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Recombinant Human Relaxin in the Treatment of Systemic Sclerosis With Diffuse Cutaneous Involvement

A Randomized, Double-Blind, Placebo-Controlled Trial

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Objective. **A phase II randomized controlled trial of recombinant human relaxin suggested that a dosage of 25 μg/kg/day was safe and clinically effective in improving skin disease and reducing functional disability in scleroderma (systemic sclerosis; SSc). We undertook a large randomized, double-blind, placebo-controlled clinical trial to compare placebo with 10 μg/kg/day and 25 μg/kg/day recombinant human relaxin, given for 24 weeks in patients with stable, diffuse, moderate-to-severe SSc.**

Methods. **Men and women ages 18–70 years with diffuse cutaneous SSc (dcSSc) were administered recombinant human relaxin (10 μg/kg/day or 25 μg/kg/day) or placebo for 24 weeks as a continuous subcuta-**

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neous infusion. There was a followup safety visit at week 28.

Results. **The primary outcome measure, the modified Rodnan skin thickness score, was similar among the 3 groups at baseline and at weeks 4, 12, and 24. Secondary outcomes such as functional disability were similar in all 3 groups, while the forced vital capacity decreased significantly in the relaxin groups. The discontinuation of both doses of relaxin at week 24 led to statistically significant declines in creatinine clearance and serious renal adverse events (defined as doubling of serum creatinine, renal crisis, or grade 3 or 4 essential hypertension) in 7 patients who had received relaxin therapy but in none who had received placebo.**

Conclusion. **Recombinant relaxin was not significantly better than placebo in improving the total skin score or pulmonary function or in reducing functional disability in patients with dcSSc. In addition, relaxin was associated with serious renal adverse events, the majority of which occurred after stopping the infusion. If relaxin is used therapeutically for any conditions other than scleroderma, close monitoring of blood pressure and renal function must be performed.**

Relaxin, a naturally occurring protein, is structurally related to the insulin family of peptides and is produced primarily by the ovary and/or placenta in pregnancy and by the prostate of mammals. Relaxin has been implicated in a number of pregnancy-related functions, including relaxation of the cervix and vagina at the time of delivery.

Relaxin has antifibrotic properties; it downregulates collagen production and increases collagen degradation (1). In vitro studies show that relaxin acts directly on transforming growth factor β1–stimulated fibroblasts to decrease myofibroblast differentiation and collagen secretion while increasing expression of the metalloproteinases—enzymes that are responsible in part for collagen degradation (2,3). Relaxin acts in synergy with interferon-γ to reduce collagen overexpression by fibroblasts isolated from patients with scleroderma (systemic sclerosis; SSc) (3). In addition, recombinant human relaxin prevents the development of bleomycin-induced pulmonary fibrosis in rodents (4).

Phase I and phase II studies of recombinant human relaxin in patients with SSc with diffuse cutaneous involvement (dcSSc) demonstrated that steady-state serum concentrations of relaxin up to 60 times higher than those seen in normal pregnancy could be safely achieved with continuous subcutaneous (SC) infusion (5,6). In addition, a previous phase II randomized controlled trial suggested that 25 μg/kg/day recombinant human relaxin was safe and well tolerated and was likely to be clinically effective in improving skin disease and reducing functional disability (5).

We report the results of a phase III randomized, double-blind, controlled trial comparing placebo with recombinant human relaxin, 10 μg/kg body weight per day and 25 μg/kg body weight per day, given for 24 weeks in patients with stable, diffuse, moderate-to-severe scleroderma. The dosage of 25 μg/kg/day was selected on the basis of pharmacokinetics and clinical efficacy results from earlier studies (5,6). On the basis of preclinical and earlier clinical studies, we hypothesized that this serum concentration would have antifibrotic effects. In order to explore further dose-response relationships, a lower dosage of 10 μg/kg/day was included. The 25 μg/kg/day dosage was used to replicate the phase II study (5).

PATIENTS AND METHODS

Patients. Before screening, all patients gave full informed voluntary written consent according to the principles of the Declaration of Helsinki and in compliance with US Food and Drug Administration requirements. Patients with scleroderma meeting the American College of Rheumatology (formerly, the American Rheumatism Association) criteria (7) were recruited through US member institutions of the Scleroderma Clinical Trials Consortium. The criteria for entry and study design were intentionally kept nearly identical to those of the previous study, which compared relaxin in dosages of 25 μg/kg/day and 100 μg/kg/day with placebo. Men and women ages 18–70 years were eligible if they had a history of dcSSc (defined as skin thickening proximal as well as distal to the elbows or knees, with or without involvement of the face and neck) for $\leq$5 years since the onset of the first sign or symptom of SSc other than Raynaud's phenomenon. A baseline modified Rodnan skin thickness score (MRSS) of at least 20, or of at least 16 if truncal involvement was present, was required for entry into the treatment phase of the study. Patients were excluded if their MRSS varied by >5 units from screening to the first treatment day.

