Livedoid Vasculopathy Associated With Plasminogen Activator Inhibitor-1 Promoter Homozygosity (4G/4G) Treated Successfully With Tissue Plasminogen Activator

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Background: Livedoid vasculopathy (LV) is an occlusive thrombotic disease that affects primarily the small blood vessels of the lower extremities and is associated with recurrent painful ulcerations. The pathogenesis of LV is unclear, but the disease is largely attributed to a hypercoagulable state. Factor V Leiden mutation, heterozygous protein C deficiency, homozygous hyperhomocysteinemia, and other inherited thrombophilias have been associated with LV. Plasminogen activator inhibitor-1 (PAI-1) is an important inhibitor of the fibrinolytic system. Elevated levels of PAI-1 are found in some patients with thrombotic diseases. Some of these patients are homozygous for an allele of PAI-1 containing a stretch of 4 guanines at base −675 in the promoter region. This variant is associated with elevated PAI-1 protein levels, impaired fibrinolysis, and increased risk of thrombosis.

Observations: A 33-year-old white woman had a 3-month history of painful enlarging ulcers on both ankles. Various therapies, including administration of oral antibiotic agents and prednisone up to 100 mg/d, to treat presumed vasculitis, were unsuccessful. Skin biopsy specimens revealed numerous thick-walled small blood vessels, many of which were filled with fibrin thrombi, in association with minimal perivascular inflammatory infiltrate, extensive epidermal necrosis, and focal ulceration. A diagnosis of thrombotic vasculopathy was made. Clinical workup revealed an elevated plasma level of PAI-1 (31 µm/mL; reference range, <25 µm/mL) and PAI-1 promoter 4G/4G homozygosity detected at DNA sequencing. Treatment with heparin sodium and tissue plasminogen activator dramatically improved the lesions, resulting in complete healing of the ulcerations. Continuation of anticoagulant therapy with warfarin sodium and episodic administration of tissue plasminogen activator was required for symptomatic control.

Conclusions: Patients with LV may have elevated plasma PAI-1 levels. This may be associated with the PAI-1 promoter 4G/4G genotype, which has not previously been linked with LV. Further studies in patients with LV are warranted to determine how frequently this genotype is present because it may identify responsiveness to fibrinolytic therapy.

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LIVEDOID VASCULOPATHY (LV) is an uncommon skin disease that is difficult to manage. The disease affects primarily the small blood vessels in the lower extremities and is typified by persistent livedo reticularis associated with recurrent painful ulcerations that heal with white atrophic scars. The histopathologic features are characteristic and include segmental hyalinization, endothelial proliferation, and thrombosis of the upper and middernal blood vessels. The pathogenesis of LV is still unclear, but the disease is considered an occlusive thrombotic process due to a hypercoagulable state. There have been reports of LV associated with factor V Leiden mutation, heterozygous protein C deficiency, homozygous hyperhomocysteinemia, and other inherited thrombophilic disorders. Successful treatment of LV with thrombolytic therapy such as heparin sodium, warfarin sodium (Coumadin), and tissue plasminogen activator (tPA) supports this hypothesis.

See also pages 1413 and 1481

Plasminogen activator inhibitor-1 (PAI-1) is an important inhibitor of the fibrinolytic system. Composed of a single-chain glycoprotein, it is the primary inhibitor of both tPA and urokinase-type plasminogen activator. Elevated levels of
PAI-1 were found in some patients with thrombotic diseases owing to impaired activation of plasminogen. Some of these patients are homozygous for an allele of PAI-1 containing 4G in the promoter region. We describe a patient with LV with an elevated plasma PAI-1 level and 4G/4G homozygosity. The patient was treated successfully with a combination of heparin and tPA but requires maintenance antiplatelet or anticoagulant therapy. To our knowledge, this is the first reported case of LV associated with PAI-1 4G/4G promoter homozygosity.

**REPORT OF A CASE**

A 33-year-old white woman was seen at our dermatology clinic with a 3-month history of painful enlarging ulcers on both ankles. Before development of the ulcerations, the patient noted a prodrome of lower extremity pain and erythema involving medial and lateral aspects of the ankles. She had been seen by several dermatologists, and multiple specimens had been obtained from lesional skin. Clinical diagnoses included necrotic spider bite reaction and leukocytoclastic vasculitis. Various treatments including oral antibiotic agents and prednisone up to 100 mg/d were unsuccessful. At physical examination, this slightly overweight patient (body mass index [calculated as weight in kilograms divided by height in meters squared], 28) had severe discomfort. There were multiple deep, well-demarcated ulcers on the medial and lateral aspects of both ankles (Figure 1).

