Altered Arachidonate Metabolism by Platelets in Patients With Myeloproliferative Disorders

By Minoru Okuma and Haruto Uchino

Platelet lipoxygenase and cyclo-oxygenase pathways were investigated by the incubation of 1-14C-arachidonic acid with washed platelets in 33 patients with myeloproliferative disorders, including 14 patients with chronic myeloid leukemia (CML), 12 with polycythemia vera (PV), 4 with essential thrombocythemia (ET), and 3 with myelofibrosis (MF). In patients with MF and CML, mean activities of the lipoxygenase pathway were significantly lower when compared with normal controls (p<0.001 and p<0.01, respectively). When a normal range of the activity was defined as mean ± 2 SD, all patients with MF, 8 with CML, 6 with PV, and 1 with ET showed decreased lipoxygenase activities, while activities of the cyclo-oxygenase pathway were decreased in one of each patient with CML, PV, and ET. In 4 of 10 patients with a selective lipoxygenase deficiency, platelets were aggregated by lower concentrations of arachidonic acid than those necessary to induce normal platelet aggregation. It is suggested that the lipoxygenase activity could modulate platelet functions through its effect on arachidonate metabolism by the cyclo-oxygenase pathway and that a selective lipoxygenase deficiency could offer a mechanism for hyperfunction of the platelet, which may lead to a thrombotic tendency, one of the common features of myeloproliferative disorders.

In human platelets, arachidonic acid (AA) is transformed by lipoxygenase and cyclo-oxygenase pathways. The former produces 12-l-hydroperoxy-5,8,10,14-eicosatetraenoic acid (HPETE) and 12-l-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), while the latter produces prostaglandin (PG) endoperoxides, which are then converted to 12-l-hydroxy-5,8,10-heptadecatrienoic acid (HHT), malondialdehyde (MDA), and thromboxane (TX) A2 as well as small amounts of primary PGs. TXB2 is finally produced from TXA2. Among these metabolites, PG endoperoxides and TXA2 mediate such platelet functions as aggregation and the release reaction, while HETE has been reported to stimulate chemotaxis and random migration of human granulocytes. Platelet cyclo-oxygenase (PCO) deficiency has been found in some patients with congenital disorders of platelet function and those with myeloproliferative disorders (MPD), whereas patients with abnormalities of platelet lipoxygenase (PLO) activity have not yet been reported except for our previous brief communication. The significance of the PLO pathway in platelet functions is currently not known. Therefore, it is of great interest to investigate arachidonate metabolism by platelets under various patho-

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 logic states, especially under those with abnormal platelet and granulocyte functions.

We report our observations that the platelets of some patients with myeloproliferative disorders (MPD) show altered arachidonate metabolism, especially deficient lipoxygenase activity and abnormal cyclo-oxygenase activities. Platelet aggregation was also investigated in most of these patients, and it is suggested that PLO activity could modulate platelet functions through an effect on the metabolism of AA by the PCO pathway.

MATERIALS AND METHODS

Blood Collection and Preparation of Platelet Suspensions

Blood from healthy donors and patients who had taken no drugs known to interfere with platelet function for at least 1 wk before venipuncture was collected from the antecubital vein and mixed with 3.8% trisodium citrate solution (9 vol blood:1 vol citrate). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared as described previously. For the preparation of washed platelets, PRP was mixed with 77 mM disodium ethylenediaminetetraacetate (EDTA), pH 7.4 (9 vol PRP:1 vol EDTA), centrifuged at 1100 g once or twice for 10 sec each to eliminate contaminated erythrocytes and leukocytes, and then centrifuged at 1700 g for 30 min. The platelet pellet was washed once with pH 7.4 Tris-buffered saline with EDTA (0.15 M NaCl/0.15 M Tris-HCl pH 7.4/77 mM EDTA pH 7.4 = 90:82, v/v) and once with 0.15 M NaCl (saline). The washed platelet pellet was routinely suspended in saline (1.5–3.0 x 10⁹/ml). All procedures were carried out with either plastic or siliconized equipment. Platelets were washed at 0°-4°C and were counted by phase contrast microscopy. Contamination by erythrocytes and leukocytes was negligible. Informed consent was obtained from all patients and healthy donors.