We also excluded patients who had limited cutaneous scleroderma (skin thickening distal but not proximal to the knees and elbows, with or without facial involvement), eosinophilic fasciitis, eosinophilic myalgia syndrome, or scleroderma in conjunction with any other definable connective tissue disease, such as rheumatoid arthritis, systemic lupus erythematosus, or polymyositis/dermatomyositis. We excluded patients with a substantial history of environmental exposure to "tainted" rapeseed oil, vinyl chloride, trichloroethylene, or silica dust. Also excluded were patients in whom the onset of renal crisis occurred in the 2 months prior to enrollment or those with chronic renal failure (serum creatinine level $\geq$2.0 mg/dl), as well as patients with severe pulmonary disease (forced vital capacity [FVC] <50% predicted and diffusing capacity for carbon monoxide [DLCO] <40% predicted), se-

vere cardiovascular disease (uncontrolled hypertension, symptomatic coronary artery disease, second or third atrioventricular nodal block and/or bifascicular block, congestive heart failure, cor pulmonale, or symptomatic pericardial effusion), gastrointestinal disease (gastrointestinal bleeding requiring blood transfusion or surgical intervention within the last 6 months or weight <70% of ideal body weight), or hematologic disease (hemoglobin concentration ≤8 mg/dl, platelet count ≤100/μl, white blood cell count ≤3,000/μl, or polymorphonuclear cell count ≤1,000/μl). Other exclusion criteria were pregnancy and current breast-feeding.

Patients were required to discontinue putative "disease-modifying" treatments for scleroderma (including immunosuppressive agents, potassium aminobenzoate, photopheresis, colchicine, or any other experimental therapy) at least 4 weeks before the study. Patients were excluded if they were receiving >10 mg of prednisone (or equivalent) per day.

Intervention. We administered recombinant human relaxin (10 μg/kg/day or 25 μg/kg/day) or placebo for 24 weeks by continuous SC infusion, using microinfusion pumps (Panomat T-series; Disetronic Medical Systems, Minneapolis, MN). Recombinant human relaxin was produced by Connetics Corporation (Palo Alto, CA) in *Escherichia coli*. The placebo was a sterile acetate buffer solution that was identical in composition to the buffer used for relaxin.

Patients were randomly assigned to receive placebo or recombinant human relaxin (10 μg/kg/day or 25 μg/kg/day) in a 2:1:2 ratio. Randomization was performed at a centralized data management organization (Pacific Research Associates, Los Altos, CA). "Biased coin" randomization was used to stratify patients on the basis of disease duration (≤2.5 years or >2.5 years to ≤5 years) and use of D-penicillamine in the previous 6 months. The same randomization procedure was used to replace patients who withdrew before completing 4 weeks of treatment. The patients were recruited between 1999 and 2000.

Patient prescriptions for the study medication were forwarded to a centralized pharmacy for preparation of blinded supplies of the study drug. Each patient's dose was based on body weight at the time of screening. The dose was adjusted only if body weight changed by ≥10% during the study. Treatment was administered 24 hours per day for 24 weeks. Continuous SC infusion was chosen as the mode of administration to eliminate the need for 6 daily SC injections, to conserve drug supply, and to mimic the constancy of relaxin concentrations usually seen during pregnancy. The infusion site and needle were changed at least every 72 hours.

Assessments. The primary measure of efficacy was the MRSS, a clinical evaluation of skin thickness in 17 body surface areas (face, chest, and abdomen, and right and left fingers, hands, forearms, upper arms, thighs, lower legs, and feet) (8). Each area was assessed for thickness on a 0–3 scale (0 = normal, 1 = mild but definite thickening, 2 = moderate skin thickening, 3 = severe skin thickening). The total score (the sum of scores from all 17 body areas) ranged from 0 to 51. The MRSS is both accurate and reproducible (with an interobserver SD of ± 4.6 units and an intraobserver SD of ± 2.5 units) (8). Before the study began, investigators underwent (re)training and standardization training.

Secondary measures of efficacy included the following: blood pressure (BP) measurement; maximal oral aperture (lip to lip); maximal hand extension (distance between the tip of the thumb and the tip of the fifth finger in maximum hand opening); tenderness and swelling of the metacarpophalangeal joints (as a unit), wrists, elbows, and knees (8 joints); number of skin ulcers; Medical Outcomes Study Short Form 36 (SF-36) health survey, version 1 (9); Health Assessment Questionnaire disability index (HAQ DI) score (10,11); patient's and investigator's assessments of global disease activity on a 0–100-mm visual analog scale; and pulmonary function tests, including FVC as % predicted and DLCO as % predicted, corrected for hemoglobin. We have previously reported the SF-36 and HAQ DI data in this clinical trial (12,13), and those results will not be reported here; the current report presents the results of the original randomized clinical trial.

Renal crisis was determined to be present when the patient's physician detected renal insufficiency (serum creatinine ≥2.0 mg/day or a doubling of serum creatinine above the value at baseline in the absence of another defined cause) and/or malignant hypertension (systolic BP ≥160 mm Hg or diastolic BP ≥110 mm Hg on at least 2 occasions a minimum of 12 hours apart) accompanied by persistent urine abnormalities (i.e., proteinuria) or evidence of microangiopathic hemolytic anemia (MAHA). The following equation may help to explain the definition of renal crisis: renal crisis = (increase in serum creatinine) and/or (increase in BP + abnormal urinalysis results or MAHA).

