Fulvestrant (Faslodex), an Estrogen Selective Receptor Downregulator, in Therapy of Women with Systemic Lupus Erythematosus. Clinical, Serologic, Bone Density, and T Cell Activation Marker Studies: A Double-blind Placebo-controlled Trial

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ABSTRACT. ***Objective.*** Estrogen plays a role in the activation of systemic lupus erythematosus (SLE) and in upregulating intracellular signals by binding to the estrogen receptor(s). Fulvestrant (Faslodex, AstraZeneca Pharmaceuticals, Wilmington, DE, USA), an estrogen selective receptor downregulator, competes for receptor binding *in vitro* and inhibits estrogen action in target cells. We evaluated the efficacy, side effects, and expression of T cell activation markers, following the administration of fulvestrant or placebo to premenopausal patients with SLE.

Methods. Twenty women with moderate SLE Disease Activity Index (SLEDAI; 7.87 ± 3.7) were enrolled. They were premenopausal with regular menstrual cycles and not taking exogenous hormones. The study was double-blind and placebo-controlled. Ten patients received 250 mg fulvestrant intramuscularly for 12 months, and 10 received the placebo. All were observed monthly and 3 months after final fulvestrant/placebo injection. Measures studied were monthly SLEDAI scores, routine and serologic markers for lupus, and serum concentrations of estrogen and fulvestrant. Expression of T cell calcineurin and CD154 mRNA in peripheral T cells was measured by polymerase chain reaction. Medications the patients were taking were recorded each visit. Bone density was obtained at baseline and at visit 12.

Results. Sixteen patients completed the 15-month study, 8 from each group. SLEDAI improved significantly in the fulvestrant group at both 12 months ($p = 0.02$) and 15 months ($p = 0.002$), but serologic markers, routine laboratory tests, and bone density did not. Serum estrogen levels were higher in the fulvestrant group and dropped when fulvestrant was discontinued; these differences were not statistically significant. Medications for therapy of lupus to the fulvestrant group were reduced, whereas the placebo group medications were unchanged or increased. Comparison of relative values at individual timepoints revealed significantly lower median values for the T cell activation markers CD154 ($p < 0.001$) and calcineurin ($p = 0.013$) in the fulvestrant arm.

Conclusion. Blocking estrogen receptors *in vivo* by an estrogen selective receptor downregulator could be considered as a new and relatively safe therapeutic approach in the management of SLE patients with moderately active disease for the 1-year study period. (First Release Mar 15 2008; J Rheumatol 2008;35:797–803)

Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS FULVESTRANT
SELECTIVE ESTROGEN RECEPTOR DOWNREGULATOR
SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY INDEX BONE DENSITY
T CELL ACTIVATION MARKERS CALCINEURIN CD154

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Supported by AstraZeneca Pharmaceutical Company (study IRUS-FULV0031) and by NIH grant A149272 and RR-16475 from the INBRE program of the National Center for Research Services, Evans Memorial Funds, Saint Luke's Hospital Foundation Research Funds, and by private funds from the Center for Rheumatic Diseases.

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Accepted for publication December 20, 2007.

Systemic lupus erythematosus (SLE) predominantly affects women (about 90%), particularly in the childbearing period[1]. Estrogen metabolism is altered in SLE, with elevated levels of a highly estrogenic metabolite, 16α-hydroxyesterone[2]. Since estrogen is a key regulator of molecules involved in inflammation, and circulating estradiol is highest during the peak time of SLE onset, estrogen could contribute to the development, progression, or severity of SLE[3,4]. At the molecular level, estrogen activates lupus T cells *in vitro* with upregulation of 2 intracellular activation signals, calcineurin and CD154[3,4]. In animal models of SLE, female mice develop an earlier aggressive disease that could be ameliorated by ovariectomy and/or androgen therapy[5]. Non-lupus-prone normal transgenic mice expressing heavy-chain anti-DNA could be induced into lupus phenotype by administering estrogen[6].