Enzyme Preparation

Arachidonate lipoxygenase in the platelet was prepared from the supernate of broken platelets. In short, normal washed platelets suspended in saline were disrupted sonically, the homogenate thus prepared was centrifuged at 105,000 g for 60 min at 4°C. Ammonium sulfate was added to the supernate to a saturation of 35%, and the precipitate was collected by centrifugation. The pellet was dissolved in saline to yield a protein concentration of 5–8 mg/ml. This solution was stored at –70°C and used as the enzyme preparation to produce HPETE and HETE as reference standards.

Reaction of AA With Platelets or With the Enzyme Preparation

Unless otherwise stated, arachidonate metabolism by the platelet was studied by incubation of 1-¹⁴C-AA with washed platelets or with the enzyme preparation in the following reaction mixture (0.1 ml): 50 mM Tris-HCl buffer, pH 7.4, 0.1 mM ¹⁴C-AA (1.4 x 10⁵ cpm) and washed platelets (10⁴) or the enzyme preparation (200 µg protein) instead of the platelets. Saline and 0.15 M Tris-HCl buffer were used to keep the reaction mixture isotonic. The reaction was initiated by the addition of ¹⁴C-AA, and the incubation was routinely performed at 37°C for 30 sec. Termination of the reaction, lipid extraction from the reaction mixture, and thin-layer chromatography (TLC) were carried out under cold conditions as described previously. TLC was routinely performed using the solvent system of ethyl ether/petroleum ether/glacial acetic acid (85:15:0.1, v/v), and radioactivity on chromatographic plates was monitored either by a Packard radiocromatogram scanner model 7201 or by exposing the thin-layer plates containing ¹⁴C-labeled compounds to Sakura Medical x-ray films for 1–5 days, depending on the amount of radioactivities on the plate.

Analysis of Reaction Product

The identity of each peak or spot of radioactivity in the thin-layer radiochromatograms or the autoradiograms of the reaction products obtained by the incubation of platelets with ¹⁴C-AA was based on: (1) a comparison of peaks or spots with those of the chromatograms reported previously (TXB₂ and HHT), or with those of the chromatograms of the reaction products obtained on incubation of ¹⁴C-AA with semipurified lipoxygenase enzyme (HPETE and HETE); (2) pharmacologic evidence
(disappearance with aspirin denotes the substances are PCO products); and (3) a comparison of \( R_i \) values of the reaction products with those of cochromatographed authentic compounds (TXB₂, PGE₂, PGF₂α, HHT, HPAA, and AA).

**Protein Determination**

Protein was measured as described previously.¹¹

**PLO and PCO Activities**

PLO and PCO activities were estimated by the radioactivities of lipid products obtained after the incubation of \( 1^{14}C\)-AA with platelets at 37°C for 30 sec. Namely, silica gel of the area on the TLC plate corresponding to the reaction products by PLO and PCO pathways was scraped off, eluted with 0.5 ml ethyl acetate in vials, combined with 5 ml of the scintillator solution of Patterson and Greene,¹³ and the radioactivity was determined by a Packard liquid scintillation spectrometer model 3385. Remaining areas on the plate were divided into 2-cm bands and treated similarly. Percent conversion to each lipid product was then obtained by relating its radioactivity to the total radioactivity recovered from the plate, and PLO and PCO activities were estimated as follows: percent PLO activity = percent radioactivities of HPETE and HETE; percent PCO activity = percent radioactivities of HHT and TXB₂.

