Increased Thromboxane Biosynthesis in Patients With Polycythemia Vera: Evidence for Aspirin-Suppressible Platelet Activation In Vivo

By Raffaele Landolfi, Giovanni Ciabattoni, Paola Patrignani, Maria A.L. Castellana, Enrico Pogliani, Bruno Bizzi, and Carlo Patrono

Increased thromboxane (TX) production and modified aspirin sensitivity has been detected in vitro in platelets isolated from patients with polycythemia vera. To verify the relevance of these capacity-related measurements to the actual rate of TXA₂ biosynthesis in vivo and its suppression by oral aspirin, we have investigated the urinary excretion of major enzymatic metabolites of TXB₂ in 17 patients with polycythemia vera and 23 gender- and age-matched controls. Urinary 11-dehydro-TXB₂ and 2,3-dinor-TXB₂ were measured by previously validated radioimmunoassays. In addition, urinary immunoreactive leukotriene (LT) E₂ was measured to explore the 5-lipoxygenase pathway of arachidonate metabolism. Polycythemic patients had significantly (P < 0.001) higher excretion rates of both 11-dehydro-TXB₂ (1033 ± 1050 v 117 ± 45 pmol/mmol creatinine; mean ± SD) and 2,3-dinor-TXB₂ (725 ± 676 v 82 ± 43 pmol/mmol creatinine) than controls. In contrast, urinary LTE₄ was not significantly different. Enhanced metabolite excretion did not correlate with the platelet count or with the hematocrit value, and was not related to the current treatment or to a clinical history of thrombotic complications. Platelet TX receptor studies did not show any significant changes in the binding characteristics of two different ligands. A platelet-selective regimen of aspirin therapy (50 mg/d for 7 to 14 days) was associated with greater than 80% suppression in metabolite excretion in nine patients. These results are consistent with abnormal stimuli operating in polycythemia vera to induce a selective enhancement in the platelet biosynthesis of TXA₂ without changes in receptor binding. This in vivo abnormality in platelet biochemistry can be largely suppressed by low doses of aspirin.

© 1992 by The American Society of Hematology.

THROMBOEMORRHAGIC diathesis is a major complication of myeloproliferative disorders and is characterized by recurrent episodes of cutaneous/mucosal bleeding and arterial and venous thrombosis. The hemostatic defect of these patients is generally attributed to platelet functional abnormalities. There is evidence suggesting that, in these syndromes, circulating platelets might undergo spontaneous activation with release of granules. In addition, the peculiar phenomenon of spontaneous platelet aggregation can be shown ex vivo. A number of studies have shown functional and biochemical abnormalities of platelets in myeloproliferative disorders. In polycythemia vera, thrombotic events are more prevalent than in other myeloproliferative disorders and represent the major cause of morbidity and mortality. Increased thromboxane (TX) A₂ production and modified aspirin sensitivity have been detected in vitro in platelets isolated from patients with polycythemia vera. To verify the relevance of these capacity-related measurements to the actual rate of TXA₂ biosynthesis in vivo and to its suppression by oral aspirin, we have investigated the urinary excretion of major enzymatic metabolites of TXB₂ in patients with polycythemia vera and healthy controls. Urinary enzymatic metabolites provide reliable, noninvasive indexes of eicosanoid biosynthesis in vivo and their measurement has been used recently to elucidate alterations in TX production associated with cardiovascular risk factors. Moreover, because platelets can efficiently convert neutrophil-derived leukotriene (LT) A₄ into the potent vasoconstrictor LTC₄ in exploring the cellular specificity of the mechanism(s) responsible for arachidonate release and oxygenation, we have also measured sulfidopeptide LT production ex vivo as well as in vivo.

MATERIALS AND METHODS

Study population. Seventeen patients with polycythemia vera (9 men, 8 women; 23 to 68 years of age) and 23 healthy volunteers (12 men, 11 women; 27 to 70 years of age) were examined on several occasions between April 1988 and January 1990. Informed consent was obtained from each subject. Patients and controls were comparable for smoking habits and blood cholesterol and blood pressure levels. One patient had type II diabetes mellitus. No alterations in renal function were detected in any of the patients. An unequivocal diagnosis of polycythemia vera was made on the basis of the criteria recommended by the Polycythemia Vera Study Group. An arterial thrombotic event previously had occurred in five patients (6 months to 5 years before the study). Another patient suffered from migratory thrombophlebitis. Four patients were examined before any treatment and 13 while undergoing phlebotomy (n = 8), chemotherapy (n = 3), or both (n = 2). The various treatment regimens were individualized to keep the hematocrit value below 50%: Hydroxyurea (Onco-Carbide, Simes, Milan, Italy) was used as the chemotherapeutic agent at doses ranging between 0.5 and 1.5 g/d. At the beginning of the study, all healthy subjects, as well as the patients, were free of drugs known to affect platelet function. A complete hematologic screening was performed shortly before collecting blood and urine samples for TXB₂ metabolite assays.

