

# Recombinant Human Relaxin in the Treatment of Scleroderma

## A Randomized, Double-Blind, Placebo-Controlled Trial

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**Background:** Relaxin is a pregnancy-related hormone that has tissue remodeling and antifibrotic effects. Systemic sclerosis (scleroderma) is characterized by fibrosis of the skin, vasculature, and internal organs.

**Objective:** To assess the efficacy, safety, and dose-response effect of recombinant human relaxin in patients with scleroderma.

**Design:** Multicenter, parallel-group, randomized, double-blind, placebo-controlled trial.

**Setting:** Academic referral centers.

**Patients:** 68 patients who had had stable, diffuse scleroderma (moderate to severe) for less than 5 years.

**Intervention:** Recombinant human relaxin, 25 or 100  $\mu\text{g}/\text{kg}$  of body weight per day, or placebo administered by continuous subcutaneous infusion over 24 weeks.

**Measurements:** Modified Rodnan skin score was the primary efficacy measure. Secondary measurements were pulmonary function, the Health Assessment Questionnaire, and other measures of scleroderma that reflected fibrosis.

**Results:** Patients who received 25  $\mu\text{g}/\text{kg}$  of recombinant human relaxin per day had significantly lower skin scores than those who received placebo (mean change,  $-3.6$  at 4 weeks [ $P = 0.021$ ],  $-7.5$  at 12 weeks [ $P < 0.001$ ], and  $-8.7$  at 24 weeks [ $P = 0.040$ ]). Similar trends were noted in other outcome measures, including forced vital capacity, measures of oral aperture and hand extension, functional status, and global assessment. Patients who received 100  $\mu\text{g}/\text{kg}$  of relaxin per day did not differ from those who received placebo. Drug-related adverse events included menometrorrhagia, reversible anemia, and complications of the subcutaneous drug administration system (site irritation and local infection).

**Conclusions:** Twenty-four weeks of recombinant human relaxin, 25  $\mu\text{g}/\text{kg}$  per day, is associated with reduced skin thickening, improved mobility, and improved function in patients with moderate to severe diffuse scleroderma.

Relaxin, a heterodimer protein with a molecular weight of 6000, is secreted by the corpus luteum and placenta during pregnancy (1, 2). It is structurally related to insulin and insulin-like growth factor I, and its principal physiologic role seems to be fostering the growth and remodeling of the uterus. Relaxin also loosens the pelvic ligaments and ripens the uterine cervix in preparation for parturition (3).

The availability of recombinant human relaxin has permitted focused investigations of its effects on connective tissue. Recombinant human relaxin alone reduces synthesis of dermal fibroblast collagen and enhances the effects of interferon- $\gamma$  (4). Relaxin attenuates the actions of profibrotic cytokines, including transforming growth factor- $\beta$  and interleukin-1 $\beta$  (5), and increases secretion of dermal fibroblast collagenase while reducing levels of tissue inhibitor of metalloproteinase (5). Of interest, the effect of relaxin on reduced secretion of collagen and tissue inhibitor of metalloproteinase is dose-dependent, whereas its effect on collagenase is optimal in a narrow range of concentrations (5). Finally, recombinant human relaxin prevents the development of bleomycin-induced pulmonary fibrosis in rodents (6), as well as dermal fibrosis in rodent irritant models (7).

In vitro and animal studies suggest that recombinant human relaxin might be therapeutically useful for diseases characterized by fibrosis. Systemic sclerosis (scleroderma) is the prototypical fibrosing disease in humans. Although the pathogenesis of systemic sclerosis is not completely understood, tissue fibrosis dominates the clinical features of the disease and largely determines its morbidity and mortality (8). Scleroderma-related fibrosis includes both the fibrotic intimal hyperplasia of small arteries and arterioles (the Raynaud phenomenon, renal "crisis," and pulmonary hypertension), as well as extravascular tissue fibrosis (skin, interstitial lung disease, and tendon involvement) (8). The long-term clinical benefit of preventing or reversing fibrosis in systemic sclerosis has not been tested, and no therapies to date have demonstrated such effects (9).