Statistical analysis. Patients who received relaxin or placebo for at least 4 weeks were considered evaluable for treatment efficacy in a last observation carried forward analysis. Baseline demographic data were evaluated for comparability of the 3 treatment groups by one-way analysis of variance for continuous variables and by Fisher's exact test for categorical variables. All efficacy and laboratory variables were evaluated by analysis of covariance, adjusting for values at week 0. Frequencies of adverse events in the treatment groups were compared using Fisher's exact test. A 2-tailed *P* value of 0.05 was considered significant for all comparisons. Continuous data are presented as the mean ± SEM, are presented and dichotomous data as the number (percent).

The prospective primary efficacy hypothesis was that relaxin therapy would reduce the MRSS by >4 units after 24 weeks of treatment. This reduction was considered clinically meaningful based on consensus by SSc experts (14), and this has been confirmed in a data-driven analysis of another early dcSSc clinical trial (15).

Based on the phase II study, it was hypothesized that a 25 μg/kg/day dosage of relaxin would result in at least 4 units greater improvement in the MRSS compared with placebo at the end of the study (5). The sample size was selected to provide confirmation of the efficacy, safety, and dose-response effects of the 25 μg/kg/day dosage compared with placebo, with 80% power to detect a significant difference between treatments with a 2-sided alpha level of 0.05.

RESULTS

Baseline characteristics. The study recruited 239 patients with dcSSc, 8 of whom did not attend the baseline visit. In the 231 patients with a baseline visit, the

Table 1. Baseline measures in the 231 patients with systemic sclerosis with diffuse cutaneous involvement*

	Placebo (n = 94)	Relaxin 10 μg/kg/day (n = 42)	Relaxin 25 μg/kg/day (n = 95)
Age, years	46.2 ± 0.7	46.1 ± 1.0	46.4 ± 1.6
Women, no. (%)	79 (84)	38 (90)	81 (85)
Ethnicity, no. (%)			
Caucasian	62 (66)	33 (79)	70 (74)
African American	19 (20)	5 (12)	8 (8)
Hispanic	10 (11)	3 (7)	14 (15)
Asian	2 (2)	0 (0)	0 (0)
Other	1 (1)	1 (1)	3 (3)
Disease duration, years	2.3 ± 0.1	1.9 ± 0.2	2.2 ± 0.1
Disease duration <2.5 years, no. (%)	55 (59)	32 (76)	60 (63)
Disease duration >2.5 years, no. (%)	39 (41)	10 (24)	35 (37)
Use of D-penicillamine within last 6 months, no. (%)	88 (94)	40 (95)	84 (88)
Past use of immunosuppressive agents, no. (%)			
Methotrexate	33 (35)	14 (33)	32 (34)
Oral prednisone	18 (19)	15 (36)	19 (20)
Minocycline	16 (17)	8 (19)	15 (16)
Hydroxychloroquine	3 (3)	5 (12)	6 (6)
Cyclophosphamide	3 (3)	3 (7)	5 (5)
Concomitant therapies, no. (%)			
ACE inhibitors or ARBs	18 (19)	12 (31)	20 (21)
Calcium-channel blockers	39 (41)	14 (33)	35 (37)
MRSS, 0–51	27.1 ± 0.6	28.6 ± 1.1	28 ± 0.8
Maximum oral aperture, mm	43.6 ± 0.9	43.8 ± 1.5	43.2 ± 1.0
Right hand extension, mm	166.6 ± 3.4	155.6 ± 4.3	162.2 ± 3.5
Left hand extension, mm	169.3 ± 3.5	163.2 ± 4.0	169.1 ± 3.3
Total musculoskeletal assessment (synovitis) score, 0–8	2.8 ± 0.4	3.4 ± 0.6	2.7 ± 0.4
Physician's global assessment, 0–100-mm VAS	49.5 ± 2.2	49.8 ± 3.3	51.6 ± 2.1
Patient's global assessment, 0–100-mm VAS	49.6 ± 2.7	49.7 ± 2.6	55.3 ± 3.7
DLCO, % predicted, corrected for hemoglobin	69.6 ± 2.1	71.4 ± 3.5	67.2 ± 2.4
FVC, % predicted	85.8 ± 1.7	87.1 ± 2.0	81.8 ± 1.6
Total number of cutaneous ulcers	0.9 ± 0.2	0.8 ± 0.3	0.5 ± 0.1
HAQ DI score	1.20 ± 0.07	1.36 ± 0.10	1.22 ± 0.080
SF-36 PCS	33.9 ± 10.3	30.8 ± 9.1	33.3 ± 11.6
SF-36 MCS	50.6 ± 9.0	47.4 ± 8.2	48.8 ± 11.3
Creatinine clearance, ml/minute	128.0 ± 4.1	115.5 ± 5.9	127.1 ± 4.9
Systolic blood pressure, mm Hg	116.5 ± 1.9	115.6 ± 2.5	120.4 ± 1.9
Diastolic blood pressure, mm Hg	71.3 ± 1.0	68.8 ± 1.6	71.1 ± 0.9
Hemoglobin, gm/dl	12.6 ± 0.1	12.9 ± 0.2	12.8 ± 0.2