Our previous results showed that T cell activation by estrogen in human lupus T cells could be blocked by *in vitro* incubation with the pure estrogen receptor antagonist fulvestrant (ICI 182,780)[3,4,7]. We therefore extended the *in vitro* data and explored the possibility that patients with SLE who have moderately active disease could improve their disease activity upon treatment with fulvestrant, and allow us to decrease the dosage of drugs (corticosteroids and immunosuppressants) used to treat their lupus. In a double-blind, placebo-controlled study of fulvestrant, we monitored the efficacy of therapy by monthly assessments using the SLE Disease Activity Index (SLEDAI), checking the serologic measures for SLE, patient's medications, and possible side effects, including changes in menstrual cycles and bone mineral density. Estrogen (estradiol 17-ß) and fulvestrant serum concentrations were monitored during the study period, as were changes in the expression of T cell activation markers of peripheral blood T cells, namely CD154 and calcineurin. We hypothesized that blockade of the estrogen receptor in women with SLE could reduce T cell activation and slow disease progression. The results were expected to provide a rationale to develop new therapies and improve quality of life for patients with SLE.

MATERIALS AND METHODS

Twenty women with SLE entered a double-blind, placebo-controlled study. They all fulfilled American College of Rheumatology criteria for SLE[8] and had moderate disease activity with mean (± SD) SLEDAI score of 7.87 ± 3.7[9]. Patients who had current or recent history of active cerebritis, nephritis, cytopenia, or severe disease with SLEDAI > 15 were excluded. Participants had regular menstrual cycles and none was taking hormone replacement therapy or oral contraceptives, or had a history of other collagen vascular diseases. No participant, at the time of study, had active renal or central nervous system disease. Patients enrolled in the study had symmetrical polyarticular non-erosive arthritis (n = 18), proteinuria with normal renal function (n = 7), pleuritis (n = 4), headache (n = 2), cognitive dysfunction (n = 2), lupus malar rash or discoid lupus rash (n = 7), oral ulcers (n = 3), Raynaud's syndrome (n = 9), scarring scalp alopecia (n = 2), history of cerebritis (n = 3), and leukopenia or hemolytic anemia (n = 4). At entry, the 16 patients who completed the study were receiving prednisone daily at a mean dose of 5.5 mg/day. Eleven patients were taking azathioprine (125 mg/day), 12 were taking daily hydroxychloroquine (400 mg/day), and 5 were taking nonsteroidal antiinflammatory drugs. Ten participants were Caucasian, 5 African American, and 1 Hispanic. All patients at study entry signed a consent form. Ten patients were randomly assigned by the study pharmacist to the fulvestrant arm and 10 patients received the placebo. Four patients were dropped from the study; 2 for having irregular menstrual cycles (1 from each arm of the study), 1 for developing headaches (placebo arm), and 1 for noncompliance (fulvestrant arm). Patients in the fulvestrant group (n = 8) who completed the study were 39 years old (mean; range 34–40 yrs) and had lupus for 11.3 years (mean; range 0.5–28 yrs). The mean SLEDAI for patients in the fulvestrant arm at study entry was 8.25 (± 2.6). Patients in the placebo group (n = 8) who completed the study were 38 years old (mean; range 29–45 yrs) and had lupus for 9.75 years (mean; range 0.5–32 yrs). The SLEDAI score for the placebo arm was 7.87 (± 4.9).

The study was approved by the Institution Review Board (IRB no. 03082) and registered with ClinicalTrials.gov (no. NCT00417430).

Our study was investigator-initiated and funded primarily by AstraZeneca Pharmaceutical Company, Wilmington, DE, USA (study no. IRUSFULV0031).

Our aim was to test the *in vivo* effect of fulvestrant versus placebo treatment on clinical disease activity as measured by SLEDAI and serological markers for SLE; determine effects of therapy on bone density and estrogen levels; measure serum levels of fulvestrant to ensure good trough levels; observe any side effects of therapy, particularly changes in the menstrual cycles or any allergic reaction; and monitor changes in patients' other therapy for SLE. In conjunction with this clinical study the same patients were evaluated for the expression of CD154 and calcineurin, markers of activation in circulating T cells.