Design of the studies. In the first study, a cross-sectional comparison of TX and LT production was performed between the patients and controls. Patients and healthy volunteers were studied...
on an outpatient basis. After an overnight fast, blood samples were obtained for measurement of TXA2, LTC4 synthesis, and TXA2 receptor studies. Urine was collected during the 24 hours before blood sampling; the samples were frozen immediately and kept at −20°C until extraction. Urine samples for LTC4 measurements were added with 1 mmol/L 4-hydroxy-TEMPO (Sigma Chemicals, St Louis, MO) as an antioxidant.

A second study was designed to examine the relative contribution of platelets to enhanced excretion of TXB2 metabolites. For this purpose, nine patients were treated with aspirin (50 mg/d for 7 to 14 days), and 24-hour urine collections and blood samples (in only three patients) were obtained before and at the end of aspirin therapy and over the following 2 weeks.

In a third study, we measured TXB2 metabolite excretion in three patients undergoing phlebotomy only and phlebotomy while on low-dose aspirin therapy. Urine was obtained before and on the first (collection started 18 hours after phlebotomy), third, and fifth days after phlebotomy, both during an aspirin-free phase and again while on low-dose aspirin therapy. The study was approved by the Internal Medicine Review Boards of our institutions.

**Whole blood studies.** TXB2 production during whole blood clotting was measured as previously described.21 The anti-TXB2 serum used was obtained in our laboratory and was described previously.22 Sulfidopeptide LT production (as detected by LTE4-like immunoreactivity) in whole blood stimulated with the calcium ionophore A23187 was measured as described for LTC4.21 The anti-LTE4 serum was a gift of Dr J. Maclouf (Hopital Lariboisière, Paris, France) and was the same used for urinary measurements (see below).

**TX receptor-binding studies.** These studies were performed in four polycythemic patients and four healthy volunteers, who had not taken any nonsteroidal anti-inflammatory drugs during the previous 10 days. Platelets were obtained by differential centrifugation from peripheral blood samples and washed three times in a modified Tyrode’s buffer. Aliquots of the platelet suspension containing 5 x 10^10 platelets were incubated for 30 minutes at 25°C with [3H] SQ29,548 (30 Ci/mmol, 5 nmol/L; Du Pont Nemours-NE'l Research Products, Boston, MA) or [3H] U46619 (15 Ci/mmol, 20 nmol/L) and varying concentrations (10^{-11} to 10^{-5} mol/L) of competing homologous and heterologous cold ligands. The reaction was terminated by the addition of 4 mL of ice-cold 50 mmol/L Tris/100 mmol/L NaCl buffer, pH 7.4, followed by rapid filtration through Whatman GF/C glass fiber filters (Whatman Inc, Clifton, NJ). Displacement curves were fitted to a nonlinear model using the LIGAND program.24 The binding parameters of radiolabeled ligands were evaluated in the same number of platelets for patients and controls. This averaged 5 x 10^10 and 10^8 platelets for the binding of [3H]-SQ29,548 and [3H] U46619, respectively, in both groups. Although platelets of polycythemia vera patients may be larger than normal platelets, the amounts of platelet proteins measured in our studies were not significantly different in the two groups. Analysis of heterologous and homologous displacement curves best fitted the experimental data to a one-site model in both patients and controls.