Slides from 2 previous biopsy specimens were obtained for review. Light microscopic examination disclosed numerous thick-walled small blood vessels, many of them filled with fibrin thrombi, in association with minimal inflammatory infiltrate and epidermal necrosis (hematoxylin-eosin, original magnification ×100); B, an incisional biopsy specimen obtained from the border of the lesion shows more prominent small and larger thickened walled blood vessels containing fibrin thrombi in the lumens and within the vessel walls (hematoxylin-eosin, original magnification ×200).

Figure 1. Purpuric lesions with well-demarcated ulcers with a reticulated background on the patient’s right (A) and left (B) ankles; C, view of the right ankle 6 months after treatment.

Figure 2. A, At light microscopy, the superficial dermis contained numerous thick-walled small blood vessels, many of them filled with fibrin thrombi, in association with minimal inflammatory infiltrate and epidermal necrosis (hematoxylin-eosin, original magnification ×100); B, an incisional biopsy specimen obtained from the border of the lesion shows more prominent small and larger thickened walled blood vessels containing fibrin thrombi in the lumens and within the vessel walls (hematoxylin-eosin, original magnification ×200).
merase chain reaction to amplify the region around the polyguanine stretch at −675 in the PAI-1 promoter. The reaction was performed in 1.5 mmol/L magnesium chloride, 800 mmol/L each of deoxyribonucleoside phosphate and 1X Taq buffer, 1.25 U of Taq polymerase, and 1.25 pmol/L each of oligonucleotide primers (sense primer, 5′-AAAGCTTTTACATGGTAAACCCTGGT-3′; and antisense primer, 5′-TGCAAGCAGGCACTT-GATTGTCTAG-3′) in a final volume of 25 µL. The polymerase chain reaction was run for 30 cycles with an annealing temperature of 60°C. This yielded the expected 257-base pair product when analyzed on an agarose gel. After cleanup of the polymerase chain reaction, the products were used directly in an automated sequencing reaction using BigDye Terminator chemistry (Perkin Elmer/Applied Biosystems Division, Foster City, Calif) and the same primers. The polymerase chain reaction product was sequenced in both directions. Each independently demonstrated a pure population of 4G molecules with no evidence of 5G molecules. This is consistent with the patient being homozygous for the PAI-1 promoter 4G polymorphism.

Other laboratory findings, including complete blood cell count; erythrocyte sedimentation rate; antinuclear antibody, antiphospholipid antibody, cryoglobulin levels; prothrombin time, partial thromboplastin time; international normalized ratio; antithrombin III, homocysteine, and proteins C and S levels, were all within normal limits. Factor V genotyping was negative for the Leiden allele. Hepatitis B and C serologic results were negative.

Because tPA infusion has been reported to arrest the progression of acute ulceration in LV,13 the patient was given tPA intravenously at a dose of 10 mg/d for 2 weeks. The lesions improved dramatically, and the patient was discharged with a 1-month course of subcutaneous heparin at a dose of 5000 U twice a day and aspirin at a dose of 81 mg/d. At 1-month follow-up, ulcerations were substantially smaller. However, with cessation of heparin therapy, the patient developed violaceous discoloration around the healing ulcers. Dipyridamole, 75 mg/d, was added to the aspirin therapy, and within the next 2 months, the ulcers completely reepithelialized, leaving white atrophic scars on a background of reticulated violaceous erythema. Maintenance treatment included aspirin and dipyridamole antiplatelet therapy, without relapse of prothrombotic symptoms or ulcerations. Antiplatelet therapy was discontinued briefly while the patient underwent a radical hysterectomy because of cervical cancer; and within the next 2 months she began to experience increasing pain, swelling, and redness of the lower extremities. Antiplatelet therapy along with aspirin and dipyridamole therapy was discontinued and treatment with warfarin sodium was initiated, but the patient continued to experience worsening of symptoms. Ultrasonographic evaluation failed to reveal evidence of deep vein thromboses, and the patient was admitted for another 2-week course of tPA infusions, which led to improvement in both symptoms and lower extremity discoloration.

After the second hospital discharge, maintenance treatment included anticoagulant therapy with warfarin sodium. However, within the next 4 months, pain and lower extremity erythema recurred in a setting of widely fluctuating international normalized ratios. The patient was readmitted for a third course of tPA infusion, and was discharged with a regimen of aspirin and dipyridamole therapy and enoxaparin subcutaneous injections of 60 mg/d. The symptoms were well controlled for the next 3 months, but lower extremity pain and erythema recurred after the patient missed 2 weeks of enoxaparin therapy. She was admitted for a fourth 2-week course of tPA infusion therapy, and symptoms remain well controlled with a combination therapy of daily aspirin, dipyridamole, and enoxaparin.

