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THROMBOSIS IN PATIENTS WITH CONNECTIVE TISSUE DISEASES TREATED WITH SPECIFIC CYCLOOXYGENASE 2 INHIBITORS

A Report of Four Cases

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Specific inhibitors of cyclooxygenase 2 (COX-2) have been approved for the treatment of osteoarthritis and rheumatoid arthritis. Unlike nonsteroidal antiinflammatory drugs, specific COX-2 inhibitors do not inhibit platelet activation. However, these agents significantly reduce systemic production of prostacyclin. As a result, theoretical concerns have been raised that specific COX-2 inhibitors could shift the hemostatic balance toward a prothrombotic state. Patients with connective tissue diseases (CTD), who may be predisposed to vasculopathy and thrombosis, often have arthritis or pain syndromes requiring treatment with antiinflammatory agents. Herein we describe 4 patients with CTD who developed ischemic complications after receiving celecoxib. All patients had a history of Raynaud's phenomenon, as well as elevated anticardiolipin antibodies, lupus anticoagulant, or a history compatible with antiphospholipid syndrome. It was possible to measure a urinary metabolite of thromboxane A_2 in 2 of the patients as an indicator of in vivo platelet activation, and this was markedly elevated in both. In addition, the patients had evidence of ongoing inflammation as indicated by elevated erythrocyte sedimentation rate, hypocomplementemia, and/or elevated levels of anti-DNA antibodies. The findings in these 4 patients suggest that COX-2 inhibitor–treated patients with diseases that predispose to thrombosis should be monitored carefully for this complication.

Prostaglandins (PG) and thromboxane (TX) are regulators of platelet and endothelial cell function. Activated platelets synthesize TXA_2, which is a potent platelet aggregant and vasoconstrictor. PGI_2, which is synthesized primarily by endothelial cells, inhibits platelet activation by elevating platelet cyclic AMP and induces vasodilation. There is evidence that endogenous PGI_2 has antithrombotic properties. Infusion of PGI_2 and enhancement of endogenous PGI_2 also provide antiplatelet activity (1).

A multienzyme pathway that includes the cyclooxygenase (COX) enzymes is responsible for synthesis of prostanoids. There are 2 COX isoforms, COX-1 and COX-2. COX-1 is constitutively expressed in most tissues and is the only isoform in platelets (2). The antiplatelet effects of aspirin and other nonsteroidal antiinflammatory drugs (NSAIDs) are mediated through inhibition of COX-1–dependent TXA_2 production (3). COX-2 expression is induced by inflammation and tissue injury (4). The majority of the antiinflammatory, analgesic, and antipyretic actions of NSAIDs are due to inhibition of COX-2 (4). Specific COX-2 inhibitors developed for use in patients with osteoarthritis, rheumatoid arthritis, and pain have efficacy similar to that of nonselective NSAIDs that inhibit both COX isoforms.

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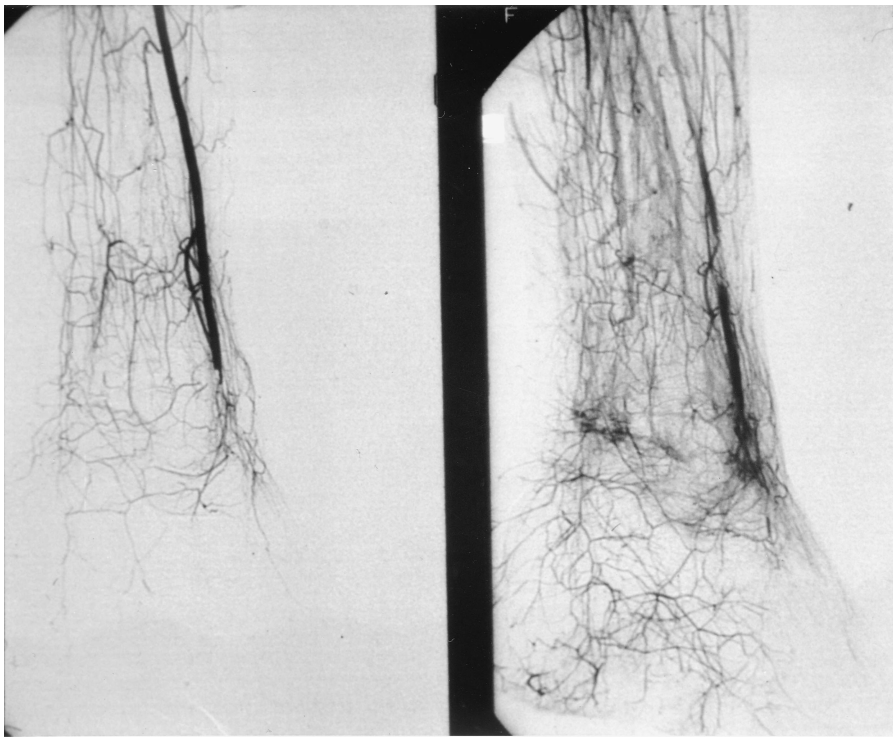