**Urinary 11-dehydro-TXB2 and 2,3-dinor-TXB2 assays.** TXB2, the chemically stable hydrolysis product of TXA2, undergoes two major pathways of metabolism in humans.25 2,3-dinor-TXB2 and 11-dehydro-TXB2 have been identified as the major urinary metabolites originating via β-oxidation and dehydrogenation of the hemiacetal alcohol group at C-11, respectively.25 Urinary excretion of both metabolites increases linearly with the rate of entry of TXB2 into the systemic circulation of healthy subjects.26

After adjusting the urine pH to 4.0 with formic acid, 2,3-dinor-TXB2 and 11-dehydro-TXB2 were extracted on SEP-PAK C18 cartridges (Waters Associates, Milford, MA) and eluted with ethyl acetate. The eluates were subjected to silicic acid column chromatography and further eluted with benzene:ethyl acetate:methanol (60:40:30). These eluates were assayed for 11-dehydro-TXB2 by radioimmunoassay (RIA), as previously described.27 The same eluates were subjected to reverse-phase high-performance liquid chromatography (RP-HPLC) with the solvent system acetonitrile:water:acetic acid (27:73:0.18) at a flow rate of 0.5 mL/min to separate TXB2 from 2,3-dinor-TXB2. The latter was measured by a previously validated RIA.28 The extraction and further purification recoveries for labeled 11-dehydro-TXB2 and 2,3-dinor-TXB2 averaged 75% ± 6% and 50% ± 7% (mean ± SD, n = 32), respectively, and urinary measurements were corrected accordingly.

**Urinary LTE4 assay.** LTE4 represents a major enzymatic derivative of LTC4 and its urinary excretion increases linearly with the rate of entry of LTC4 into the systemic circulation of healthy subjects.29

Immunoreactive LTE4 was extracted from 10- to 20-mL aliquots of urine from 10 patients and 8 controls, on SEP-PAK C18 cartridges and eluted with methanol. After evaporation of the methanol to dryness, the extracts were reconstituted with 150 µL of methanol/water (1:1, vol/vol) and injected into a Nova-Pak C18 column (3.9 mm x 15 cm; Waters Associates) and eluted with a solvent system methanol/water:acetic acid (5842:0.1, vol/vol) containing 1 mmol/L EDTA adjusted to pH 5.6 with ammonium hydroxide, at a flow rate of 1 mL/min. Fractions (1 mL) eluting with similar retention times to those of authentic LTC4 and LTE4 were collected, evaporated to dryness, and reconstituted with 0.5 mL of phosphate buffer (0.02 mol/L, pH 7.4). Radioactivity in these HPLC fractions corresponding to the retention times (11 minutes) of [3H]LTE4 (168 Ci/mmol, 6,000 disintegrations per minute [dpm] added to each urine sample) was measured by scintillation counting to determine recovery, whereas those fractions with a retention time close to that of authentic LTE4 (26 minutes) were assayed for immunoreactivity by RIA. [3H]LTC4 and [3H]LTE4 (180 Ci/mmol) added to urine showed similar recovery of approximately 60%. RIA was performed by addition of 10 to 50 µL of the HPLC fractions to 1.5 mL volume of 0.02 mol/L phosphate buffer, pH 7.4; 4,000 dpm of [3H]LTE4 and an anti-LTE4 serum29 (showing 32% cross-reaction with LTC4) diluted 1:50,000 were added and the mixture incubated for 18 to 24 hours at 4°C. Separation of the antibody-bound from free [3H]LTE4 was performed by rapidly adding 0.05 mL of human plasma and 0.1 mL of a charcoal suspension (100 mg/mL), followed by subsequent centrifugation at 3,000 rpm for 10 min at 4°C. To further validate these measurements, the LTE4-like immunoreactivity detected in some samples was analyzed with a different monoclonal antibody directed against LTC4 (a gift of Dr J. Rokach, Merck Frosst, Canada) that showed 35% cross-reaction with LTE4. A highly significant correlation (r = .93, n = 15, P < .001) was found between measurements of urinary LTE4 using the two antisera. The IC50 values for LTE4 for the two antisera were 39 and 38 pg/mL of incubation mixture, respectively. LTE4 concentration in each urine sample was corrected by recovery of [3H]LTC4.

**Other assays.** The serum and urinary levels of creatinine were measured by the Jaffe’s method without deprotonization. Serum levels of creatinine, measured on the occasion of the study, averaged 89.0 ± 16.6 µmol/L (range, 69.0 to 123.8) in the 17 patients.

