Systemic Toxicity Following Administration of Sirolimus (Formerly Rapamycin) for Psoriasis

Association of Capillary Leak Syndrome With Apoptosis of Lesional Lymphocytes

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Background: Sirolimus (formerly rapamycin) is an immunosuppressive agent that interferes with T-cell activation. After 2 individuals with psoriasis developed a capillary leak syndrome following treatment with oral sirolimus, lesional skin cells and activated peripheral blood cells were analyzed for induction of apoptosis.

Observations: A keratome skin specimen from 1 patient with sirolimus-induced capillary leak syndrome had a 2.3-fold increase in percentage of apoptotic cells (to 48%) compared with an unaffected sirolimus-treated patient with psoriasis (21%). Activated peripheral blood T cells from patients with psoriasis tended to exhibit greater spontaneous or dexamethasone-induced apoptosis than did normal T cells, particularly in the presence of sirolimus.

Conclusions: Severe adverse effects of sirolimus include fever, anemia, and capillary leak syndrome. These symptoms may be the result of drug-induced apoptosis of lesional leukocytes, especially activated T lymphocytes, and possibly release of inflammatory mediators. Because patients with severe psoriasis may develop capillary leak from various systemic therapies, clinical monitoring is advisable for patients with inflammatory diseases who are treated with immune modulators.

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Psoriasis may be caused by pathogenic blood-derived immunocytes inducing secondary activation and deranged growth of keratinocytes and vascular endothelium.1-2 Psoriatic lesions contain activated CD4+ T helper cells.3-4 T-lymphocyte clones from lesional skin release growth factors that induce keratinocyte proliferation.3 Adhesion molecules and other skin cell activation markers are up-regulated in the skin of patients with psoriasis.6-8 T cells in psoriasis exhibit a Th1-like cytokine secretion profile.9-10 Serum interleukin 2 (IL-2) and IL-2 receptor (IL-2R) levels are elevated in patients with chronic plaque psoriasis.11,12 Sirolimus (formerly rapamycin) is a macrolide immunosuppressant that interferes with T cells by impairing the activation response to lymphokines IL-2, IL-4, and IL-12.13-15 Sirolimus prevents allograft rejection16 and shows suppressive activity in experimental models of autoimmune diseases.13,17

Although the immunosuppressive activity of sirolimus is commonly attributed to its antiproliferative effect on lymphoid cells, the drug affects a number of other cellular functions.18,19 Sirolimus inhibits proliferating cell nuclear antigen (PCNA) expression, thereby blocking the cell cycle in the G1 phase in human keratinocytes,20 and selectively blocks IL-2-induced PCNA gene expression in T lymphocytes.21 Sirolimus also blocks the activation of p70 S6 kinase, thus preventing a downstream cascade of events critical for cell growth.22

Inhibition of signal transduction may play an important role in the susceptibility of cells to apoptosis. Programmed cell death has been induced by sirolimus23,24 in many but not all malignant cell lines.25

We report 2 cases of systemic capillary leak syndrome characterized by clinical signs of vascular leakage with fever and anemia in patients with psoriasis after oral administration of sirolimus. We hypothesized that sirolimus might trigger this systemic toxic reaction by inducing programmed cell death in activated T cells in psoriatic skin lesions and blood. Lesional cells and activated peripheral blood T cells from patients with psoriasis and healthy controls were therefore studied for evidence of induction of apoptosis by sirolimus.
METHODS

As part of a phase I pharmacokinetic clinical trial at the University of Michigan, Ann Arbor, 6 patients with psoriasis (including patient 1 in the “Results” section) received 3, 5, or 8 mg/m² body surface area per day of oral sirolimus. Keratome skin biopsy specimens from the hip or buttock area of these patients were taken before treatment (day 0), 2 days into treatment, and on the seventh day of treatment. Patients had used neither systemic immunosuppressives for at least 4 weeks nor systemic retinoids, corticosteroids, or phototherapy for at least 3 weeks before entry into the study. Patient 2 participated in a subsequent study at the University of California-Irvine (similar skin biopsy specimens were not obtained). All procedures were approved by each institution’s internal review board, and informed consent was obtained from each subject.