**Platelet Aggregation**

Platelet aggregation was monitored using a standard nephelometric technique¹⁴ as described previously.¹⁵ Routinely, platelet counts in PRP were adjusted to 300,000/µl by diluting the original PRP with autologous PPP. Plasma samples were kept at 20°C in tightly stoppered polycarbonate tubes and used for testing platelet aggregation within 3 hr after venesection. Usually, 2 concentrations of aggregating agents, namely, 0.5 \( \mu M \) and 2 \( \mu M \) adenosine diphosphate (ADP), 50 nM and 5 \( \mu M \) epinephrine, 0.5 \( \mu g/ml \) and 2 \( \mu g/ml \) collagen, and 0.1 \( mM \) and 1.5 \( mM \) AA were used to detect abnormalities of platelet aggregation induced by these aggregating agents. They were graded as normal, increased, decreased, or absent by the following criteria, based on the observation of patterns of each platelet aggregation in 20 normal subjects. The appearance of second-phase aggregation by 0.5 \( \mu M \) ADP, by 50 nM epinephrine, more than 50% changes in light transmission induced by 0.5 \( \mu g/ml \) collagen, or by 0.1 \( mM \) AA were considered as increased platelet aggregation induced by respective aggregating agents. On the other hand, decreased reactions were defined by the decreased maximal changes in light transmission when the higher concentration of each aggregating agent was used (see above) as follows: less than 10% change by ADP, less than 5% (first phase) and 43% (second phase) changes by epinephrine, less than 50% changes by collagen and AA.

**Patients and Normal Controls**

Thirty-three patients with MPD were studied, including 14 patients with chronic myeloid leukemia (CML), 12 patients with polycythemia vera (PV), 3 patients with myelofibrosis (MF), and 4 patients with essential thrombocythemia (ET). They were 24–74 yr old and consisted of 20 men and 13 women. Diagnosis was determined by standard methods, including peripheral blood picture, bone marrow examination, chromosome analysis, blood volume, and leukocyte alkaline phosphatase. Twenty normal volunteers (13 men and 7 women between 21 and 68 yr old) were also studied as normal controls.

**Materials**

\( 1^{14}C\)-AA (58 mCi/mmole) was purchased from the Radiochemical Centre, Amersham, England. ADP and AA were obtained from Sigma Chemical, St. Louis, Mo.; epinephrine from Daichi Pharmaceutical, Tokyo, Japan; aspirin from Yoshitomi Pharmaceutical, Osaka, Japan; and collagen from Hormon-Chemie, Muenchen, West Germany. PCE₂, PGF₂α, TXB₂, and HPAA were donated by the Central Research Institute, Ono Pharmaceutical, Osaka, Japan. Precoated silica gel 60 F₂₅₄ glass plates were obtained from E. Merk, Darmstadt, West Germany. HHT was kindly donated by Dr. Shozo Yamamoto, Department of Medical Chemistry, Kyoto University Faculty of Medicine, Kyoto, Japan.
RESULTS

Lipid Products by the Incubation of $^{14}$C-AA With Normal Human Platelets

The incubation of AA with normal human platelets for 30 sec at 37°C gave 4 main products: compounds I, II, III, and IV (Fig. 1A). Compounds I and II, which comigrated with authentic TXB$_2$ and HHT, respectively, were not produced by washed platelets that had been incubated with aspirin (Fig. 1B). Although authentic PGE$_2$ and PGF$_{2\alpha}$ migrated close to TXB$_2$ with the solvent system used in this experiment, and these PGs could be included in the area corresponding to compound I, neither of them were detected in the lipid products obtained by elution with ethylacetate of silica gel in that area and rechromatography of the eluted lipid with a TLC solvent system (benzene/dioxane/acetic acid—60:30:3, v/v)$^{15}$ clearly resolved TXB$_2$, PGE$_2$ and PGF$_{2\alpha}$, suggesting that the chemical decomposition products, PGE$_2$ and PGF$_{2\alpha}$, were not formed during these experimental conditions. Based on these findings, compounds I and II were identified as TXB$_2$ and HHT, respectively. Compounds III and IV, which migrated close to HPAA (Fig. 1A),