Before porcine relaxin was withdrawn from the market in the early 1960s in response to reformed policies of the U.S. Food and Drug Administration

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**Table 1. Demographic Characteristics of 64 Evaluable Patients with Scleroderma, according to Treatment Assignment**

| Characteristic       | Patients Receiving Placebo<br>(n = 19) | Patients Receiving Relaxin,<br>25 $\mu\text{g}/\text{kg}$ per Day<br>(n = 21) | Patients Receiving Relaxin,<br>100 $\mu\text{g}/\text{kg}$ per Day<br>(n = 24) | P Value |
|----------------------|--|---|--|---------|
| Sex, n (%)           |  |   |  |         |
| Male                 | 3 (16)                                 | 0 (0)   | 5 (21)   | 0.080*  |
| Female               | 16 (84)                                | 21 (100)  | 19 (79)  |         |
| Age, y               |  |   |  |         |
| Mean $\pm$ SE        | 44.2 $\pm$ 2.4                         | 45.3 $\pm$ 3.0  | 44.8 $\pm$ 2.3   | >0.2†   |
| Median (range)       | 43.7 (28–64)                           | 41.8 (27–70)  | 43.2 (15–66)   |         |
| Ethnicity, n (%)     |  |   |  |         |
| Asian                | 2 (11)                                 | 2 (10)  | 1 (4)  | >0.2*‡  |
| Black                | 1 (5)                                  | 7 (33)  | 4 (17)   |         |
| White                | 14 (74)                                | 12 (57)   | 17 (71)  |         |
| Hispanic             | 2 (11)                                 | 0 (0)   | 2 (8)  |         |
| Disease duration, y§ |  |   |  |         |
| Mean $\pm$ SE        | 2.7 $\pm$ 0.06                         | 2.4 $\pm$ 0.2   | 2.5 $\pm$ 0.3  | >0.2†   |
| Median (range)       | 2.0 (0.5–11.9)                         | 2.3 (0.5–4.4)   | 2.5 (0.7–5.0)  |         |

\* Determined by using the Fisher exact test.

† P value for treatment comparison derived from one-way analysis of variance.

‡ Calculated after combining Asian, black, and Hispanic into one category.

§ Years since onset of first non-Raynaud sign or symptom of scleroderma.

|| One patient with a disease duration of 11.9 years was included as a protocol exception.

(FDA), open case studies showed that it improved scleroderma-related skin change and healed cutaneous ulcers (10). Phase I studies of recombinant human relaxin in patients with diffuse scleroderma have 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 infusion (11, 12). The most common drug-related adverse events associated with relaxin treatment have been menometrorrhagia and moderate reversible reductions in hemoglobin. In phase I studies, extent and severity of skin thickening as well as patient global assessment and functional status improved over periods of up to 1 year. However, interpretation of these findings has been hampered by short duration of treatment (11) or inadequacies of open-label design (12).

We report the results of a randomized, double-blind, controlled clinical trial comparing placebo with recombinant human relaxin, 25  $\mu\text{g}/\text{kg}$  of body weight per day and 100  $\mu\text{g}/\text{kg}$  per day, given for 24 weeks in patients with stable, diffuse, moderate to severe scleroderma.

## Methods

### Patients

Before screening, all patients gave informed consent according to the principles of the Declaration of Helsinki and in compliance with FDA requirements. Patients were recruited through 13-member institutions of the Scleroderma Clinical Trials Consortium. Men and women 18 to 70 years of age were included if they had a history of systemic sclerosis with diffuse scleroderma (defined as skin involvement proximal to the elbows or knees, exclud-

ing the face and neck) and less than 5 years had elapsed since onset of the first non-Raynaud sign or symptom. A baseline modified Rodnan skin score of at least 20, or of at least 16 in the case of truncal involvement, was required for inclusion in the treatment phase of the study. Patients were excluded from this phase if their skin score varied by more than 5 points from screening to the first treatment day.