Each patient received either fulvestrant or placebo monthly for 12 months. Fulvestrant 250 mg (Faslodex, AstraZeneca Pharmaceuticals, Wilmington, DE, USA) was administered intramuscularly monthly (5 ml divided at 2 sites, 2.5 ml each) between the 4th and 10th day of the menstrual cycle, when estradiol in circulation is still low[4,7]. Five milliliters of the placebo was given intramuscularly to the placebo group. Each patient was examined monthly. Patients had all serologic and T cell activation marker laboratory tests done monthly prior to administration of the monthly fulvestrant or placebo. The same patients were reevaluated at the 15th month, 3 months after the last fulvestrant or placebo injection. All patients had bone density measurement of femoral neck and lumbar spine at study entry and at the 12th month using the dual-energy x-ray absorptiometry machine.

Serologic markers. Antinuclear antibody (ANA) was assessed by the routine immunofluorescent assay. Anti-double-stranded DNA, C3, C4, and estrogen levels were evaluated by ELISA. Fulvestrant serum levels were determined blindly in triplicate at the AstraZeneca research laboratory using liquid chromatography/mass spectrophotometry[10]. Serum fulvestrant levels < 0.25 ng/ml are considered negative. Estrogen serum levels were measured in triplicate, using a commercial ELISA plate (Estradiol ELISA Kit, lot no. 298, Neogen Corp., Lexington, KY, USA). Reference 17ß-estradiol standards were provided on each plate. Normal mean value of estradiol in healthy cycling women is 17 ng/ml (range 6–44 ng/ml) depending on the phase of their menstrual cycle.

Collection of T cell enriched peripheral blood mononuclear cells. Blood samples (~100 ml) were collected prior to treatment and thereafter monthly for a total of 11 months. Three months after the last injection, blood was drawn to determine T cell activation marker (CD154, calcineurin) expression after removal of fulvestrant or placebo. T cell enriched mononuclear cells were separated from blood samples by density gradient (Histopaque, Sigma, St. Louis, MO, USA). The lymphocytes were isolated, washed in serum-free medium (RPMI 1640, Fisher Scientific, Hanover Park, IL, USA), and residual red blood cells were lysed (H-Lyse buffer, R&D

Systems, Minneapolis, MN, USA). T cells were purified by negative selection through T cell isolation columns (Human T Cell Enrichment Columns, R&D Systems). We have shown[4] that T cell purity using these methods is greater than 95% as assessed by flow cytometry.

T cell extracts. T cell extracts were prepared by incubating the T cells for 30 min on ice in a lysis solution (10 mM Tris-H1, pH 7.5, 30 mM sodium pyrophosphate, 50 mM sodium chloride, 50 mM sodium fluoride, 5 mM EDTA, 1% Triton X-100, 1 mM sodium orthovanadate), containing protease inhibitors as described[3,4]. Lysates were clarified by centrifugation at 10,000 × g for 10 min at 4°C. Purified T cell extracts were stored at –80°C.

Measurement of calcineurin and CD154 mRNA. Semiquantitative polymerase chain reaction (PCR) amplification using a Peltier Thermocycler (MJ Research, San Francisco, CA, USA) was employed to measure calcineurin and CD154 mRNA. Total RNA was isolated from SLE T cells. To ensure removal of genomic DNA, samples were treated with DNAse I according to the manufacturer's protocol (DNA-free, Ambion, Austin, TX, USA). cDNA was synthesized in 20 µl from 4 µg of RNA using an oligo(dT) primer (0.5 µg/µl) and MMLV reverse transcriptase as described[3,7]. The primers for glyceraldehyde phosphate dehydrogenase (G3PDH; sense, 5'-GAG TCA ACG GAT TTG GTC GT-3'), calcineurin (sense, 5'-TTG ATT GCC ACT GTA GTT TGG T-3'), and CD154 (sense, 5'-ACA TAC AAC CAA ACT TCT CC-3') were verified in our laboratory by sequence analysis of the resulting PCR products[3,7]. PCR for CD154 were amplified at 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min for 24 cycles. PCR for calcineurin were carried out at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 26 cycles. PCR amplification was within the linear range under the conditions used. Control reactions were amplified without template and no product was evident. Amplified products were separated on 2% agarose gels containing ethidium bromide. A 100 base-pair DNA size standard (Promega, Madison, WI, USA) was electrophoresed on the same gel to determine product size. The gel was photographed and the amount of calcineurin and CD154 from each sample was analyzed by scanning densitometry. To normalize the results, the amount of calcineurin and CD154 was divided by the amount of G3PDH amplified from the same template. To compare the reproducibility of monthly assays with values obtained in a single PCR assay, 12 cDNA templates from a single patient that had been collected and analyzed monthly were amplified in a single PCR assay at the end of the study period. CD154 values were normalized to G3PDH and compared with the monthly data obtained from the same cDNA templates. The variance was 8.5% between the 2 methods (data not shown).