![Fig. 1. Thin-layer radiochromatograms of products isolated after incubation of $^{14}$C-AA with platelet suspension and with the semipurified PLO enzyme preparation. The condition of incubation, isolation of lipid products, and method of TLC were described in Materials and Methods. (A) A 30-sec incubation with washed platelets ($10^9$). (B) Washed platelets ($10^9$) were incubated with 0.3 mM aspirin for 5 min at 37°C and then incubated with $^{14}$C-AA for 30 sec at the same temperature. (C) Incubation for 1-min with the PLO enzyme preparation (200 μg protein). (D) The reaction and extraction of lipid products were performed as described in experiment C. The solvent (70 μl) containing lipid products was then evaporated under N$_2$, the residue dissolved in 70 μl of 1% SnCl$_2$ in ethanol/0.5 M Tris-HCl, pH 7.4 (9:1, v/v), was left standing at 20°C for 2 min, and TLC was carried out as usual. Authentic samples of AA, HPAA, HHT, TXB$_2$, PGE$_2$, and PGF$_{2\alpha}$ (F$_{2\alpha}$) were run as shown at the top of A.](image-url)
were produced by the aspirin-treated platelets (Fig. 1B), indicating that these compounds were not PCO products but were PLO products. In order to confirm the identity of compounds III and IV with HETE and HPETE, respectively, \( R_f \) values of compounds III and IV were compared with those of HETE and HPETE synthesized by the incubation of AA with PLO that had been partially purified from the soluble fraction of the platelet. As shown in Fig. 1C, two products that showed the same \( R_f \) values as those of compounds III and IV were synthesized by this enzyme preparation, and treatment of the lipid products with stannous chloride converted a product corresponding to compound IV to another product corresponding to compound III (Fig. 1D). This observation was identical with the report that stannous chloride reduction converted HPETE to HETE. Based on these findings, compounds III and IV were identified as HETE and HPETE, respectively.

**Time Course of the Reaction**

Figure 2 shows a typical experiment in which PCO and PLO products were determined at varying times of the incubation of \( 1^{-14}C\)-AA with normal human platelets. Time courses for the production of HHT and of TXB\(_2\) appeared to be similar and different from those observed for the production of HPETE and HETE, respectively. Although the production of HPETE was observed only after a short incubation period, HETE became predominant after longer periods of time. When the semipurified PLO enzyme was used as an enzyme source in another experiment, HPETE was produced for the initial 5 min, while a much larger amount of

![Graph showing time course of production of arachidonate metabolites](image-url)
PLATELET ARACHIDONATE METABOLISM

Table 1. Arachidonate Metabolites Produced by PCO and PLO Pathways
in Normal Subjects and MPD Patients

<table>
<thead>
<tr>
<th></th>
<th>PCO Products</th>
<th></th>
<th>PLO Products</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>TXB_2</td>
<td>HHT</td>
<td>Total</td>
<td>HETE</td>
<td>HPETE</td>
</tr>
<tr>
<td>Normal</td>
<td>19</td>
<td>14.9 ± 2.7</td>
<td>13.6 ± 3.5</td>
<td>28.4 ± 5.8</td>
<td>15.5 ± 3.3</td>
<td>10.0 ± 5.3</td>
</tr>
<tr>
<td>CML</td>
<td>14</td>
<td>12.3 ± 3.8*</td>
<td>11.7 ± 3.7</td>
<td>23.9 ± 7.1</td>
<td>8.1 ± 8.9†</td>
<td>4.7 ± 4.7†</td>
</tr>
<tr>
<td>PV</td>
<td>12</td>
<td>12.8 ± 4.3</td>
<td>12.0 ± 5.3</td>
<td>24.8 ± 9.5</td>
<td>15.3 ± 10.9</td>
<td>5.6 ± 4.8*</td>
</tr>
<tr>
<td>MF</td>
<td>3</td>
<td>12.5 ± 2.5</td>
<td>12.3 ± 2.1</td>
<td>24.8 ± 4.5</td>
<td>2.5 ± 1.7‡</td>
<td>1.2 ± 0.4‡</td>
</tr>
<tr>
<td>ET</td>
<td>4</td>
<td>9.2 ± 4.9*</td>
<td>8.5 ± 5.1</td>
<td>17.7 ± 10.0</td>
<td>12.7 ± 7.5</td>
<td>7.0 ± 5.1</td>
</tr>
</tbody>
</table>