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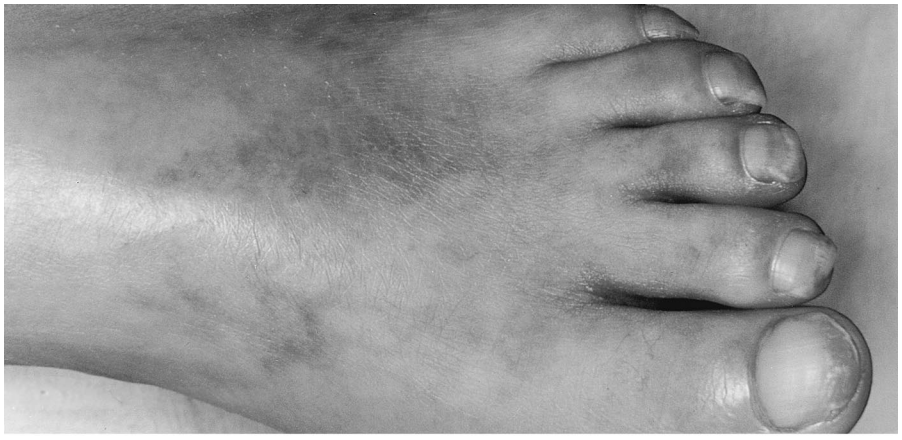

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Figure 1. A, Early-phase (left panel) and late-phase (right panel) lower extremity angiography of the left lower leg of patient 1, demonstrating abrupt occlusion of the anterior tibial artery at the level of the ankle. There is little collateral flow and no perfusion distal to the metatarsals. **B,** Dorsal aspect of the left foot of patient 1, demonstrating ischemic change.

Inhibition of both COX-1 and COX-2 by anti-inflammatory doses of aspirin and NSAIDs leads to a simultaneous decrease in platelet TXA_2 and endothelial cell PGI_2, resulting in a balanced reduction of prostanoids with opposing actions. In contrast, specific COX-2 inhibitors have no effect on platelet TXA_2, and thus do not inhibit platelet function (5,6). Since specific COX-2 inhibitors block production of systemic PGI_2, the question has been raised as to whether these agents may be prothrombotic (7,8). Herein we report the cases of 4 patients with connective tissue diseases (CTD) in whom administration of

Table 1. Summary of the results of serologic and coagulation studies in the 4 patients*

	Patient 1	Patient 2	Patient 3	Patient 4
ANA, titer and pattern	>1:2,560, speckled†	>1:2,560, homogeneous†	1:2,560, nucleolar†	1:160, homogeneous†
Anti-dsDNA, IU/ml (normal 0.0–0.7)	52.1†	129.9†	−	17.3†
ENA	Sm+, RNP−, Ro/SSA−, La/SSB−	Sm−, RNP−, Ro/SSA+, La/SSB+	Sm−, RNP+, Ro/SSA+, La/SSB−	Sm−, RNP−, Ro/SSA−, La/SSB−
Anti–Scl-70	+†	−	ND	ND
RF, IU/ml (normal 0–30)	250†	ND	ND	<20
C3, mg/dl (normal 83–240)	66†	69†	55.6†	61†
C4, mg/dl (normal 13–60)	<10†	12†	12†	13
ESR, mm/hour (normal 0–20)	67†	17	100†	91†
CRP, mg/dl (normal 0.0–0.6)	1.6†	0.9†	ND	2.1†
IgG ACA, GPL (normal 0–22)	52†	42†	−	10
IgM ACA, MPL (normal 0–10)	25†	2	−	2
DRVVT, seconds (normal 25.9–34.7)	ND	33.9	ND	37.2†
PT, seconds (normal 9.3–10.9)	11.9†, INR 1.2 (on heparin)	9.6, INR 1.0	18.3†, INR 2.6 (on warfarin)	9.6, INR 1.0
APTT, seconds (normal 20.3–27.4)	84† (on heparin)	23.4	40†	20.1

* ANA = antinuclear antibody; anti-dsDNA = anti–double-stranded DNA; ENA = extractable nuclear antigen; ND = not done; RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; ACA = anticardiolipin antibody; DRVVT = dilute Russell viper venom time; PT = prothrombin time; INR = international normalized ratio; APTT = activated partial thromboplastin time.
† Abnormal value.

celecoxib, a specific COX-2 inhibitor, was temporally associated with thrombosis.