DNA STAINING OF DERMAL CELLS

Keratome specimens were placed into a neutral protease solution (Dispase; Collaborative Research Inc, Boston, Mass) for overnight incubation at 4°C. The dermis was separated from the epidermis and a single-cell suspension was prepared using collagenase, hyaluronidase, and deoxyribonuclease. Cells were fixed in 70% ethanol and stained with propidium iodide (PI), 50 µg/mL, plus RNAse, 100 U/mL for DNA content. Flow cytometry was performed using a flow cytometer (Epics Elite; Coulter Cytometry, Hialeah, Fla). Light scatter (forward and 90°) was used to gate out debris. Cell aggregates were eliminated from the DNA analysis based on the ratio of integrated to peak fluorescence of PI. Listmode data were analyzed using Elite software (Coulter Cytometry, version 2.0) and for cell-cycle analysis, ModFit (Verity Software House Inc, Topsham, Me, version 2.0).

CULTURE OF PERIPHERAL BLOOD LYMPHOCYTES AND DETECTION OF APOPTOSIS

Peripheral blood was obtained from 5 healthy volunteers (mean age, 32 years; age range, 19-50 years) and 4 patients with psoriasis (mean age, 52 years; age range, 40-57 years). Three of the patients had no adverse effects consistent with capillary leak syndrome, and the fourth was patient 1; the blood from patients was obtained 3 to 5 months after they had stopped sirolimus treatment.

Peripheral blood mononuclear cells were isolated by centrifugation over Ficoll-Hypaque and washed 3 times. Peripheral blood mononuclear cells were cultured in medium (RPMI 1640 supplemented with 10% fetal bovine serum, 2% glutamine, 1% penicillin/streptomycin) and phytohemagglutinin (0.5 µg/mL), at a concentration of 1 × 10⁶ cells per milliliter. At day 3 or 4 and every 3 to 4 days thereafter, cells were washed and resuspended in lectin-free medium (1 × 10⁶ cells per milliliter) supplemented with IL-2 (10-20 U/mL). At day 14 to 18 of culture, cells were washed and subcultured for 18 hours in medium alone, IL-2 (20 U/mL), dexamethasone (10⁻³ mol/L), sirolimus (1, 10, 25, or 100 nmol/L), or combinations of these agents. The cells were fixed in 1% formaldehyde for 15 minutes at 4°C, washed with phosphate-buffered saline (PBS), and permeabilized with 70% ethanol.

DNA strand breaks associated with apoptosis were detected by flow cytometry. DNA was labeled with biotinylated deoxyuridine triphosphate using exogenous terminal deoxynucleotidyl transferase, and the incorporated biotinylated deoxyuridine triphosphate was detected by fluoresceinated avidin. Concurrent staining with PI was also done to allow determination of position in the cell cycle of apoptotic cells, and to detect cells with a subdiploid amount of DNA. For such experiments, 1 million cells were resuspended in 0.05 mL of cadocyte buffer (0.2 mol/L potassium cacodylate, 25 mmol/L tris-hydrochloride [pH 6.6], 2.5 mmol/L cobalt chloride, 0.25 mg/mL bovine serum albumin, 100 U/mL terminal deoxynucleotidyl transferase, 0.5 nmol/L boitin-16-deoxyuridine triphosphate) and incubated for 30 minutes at 37°C. Cells were rinsed in PBS, resuspended in 100 µL of saline citrate buffer (2.5 µg/mL fluoresceinated avidin, 0.1% Triton X-100, 5% wt/vol ratio nonfat dry milk, 0.6 mol/L sodium chloride, and 0.06 mol/L sodium citrate), and incubated for 30 minutes at room temperature in the dark. Cells were rinsed in PBS containing 0.1% Triton X-100 and resuspended in 1 mL of PI buffer (PBS, 5 µg/mL, PI, 0.1% RNAse A). For each condition, a control sample was stained in an identical manner, except that the terminal deoxynucleotidyl transferase was omitted. Staining was analyzed by flow cytometry using a Coulter Elite instrument (Coulter Cytometry). The results were adjusted after background was subtracted.

STATISTICAL ANALYSIS

Student t test corrected for repeated measures was used. A 2-tailed P≤.07 was considered significant.