COMMENT

Livedoid vasculopathy is an uncommon skin condition that poses a diagnostic and therapeutic challenge for dermatologists and dermatopathologists. For some time, LV has been considered a vasculitic process. In many patients, like ours, LV was misdiagnosed as vasculitis. However, it is a common observation that, at histopathologic examination, most lesions of LV lack neutrophilic infiltrate of the blood vessel walls and fibrinoid necrosis, hallmark features of true vasculitis. A study by Papi et al14 demonstrated that in cutaneous small vessel vasculitis, the serum levels of proinflammatory cytokines, including interleukin (IL) 1β, tumor necrosis factor-α, IL-8, IL-2, and soluble IL-2 receptor are high, whereas in LV, the levels of inflammatory mediators are in the normal range. Platelet and lymphocyte activation is present, which suggests that LV is primarily a noninflammatory occlusive thrombotic vascular disease. Although the pathogenesis of LV is still unclear, most authors consider a hypercoagulable state as the primary pathogenic mechanism. This is suggested by the reports of LV associated with inherited thrombophilias, including activated protein C resistance owing to a heterozygous factor V Leiden mutation,3 heterozygous protein C deficiency,4 and hyperhomocysteinemia secondary to the homozygous C677T mutation in the MTHFR gene.5 This hypothesis is supported by several reports of clinical improvement in skin lesions in patients with LV receiving fibrinolytic and anticoagulant therapies, such as heparin and tPA.6,8 Recently, Browning and Callen9 described LV associated with cryofibrinogenemia and hyperhomocysteinemia. The recurrent painful skin ulcers were refractory to treatment with multiple other medications but improved dramatically with warfarin therapy that was incidentally prescribed to treat atrial fibrillation.

Plasminogen activator inhibitor-1 is an important inhibitor of the fibrinolytic system. It is composed of a single-chain glycoprotein synthesized in the liver, adipose tissue, and endothelial cells. Plasminogen activator inhibitor-1 is the primary inhibitor of both tPA and urokinase-type plasminogen activator.10 Synthesis of PAI-1 is inducible under the regulation of physical mediators, including endothelin, IL-1, tumor necrosis factor-α, fibroblast growth factor-2, and lipids.11 Elevated levels of PAI-1 have also been found in association with chronic venous ulcers.12

The discovery of polymorphisms in the PAI-1 promoter region has shed more light on the role of PAI-1. The gene for PAI-1 has several polymorphic loci. The most important is the 4G/5G insertion-deletion 675 base pairs...
from the start of the promoter, affecting the binding of nuclear proteins involved in the regulation of PAI-1 gene transcription. The 4G allele appears to bind only the enhancer; thus the 4G/4G genotype gives rise to higher PAI synthesis, while the 5G allele binds both the enhancer and the suppressor, resulting in a lower level of transcription.13 Studies have shown that individuals with the 4G/4G genotype have levels of PAI-1 approximately 25% higher that in those with the 5G/5G phenotype.14,15 A recent prospective study from Italy in 111 consecutive patients undergoing elective coronary bypass surgery showed that carriers of the 4G allele, compared with those homozygous for the 5G/5G genotype, have approximately 20% more PAI-1 activity and antigen both before and after surgery.16 A more recent study from the Netherlands showed that the 4G allele in the PAI-1 gene increases the risk of cerebral ischemia after aneurysmal subarachnoid hemorrhage and probably also increases the risk of poor outcome.17 Although the evidence for the relationship between PAI-1 level and the risk of venous thrombolism is conflicting, most studies have related homozygosity for the 4G allele to increased risk of venous thrombolism and poor outcome of stroke.

To our knowledge, the case described herein is the first patient reported with LV and skin ulcerations associated with elevated serum PAI-1 activity and homozygosity for the PAI-1 promoter 4G polymorphism. Treatment with heparin and tPA dramatically improved the lesions, which suggests that elevated serum PAI-1 activity associated with PAI-1 4G/4G promoter homozygosity may be the underlying cause of LV in this patient. We found no similar case in the literature, probably because of lack of investigation rather than rarity of the association. To date, there is insufficient information to recommend testing of PAI-1 plasma levels or PAI-1 promoter genotype in every patient with LV. However, it is certainly prudent to screen for an inherited hypercoagulable state in patients with LV and skin ulcers because the correct diagnosis will lead to proper therapies and dramatic improvement in the skin lesions.

Other studies in the literature elucidate the role of PAI-1 in tumoral angiogenesis and cancer biology.18,19 In our patient, cervical cancer was diagnosed a year after the presence of skin lesions that were confined to the lower extremities and still recurrent after discontinuation of tPA therapy 1 year after surgical removal of the tumor. Although we can speculate that there may be a connection between PAI-1 homozygosity and cervical cancer in our patient, there is no diagnostic test to prove the relationship. More epidemiologic and clinical studies will need to be done to establish the relationship.

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Author Contributions: Dr Gaspari had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Deng and Gaspari. Acquisition of data: Deng, Gocke, Hess, Paltiel, and Gaspari.

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REFERENCES