Data collection and statistical analysis. The results were unblinded after all the data were collected. For each measure tested, the fulvestrant patients' data were compared for changes at the 12th month and 15th month to measures tested prior to entry into the study. Data were analyzed by a mixed model and Wilcoxon signed-rank test[11]. Due to the large amount of data, we report the prestudy data and data at the 12th and 15th months for SLEDAI, serologic markers, estrogen, and fulvestrant serum levels and for changes in therapy. However, due to fluctuations in T cell activation marker gene expression, values for CD154 and calcineurin are reported monthly. Values for T cell calcineurin and CD154 are expressed relative to the baseline values (i.e., prior to the first injection) at each timepoint. Differences in calcineurin and CD154 expression were assessed using the nonparametric Mann-Whitney test. A p value < 0.05 was considered statistically significant.

RESULTS

All routine blood and urine measures in both the fulvestrant and placebo groups that were tested for all patients on a monthly basis did not show any significant changes; this included white cell counts with differential, hemoglobin, platelet counts, liver enzymes (AST and ALT), serum creatinine, and urinalysis (not shown in the tables).

Effect of fulvestrant on SLEDAI scores (Table 1). In the fulvestrant group, SLEDAI scores improved significantly at the 12th month. Prestudy mean SLEDAI score was 8.25 ± 2.6, and score at the 12th month was 3.75 ± 3.6 (p = 0.02) and at the 15th month 3.5 ± 2.9 (p = 0.002). There was no statistically significant difference between SLEDAI score at the 12th month compared to the 15th month. Although the drop of the SLEDAI in the fulvestrant group was noted 1 month after the first dose, the maximum decline was not achieved before the 7th month of treatment. This reduction of SLEDAI was sustained up to the 12th month. In the placebo group, SLEDAI scores did not change significantly at the 12th or 15th month. SLEDAI score of the placebo group prestudy was 7.87 ± 4.9, at the 12th month 7.0 ± 3.7 (p = 0.4), and the 15th month 6.62 ± 3.1 (p = 0.4). There was no significant difference in the scores between the 12th and 15th months (p = 0.7).

Effect of fulvestrant on serologic markers (Table 2). All 16 patients who completed the study had monthly measurements, for 12 months, of ANA, anti-double-stranded DNA, total hemolytic complement (CH_{50}), C3, and C4; and the same measures were done at the 15th month at the termination of the study. There was no statistically significant difference in all the serologic values, prestudy versus 12th and versus 15th month. All p values were > 0.5.

Effect of therapy on estrogen and fulvestrant serum levels (Table 3). All samples were frozen from both fulvestrant and placebo groups at –70°C and tested at the termination of the 15th month of the study to avoid bias in interpreting the data.

Changes in the levels of circulating estrogen were not significantly different at the timepoints measured. However, mean values increased at the 12th month after the monthly fulvestrant therapy; the increase was not statistically significant (p = 0.09) compared to mean estrogen values prior to starting fulvestrant injections. The lack of significance may be due to the large variation and small sample size. The increase of estrogen serum levels in the fulvestrant group was gradual each month, indicating possible cumulative

Table 1. Efficacy of fulvestrant on moderate active SLE disease. Clinical response measured by SLE Disease Activity Index (SLEDAI).

