities to the various aggregating agents, accounting for these variable nonspecific abnormalities.

In spite of severe impairment of arachidonic acid metabolism, in none of the cases was the bleeding time prolonged and in only one case (patient 5) was spontaneous bleeding encountered. Previous investigators have noted the lack of correlation between abnormalities of platelet function and bleeding complications in CML. The results of our study suggest that arachidonic acid, and its metabolites may not be essential for promoting platelet function or primary hemostasis in these patients. Whether other factors, such as vessel wall prostacyclin, are most important in determining primary hemostasis in these patients remains to be studied.

REFERENCES

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