**Statistical analyses.** The results were evaluated by means of a parametric analysis of variance (ANOVA) for multiple comparisons and by Student’s t-test for single comparison. Moreover, the associations of eicosanoid measurements with other biochemical and hematologic variables were assessed by stepwise regression analysis and multiple linear regression. All values are reported as mean ± SD. Statistical significance was defined as P < .01.
RESULTS

Polycythemic patients had significantly \((P < .001)\) higher 11-dehydro-TXBz excretion than sex- and age-matched controls \((1.033 \pm 1.050 \text{ pmol/mmol creatinine}; \text{mean} \pm \text{SD})\). Table 1 details the individual measurements of 11-dehydro-TXBz in relation to hematologic variables, treatment, and history of thrombotic complications. All 17 patients with polycythemia vera had 11-dehydro-TXBz excretory rates 2 SD higher than the normal mean. Enhanced metabolite excretion did not correlate with platelet count or with the hematocrit value, and was not related to the current treatment or to a clinical history positive for thrombotic complications. The rate of excretion of 11-dehydro-TXBz was relatively stable when assessed repeatedly on different days (intrasubject coefficient of variation: 18.4% \(\pm \) 9.4%; mean \(\pm\) SD; \(n = 8\)). The urinary excretion of 2,3-dinor-TXBz was also significantly \((P < .001)\) higher in patients than controls \((725 \pm 676 \text{ pmol/mmol creatinine})\). A highly significant linear correlation was found between the excretion rates of 11-dehydro-TXBz and 2,3-dinor-TXBz (Fig 1). This finding suggests that enhanced metabolite excretion in patients with polycythemia vera reflects increased TXA2 biosynthesis rather than alterations in its metabolic disposition.

At variance with TXB2 metabolite excretion, urinary LTA4 was not significantly different in patients \((9.3 \pm 6.1\ \text{pmol/h}; \ n = 10)\) and controls \((10.9 \pm 7.3\ \text{pmol/h}; \ n = 8)\), thus implying a selective alteration of arachidonate metabolism via the cyclooxygenase pathway. Enhanced TXA2 biosynthesis might be a consequence of (1) alterations in platelet biochemistry (eg, increased substrate availability) or number; (2) abnormal stimuli to platelet activation; or (3) increased extraplatelet production of TXA2. Whereas (1) would be reflected by changes detectable ex vivo, (2) and (3) would be compatible with unchanged capacity of platelets to synthesize TXA2 in vitro.

To assess the biosynthetic capacity of the patients platelets, we measured TXB2 production during whole blood clotting. This reflects the virtually maximal production of TXA2 by platelets exposed to endogenous thrombin.21 As shown in Table 2, serum TXB2 concentrations in patients were twofold higher than previously measured in a large \((n = 177)\) population of healthy controls.31 However, when corrected for the platelet count, an identical value of TXB2 production was found in patients and controls. That enhanced TXB2 metabolite excretion found in polycythemia vera does not merely reflect increased platelet numbers is indicated by the lack of any statistically significant correlation between platelet counts and urinary 11-dehydro-TXBz \((r = .055)\) or 2,3-dinor-TXBz \((r = .075)\). Moreover, the production of LTA4 in whole blood—a reflection of transcellular metabolism of neutrophil-derived LTA4 by other blood cells, including platelets92—was similar in polycythemic patients \((10.8 \pm 14.3\ \text{pmol/10^6 WBCs})\) and controls \((11.1 \pm 7.3\ \text{pmol/10^6 WBCs}; \ n = 8)\).

To characterize the platelet versus nonplatelet origin of enhanced TXB2 metabolite excretion, we evaluated the short-term effects of a platelet-selective regimen of aspirin therapy \((50 \text{ mg/d for 7 to 14 days})\) on the extent of suppression and time-course of recovery of metabolite

| Table 1. Individual Measurements of 11-Dehydro-TXB2 Excretion in 17 Patients With Polycythemia Vera, in Relation to Hematologic Variables, Treatment, and History of Thrombotic Complications |
|---|---|---|---|---|
| Patient No. | Platelet Count | Hematocrit (L) | Urinary 11-Dehydro-TXB2 (pmol/mmol creatinine)* | Treatment |
| | \(10^9/\text{L}) | | | Thrombotic Complications |
| 1 | 250 | 0.45 | 756 | C | Yes |
| 2 | 500 | 0.47 | 210 | P | No |
| 3 | 180 | 0.50 | 4,565 | P + C | Yes |
| 4 | 179 | 0.50 | 825 | P | No |
| 5 | 180 | 0.48 | 1,201 | P | No |
| 6 | 450 | 0.51 | 1,270 | P + C | Yes |
| 7 | 400 | 0.47 | 1,208 | P | No |
| 8 | 145 | 0.50 | 831 | P | No |
| 9 | 670 | 0.42 | 809 | C | Yes |
| 10 | 680 | 0.49 | 372 | — | No |
| 11 | 225 | 0.48 | 310 | — | Yes |
| 12 | 128 | 0.50 | 371 | P | No |
| 13 | 721 | 0.45 | 1,192 | P | Yes |
| 14 | 1,097 | 0.46 | 2,324 | — | No |
| 15 | 696 | 0.49 | 426 | P | No |
| 16 | 360 | 0.49 | 567 | P | No |
| 17 | 950 | 0.47 | 339 | — | No |