Month of Study	SLEDAI Scores Fulvestrant Group, n = 8	 Placebo Group, n = 8
Prestudy (before drug or placebo)	8.25 ± 2.6	7.87 ± 4.9
Month 12 (taking drug or placebo for 12 mo)	3.75 ± 3.6*	7.0 ± 3.7†
Month 15 (not taking drug or placebo for 3 mo)	3.5 ± 2.9**	6.62 ± 3.1††

* p < 0.02 compared to prestudy scores. ** p < 0.002 compared to prestudy scores. † p = 0.4 compared to prestudy scores. †† p = 0.4 compared to prestudy scores.

Table 2. Efficacy of therapy with fulvestrant on serologic markers of SLE. All patients were tested before entering the study, at the 12th month after completing 12 monthly injections, and at the 15th month, being off fulvestrant or placebo for 3 months. Values are mean (range).

	Fulvestrant Group, n = 8					Placebo Group, n = 8				
Month of Study	ANA* Titer	Anti-DNA**, IU/ml	CH_{50}***, U/ml	C3, mg/dl†	C4, mg/dl††	ANA* Titer	Anti-DNA**, IU/ml	CH_{50}***, U/ml	C3, mg/dl†	C4, mg/dl††
Prestudy	1:320 (1:20–1:1280)	9.25 (3–18)	162 (120–209)	106 (68–148)	20 (10–35)	1:320 (1:20–1:1280)	12.4 (3–48)	156 (124–193)	113 (66–144)	19 (12–31)
Month 12	1:320 (1:20–1:1280)	9.25 (3–20)	138 (90–217)	112 (75–154)	22 (10–36)	1:320 (1:160–1:1280)	14.2 (3–46)	135 (102–193)	110 (66–139)	15 (8–26)
Month 15	1:160 (1:20–1:1280)	9.12 (3–22)	156 (113–208)	123 (74–184)	23 (9–35)	1:640 (1:160–1:1280)	14.0 (3–49)	127 (92–176)	106 (52–149)	17 (8–24)

* ANA < 1:20 is considered negative. ** Normal range of anti-DNA 0–5 IU/ml. *** CH_{50}, total hemolytic complement normal range is 142–279 U/ml. † Normal range of C3 83–172 mg/dl. †† Normal range of C4 18–51 mg/dl.

Table 3. Effects of therapy with fulvestrant estrogen or fulvestrant serum levels. All patients were tested before entering the study and at the 12th month after receiving fulvestrant or placebo monthly, and then were reevaluated at the 15th month, being off fulvestrant or placebo for 3 months.

	Fulvestrant Group, n = 8		Placebo Group, n = 8	
Month of Study	Estrogen*, ng/ml	Fulvestrant, ng/ml	Estrogen*, ng/ml	Fulvestrant, ng/ml
Prestudy	53 ± 43	< 0.25	50 ± 29	< 0.25
Month 12	90 ± 27	6.16 ± 1.9	62 ± 19	< 0.25
Month 15	59 ± 37	3.44 ± 0.7**	30 ± 23	< 0.25

* No significant differences were seen in either group at any of the study intervals. ** Fulvestrant serum levels at the 15th month were significantly lower ($p < 0.02$) than values of the fulvestrant group at the 12th month.

increase of fulvestrant as the injections were repeated monthly during the 12 months of the study (not shown in Table 3). Estrogen serum levels in the placebo group did not change significantly at the 12th and 15th months compared to prestudy estrogen serum levels. As expected, fulvestrant levels in the placebo group were all undetectable (< 0.25 ng/ml) during the study period (Table 3). In the fulvestrant group, fulvestrant levels gradually increased each month and reached 6.16 ± 1.9 ng/ml by the 12th month. Fulvestrant levels did, however, drop significantly ($p < 0.02$) at the 15th month (Table 3).

Effects of fulvestrant on bone density T scores (Table 4). All 16 patients had bone density measurements prior to entering the study, and then at the 12th month after they completed the monthly fulvestrant or placebo injections. We monitored their T scores at the femoral neck and lumbar spine.

