Tamoxifen-Associated Venous Thrombosis and Activated Protein C Resistance Due to Factor V Leiden

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BACKGROUND. Thromboembolic events are well recognized complications of cancers and their treatment. Tamoxifen, an antiestrogen used in the treatment of breast carcinoma and other malignancies, has been associated with thrombotic events. Activated protein C resistance due to Factor V Leiden is the most prevalent inherited prothrombotic defect in populations of European descent and has been reported as a major cofactor in the development of thrombosis in women receiving estrogens.

METHODS. The authors report three patients who developed thromboembolic complications while receiving tamoxifen. These patients were studied for the presence of activated protein C resistance by coagulation assay and the presence of Factor V Leiden by molecular analysis.

RESULTS. All three patients had resistance to activated protein C by coagulation assay and were determined to be heterozygous for Factor V Leiden by molecular analysis.

CONCLUSIONS. The authors propose that inheritance of Factor V Leiden significantly increases the risk of thrombosis in patients who receive tamoxifen therapy. All patients prescribed tamoxifen should be carefully questioned regarding personal and family histories of thrombosis and, when indicated, screened for Factor V Leiden. ***Cancer* 1997;79:2024–7.** © *1997 American Cancer Society.*

KEYWORDS: tamoxifen, thrombosis, activated protein C resistance, Factor V Leiden.

Venous and arterial thromboses are well recognized complications in patients with malignancies. As initially observed by Trousseau in 1865[1] and more recently reviewed,[2–4] thromboembolic complications have been described in patients with overt as well as occult adenocarcinomas. Thromboses have also been described in the setting of cancer treatment. Tamoxifen, an antiestrogen, is commonly used in the treatment of carcinoma of the breast, melanoma, and gliomas. Tamoxifen therapy has been reported to be associated with thromboembolic events, with an incidence reported to be between 3.8–6.8%.[5–10] In this article, the authors report three patients who developed thrombotic complications while receiving tamoxifen therapy. Laboratory evaluation for underlying prethrombotic hemostatic defects in these patients disclosed activated protein C resistance (APCR) due to Factor V Leiden. The authors propose that this common prothrombotic genetic defect significantly increases the risk of thrombosis in patients receiving tamoxifen.

Case Reports

Case 1

A 67 year-old white female presented in November 1993 with a 2-cm left breast mass. She underwent lumpectomy followed by radiation

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therapy for a T1N0M0 (based on the American Joint Committee on Cancer Staging System) infiltrating ductal adenocarcinoma. The tumor was estrogen receptor (ER) positive and progesterone receptor (PR) and the patient was started on tamoxifen, 10 mg twice daily. The patient did well until December 1994, when she reported painful swelling of the right leg. Venous doppler of the lower extremities was positive for an acute venous occlusion of the femoral vein. The patient was anticoagulated with heparin followed by warfarin with resolution of her symptoms. Tamoxifen was discontinued. The patient's antithrombin III level was 88% (normal, 70–120%) and 29 mg/dL (normal, 22 to 39 mg/mL), protein C was 134% (normal, 67–120%), and protein S was 118% (normal, 70–125%). The tissue factor-dependent factor V assay for APCR assay was positive and molecular analysis demonstrated that the patient was heterozygous for Factor V Leiden. At last follow-up, the patient had no evidence of disease.

Case 2

A 54-year-old white female presented with an abnormal mammogram of the right breast. A lumpectomy revealed a T1N0M0 infiltrating ductal carcinoma. Her ER was 0 and her PR was 20. She had been receiving conjugated estrogens and medroxyprogesterone acetate for several years prior to the development of the mass; these medications were discontinued and tamoxifen, 10 mg twice daily, was given. She also received radiation therapy to the right breast. In September 1995 the patient noticed a lump on her right upper chest. Ultrasound revealed a solid mass that was then excised. The pathology was compatible with recurrent carcinoma of the breast. Chest X-ray, bone scan, and computed tomography scan of the abdomen were negative for metastatic disease. In November 1995 tamoxifen was discontinued and the patient was treated with cyclophosphamide doxorubicin, and 5-fluorouracil. In early December 1995 the patient was admitted to the hospital after reporting the sudden onset of shortness of breath. The chest X-ray did not show any infiltrates or nodules. A ventilation-perfusion lung scan revealed significant perfusion defects to the right upper lobe as well as to the left mid- and lower lung fields that was highly suggestive of pulmonary embolism. Anticoagulation was begun with heparin and the patient was discharged on warfarin. At last follow-up, she was clinically free of disease. The patient's antithrombin III was 82%, her protein C was 74%, her protein S was 111%, and her APCR assay was positive. Molecular analysis demonstrated that she was heterozygous for Factor V Leiden.

Case 3

A 40-year-old white male presented in May 1994 with a mole on the right chest wall. Biopsy revealed a Clark's level IV melanoma. The patient underwent a wide excision. In March 1995 he developed an axillary mass. Excisonal biopsy confirmed recurrent melanoma. Tissue was taken for autologous vaccine preparation. In August 1995 he developed diffuse cutaneous metastases as well as liver and lung metastases. With the exception of 2-mm lesion on the lateral aspect of the left leg, his cutaneous disease was entirely above the waist. He was treated from August 1995 until October 1995 with cyclophosphamide followed by an autologous melanoma vaccine, but did not have a significant response. He was then treated with combination chemotherapy (Cisplatin, Vinblastine, and decarbazine), immunotherapy (interleukin-2 and interferon), and tamoxifen. At the end of February 1996, the patient presented with acute onset of pain and swelling of the left leg. Clinical and laboratory evidence was compatible with a deep venous thrombosis. The patient was anticoagulated with heparin with rapid resolution of his symptoms. His antithrombin III was 72%, protein C was 86%, protein S was 93%, D-dimers were 2000–4000 ng/mL and his APCR assay was positive. Molecular analysis confirmed that the patient was heterozygous for Factor V Leiden.