We excluded patients who had systemic sclerosis with limited scleroderma (skin involvement restricted to face and neck and sites distal to elbows and knees); eosinophilic fasciitis; eosinophilia myalgia syndrome; or scleroderma in conjunction with any other definable connective tissue disease, such as rheumatoid arthritis, systemic lupus erythematosus, polymyositis, or dermatomyositis. We also excluded patients with a substantial history of environmental exposure to tainted rapeseed oil, vinyl chloride, trichloroethylene, or silica. In addition, patients with renal crisis in the previous 6 months; chronic renal failure; or severe cardiovascular, gastrointestinal, or pulmonary disease were excluded.

Patients were required to discontinue putative “disease-modifying” treatments for scleroderma (including D-penicillamine, cyclophosphamide, cyclosporine, azathioprine, methotrexate, potassium aminobenzoate, photopheresis, colchicine, or any other experimental treatment) at least 4 weeks before beginning treatment with the study drug. Patients were excluded if they were receiving more than 10 mg of prednisone per day or an equivalent dose of another glucocorticoid.

### Intervention

We administered recombinant human relaxin, 25  $\mu\text{g}/\text{kg}$  per day or 100  $\mu\text{g}/\text{kg}$  per day, or placebo for 24 weeks by continuous subcutaneous infusion, us-

ing microinfusion pumps (Panomat T-Series 5 mL, Disetronic Medical Systems, Inc., Minneapolis, Minnesota). Recombinant human relaxin was produced by Connetics Corp. (Palo Alto, California) in *Escherichia coli* (13). 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 (25  $\mu\text{g}/\text{kg}$  per day or 100  $\mu\text{g}/\text{kg}$  per day). Randomization was performed at a centralized data management organization (Pacific Research Associates, Los Altos, California). "Biased coin" randomization (14, 15) was used to stratify patients on the basis of disease duration ( $\leq 2.5$  years or  $> 2.5$  to 5 years) and use of D-penicillamine in the previous 6 months (16). The same randomization procedure was used to replace patients who withdrew before completing 4 weeks of treatment.

Patient prescriptions for the study medication were forwarded to a centralized pharmacy (Coram Healthcare of Northern California, Hayward, California) for preparation of blinded supplies of the study drug. Each patient's dose was based on screening body weight. The dose was adjusted only if body weight changed by 10% or more during the study. Treatment was administered over 24 hours for 24 weeks. The infusion site and needle were changed at least every 72 hours.

The dosage of 25  $\mu\text{g}/\text{kg}$  per day was selected on the basis of pharmacokinetic results from earlier studies. We anticipated that it would be safe and well tolerated and would produce steady-state serum concentrations of relaxin that were approximately three- to fivefold greater than those found in human pregnancy (11). On the basis of preclinical

and earlier clinical studies, we hypothesized that this serum concentration would have antifibrotic effects. To measure the potential for a dose-response effect, we selected the dosage of 100  $\mu\text{g}/\text{kg}$  per day on the basis of safety and tolerability data from earlier clinical studies (11, 12). Continuous subcutaneous infusion was chosen as the mode of administration to eliminate the need for six daily subcutaneous injections, to conserve drug supply, and to mimic the constancy of relaxin concentrations that are usually seen in pregnancy (11).

### Study Design

The objectives of the study were to assess the efficacy, safety, and dose-response effect of recombinant human relaxin in patients with diffuse scleroderma. The study was conducted as a randomized, double-blind, placebo-controlled, parallel-treatment clinical trial.