Abbreviations: C, chemotherapy; P, phlebotomy (urine collection was started at least 18 hours after phlebotomy).

**Normal mean is 117 \(\pm\) 45 pmol/mmol creatinine (range, 53 to 210).**

Fig 1. The correlation between urinary excretion rates of 11-dehydro-TXBz and 2,3-dinor-TXBz in patients with polycythemia vera. Thirty-five urine samples obtained from 17 patients were assayed for both metabolites.
excretion in nine patients. Before aspirin administration, urinary 11-dehydro-TXB\(_2\) and 2,3-dinor-TXB\(_2\) averaged 688 ± 692 and 467 ± 428 pmol/mmol creatinine, respectively. The drug was well tolerated by all patients and no untoward effects were recorded during the study. As shown in Fig 2, aspirin administration was associated with greater than 80% reduction in metabolite excretion and virtually maximal suppression in platelet TXB\(_2\) production. The time-course of recovery of TXA\(_2\) biosynthesis was linear over the next 2 weeks, consistent with the time-dependent maximal suppression in platelet TXB\(_2\) production. The biosynthetic capacity to the systemic circulation on return of unacetylated platelet cyclooxygenase and TXA\(_2\) respectively. The drug was well tolerated by all patients and no other abnormalities were recorded during the study.

Because all of the previously mentioned findings are consistent with enhanced platelet biosynthesis and release of TXA\(_2\) in response to stimuli operating in vivo, we assessed the interaction of two different ligands with the platelet TXA\(_2\) receptor(s) of patients and controls with the aim of detecting a possible downregulation of TXA\(_2\) receptor binding. As detailed in Table 3, washed platelets from polycythemic patients were characterized by similar equilibrium dissociation constants (kd) and binding capacity (B\(_{max}\)) for the receptor antagonist SQ29,548 and for the agonist U46619 as measured in healthy controls.

To clarify the nature of stimuli to platelet activation in polycythemia vera, we also evaluated the short-term effects of phlebotomy on 11-dehydro-TXB\(_2\) and 2,3-dinor-TXB\(_2\) excretion in three patients. As shown in Fig 3, metabolite excretion was only marginally reduced after phlebotomy, whereas it was completely suppressed during low-dose aspirin treatment.

**DISCUSSION**

When compared with other myeloproliferative disorders, patients with polycythemia vera are particularly prone to develop thrombotic complications. This is especially true in patients over 70 years old with a prior thrombotic event. A number of factors have been suggested to contribute to such increased thrombotic risk. These factors include an elevated hematocrit value, increased blood viscosity, thrombocytosis, as well as qualitative platelet abnormalities. In addition to several platelet receptor defects, alterations in arachidonate metabolism have been reported. Thus, Shafer described a selective deficiency of platelet lipoxygenase activity in 10 of 22 patients with polycythemia vera, whereas this enzyme activity was normal in patients with reactive thrombocytosis or secondary polycythemia. Interestingly, lipoxygenase-deficient platelets generated higher levels of TXA\(_2\) than control platelets, a finding possibly related to increased substrate availability for the cyclooxygenase pathway and to diminished formation of 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid. Because we did not measure 12-lipoxygenase activity, our measurements of platelet TXB\(_2\) production ex vitro cannot be directly compared with those in the study of Shafer. Moreover, the nature of the stimulus used in the latter and in the present studies was different, ie, exogenously added arachidonate versus endogenously released substrate, respectively. In the patient studied by Mehta et al, serum TXB\(_2\) was numerically increased when expressed in nanograms per milliliter, but perfectly normal when corrected for the high platelet count. In vitro studies of platelet function have shown either diminished or enhanced aggregation to various stimuli. The latter finding is consistent with increased platelet-fibrinogen affinity, as shown by both the aggregometric technique and by measuring the platelet binding of \({}^{125}\)I-labeled fibrinogen.