MATERIALS AND METHODS

Laboratory studies were performed on blood samples collected in 3.2% sodium citrate from which platelet poor plasma was prepared by centrifugation at 10,000 revolutions per minute at 4 °C for 15 minutes. Plasma samples were stored at −20 °C until tested. Genomic DNA was isolated from 200 μL of the cellular pellet remaining after removal of plasma for coagulation studies using the QAIamp Blood Kit (Qiagen, Chatsworth, CA). General coagulation assays and functional assays for antithrombin III, protein C, and protein S were performed using commercial assay kits (protein C: Proclot Protein C, ACL Instrument Laboratories, Lexington, MA, and protein S: Staclot Protein S, Diagnotica Stago, Asnieres-sur-Seine, France) and as previously reported.[11] Coagulation assay for APCR and the molecular assay for Factor V Leiden were performed as recently reported by the authors' laboratory.[12]

DISCUSSION

Thrombotic complications associated with cancers and cancer treatment are well described.[1–4] The mechanisms are complex. Laboratory evidence of low grade disseminated intravascular coagulation is the hallmark of Trousseau's syndrome.[1–4] Callander and Rapaport proposed that this is secondary to exposure of the

blood to tissue factor from neoplastic cells.[4] However, thrombotic events also occur in patients receiving adjuvant treatment with minimal or no clinically evident active tumor. Levine et al.,[5] studied 205 patients with Stage II breast carcinoma treated with adjuvant chemotherapy with and without tamoxifen and found an overall incidence of thrombosis of 6.8%. In addition, several reports have suggested that the addition of tamoxifen may enhance the thrombogenicity of combination chemotherapy.[5–10] In two large Eastern Cooperative Oncology Group adjuvant breast carcinoma trials, Saphner et al.[6] found an increased incidence of thrombotic events in both pre- and postmenopausal women receiving tamoxifen in combination with chemotherapy. Tamoxifen, although considered an antiestrogenic hormone, has overlapping estrogenic effects. Estrogen has well documented effects on the coagulation system. Hypercoagulability due to increased levels of von Willebrand's factor[13,14] factor VIII,[13,14] fibrinogen,[13,14] and mildly decreased levels of antithrombin III,[14] has been demonstrated in women during pregnancy and while receiving estrogen for a wide variety of reasons. Levels of antithrombin III have been reported to decrease in patients receiving tamoxifen, but, as reported by Jordan et al.[15] and Auger et al.[16] these decreases were modest and may not be clinically significant.

In 1993, Dahlback et al.[17] identified a new mechanism responsible for many cases of unexplained familial thrombophilia. APCR has rapidly emerged as the most common cause of familial thrombophilia, with an incidence of 2–5% in the white population.[18–21] The molecular abnormality has been characterized as a point mutation in the factor V genome (1691, A for G).[22–24] The abnormal factor V produced as a result of the Arg 506-Gln substitution, Factor V Leiden, has lost an activated protein C cleavage site, thus rendering the protein resistant to activated protein C degradation.[22] The abnormality is inherited in an autosomal dominant fashion with variable penetrance. The mean age of presentation of initial thrombosis is 25 years in homozygous patients. In heterozygous patients, the mean age of presentation is reported as 44 years.[25] The risk for thrombosis in homozygous patients is 50–100 fold, and it is estimated at 5–10 fold in heterozygous patients. The estrogenic effects of oral contraceptives and pregnancy may increase the risk of thrombosis significantly. Recent studies of thrombosis associated with pregnancy and the use of estrogen-containing birth control pills have demonstrated a significant increased risk of thrombosis in patients with Factor V Leiden.[26,27] However, Rosendaal et al.[25] noted that even some homozygous patients had experienced previous pregnancies or surgeries without thrombosis.

In this article, the authors report three patients with APCR who developed thromboses while receiving tamoxifen. Two of the patients were receiving adjuvant tamoxifen for the treatment of carcinoma of the breast. The first patient had Stage I disease with no identifiable risk factors for thrombosis other than the use of tamoxifen. The second patient had a local recurrence, but was free of gross disease at the time of the thrombotic event. Tamoxifen had been stopped 4 weeks prior to the onset of the thrombosis. However, studies on the thrombotic risks associated with pregnancy and birth control pills have shown a continued risk of thrombosis 4 to 6 weeks after delivery or discontinuation of estrogen-containing contraception.[28] The third patient had advanced melanoma, a malignancy not reported to have a significantly increased risk of thrombosis. In a retrotrospective analysis of 182 cases of malignancy associated with Trousseau's syndrome, Sack et al.[2] reported only 1 patient with melanoma and thrombosis.

APCR due to Factor V Leiden has emerged as the most frequently inherited risk factor for thrombosis in individuals of European descent. There have been an increasing number of reports regarding the synergistic effects of this defect when combined with other inherited or acquired prothrombotic defects. Although the thrombotic risk for patients taking estrogen-containing birth control pills is estimated to be 4-fold greater than the general population birth control pills in patients with APCR increases the risk to 30-fold.[27] The authors believe that similar risks exist in women with APCR who are taking tamoxifen. Therefore, physicans prescribing tamoxifen as either primary or adjuvant cancer therapy should extract careful histories regarding prior thromboembolic events or family histories of thrombosis. If patients histories are ''suggestive'' of a prothrombotic risk, the patients should be screened for APCR. Patients with APCR should be counseled regarding their thrombotic risk. The decision regarding the use of tamoxifen in these patients should be made with consideration to the overall risk to the patient. In selected patients, the use of tamoxifen with prophylactic anticoagulation may be justified.

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