CASE REPORTS

Patient 1. Patient 1, a 42-year-old woman, was admitted to the University of Michigan Medical Center (UMMC) with a painful, cold, and cyanotic left foot. She had a 23-year history of arthritis, Raynaud's phenomenon (RP), and fatigue treated intermittently with prednisone and hydroxychloroquine (HCQ). She had a 45–pack-year smoking history. Two weeks prior to admission, celecoxib in 200-mg capsules had been prescribed, to be taken up to twice daily on an as-needed basis. Her symptoms had developed acutely after 2 doses of celecoxib. She presented to an outside hospital, where angiography revealed diffuse aortoiliac and common femoral atherosclerotic disease, chronic occlusion of the left posterior tibial and peroneal arteries at the level of the mid-calf, and abrupt occlusion of the anterior tibial artery at the level of the ankle, consistent with acute thromboembolic disease (Figure 1A). She was administered 1 dose of intravenous methylprednisolone (IV MP). Treatment with unfractionated heparin was begun, and she was transferred to UMMC.

On admission, the patient's left forefoot showed blue mottling (Figure 1B) and was cool to touch. The dorsalis pedis and posterior tibial pulses were diminished. Heparin and IV MP were continued, and treatment with an oral calcium channel blocker was added to alleviate vasospasm. Findings on a surface echocardiogram were normal. The results of serologic and coagulation studies are presented in Table 1. Urokinase infusion failed to reestablish blood flow, and the patient underwent an embolectomy, which resulted in partial restoration of arterial flow. Just prior to discharge, a spot urine collection was obtained for analysis of the urinary metabolites of TXA_2 (11-dehydro TXB_2) and PGI_2 (2,3-dinor-6-keto $PGF_{1\alpha}$) (Table 2). The patient was discharged on a regimen of daily warfarin, prednisone, and HCQ. At followup 1 month after discharge, arterial flow to the left forefoot remained diminished, with a pulse detectable only by doppler. She had developed gangrene of the distal half of the left great toe and an ischemic ulcer on the dorsum of the foot.

Patient 2. Patient 2 was a 37-year-old woman who was admitted to UMMC with pain and cyanosis of the toes of the right foot and interdigital ulceration. Systemic lupus erythematosus (SLE) had been diagnosed 7 years previously. Her disease was characterized by RP, arthritis, sicca symptoms, and, more recently, cerebritis necessitating monthly treatment with IV cyclophosphamide. Other medications included daily MP (16 mg) and HCQ (400 mg). She had previously undergone right upper extremity sympathectomy for refractory RP. Celecoxib (100 mg twice daily) had been prescribed 3 weeks prior to admission. Within 1 week of beginning this treatment, she developed swelling of the right foot. After an additional week of treatment, she developed

Table 2. Urinary metabolites of systemic thromboxane A_2 (TXA_2) and prostaglandin I_2 (PGI_2)*

	Patient 1	Patient 3
Urine 11-dehydro TXB_2	1.89 ng/mg creatinine (normal <1.00; matched control 0.18)†	4.11 ± 0.17 ng/mg creatinine (normal <0.65)
Urine 2,3-dinor-6-keto $PGF_{1\alpha}$	0.348 ng/mg creatinine (normal <0.200; matched control 0.025)	0.472 ± 0.032 ng/mg creatinine (normal <0.29)

* Urine 11-dehydro TXB_2 is the metabolite of extrarenal TXA_2, and urinary 2,3-dinor-6-keto $PGF_{1\alpha}$ is the metabolite of PGI_2 produced outside the kidney. Metabolites were measured by gas chromatography negative ion chemical ionization mass spectometry using authentic deuterated standards (8). Patient 2 had been treated with low-dose aspirin, and values were in the normal range (data not reported). Urine for measurement of eicosanoid metabolites was unavailable from patient 4.
† Control was matched by age and sex.

purplish discoloration of the toes of the right foot, with pain and swelling that prompted her to discontinue the celecoxib. She was admitted because of worsening pain and cyanosis.