A major limitation of platelet studies performed in vitro is that they only examine capacity-related indexes of platelet dysfunction.
THROMBOXANE BIOSYNTHESIS IN POLYCYTHEMIA VERA

...generate approximately the same amount of TXB2 pro-

vera by measuring two major urinary enzymatic metabo-

teter biochemistry and function, such as TXA2 production

and TXA2-dependent aggregation. Despite a very substan-

tial biosynthetic capacity of human platelets to produce

TXA2 when challenged in vitro, the actual rate of TXA2

biosynthesis in vivo is very low under physiologic circum-

stances, possibly because of the low frequency and/or

intensity of stimuli to its production.33 The discrepancy

between the two is several thousand fold, with the platelets

contained in 1 mL of human whole blood being capable of

generating approximately the same amount of TXB2 pro-

duced by the whole body, ie, 420 ng/h. Thus, capacity-

related indexes do not necessarily reflect changes in the

stimuli to TXA2 biosynthesis operating in vivo. Moreover,

as recently reviewed by George and Shattil,34 in addition to

technical variables, platelet aggregation responses among

normal persons can vary with mental stress, age, sex, race,

diet, and hematocrit level, and a person may have different

responses on repeated determinations.

Thus, the present study sought to determine the actual

rate of TXA2 biosynthesis in patients with polycythemia vera by measuring two major urinary enzymatic metabo-

lites, ie, 11-dehydro-TXB2 and 2,3-dinor-TXB2, noninva-

sive indexes of arachidonate metabolism via the cyclooxy-

gense/TX-synthase pathway.16 All of our patients excreted 2
to 40 times the level of metabolites excreted by controls

matched for sex and age. The finding of a linear correlation

between the two metabolites (Fig 1), originating through the

11-hydroxy-dehydrogenase and the β-oxidation path-

ways, respectively, suggests that the enhanced metabolite

excretion detected in patients with polycythemia vera re-

flects a change in the biosynthesis of TXA2 rather than a

shift in its metabolic disposition. The finding of unchanged

excretion of LTE4, a major metabolite of LTC4 in hu-

mans,29,35 excludes a generalized abnormality of arachido-

nate metabolism by blood cells of polycythemic patients.

Moreover, our observation of similar LTE4 production in

whole blood samples of patients and controls is consistent

with the recent finding of Stenke et al36 of unaltered LTC4

production in WBC preparations from polycythemia vera

patients. Enhanced TXA2 biosynthesis in our patients was

associated with unchanged biosynthetic capacity of circulat-

ing platelets, as assessed by measurement of TXB2 produc-

tion during whole blood clotting (Table 2). Moreover,

TXB2 metabolic excretion did not correlate with the platelet

count to any statistically significant extent, thus exclud-

ing thrombocytosis as the primary cause of this abnormality.

Urinary metabolites do not necessarily reflect a specific

site of eicosanoid biosynthesis.37 To distinguish between

platelet and nonplatelet sites of TXA2 synthesis, we ex-

ploited the capacity of aspirin to acetylate platelet pro-

staglandin G/H-synthase selectively when it is administered
daily in low doses.32 Other sites of cyclooxygenase activity,
such as the kidney, that can be involved in enhanced TXA2

production under pathophysiologic circumstances are

largely unaffected by doses of aspirin in the range of 30 to

50 mg/d.23 The greater than 80% reduction in TXB2

metabolite excretion in nine patients after they received

low doses of aspirin for 1 to 2 weeks as well as the slow rate

of recovery of TXA2 production on withdrawal of aspirin

administration are consistent with a role for platelets as the

major source of enhanced TXA2 biosynthesis in poly-

cythemia vera. This degree of suppression of TXB2 metabo-
lite excretion is comparable to that achieved by daily doses

of aspirin (40 to 80 mg) in healthy subjects,36 as well as in

patients with episodically39 or persistently17,18 enhanced TX

biosynthesis. The fact that enhanced TXA2 biosynthesis

was not related to a clinical history of thrombotic complica-

tions is not surprising, inasmuch as these had occurred to

five patients 6 months to 5 years before the study.