* Except where indicated otherwise, values are the mean ± SEM. There were no significant differences. ACE = angiotensin-converting enzyme; ARBs = angiotensin II receptor blockers; MRSS = modified Rodnan skin thickness score; VAS = visual analog scale; DLCO = diffusing capacity for carbon monoxide; FVC = forced vital capacity; HAQ DI = Health Assessment Questionnaire disability index; SF-36 = Medical Outcomes Study Short Form 36 health survey; PCS = Physical Component Summary; MCS = Mental Component Summary.

mean ± SEM disease duration (from the first non–Raynaud's phenomenon symptom) was 2.2 ± 0.1 years; 147 patients (64%) had a disease duration of ≤2.5 years. The majority of patients were female (86%) and Caucasian (71%), with a mean ± SEM age of 46.9 ± 0.7 years. There were no statistically significant differences in the past use of immunosuppressive agents and in the current use of oral vasodilators (Table 1). The mean ± SEM baseline MRSS was 27.7 ± 0.5 units, and the mean ± SEM baseline HAQ DI score was 1.24 ± 0.05 units, indicating moderate-to-severe disease (11). There were no significant differences in the baseline characteristics between the placebo, 10 μg/kg/day, and 25 μg/kg/day groups (Table 1).

Course of study. All 231 SSc patients attended the 4-week followup visit and were included in the efficacy and safety analyses. One hundred ninety-five patients completed the 24-week study, 21 dropped out upon request or due to noncompliance, 14 withdrew due to adverse events, and 1 person died (for further information, please contact the corresponding author).

Primary outcome measure. The MRSS was similar among the 3 groups at weeks 4, 12, and 24 (Table 2 and Figure 1). The average MRSS declined over the

Table 2. Mean change in outcome measures from baseline to week 24 in the 231 patients who completed the study*

	Placebo (n = 94)	Relaxin 10 μg/kg/day (n = 42)	P†	Relaxin 25 μg/kg/day (n = 95)	P‡
MRSS, 0–51	−4.9 ± 0.7	−4.3 ± 1.3	0.72	−5.2 ± 0.7	0.365
Maximum oral aperture, mm	0.1 ± 0.6	0.7 ± 1.2	0.629	1.3 ± 0.8	0.258
Right hand extension, mm	−3.3 ± 1.3	−0.1 ± 2.1	0.358	−0.6 ± 2.5	0.52
Left hand extension, mm	−1.9 ± 1.9	−3.8 ± 2.3	0.334	−3.0 ± 2.4	0.69
Total musculoskeletal assessment (synovitis) score, 0–8	−0.3 ± 0.4	−0.7 ± 0.5	0.848	−1.1 ± 0.3	0.17
Physician's global assessment, 0–100-mm VAS	−7.2 ± 2.2	−3.4 ± 3.4	0.182	−8.0 ± 2.3	0.879
DLco, % predicted, corrected for hemoglobin	0.2 ± 1.5	2.3 ± 2.1	0.361	0.3 ± 1.1	0.93
FVC, % predicted	−0.6 ± 0.9	−4.3 ± 1.3	0.033	−2.3 ± 1.0	0.016
Total number of cutaneous ulcers	−0.1 ± 0.2	1.1 ± 0.6	0.017	0.1 ± 0.1	0.758
HAQ DI score	−0.01 ± 0.05	0.08 ± 0.06	0.19	0.07 ± 0.04	0.225
Creatinine clearance, ml/minute	−5.6 ± 3.1	8.2 ± 6.3	0.046	6.1 ± 3.4	0.015
Systolic blood pressure, mm Hg	−1.0 ± 1.7	2.1 ± 2.6	0.483	−2.7 ± 1.9	0.688
Diastolic blood pressure, mm Hg	0.0 ± 1.1	0.8 ± 1.8	0.455	−2.9 ± 1.3	0.018
Hemoglobin, gm/dl	−0.41 ± 0.13	−1.24 ± 0.2	0.001	−1.41 ± 0.1	<0.001

* Values are the mean ± SEM. See Table 1 for definitions.
† Placebo versus 10 μg/kg/day relaxin.
‡ Placebo versus 25 μg/kg/day relaxin.

course of 24 weeks. Subanalysis of groups by disease duration (≤2.5 years and >2.5 years) showed similar patterns of improvement in the MRSS (for ≤2.5 years of disease duration, –4.6 ± 0.8 in the placebo arm versus –4.4 ± 1.0 in the 25 μg/kg/day arm [$P = 0.57$]; for >2.5 years of disease duration, –5.4 ± 1.2 in the placebo arm versus –6.6 ± 0.9 in the 25 μg/kg/day arm [$P = 0.29$]).