RESULTS

CASE REPORTS

Patient 1

A 53-year-old woman with a 3-year history of severe psoriasis and no other notable medical problems had been treated in the past with cyclosporine, sulfasalazine, and topical corticosteroids without serious adverse effects. She received a single dose of sirolimus (8 mg/m² per day) 1 week prior to beginning treatment with consecutive daily doses. After the third daily dose (also 8 mg/m² per day), she developed fever (temperature 39.4°C), progressive severe lower extremity edema, orthopnea, weight gain of 5 kg, and hypotension. Sirolimus treatment was stopped. A complete blood cell count revealed normocytic anemia (hemoglobin, 90 g/L; hematocrit, 0.26; mean corpuscular volume, 83 pg) and leukocytosis with eosinophilia. Direct and indirect Coombs test results were negative, and no evidence of hemolysis was found; iron profile studies were consistent with anemia of chronic disease. A chest radiograph was consistent with pulmonary congestion and cardiomegaly. She was treated empirically with antibiotics without defervescence while an extensive evaluation was done, including blood and urine profile studies consistent with anemia of chronic disease. A chest radiograph was consistent with pulmonary congestion and cardiomegaly. She was treated empirically with antibiotics without defervescence while an extensive evaluation was done, including blood and urine
cultures, upper and lower gastrointestinal tract endoscopies, antinuclear antibody testing, rheumatoid factor testing, parvovirus B19 serologic testing, transesophageal echocardiogram, lower extremity Doppler studies, and ventilation-perfusion lung scan, all of which findings and results were negative or normal. The diagnosis of capillary leak syndrome due to sirolimus was made. Her clinical status began to improve 72 hours after the discontinuation of therapy with the drug; she had no sequelae. **Figure 1** shows selected clinical features observed in this patient.

**Patient 2**

A 58-year-old man with a history of severe psoriasis and psoriatic arthritis received sirolimus 1 mg/m² per day (no preliminary dose was administered). Concurrent medications included ibuprofen, the combination drug trimethoprim-sulfamethoxazole, and acetaminophen. After 1 month of sirolimus treatment, he noticed low-grade fevers at night, progressive dizziness, and fatigue. He was found to have orthostatic hypotension, lower extremity edema, and a decreased hematocrit (hemoglobin, 74 g/L; hematocrit, 0.22; mean corpuscular volume, 84 pg). Direct and indirect Coombs test results were negative, and there was no evidence of hemolysis on the blood smear. Iron profile studies were consistent with anemia of chronic disease. Parvovirus-B19 serologic test results were negative. A bone marrow biopsy specimen showed trilineal hematopoiesis, 50% cellularity, normal megakaryocytes, a myeloid-erythroid ratio of 5.1, normal maturation of the myeloid series, and slight dyserythropoiesis with unusual vacuolation of the erythroblasts and normal maturation of the myeloid series. There was no evidence of an infiltrative process, and sideroblasts were not seen. Sirolimus treatment was discontinued. The patient was treated with intravenous furosemide and transfusion of packed red blood cells. His condition improved; follow-up revealed no sequelae.

**LABORATORY FINDINGS**

Skin biopsy specimens were taken from psoriasis lesions of patients while treated with daily sirolimus doses of 3 mg/m² (2 patients), 5 mg/m² (2 patients), or 8 mg/m² (2 patients), and cell suspensions were analyzed by flow cytometry. The percentage of cells with sub-G₀/G₁ DNA content, indicating apoptotic cells, was similar in all epidermal and dermal samples from days 0, 2, and 7 among the 6 patients (data not shown) with the exception of patient 1. Two days after initiation of sirolimus treatment, 48% of the dermal cells of patient 1 had sub-G₀ DNA content, a 2.3-fold higher percentage than the other patient who also received 8 mg/m² per day of sirolimus (21%) (**Figure 2**). The percentage of cells from patient 1 was 1.8-fold higher than in her day 0 sample, and coincided with the occurrence of clinical symptoms. Peripheral blood lymphocytes from patients with psoriasis and normal controls were cultured and induction of apoptosis was measured in the presence or absence of sirolimus. The results are shown as the percentage of cells with staining for DNA strand breaks above background levels (**Figure 3**).