As shown in Table 4 there was no significant difference between the fulvestrant and placebo groups in either femoral neck or lumbar spine score before entering the study and also when scores were compared at 12 months. Moreover, no patient reported bone fractures during the study. All patients were taking calcium 1000–1200 mg daily and vitamin D 400 units daily since they were receiving corticosteroid therapy for their lupus.

Table 4. Effects of therapy with fulvestrant on bone density T-scores. All patients were tested before entering the study and then at the 12th month after receiving fulvestrant or placebo monthly.

		Bone Density T-Score, mean (range)*	
Month of Study	Bone Density Site	Fulvestrant Group, n = 8	Placebo Group, n = 8
Prestudy	Femoral neck	+0.1 (+1.6 to –1.3)	0.0 (+2.8 to –1.50)
	Lumbar spine	+2.06 (+2.53 to –1.27)	+1.31 (+4.37 to –0.03)
Month 12	Femoral neck	+2.6 (+2.8 to –1.50)	+2.2 (2.7 to –0.6)
	Lumbar spine	+1.3 (+2.24 to –1.72)	+1.9 (+4.21 to –2.19)

* No significant differences were seen in either group at both sites studied. Prestudy T-score values were not significantly different from values at the 12th month.

Changes of therapy for lupus during the study (Table 5). All 16 patients who completed the study had all their medications recorded each month. Decisions about drug therapy for lupus were made by the patient's individual rheumatologist, who was blinded to the patient's study arm assignment. As shown in Table 5, average prednisone dose was reduced at the 12th and 15th months in the fulvestrant group and was increased in the placebo group. The dose of hydroxychloroquine and azathioprine therapy had the same trend as the prednisone dose. Due to the wide range of doses and the small number of patients (n = 8 in each group) the changes were not significantly different.

Effect of fulvestrant on CD154 expression. For individual timepoints (visit 2 through visit 12) comparison of relative values between placebo and fulvestrant groups revealed 3 instances where there was a significant difference in CD154 expression ($0.01 < p < 0.05$, without correction for multiple comparisons), even though the median values at all timepoints were lower in the fulvestrant group than in the placebo group. This is shown in Figure 1, where the median value in each treatment group is displayed as a function of time after initiation of treatment. For individual CD154 timepoints at visit 2 through visit 12 comparison of values revealed a limited distribution of relative values over time,

Table 5. Changes of therapy during the study. Sixteen of 16 patients were taking prednisone; 12/16 patients were taking hydroxychloroquine; 11/16 patients were taking azathioprine.

Therapy	Fulvestrant Group, n = 8			Placebo Group, n = 8		
	Prestudy	Month 12	Month 15	Prestudy	Month 12	Month 15
Prednisone, mg/day	5.75	2.5	2.5	5.25	10.25	10
Hydroxychloroquine, mg/day	400	400	200	200	400	400
Azathioprine, mg/day	125	100	100	100	150	150

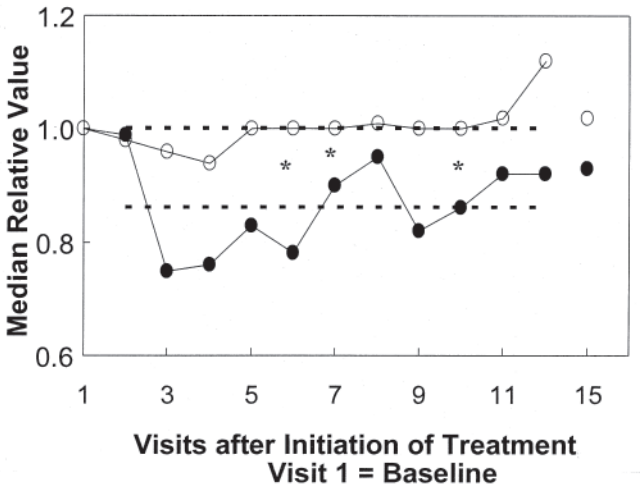

Figure 1. Median values for CD154 are lower at all timepoints in the fulvestrant arm (●) compared with the placebo arm (○) of the study. For individual CD154 measures at visit 2 through visit 12, comparison shows a limited distribution of relative values over time (range 0.4–2.8). Values at visit 15 were measured 3 months after the last injection. *Values at visits 6, 7, and 10 were significantly lower in the fulvestrant arm. Broken lines indicate median values for the 2 groups, using all values except baseline (visit 1) and visit 15.