Secondary outcome measures. Other efficacy measures, including change in DLco % predicted, HAQ DI score, and physician's global assessment, were similar among the 3 groups (Table 2). The mean decline in FVC % predicted was significantly greater in the 10 μg/kg/day and 25 μg/kg/day groups (–4.3% and –2.3%, respectively) than in the placebo group (–0.6%) ($P = 0.033$ for placebo group versus 10 μg/kg/day group; $P = 0.016$ for placebo group versus 25 μg/kg/day group). The numbers of patients with baseline FVC ≤70% predicted were similar in the 3 groups ($P = 0.13$), and the declines in their FVC % predicted were similar at 24 weeks ($P = 0.27$). Patients were not screened for active interstitial lung disease either by high-resolution computed tomography or by bronchoalveolar lavage.

Safety. Both doses of relaxin were associated with an increase in creatinine clearance from baseline (mean change 8.2 ml/minute with the 10 μg/kg/day dosage and 6.1 ml/minute with the 25 μg/kg/day dosage) compared with a decline in the placebo group (mean change –5.6 ml/minute; $P < 0.05$ versus both relaxin doses). In

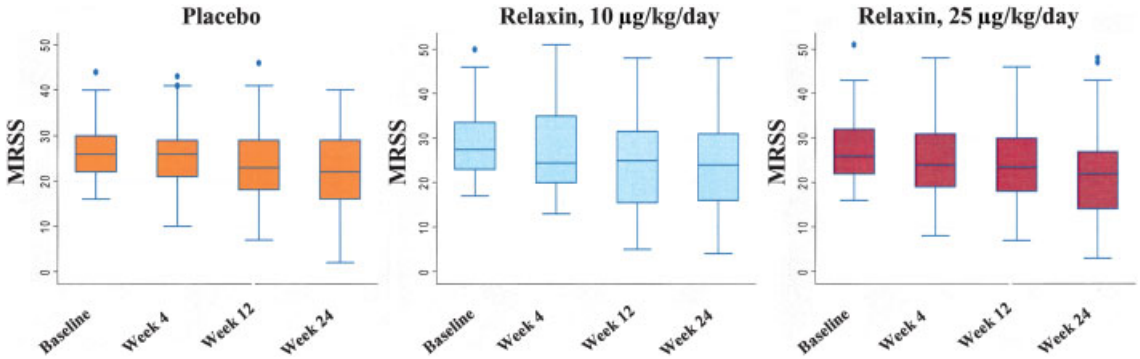

Figure 1. Course of the modified Rodnan skin thickness score (MRSS) over 24 weeks. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the minimum value to the 25th percentile and the 75th percentile to 1.5 times the interquartile range from the third quartile. Circles represent outliers. Despite individual variability at each time point, the MRSS decreased over 24 weeks, and there were no statistically significant differences among the 3 groups during the trial.

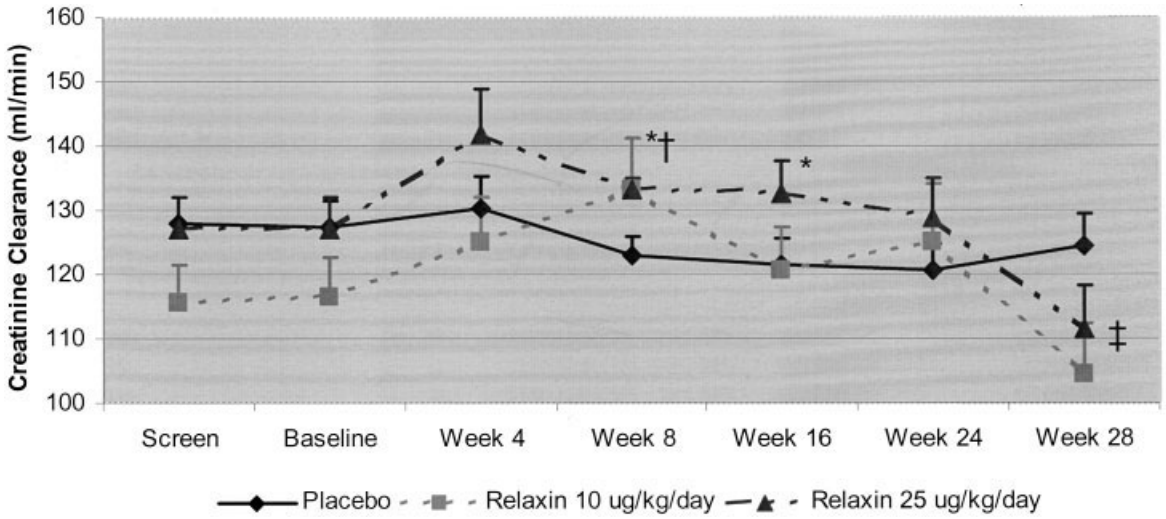

Figure 2. Course of creatinine clearance over 24 weeks. Therapy with relaxin was associated with an increase in creatinine clearance during the trial and an abrupt decline after the therapy was discontinued. Data are presented as the mean and SEM. $* = P < 0.05$ for 25 μg/kg/day relaxin versus placebo; † = $P < 0.05$ for 10 μg/kg/day relaxin versus placebo; ‡ = $P = 0.03$ for both relaxin groups combined versus placebo, all adjusted for baseline value.

addition, the 25 μg/kg/day dosage was associated with a statistically significant decline in diastolic BP (mean change –2.9 mm Hg versus 0.0 mm Hg with placebo; $P = 0.018$) but with no significant impact on systolic BP ($P = 0.688$) (Table 2). There was no significant difference between placebo and the 10 μg/kg/day relaxin dosage regarding change in either systolic or diastolic BP ($P > 0.05$).