On presentation, the dorsalis pedis pulses were strong and equal bilaterally. There was no lower extremity edema. The toes of the right foot were cyanotic, and there were ulcerations in the third and fourth interdigital spaces. Serologic and coagulation results are shown in Table 1. She was treated with IV MP, 1 gm daily for 3 consecutive days, and discharged on a regimen of oral MP 20 mg and aspirin 325 mg daily, later reduced to 80 mg daily. With aspirin treatment, measured values of urinary PGI_2 and TXA_2 metabolites were within the normal range, as expected (data not shown). After the patient failed to improve clinically, she was readmitted 2 weeks later to receive an IV bolus of cyclophosphamide. On followup 2 weeks later, the ulcerated areas had healed, but there was persistent digital ischemia with constant pain, bluish discoloration, and coolness to touch.

Patient 3. This patient, a 56-year-old woman with systemic sclerosis (SSc) and lupus anticoagulant (LAC), was admitted to the Medical University of South Carolina with shortness of breath. She was diagnosed as having SSc associated with pulmonary hypertension and RP in 1995. She developed an ulnar artery thrombosis in May 1997 and was prescribed warfarin after the LAC was detected. Her prothrombin time international normalized ratio was maintained in the 2.0–2.5 range rather than the recommended range of >3 since she had previously had excessive vaginal and gastrointestinal bleeding. In April 1999, she developed leg pain and was prescribed celecoxib (200 mg once or twice daily). After 2 days, she developed dyspnea. She presented to the emergency room 2 days after the dyspnea developed.

A V/Q scan identified at least 3 mismatched defects in the right upper lobe, right lower lobe, and left upper lobe, leading to interpretation as a high probability for pulmonary embolus. Cardiac and lower extremity ultrasound failed to reveal a thrombotic source. She was treated with heparin, and a followup V/Q scan before discharge revealed no mismatched defects. Findings of serologic and coagulation studies are shown in Table 1. After resolution of the thrombus, discontinuation of celecoxib and heparin, and reinitiation of warfarin, spot urine samples were collected for measurement of urinary metabolites of TXA_2 and PGI_2 (Table 2).

Patient 4. Patient 4, a 41-year-old woman with a history of SLE, was admitted to UMMC with a cold, painful, cyanotic right foot. The patient had an earlier history of bilateral deep venous thromboses, a miscarriage occurring at 7 months into the pregnancy, and elevated IgG anticardiolipin antibody (ACA). She had been treated with warfarin for ~7 years, but it had been discontinued 10 years prior to admission. Past manifestations of SLE also included lupus nephritis diagnosed by renal biopsy, myositis, RP, and synovitis. She had been treated with methotrexate (15 mg/week) and prednisone (10 mg/day). Her antiinflammatory drug was changed to celecoxib (200 mg twice daily) 5 months prior to admission. Approximately 2 months after the initiation of celecoxib treatment she presented to the emergency room with bluish mottling and pain in her right foot. The symptoms were attributed to vasculitis. The next month she again developed bluish discoloration of her right foot with ulcer formation on the toes. She was admitted to UMMC with a diagnosis of vasculitis.

Serologic and coagulation findings are shown in Table 1. Ankle–brachial arterial indices were normal, and a surface echocardiogram failed to reveal valvular vegetations. The patient was treated with IV MP and discharged on a regimen of prednisone in an increased dosage (60 mg/day). Her treatment with celecoxib was continued. She returned 1 week later with an ischemic, pulseless right foot. Arteriography revealed a large, elongated thrombus of the distal right common iliac artery extending to and occluding the right internal iliac

Table 3. Temporal relationship between initiation of cyclooxygenase 2 inhibition treatment and development of thrombotic symptoms

	Patient 1	Patient 2	Patient 3	Patient 4
Duration of treatment prior to symptoms	2 weeks (2 doses)	1 week	2 days (3 doses)	2–5 months*
Prescribed dosage	200 mg twice daily as needed	100 mg twice daily	200 mg once or twice daily	200 mg twice daily

* Symptoms attributed to vasculitis 2 months after initiation of treatment in this patient may, in retrospect, have been due to thrombosis.

artery. Occlusive thrombus was also present within the distal right popliteal artery above the level of the knee. Occlusive emboli involved the proximal right anterior tibial, proximal peroneal, and proximal posterior tibial arteries. There was no evidence of atherosclerotic disease. The patient was treated with thrombolytic infusion therapy that resulted in some improvement; however, surgical embolectomy was needed for restoration of blood flow to the pedal vessels. Long-term warfarin therapy was instituted prior to discharge.