Thus, the present findings provide the following novel

information on the in vivo arachidonate metabolism in poly-
cythemia vera: (1) a biochemically selective alteration exists

in all the examined patients, involving the cyclooxygenase/

TX-synthase pathway; (2) platelets provide the major

source of enhanced TXA2 biosynthesis; and (3) this aspirin-
suppressible persistent abnormality is not related to in-

creased platelet count and is likely to reflect stimuli to

platelet activation operating in vivo. Atherosclerotic vascu-

lar disease may be associated with accelerated platelet-

vascular interactions and provide triggers to platelet activa-

tion in vivo. However, it should be noted that stable

manifestations of coronary40 and peripheral39 vascular dis-

case are not associated with detectable changes in TXB2

metabolite excretion. Because none of our patients had

acute manifestations of vascular occlusion at the time of the

study, it is unlikely that vascular disease contributed to the

observed changes in platelet arachidonate metabolism.

Persistently elevated platelet TXA2 biosynthesis has been

characterized in association with several cardiovascular risk

factors, such as cigarette smoking,41 non-insulin-dependent
diabetes mellitus,17 and type IIA hypercholesterolemia.18

Thus, TXA2-dependent platelet activation may represent a

transduction mechanism linking these various risk factors
to the enhanced risk of vascular occlusive complications.42

An elevated hematocrit value and increased blood viscos-

ity clearly play a role in the pathogenesis of thrombotic

complications in polycythemia vera. As reviewed by Sha-
fer,1 an increased hematocrit value may permit platelets to

achieve more intimate contact with the vessel wall as a

function of the axial migration of red blood cells. In the

present study, TXA2 biosynthesis was not related to the

hematocrit, although in a relatively narrow range of values

(Table 1). Moreover, a 5% to 6% reduction in the hemato-
crit value associated with phlebotomy did not modify appreciably the rate of TXB2 metabolite excretion in three

patients studied repeatedly (Fig 3). Although phlebotomy

may acutely and transiently increase TXA2 production as a

result of the attending trauma and rapid intravascular

volume depletion, this is unlikely to be reflected in metabo-
lite excretion measured 18 hours after the procedure, given

the 45-minute half-life of 11-dehydro-TXB2 in the human

circulation.43 Thus, the mechanisms responsible for en-
hanced platelet synthesis and release of TXA2 in poly-
cythemia vera remain to be investigated further. Verifica-
tion of the pathophysiologic significance of this abnormality
would require a controlled trial of low-dose aspirin prophyl-
axis.
The potential antithrombotic effect of aspirin has been evaluated previously by the Polycythemia Vera Study Group. Thus, 166 patients were randomized to receive either phlebotomy plus aspirin (300 mg three times a day) and dipyrindamole or placebo. There were nine “severe thrombotic complications” in the entire group (seven in the aspirin-treated patients) after a maximum of 3.4 years of follow-up (median, 1.2 years), which is an overall incidence of approximately 5%. Without addressing the issue of the adequacy of the control group, it is obvious that the sample size was too small to test any realistic hypothesis of risk reduction by aspirin (eg, 20% to 30%, as suggested by the overview of the Antiplatelet Trialists’ Collaboration). The apparently negative efficacy data and the excess of gastrointestinal hemorrhagic complications reported in this study have discouraged them and other investigators from reassessing the efficacy and safety of aspirin in polycythemia vera. In this context, we believe that the present findings are useful in establishing (1) that these patients do have enhanced TXA₂ biosynthesis in vivo; and (2) that such an abnormality can be corrected by a platelet-selective dosage of aspirin that is only a small fraction (50 v 900 mg/d) of that used by the Polycythemia Vera Study Group. The recent demonstration that the antithrombotic effect of doses as low as 30 to 75 mg is comparable to that of much higher doses of aspirin and is associated with reduced gastrointestinal toxicity and bleeding provides the conceptual and practical framework for reassessing the efficacy and safety of antiplatelet therapy in polycythemia vera.

ACKNOWLEDGMENT

The authors thank Maria Luisa Bonanomi and Giuseppina Protasoni for expert editorial assistance.

REFERENCES
Increased thromboxane biosynthesis in patients with polycythemia vera: evidence for aspirin-suppressible platelet activation in vivo [see comments]

R Landolfi, G Ciabattoni, P Patrignani, MA Castellana, E Pogliani, B Bizzi and C Patrono