Discontinuation of relaxin at week 24 was associated with a decline in creatinine clearance compared with placebo (Figure 2). At the poststudy visit (week 28), the mean declines in creatinine clearance in the 10 μg/kg/day and 25 μg/kg/day relaxin groups were –8.4 ml/minute and –9.8 ml/minute, respectively, compared with a decline of –0.9 ml/minute in the placebo group ($P = 0.07$ for the placebo group versus each relaxin group; $P = 0.03$ for the placebo group versus the combined relaxin groups). This decline in creatinine clearance from baseline values reached statistical significance for the 25 μg/kg/day group ($P = 0.02$) and showed a trend for the 10 μg/kg/day group ($P = 0.09$). In addition, at week 28, compared with the placebo group, all patients who received relaxin had borderline significant combined elevations in systolic BP (mean ± SEM 3.0 ± 1.7 mm Hg versus –0.7 ± 1.9 mm Hg; $P = 0.053$) and nonsignificant combined elevations in diastolic BP (mean ± SEM 2.5 ± 1.1 mm Hg versus 0.6 ± 1.3 mm Hg; $P = 0.27$).

This change in renal function was accompanied by serious renal adverse events (defined as doubling of the serum creatinine concentration, renal crisis, or grade 3 or 4 essential hypertension) in both relaxin groups. Grade 3 hypertension was defined as requiring ≥1 antihypertensive medication, and grade 4 hypertension was defined as life-threatening consequences due to hypertension (e.g., hypertensive crisis). After relaxin was stopped, 2 patients in each of the relaxin groups had new-onset renal crisis, 2 patients in the 25 μg/kg/day group doubled their serum creatinine concentration, and 1 patient in the 25 μg/kg/day group had grade 3 or 4 hypertension (Table 3). In comparison, during the trial, 1 patient receiving placebo and 1 patient receiving 25 μg/kg/day relaxin had doubling of their serum creatinine concentration, and 1 patient in the placebo group experienced new-onset renal crisis. This unusual number of serious renal adverse events (6 events in 169 patients [3.6%]) 1–23 days after the relaxin infusion was stopped led the investigators to recommend daily BP monitoring and gradual discontinuation of relaxin therapy. Subsequent to implementation of this recommendation, only 1 serious renal adverse event in the remaining 62 patients (1.6%) was noted in the relaxin treatment arm after drug discontinuation.

Another adverse event that differed significantly among the 3 groups was a decline in the mean ± SD serum hemoglobin concentration in the relaxin groups compared with that in the placebo group at week 24 (–1.36 ± 0.09 gm/dl versus –0.41 ± 0.13 gm/dl; $P <$

Table 3. Renal adverse events during the trial and 4-week safety period*

	Placebo (n = 94)	Relaxin 10 μg/kg/day (n = 42)	Relaxin 25 μg/kg/day (n = 95)	All relaxin therapy
During treatment				
Renal crisis	1	0	0	0
Doubling of serum creatinine	1	0	1	1
Grade 3 or 4 hypertension	0	0	0	0
Any renal event	1	0	1	1
After treatment				
Renal crisis	0	2	2	4
Doubling of serum creatinine	0	0	2	2
Grade 3 or 4 hypertension	0	0	1	1
Any renal event	0	2	5	7

* Values are the number of events. Grade 3 hypertension was defined as requiring ≥1 antihypertensive medications, and grade 4 hypertension was defined as life-threatening consequences due to hypertension (e.g., hypertensive crisis). The only significant difference ($P = 0.04$) was for the placebo group versus the combined relaxin groups, for any renal event after treatment.

0.001). The decrease in hemoglobin concentration was found predominantly in the female patients receiving relaxin (mean ± SD –1.33 ± 1.02 gm/dl, versus –0.32 ± 0.94 gm/dl in female patients receiving placebo; $P < 0.001$) and not in the male patients receiving relaxin (–1.04 ± 1.10 gm/dl, versus –0.99 ± 2.03 gm/dl in male patients receiving placebo; $P = 0.9$). This difference was no longer significant at the week 28 followup visit after discontinuation of relaxin and placebo (–0.52 ± 0.12 mg/dl in the placebo group versus –0.70 ± 0.11 mg/dl in the combined relaxin groups; $P = 0.43$). Menorrhagia was reported in 27.3% and 30.4% of female patients in the 10 μg/kg/day and 25 μg/kg/day relaxin groups, respectively, compared with 6.3% of female patients in the placebo group ($P = 0.0004$ and $P < 0.0001$, respectively). Most other adverse events occurred with similar frequency among the 3 groups (Table 4).