### Assessments

The primary measure of efficacy was the modified Rodnan skin score, a clinical evaluation by palpation of skin thickness in 17 body areas (face, chest, abdomen, right and left fingers, hands, forearms, upper arms, thighs, legs, and feet). Each area receives a score of 0 to 3 for degree of thickness (0 = normal, 1 = mild thickening, 2 = moderate thickening, and 3 = severe thickening). The total score ranges from 0 to 51. The modified Rodnan skin score has been the standard measure of outcome in recent clinical trials involving scleroderma (16–18). Many recent studies have confirmed that total skin scoring is both accurate (with an interobserver variability of  $\pm 4.6$  units) and reproducible (with an intraobserver variability of  $\pm 3.1$  units) (19, 20). Skin

**Table 2. Change in Modified Rodnan Skin Score in 64 Evaluable Patients with Scleroderma, according to Treatment Group\***

| Variable  | Patients Receiving Placebo<br>(n = 19) | Patients Receiving Relaxin,<br>25 $\mu\text{g}/\text{kg}$ per Day<br>(n = 21) | Patients Receiving Relaxin,<br>100 $\mu\text{g}/\text{kg}$ per Day<br>(n = 24) |
|---|--|---|--|
| Score at screening<br>P value†                          | 26.7 $\pm$ 1.4<br>>0.05                | 27.5 $\pm$ 1.6  | 26.3 $\pm$ 1.6   |
| Score at week 0<br>P value†                             | 26.8 $\pm$ 1.3<br>>0.05                | 27.5 $\pm$ 1.6  | 27.5 $\pm$ 1.7   |
| Score at week 4<br>Mean change from week 0<br>P value‡  | 26.0 $\pm$ 1.4<br>-0.8 $\pm$ 1.0       | 23.9 $\pm$ 1.7<br>-3.6 $\pm$ 0.6<br>0.021                                     | 25.0 $\pm$ 1.8<br>-2.5 $\pm$ 0.7<br>0.136                                      |
| Score at week 12<br>Mean change from week 0<br>P value‡ | 24.4 $\pm$ 1.5<br>-2.5 $\pm$ 0.9       | 20.0 $\pm$ 1.9<br>-7.5 $\pm$ 0.8<br><0.001                                    | 24.5 $\pm$ 2.1<br>-3.0 $\pm$ 0.9<br>>0.05                                      |
| Score at week 24<br>Mean change from week 0<br>P value‡ | 21.9 $\pm$ 1.8<br>-4.9 $\pm$ 1.4       | 18.8 $\pm$ 2.0<br>-8.7 $\pm$ 1.0<br>0.040                                     | 24.1 $\pm$ 2.3<br>-3.4 $\pm$ 1.2<br>>0.05                                      |

\* Data are presented as the mean  $\pm$  SE.

† Mean comparison of treatment groups by one-way analysis of variance.

‡ Comparison of treatment group with placebo group by analysis of covariance, adjusting for value at week 0.



**Table 3. Post hoc Frequency Analysis of Modified Rodnan Skin Score in 64 Patients with Scleroderma**

| Skin Score Response        | Patients Receiving Placebo<br>(n = 19) | Patients Receiving Relaxin,<br>25 µg/kg per Day<br>(n = 21) | Patients Receiving Relaxin,<br>100 µg/kg per Day<br>(n = 24) |
|----------------------------|--|---|--|
|                            | ←————— n (%) —————→                    |   |  |
| Any worsening              | 4 (21)                                 | 0 (0)   | 5 (21)   |
| Improvement of ≥7 points*  | 7 (37)                                 | 15 (71)   | 9 (38)   |
| Improvement of ≥10 points† | 5 (26)                                 | 11 (52)   | 5 (21)   |

\*  $P = 0.03$  by the chi-square test ( $2 \times 3$  table).

†  $P = 0.06$  by the chi-square test ( $2 \times 3$  table).

line, and 1 developed scleroderma renal crisis between screening and baseline). The remaining 68 patients received treatment and are included in the safety analysis. Of these, 4 patients (2 in the 25-µg/kg group and 2 in the 100-µg/kg group) withdrew from the study at a very early stage. Three withdrew consent after 3, 4, and 10 days of therapy, citing anxiety, difficulty sleeping with the infusion pump, and inability to cope with the logistical and time demands of the study, respectively; 1 withdrew after 15 days of therapy because of an adverse event (abdominal wall cellulitis). No additional measures of efficacy were performed on patients who withdrew. Therefore, 64 patients were considered evaluable for the efficacy analysis (21 in the 25-µg/kg group, 24 in the 100-µg/kg group, and 19 in the placebo group).