Results (mean ± SD) are shown as percentage of total radioactivity measured, as described in Materials and Methods. Radioactivities recovered in TXB_2, HHT, HETE, HPETE, and AA were always more than 92% of the total radioactivity present on the TLC plate.

Statistical analysis was performed by Student's t test as compared with each corresponding value of normal subjects.

*p < 0.05
†p < 0.01
‡p < 0.001

HETE was detectable after 10 min at 37°C. Time courses for the formation of PCO products were faster than those observed for the formation of PLO products.

PLO and PCO Activities and Their Alteration in Patients With MPD

Arachidonate metabolites produced by PLO and PCO pathways in normal subjects and in the patients with MPD are shown in Table 1. In normal subjects, total amount of PCO products or PCO activity was similar to that of PLO products or PLO activity. Mean PLO activities in patients with MF and CML were significantly lower than that in normal controls (p<0.001 for MF and p<0.01 for CML). Mean PLO activities in the patients with PV and ET and mean PCO activities in all types of MPD were not significantly different from those in normal controls. However, when normal ranges were considered as mean ± 2 SD of normal values, abnormal activities of PLO and PCO were detected in some patients with all types of MPD (Figs. 3 and 4). A deficiency of PLO activity was found in all of 3
patients with MF, 8 of 14 patients with CML, 6 of 12 patients with PV, and 1 of 4 patients with ET, while that of PCO activity was detected in 3 of all 33 patients investigated. Enhanced activity in either pathway was rarely detected in patients with PV and CML. Thin-layer radiochromatograms prepared from one of the patients with a selective PLO deficiency are shown in Fig. 5. The incubation of
radioactive AA with the patient’s platelets, which had been incubated with aspirin, did not produce any detectable arachidonate metabolites at all. Both parents of this patient showed normal PLO and PCO activities.

The clinical and laboratory data together with PLO and PCO activities of the 18 patients with PLO deficiency are summarized in Table 2. Three of these patients also showed evidence of PCO deficiency. The patients were 26–74 yr old, and 8 were receiving drugs, including antitumor agents, antibiotics, corticosteroid, and allopurinol, when the arachidonate metabolism was investigated. These drugs were also given to other MPD patients who had normal PLO and PCO activities. Although aspirin and indomethacin were taken by 2 PV patients 10 days and 7 days before venipuncture, respectively, PCO and PLO activities in these patients were within normal ranges. All 3 patients who had low PCO activities were off medications more than 2 wk, except for a PV patient (case 14 in Table 2) who was given busulfan. Of the 18 patients, 10 (56%) had an elevated platelet count and no thrombocytopenia was observed at the time of our platelet study. Megakaryocytes in the bone marrow were increased in 10 of the 15 patients investigated, normal in 3, and decreased in 2 patients with the blastic phase of CML. There were no specific abnormalities of megakaryocytes and platelets in patients with decreased PLO activities. Three patients had a bleeding tendency, and one of them had a thrombotic episode. In the 15 patients with normal enzyme activities, a PV patient had episodes of cervical vein thrombosis, an ET patient had recurrent painful toes, and another PV patient suffered from excessive gingival bleeding after teeth extraction. Estimation of PLO and PCO activities was repeated 3–10 mo later in 5 patients with PLO deficiency (cases 1, 2, 3, 9, and 10 in Table 2) and the same abnormality persisted. During this period, no significant hematologic changes were observed in these patients, except for case 2, in whom the platelet count increased from 1,340,000 to 2,960,000/μl.