In 14 patients, adverse events led to discontinuation of the study medication. In the placebo group, 2 patients discontinued due to anemia related to gastric antral vascular ectasia, 1 patient had anemia of unknown cause, and 1 patient had new-onset renal crisis (4 patients overall). In the 10 μg/kg/day group, 1 patient discontinued due to anemia of unknown cause, 1 patient had congestive heart failure and pulmonary hypertension, 1 patient had fatigue and dizziness, and 1 patient

Table 4. Adverse events during the 24-week trial*

	Placebo (n = 94)	Relaxin 10 μg/kg/day (n = 42)	P†	Relaxin 25 μg/kg/day (n = 95)	P‡
Body as a whole	77 (81.9)	33 (78.6)	0.64	76 (80.0)	0.10
Cardiovascular	26 (27.7)	16 (38.1)	0.22	45 (47.4)	0.05
Chest pain	5 (5.3)	4 (9.5)	0.46	9 (9.5)	0.28
Peripheral vascular disease	5 (5.3)	0 (0.0)	0.32	9 (9.5)	0.28
Tachycardia	5 (5.3)	1 (2.4)	0.67	10 (10.5)	0.19
Digestive system	47 (50.0)	27 (64.3)	0.12	56 (58.9)	0.21
Gastrointestinal hemorrhage	5 (5.3)	1 (2.4)	0.67	6 (6.3)	0.77
Melena	5 (5.3)	1 (2.4)	0.67	6 (6.3)	0.77
Hemopoietic system	20 (21.3)	15 (35.7)	0.08	34 (35.8)	0.03
Anemia	11 (11.7)	11 (26.2)	0.03	33 (34.7)	0.0002
Lymphadenopathy	7 (7.4)	1 (2.4)	0.43	3 (3.2)	0.21
Metabolic	1 (1.1)	1 (2.4)	0.56	3 (3.2)	0.32
Musculoskeletal	42 (44.7)	20 (47.6)	0.12	37 (38.9)	0.42
Respiratory system	58 (61.7)	29 (69.0)	0.41	61 (64.2)	0.72
Skin	51 (54.2)	26 (61.9)	0.41	54 (56.8)	0.72
Urogenital system	32 (34.0)	24 (57.1)	0.01	44 (46.3)	0.08
Menorrhagia	6 (6.4)	12 (28.6)	0.0004	28 (29.5)	<0.0001
Metrorrhagia	12 (12.8)	12 (28.6)	0.03	14 (14.7)	0.69

* Values are the number (%). The number (%) in major categories include the number (%) in minor categories.
† Placebo versus 10 μg/kg/day relaxin.
‡ Placebo versus 25 μg/kg/day relaxin.

had an allergic reaction to contrast dye with cardiac arrest (4 patients overall). In the 25 μg/kg/day group, 2 patients discontinued due to severe anemia of unknown cause, and 1 patient each had menorrhagia, pericardial and pleural effusion, worsening lung function, and nausea/vomiting (6 patients overall).

Two deaths were reported in the 25 μg/kg/day group. One patient died after treatment for 22 weeks; she developed uncontrolled congestive heart failure and died of cardiac arrest. Her condition was complicated by anemia, gastrointestinal bleeding, and hypertension. Another patient died of acute scleroderma renal failure 3 weeks after stopping the relaxin therapy; this patient also had a history of pericarditis during her scleroderma renal crisis.

DISCUSSION

We report the results of a phase III study of recombinant relaxin in dcSSc. This study was undertaken based on encouraging results of the phase II study and in vitro studies showing the antifibrotic potential of relaxin (5,6). The phase II study randomized patients with dcSSc into 3 groups—placebo, 25 μg/kg/day relaxin, and 100 μg/kg/day relaxin. The 25 μg/kg/day relaxin group had significantly improved total skin scores and functional outcomes compared with the placebo and 100 μg/kg/day groups (5).

Unlike the phase II study, the current phase III study did not find any difference among the placebo-, 10 μg/kg/day relaxin–, and 25 μg/kg/day relaxin–treated patients with regard to change in skin score, DLCO, or functional disability at 24 weeks. The primary outcome, the MRSS, declined statistically equally in all 3 groups over the course of the 24-week clinical trial. A decline in total skin score for all groups after entry into clinical trials has previously been reported in dcSSc (16,17). This change likely represents the natural history of dcSSc in patients entering SSc clinical trials.

The present trial incorporated patients with early dcSSc (16,18). Subgroup analysis stratified by disease duration (≤2.5 years versus >2.5 years) showed no differences in the MRSS, DLCO, or functional disability between the placebo and relaxin treatment arms (data not shown).

There were also statistically significant, but not clinically meaningful, decreases in the FVC % predicted that favored placebo (mean differences compared with placebo of –3.7% for the 10 μg/kg/day relaxin group [$P = 0.033$] and of –1.7% for the 25 μg/kg/day relaxin group [$P = 0.016$]). These data support the data from the other secondary outcomes, indicating that relaxin had no positive effects in this clinical trial.