DISCUSSION

This is the first report of thrombosis temporally associated with administration of a specific COX-2 inhibitor (Table 3). The findings in these patients raise the possibility that specific inhibition of COX-2 may shift the hemostatic balance toward a prothrombotic state in some patients. Specific inhibition of COX-2 decreases systemic PGI_2, a significant proportion of which is likely derived from the vascular endothelium (7,8). PGI_2 is a potent inhibitor of platelet function and vascular tone (9), and decreased PGI_2 production results in the loss of a natural inhibitor of platelet activation. Reduced PGI_2 synthesis may act in concert with other thrombotic risk factors occurring in a given patient to precipitate acute vascular occlusion. This risk is likely increased in patients whose platelet TXA_2 synthesis is already elevated.

Recent studies have shown that COX-2 is the primary isoform responsible for the systemic biosynthesis of PGI_2 under physiologic conditions in humans (7,8). This finding is consistent with in vitro data showing that laminar, but not turbulent, shear stress induces selective and sustained up-regulation of COX-2 in macrovascular endothelial cells (10). Endothelial COX-2 expression may also be increased in the presence of atherosclerotic disease or proinflammatory cytokines, both of which can be present in patients with CTD (11,12).

The influence of celecoxib on PGI_2 production in these patients occurred in the context of risk factors that collectively predispose to thrombosis. Two patients had elevated levels of ACA, 1 had LAC, and 1 had previously elevated ACA with a history of thrombosis and miscarriage typical of the antiphospholipid syndrome (APS). ACA and LAC are part of the spectrum of antiphospholipid antibodies (aPL) that predispose to arterial and venous thrombosis (13). The mechanism of vascular thrombosis in patients with APS is not completely known, but it is likely multifactorial (13). There is evidence that alterations of eicosanoid generation may be involved. Patients with aPL have increased TXA_2 levels, suggesting a role for platelet activation in the pathophysiology of thrombotic events (13,14). In fact, an imbalance of thromboxane/prostacyclin biosynthesis based on measurement of urinary metabolites has been previously proposed as being crucial to development of thrombosis in patients with LAC (15).

In a recent study of patients with SLE, enhanced excretion of urinary TXA_2 metabolites was highly associated with the presence of aPL and evidence of endothelial perturbation, as determined by elevated urinary excretion of von Willebrand factor and tissue plasminogen activator (16). Over a median followup period of 48 months, all patients who developed vascular complications of myocardial infarction, stroke, or deep venous thrombosis had elevated urinary 11-dehydro-TXB_2 excretion (16). Further evidence for the importance of eicosanoid production in patients with aPL comes from in vitro studies that demonstrate increased platelet TXA_2 production and aggregation when platelets are cultured with β_2-glycoprotein I and ACA (17).

The patients described herein had elevated urinary metabolites of systemic TXA_2 and PGI_2. This finding corroborates studies demonstrating that platelet activation is present in patients with aPL (16). The increased excretion of 2,3-dinor-6-keto $PGF_{1\alpha}$ in these patients is consistent with the concept that the production of PGI_2 is an important restraint on the excessive activation of platelets. It also suggests that patients with a known prothrombotic state and elevated platelet TXA_2 production may be at risk for thrombosis when selective COX-2 inhibitors are administered.

Three of these patients had reduced levels of free protein S antigen (patient 1 21%, patient 2 26%, patient

4 40%; normal 43–132%); this was not measured in patient 3. Protein S is a required cofactor for activated protein C to function as an anticoagulant. We suspect that in these patients, reduced free protein S resulted from the inflammatory state that elevated C4b-binding protein levels, leading to a shift of free protein S to the complexed, inactive form. Although a reduced protein S level alone may not have been sufficient to trigger thrombus formation, when combined with other risk factors it may have contributed to the clinical presentation.

Independent of the prothrombotic risk factors described above, these patients also had abnormal vascular endothelial cell function that could have interfered with the constitutive anticoagulant nature of the endothelium. The patients with SLE had active disease with hypocomplementemia and elevated levels of anti-DNA antibodies, suggesting circulating immune complexes. The patient with SSc had reduced complement levels as well. Patient 1 also smoked cigarettes and had evidence of atherosclerotic disease by arteriography and by pathologic examination after embolectomy.

A causal relationship between the initiation of treatment with a specific COX-2 inhibitor and these thrombotic events cannot be established on the basis of the available evidence, even though the temporal relationship is impressive and the pathophysiologic rationale well-founded. These findings are, however, consistent with a hypothesis that thrombosis is an adverse consequence of inhibition of prostacyclin biosynthesis in patients with a prothrombotic disorder. Additional evidence would be needed to support or refute this hypothesis. Certainly these observations, together with the knowledge that COX-2 inhibitors selectively block prostacyclin biosynthesis, suggest the need for heightened surveillance of the consequences of specific COX-2 inhibition in patients with diseases that predispose to thrombosis.

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