The correlation between PLO and PCO activities in all patients is shown in Fig. 6. Although all patients with reduced PCO activity showed reduced PLO activity, patients with reduced PLO activity did not always show reduced PCO activity. Correlation coefficients between PLO and PCO activities for normal controls, all patients with PLO deficiency, and all patients with selective PLO deficiency were −0.288, −0.030, and −0.160, respectively. However, amounts of TXB₂ were similar to those of HHT in all subjects examined (Table 1).

Platelet Aggregation in Patients With the Altered Arachidonate Metabolism

Platelet aggregation evaluated in 12 PLO-deficient patients is summarized in Table 3. In such patients, platelet aggregation induced by ADP, epinephrine, and collagen was not uniform but varied from one patient to another as in those with normal PLO activities. In cases 1, 3, 9, and 17, however, platelet aggregation induced by AA was increased. Other characteristics common to these 4 patients were either normal or increased aggregation induced by ADP, epinephrine, or collagen and normal PCO activity. Increased AA-induced aggregation in case 1 is shown in Fig. 7. In case 15, whose PCO and PLO activities were most defective, arachidonate-induced platelet aggregation was decreased and the aggregation induced by other aggregating agents was either absent or decreased. In cases 2, 4, 5, 11, 12, 16, and 18, arachidonate-induced platelet aggregation was normal; in cases 4, 5, and 18, the aggregations induced by any of the aggregating agents used
Table 2. Clinical and Laboratory Data of Patients With PLO and PCO Deficiencies

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Drugs Given</th>
<th>Blood Platelets (10^3/μl)</th>
<th>Bone Marrow Megakaryocytes</th>
<th>PLO* (%)</th>
<th>PCO* (%)</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>F</td>
<td>CML</td>
<td>Prednisone, cefazolin sodium</td>
<td>540</td>
<td>Increased</td>
<td>2.9</td>
<td>31.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>F</td>
<td>CML</td>
<td>-</td>
<td>1,340</td>
<td>Increased</td>
<td>2.0</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>F</td>
<td>CML</td>
<td>-</td>
<td>1,285</td>
<td>Increased</td>
<td>4.8</td>
<td>22.9</td>
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<tr>
<td>4</td>
<td>40</td>
<td>F</td>
<td>CML</td>
<td>Busulfan</td>
<td>1,120</td>
<td>Increased</td>
<td>3.4</td>
<td>19.2</td>
<td></td>
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<tr>
<td>5</td>
<td>33</td>
<td>M</td>
<td>CML</td>
<td>-</td>
<td>435</td>
<td>Increased</td>
<td>4.4</td>
<td>31.3</td>
<td></td>
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<td>6</td>
<td>33</td>
<td>F</td>
<td>CML†</td>
<td>-</td>
<td>200</td>
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<td>5.0</td>
<td>34.5</td>
<td>Purpura</td>
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<td>7</td>
<td>51</td>
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<td>CML†</td>
<td>Prednisone, cephalothin sodium</td>
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<td>38</td>
<td>M</td>
<td>CML†</td>
<td>Methotrexate</td>
<td>166</td>
<td>Decreased</td>
<td>1.0</td>
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<td>9</td>
<td>60</td>
<td>M</td>
<td>PV</td>
<td>-</td>
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<td>74</td>
<td>M</td>
<td>PV</td>
<td>Busulfan</td>
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<td>-</td>
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<tr>
<td>11</td>
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<td>25.3</td>
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<td>39</td>
<td>M</td>
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<td>-</td>
<td>348</td>
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<td>21.6</td>
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<td>13</td>
<td>69</td>
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<td>474</td>
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<td>14</td>
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<td>PV</td>
<td>Busulfan</td>
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<td>15</td>
<td>41</td>
<td>M</td>
<td>ET</td>
<td>-</td>
<td>3,840</td>
<td>Increased</td>
<td>3.3</td>
<td>3.0</td>
<td></td>
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<td>16</td>
<td>66</td>
<td>M</td>
<td>MF</td>
<td>Allopurinol</td>
<td>590</td>
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<td>73</td>
<td>F</td>
<td>MF</td>
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<td>Increased</td>
<td>6.0</td>
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<td>18</td>
<td>50</td>
<td>M</td>
<td>MF</td>
<td>Dibromomannitol</td>
<td>246</td>
<td>Increased</td>
<td>2.0</td>
<td>19.8</td>
<td></td>
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</table>

†Blastic phase.