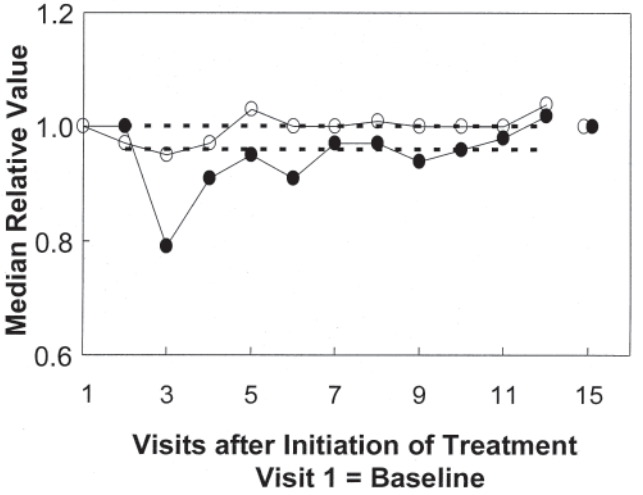

Figure 2. Median values for calcineurin are lower at 10 timepoints in the fulvestrant arm (●) compared with the placebo arm (○) of the study. For individual calcineurin measures at visit 2 through visit 12, comparison shows a limited distribution of relative values over time (range 0.5–2.0). Values at visit 15 were measured 3 months after the last injection. Broken lines indicate median values for the 2 groups, using all values except baseline (visit 1) and visit 15.

with a range from 0.4 to 2.8. There was a definite and significant difference between the 2 groups for these median values for CD154 ($p < 0.001$, Mann-Whitney test).

Effect of fulvestrant on calcineurin expression. For individual timepoints at visit 2 through visit 12, the relative value of calcineurin was 10 times lower in the fulvestrant arm compared with the placebo arm. This is shown in Figure 2, where the median value in each treatment group is displayed as a function of time after initiation of treatment. For individual calcineurin timepoints, comparison of values revealed a limited distribution of relative values over time, with a range from 0.5 to 2.0. There was a significant difference between the 2 groups for these median values ($p = 0.013$, Mann-Whitney test). Global regression analyses confirmed these findings by indicating an effect of treatment assignment, but not time, after initiation of treatment for CD154 ($p < 0.001$) and for calcineurin ($p < 0.003$).

DISCUSSION

The purpose of our study was to evaluate — in a double-blind, placebo-controlled design — the *in vivo* effects of monthly administration of fulvestrant on lupus disease activity, serologic markers, changes in T cell activation marker gene expression, and possible side effects of the drug on menstrual cycles and bone density. There is compelling evidence for the role of estrogen in women with SLE[2-7]. Lupus in susceptible women usually manifests itself after puberty and symptoms may increase in severity during the childbearing years[12]. There have been reports of flares of lupus during pregnancy[12] and, in many patients, lupus disease activity ameliorates and becomes easier to control after menopause[13]. Administering estrogen to postmenopausal women induced mild to moderate disease activity in some patients[14,15].

Our previous investigations indicated activation of intracellular signals (calcineurin and CD154) of human lupus T cells when incubated *in vitro* with 17ß-estradiol[3,4,7,16]. Activation was estrogen-specific, was not seen in normal T cells, and could be blocked by the estrogen receptor blocker ICI 182,780, now called fulvestrant[3,7]. We therefore investigated an *in vivo* extension of the *in vitro* data using the drug fulvestrant.