Changes in renal physiology associated with relaxin therapy were noted and were reminiscent of those seen in pregnancy: increase in creatinine clearance, lowering of systolic and diastolic BPs, and decreases in the hemoglobin concentration (possibly related to dilutional effects of increased blood volume) (1,19). Relaxin causes renal vasodilatation and hyperfiltration in pregnancy by increasing nitric oxide (NO) production via stimulation of type 2 NO synthase (1,19). In addition, relaxin acts on endothelin by binding to the endothelin B receptor, which is involved in renal vasodilatation, hyperfiltration, and reduced myogenic reactivity of small renal arteries. Therefore, it was not unexpected that the effects of renal vasodilatation and hyperfiltration disappeared when relaxin was withdrawn.

What was unexpected, however, was the abrupt appearance of severe hypertension and renal impairment in a disproportionate number of the patients who abruptly stopped active relaxin therapy, since no such signal was seen in the phase II study. There are recognized abnormalities in SSc renal physiology that may have predisposed the SSc patients to such renal events. Many patients with SSc have reduced renal blood flow and higher plasma renin levels, either reclining or sitting at rest, after exposure to cold or with sodium depletion (20). This suggests that the renovascular systems of patients with SSc are sensitive to changes in blood flow and other stimuli and may have contributed to the new-onset hypertension and in some cases full-blown renal crisis.

Although adverse renovascular effects of relaxin have been reported only among patients with SSc, only a small number of individuals without scleroderma have received treatment with relaxin over any prolonged period of time (21). Until there has been significantly more experience in healthy controls or in patients with circulatory or renal abnormalities, we recommend that caution be employed in the use of relaxin. At a minimum, we suggest that patients who receive relaxin therapy have their BP monitored daily during relaxin treatment and for several weeks following withdrawal of relaxin, as a means of monitoring for new-onset hypertension or acute renal impairment. If hypertension appears, then serial measurement of serum creatinine and control of BP are mandatory. Such monitoring seems to have been successful in this study, since only 1 additional serious renal adverse event was noted after relaxin withdrawal when the above precautions were taken.

There was a statistically significant decline in the

hemoglobin concentration in the relaxin treatment arms compared with placebo, a pattern of anemia also observed in previous studies of relaxin for treatment of SSc (5,6). In addition, menorrhagia was reported in 29% of patients in the relaxin groups compared with 6.3% in the placebo group ($P < 0.001$). Relaxin induces the expression of an angiogenic agent, vascular endothelial growth factor (VEGF) (22,23), and VEGF may cause menorrhagia by inducing neovascularization of the endometrial lining (22). In the present study, a decline in the hemoglobin concentration may have been due to relaxin-induced menorrhagia or some other unknown factor (e.g., dilution). The decrease in hemoglobin no longer differed significantly between the placebo and relaxin treatment arms at week 28 (4 weeks after relaxin was stopped). The effects of relaxin on creatinine clearance, BP, and menorrhagia suggest that a biologic effect was indeed achieved in this clinical trial.

There is renewed interest in assessing the biologic effects of relaxin in different experimental models, since relaxin down-regulates collagen production and increases collagen degradation (1). For example, relaxin-deficient mutant mice show an age-related progression of dermal fibrosis and skin thickening along with internal organ fibrosis similar to scleroderma; treatment with recombinant human gene-2 relaxin reverses the dermal fibrosis in early disease (24). Recent in vitro and in vivo experiments have assessed the role of relaxin in modulating fibroblast function and collagen production in pulmonary, liver, kidney, and cardiac fibrosis (25–27). Recent studies characterizing relaxin receptors in tissue (leucine-rich repeat–containing G protein–coupled receptors 7 and 8 and G protein–coupled receptors 135 and 142) have emphasized the pleiotropic actions of this family of peptides on tissue fibrosis, angiogenesis, and vascular tone (28). These basic and translational research data have led to clinical trials in humans. In fact, www.clinicaltrials.gov (accessed June 11, 2008) lists 5 phase II clinical trials using relaxin for heart failure, preeclampsia, or induction of labor. These ongoing investigations highlight the need to report the present large clinical trial, especially since the adverse events seen in this study should inform the design of future clinical trials and should, in our view, require close followup of patients after they cease relaxin therapy.

In conclusion, recombinant human relaxin given by continuous SC infusion over a 24-week period was not significantly better than placebo in improving the total skin score, pulmonary function, or function (as measured by the HAQ DI) in patients. In addition, withdrawal of relaxin was associated with serious renal adverse events.

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AUTHOR CONTRIBUTIONS

Dr. Khanna 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.

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Acquisition of data. Clements, Furst, Korn, Ellman, Rothfield, Wigley, Moreland, Silver, Kim, Steen, Kavanaugh, Weisman, Mayes, Collier, Csuka, Simms, Merkel, Medsger, Sanders, Seibold.

Analysis and interpretation of data. Khanna, Clements, Furst, Korn, Moreland, Firestein, Weisman, Mayes, Merkel, Sanders, Maranian, Seibold.

Manuscript preparation. Khanna, Clements, Furst, Wigley, Moreland, Kim, Steen, Firestein, Kavanaugh, Weisman, Mayes, Simms, Merkel, Medsger, Sanders, Seibold.

Statistical analysis. Khanna, Clements, Sanders, Maranian, Seibold.

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ROLE OF THE STUDY SPONSOR

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