Dexamethasone enhanced apoptosis in cultured activated T cells in both patients (**P = .05**) and controls (**P = .04**). Interleukin 2 tended to protect the cells of control subjects from apoptosis, but not the cells from patients with psoriasis (**Figure 3**). Sirolimus seemed to protect normal T cells from spontaneous or dexamethasone-induced apoptosis. Similar protection did not occur with psoriatic T cells, and sirolimus augmented dexamethasone-induced apoptosis in cultures from some patients (**Figure 3**). This effect was most evident when the mean channel fluorescence was measured (**P = .06** (**Figure 3**, bottom). However, the variability of results in both controls and patients with

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**Figure 1.** Clinical features of patient 1 who developed a capillary leak syndrome after receiving daily sirolimus (formerly rapamycin) treatment. Arrowheads show when sirolimus treatment was started and discontinued.

**Figure 2.** Cell cycle analysis of lesional dermal cells for apoptosis based on propidium iodide staining from patient 1 (right) and from a patient without clinical adverse effects (left), each on the second day of sirolimus (formerly rapamycin) treatment. DNA content is shown as propidium iodide fluorescence. The solid lines represent the histogram of the experimental data, and shaded areas represent the best curve fit. Number of cells (y-axis) in sub-G₀/G₁ are shown in channels 0 through 80 (x-axis) and represent cells demonstrating apoptosis. Patient 1 has a greater percentage of total cells in the part of the curve between 0 and 80 (note differences in the Y scales). Cells in G₀/G₁, S, and G₀/M phases of the cell cycle are shown to the right of channel 80.
We describe 2 patients with psoriasis who developed capillary leak syndrome, fever, and anemia after being exposed to sirolimus, with resolution after stopping treatment with the drug. In both patients, no other etiologies for the fluid retention, peripheral edema, progressive drop of hematocrit, and fever were found. The clinical findings were similar to the cases of capillary leak syndrome induced by endogenous cytokine release or exogenous administration of cytokines (eg, cancer chemotherapy with interleukin 2). Furthermore, sirolimus promotes pros tacyclin release, which could play a role in the vasodilation observed in our patients who developed capillary leak.

No clear etiology for the anemia in our patients was found. Sirolimus blocks the proliferative response of cell lines to a variety of hematopoietic growth factors. In addition, IL-1 and tumor necrosis factor α inhibit bone marrow hematopoiesis and erythropoietin formation; therefore, the triggering of a cytokine release syndrome by sirolimus could have contributed to the drop in the hematocrit.

The incidence of this form of sirolimus toxicity is not known. However, besides patient 1, 3 other individuals of the 34 patients with psoriasis who received treatment with sirolimus at the University of Michigan developed adverse effects that included lower extremity edema and a drop in hematocrit. Two of them discontinued sirolimus treatment and later restarted it without recurrence of the symptoms (C.N.E., unpublished data, October 2, 1998). These findings may represent milder forms of the syndrome. We are unable to identify factors that predispose individuals to this reaction.

Dermis from patient 1 showed a significant increase in the percentage of apoptotic cells compared with other sirolimus-treated subjects with psoriasis, including one who received an identical dose. This supports the hypothesis that sirolimus can trigger programmed cell death and probably cytokine release from cells in psoriatic lesions in certain individuals. Sirolimus was associated with greater apoptosis of cultured T cells in patients with psoriasis (including patient 1) than in control subjects when dexamethasone was added.

Sirolimus may induce apoptosis by altering signal transduction pathways or inhibiting cell survival signals. Blocking the activation of p70 S6 kinase with the administration of sirolimus may be important because a downstream target of p70 S6 kinase is p34cdc2 kinase, which regulates apoptosis of cells exposed to cytotoxic agents.

Capillary leak syndromes have been reported previously among patients with severe psoriasis. In 5 of 7 patients, a systemic therapy (etretinate in 4 and methotrexate in 1) was begun shortly before the syndrome developed; in the other 2 cases, the timing of the syndrome in relation to the patients’ etretinate and cyclosporine therapies is not clear. All of these therapeutic agents induce cell apoptosis in certain situations, and retinoids and cyclosporine have been associated with capillary leak syndrome in subjects without psoriasis. Our findings suggest a new explanation for capillary leak syndrome in patients with psoriasis not recognized by previous authors; namely, it is possible that in some patients the administration of systemic therapy triggers apoptosis that results in substantive cytokine release. The capillary leak syndrome could be an adverse effect of current and future systemic therapies for psoriasis.

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