N, normal; I, increased; D, decreased; A, absent.

Fig. 6. Correlation between PLO and PCO activities in patients with CML(Ω), MF(Δ), PV(O), and ET(Δ). Normal range (dotted areas): Mean ± 2 SD of normal controls (N = 19).

Table 3. Platelet Aggregation in PLO-Deficient Patients

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>PLO† (%)</th>
<th>PCO† (%)</th>
<th>Platelet Aggregation by</th>
<th>ADP</th>
<th>Epinephrine</th>
<th>Collagen</th>
<th>AA</th>
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<tr>
<td>1</td>
<td>CML</td>
<td>2.9</td>
<td>31.3</td>
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<td>N‡</td>
<td>I</td>
<td>I</td>
<td>I</td>
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<tr>
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<td>CML</td>
<td>2.0</td>
<td>10.5</td>
<td></td>
<td>N</td>
<td>N</td>
<td>D</td>
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*Each case no. corresponds to that in Table 2.
†Normal ranges (mean ± 2 SD): PLO = 25.6 ± 11.2, PCO = 28.4 ± 11.6.
‡N, normal; I, increased; D, decreased; A, absent.
DISCUSSION

Investigations of AA metabolism by human platelets and enzyme preparations prepared from them have demonstrated that AA is transformed via two main pathways, i.e., the PLO and PCO pathways.\(^1\) The initial step in the PLO pathway is catalyzed by a lipoygenase enzyme, producing HPETE, which is transformed to HETE as an end-product.\(^1\) Results of the present investigation on the PLO pathway are in agreement with reports that HPETE is transiently produced at first by the platelets and particularly by the semipurified PLO enzyme, followed by its conversion to a single stable product termed HETE.\(^1\) Therefore, total radioactivity of HPETE and HETE was utilized as an indicator of the activity of PLO pathway. In the PCO pathway, which is inhibited by aspirin,\(^19\) AA is transformed by PCO into PG endoperoxides, which are almost exclusively converted into HHT plus MDA and TXB\(_2\), a stable end-product of the highly active TXA\(_2\).\(^1\) Only the main products of this pathway, HHT and TXB\(_2\), were detected by the experimental conditions used in the present investigation. Small amounts of PGE\(_2\), PGD\(_2\), and PGF\(_{2\alpha}\) were thought to be chemical degradation products of PG endoperoxides,\(^16\) and the absence of these PGs in the present investigation could be due to a short incubation time of AA with platelets as well as cold conditions carefully employed throughout the extraction and separation of the lipid products. Therefore, total radioactivity of HHT and TXB\(_2\) was employed as an indicator of the activity of PCO pathway.

Alterations in AA metabolism by human platelets have been demonstrated only in patients with PCO deficiency,\(^6\) and a possible defect of PG synthesis was reported in MPD that was associated with defective platelet lipid peroxidation.\(^20\) As intermediate metabolites of AA via the PCO pathway, i.e., PG endoperoxides and TXA\(_2\), are mediators of platelet functions,\(^1,2\) a PCO deficiency or a defect of PCO pathway could be associated with hypoactive platelets or with a bleeding tendency,\(^6\) whereas it has been reported that increased PG synthesis could be related to hyperactive platelets or thrombosis.\(^21,22\) Qualitative platelet abnormalities have been described in MPD and may, in some cases, account for the bleeding or thrombotic tendency, which is a common feature of these diseases.\(^20,23-25\) The platelet abnormalities may not be identical in all cases, and the basic defects that underlie the abnormalities in platelet function in MPD remain to be determined.\(^26\)

PLO deficiency was detected in 18 of 33 MPD patients, and 3 of the defective patients had an associated PCO deficiency. Although drugs were being taken by some of the PLO-deficient patients, such drugs were also given to other patients whose platelets were shown to have normal enzyme activities. Ten of the 18 patients had not taken any drugs for at least 15 days before the platelet study. These results lessen the possibility that the PLO activity was inhibited by the drugs taken by the.