Fulvestrant is known to be a specific estrogen receptor antagonist without an agonist effect and is now referred to as a selective estrogen receptor downregulator[17]. As shown in the Results section, fulvestrant was effective and significantly decreased the SLEDAI score, an effect that was sustained for 3 months after discontinuation of fulvestrant. Therapy with fulvestrant also significantly decreased CD154 and calcineurin, activation markers of circulating blood T cells. However, we were not able to demonstrate in this 12-month study evidence of improvement of the serologic markers anti-double-stranded DNA, complement levels, and ANA, most likely due to the lag of serological improvement following the clinical response. Except for discomfort at the site of the injections, administration of fulvestrant was relatively safe, without notable deterioration of bone density and no significant changes in the menstrual cycles between the 2 groups. Indeed, somewhat surprisingly, serum estrogen levels were almost 2-fold higher in the fulvestrant arm (Table 3). Maintaining estrogen serum levels will prevent corticosteroid-induced increased rate of bone remodeling[18]. The observed increase of estrogen serum level in our study may be due to alterations in a negative feedback mechanism secondary to the blocking effect on the estrogen receptor by the estrogen receptor downregulator. The lack of side effects on bone density and changes in menstrual cycles over the 1-year study period was an important consideration (FDA discussion prior to approval of our study).

Therapy of SLE needs a new approach — other than using corticosteroids or immunosuppressive drugs — using a drug to modulate the effect of estrogen on immune cells. This preliminary study complements these efforts by specifically blocking the estrogen receptor without any agonist effect on the estrogen receptor[17,19-21]. It is not known yet if blocking the estrogen receptor by itself will be sufficient to control the disease. Moreover, we do not know the effect on bone density of longterm blockage of the estrogen receptors beyond 1 year. Due to adherence to the study protocol, we were unable to alter the dose or frequency of fulvestrant injections.

Future study protocols that deal with modulation of the estrogen receptor in cycling young lupus patients would be more efficient if the estrogen receptor blocker was administered at a certain period of the menstrual cycle, namely, as in this study, day 4 to 10, considering day 1 as the first day of the menstrual cycle. The levels of estradiol are relatively low during this phase of the menstrual cycle, and blockade of the estrogen receptor may be favored when estradiol levels are low[22,23].

Our study, for the first time, ties together a molecular mechanism by which estrogen, working through the estrogen receptor, contributes to hyperactivation of SLE T cells and disease activity in women with SLE. Antagonism of the estrogen receptor decreased expression of these T cell activation markers and led to a reduction in corticosteroid administration and lupus disease activity. It is somewhat surprising that the serological measures did not improve over this same study period. A prolonged period of observation (> 15 months) may be necessary to see changes in these serological indicators. Participants in our study received a single dose of fulvestrant monthly; different doses and/or the timing of administration need refinement. We suggest that this preliminary study needs to be extended to a large number of lupus patients given different doses of fulvestrant for different time periods. If that proves to be effective, the study needs to be extended to female SLE patients with severe and active disease. Further studies are now required to determine the status of estrogen receptors in circulating T cells following fulvestrant administration (work in progress) and the consequences of blocking estrogen receptors in SLE T cells in studies > 15 months. Such information will provide insight into a mechanistic basis of sex bias in SLE and for development of new treatments. Patients with lupus need a more specific therapeutic approach targeted toward the basic hormonal-immune dysregulation and not globally suppressing their immune system.

Our report shows that in a 1-year double-blind placebo-controlled study in patients with moderately active SLE disease, fulvestrant, an estrogen receptor antagonist, improved SLEDAI and decreased expression of CD154 and calcineurin in circulating T cells. Fulvestrant did not affect bone density and allowed reduction of the drugs used to treat lupus.

ACKNOWLEDGMENT

We thank our patients, who volunteered to participate, for their compliance and their dedication to assist us in completing the study. We thank our institution biostatisticians, Phil Jones and Dr. Xiao Lan, for the statistical analysis; Phyllis Smotherman for technical assistance, Hope Schreck for secretarial assistance, and Becky Warfield for the bone density study. We are grateful to the physicians of the Center for Rheumatic Disease for allowing us to include some of their patients in our study.

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