**Fig. 7.** AA-induced platelet aggregation in a CML patient with a selective PLO deficiency (A) and in a normal subject (B). Concentrations (mM) of AA added to PRP (300,000 platelets/μl) are shown below each platelet aggregation curve.
patients. Patients with reduced activities of the PLO pathway were shown to have either normal or decreased activities of the PCO pathway by the experiment in which 0.1 mM radioactive AA was added to the washed platelets suspended in the artificial medium. In all patients with a selective PLO deficiency, however, platelet aggregation induced by AA was not decreased, but normal or rather increased. In some of these selective PLO-deficient patients, platelets were aggregated by lower concentrations of AA than those necessary to induce normal platelet aggregation. This could be explained by more efficient availability of AA to the PCO pathway, because no AA was consumed by the PLO enzyme, which is deficient in these patients, and by the defective production of HPETE, which is reported to inhibit TX synthetase in the microsomes of human platelets, thus resulting in more efficient production of TXA₂, which is a highly potent mediator of platelet aggregation. Therefore, an alteration in the AA metabolism in human platelets secondary to selective PLO deficiency offers a possible mechanism for hyperfunction of the platelet, which may lead to a thrombotic tendency, one of the common features of MPD.

The failure to demonstrate increased amounts of TXB₂ and HHT (PCO products) even when reduced amounts of HETE and HPETE (PLO products) were found might be due to a sufficient amount of exogenous AA available to the PCO pathway irrespective of the activity of the PLO pathway at the experimental condition in which 0.1 mM ¹⁴C-AA was added to the washed platelets. If a threshold (i.e., minimal) concentration of the substrate was added to the washed platelets and the metabolites through the two pathways were analyzed, increased amounts of the PCO products could be detectable in patients with PLO deficiency. Secondly, even if PCO products could be increased by a PLO deficiency in the presence of normal enzyme activities of the PCO pathway, a possibly coexistent decrease in the activity of PCO pathway in MPD patients could mask the increasing effect of a PLO deficiency on the formation of PCO products, resulting in the formation of apparently normal amounts of TXB₂ and HHT. Such a “latent” deficiency in the activity of the PCO pathway could not be detected, and the PCO activity in these situations might be regarded as normal (i.e., as selective PLO deficiency). Another possibility involves the time course for the production of the metabolites via the two pathways. The PLO pathway is activated later than the PCO pathway and might therefore only minimally influence the latter.

The incidence of thrombosis in the MPD patients, including those with low PLO levels and enhanced platelet aggregability, was so low that no association between platelet hyperactivity in PLO-deficient patients and thrombosis could be demonstrated. However, the consideration that PLO activity could modulate platelet functions through its effect on the endoperoxide pathway suggests an important role of the lipoxygenase pathway in human platelets.

In patients with a PLO deficiency, platelet aggregation induced by aggregating agents other than AA did not show a uniform pattern but was different from one patient to another, as reported by many authors in MPD. In MPD patients, defective platelet lipid peroxidation, changes in distribution of platelet membrane glycoproteins, defective binding of thrombin to platelets, platelet storage pool deficiency, and a deficiency of platelet α-adrenergic receptors have recently been reported as the basic defects possibly contributing to hypofunction of platelets,
while platelet resistance to PGD₂ was described as a possible factor contributing to the high incidence of thrombosis. These defects or abnormalities could be present in association with altered arachidonate metabolism, resulting in the various abnormal patterns of platelet aggregation in such patients.

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REFERENCES


Altered arachidonate metabolism by platelets in patients with myeloproliferative disorders

M Okuma and H Uchino