### Demographic Characteristics

Demographic data on the evaluable study sample are shown in **Table 1**. No men were randomly assigned to the 25-µg/kg group; however, men made up 16% of the placebo group (3 of 19) and 21% of the 100-µg/kg group (5 of 24). No statistically significant differences were seen among the groups for any of the demographic variables.

### Primary Efficacy Variable: Modified Rodnan Skin Score

**Table 2** shows the change in modified Rodnan skin score. The three study groups had similar scores at screening and at week 0 (baseline). At weeks 4, 12, and 24, the 25-µg/kg group had greater reductions in skin scores than the placebo group; these reductions were statistically significant ( $P = 0.021$ ,  $P < 0.001$ , and  $P = 0.040$ , respectively). The skin scores for the 100-µg/kg group did not differ significantly from the scores of the placebo group at any of the assessments.

The **Figure** shows the individual changes in total skin score from baseline to the 24-week evaluation point. Skin score did not worsen in any patient in the 25-µg/kg group. However, scores worsened in 4 patients receiving placebo (21%) and in 5 patients receiving 100 µg/kg of relaxin per day (21%). Post

hoc analysis of frequency of response was based on the reported intraobserver variability of the modified Rodnan skin score ( $\pm 3.1$  units) (19, 20). Improvements of 7 and 10 units, respectively, represented twofold and threefold multiples of the variability of the measure. Improvements of this magnitude occurred approximately twice as frequently in patients receiving 25 µg/kg of relaxin per day than in those receiving 100 µg/kg per day or placebo (**Table 3**). When we controlled for disease duration and previous treatment with D-penicillamine, analysis of skin score changes across treatment groups showed that these two variables did not affect treatment outcome (data not shown).

### Secondary Assessments

**Table 4** shows the results of secondary efficacy assessments. The active treatment groups and the placebo group did not differ significantly for any of these measures at week 24 (with the exception of the eating component of the HAQ).

Secondary assessment measures tended to show improvement in patients who received 25 µg/kg of relaxin per day. These measures included objective measures of skin fibrosis (oral aperture, right and left hand extension) and the HAQ disability index, particularly the four components that measured upper-extremity function (**Table 4**). At 12 and 24 weeks, a smaller decrease in forced vital capacity (both actual and percentage predicted) was seen in the 25-µg/kg group than in the placebo group. The forced vital capacity decreased from baseline by a mean of 1% predicted in the 25-µg/kg group and by a mean of 3.8% predicted in the placebo group. The 100-µg/kg group was similar to the placebo group. No effects of treatment on lung diffusion capacity were observed.

Antirelaxin antibodies were detected in 0% of patients in the placebo group, 33% of patients in the 25-µg/kg group, and 58% of patients in the 100-µg/kg group. Development of antirelaxin antibodies did not affect efficacy as determined by change in skin score (data not shown).

**Table 4. Mean Change in Secondary Measures of Outcome from Baseline to Week 24 in 64 Evaluable Patients with Scleroderma\***

| Outcome Measure   | Patients Receiving Placebo<br>(n = 19) | Patients Receiving Relaxin,<br>25 $\mu\text{g}/\text{kg}$ per Day<br>(n = 21) | Patients Receiving Relaxin,<br>100 $\mu\text{g}/\text{kg}$ per Day<br>(n = 24) |
|---|--|---|--|
| Maximum oral aperture, <i>mm</i> <sup>†</sup>   | -1.6 $\pm$ 1.4                         | 0.4 $\pm$ 1.0   | -1.2 $\pm$ 1.2   |
| <i>P</i> value  |  | >0.2  | >0.2   |
| Right hand extension, <i>mm</i>   | -4.5 $\pm$ 2.4                         | 1.6 $\pm$ 3.0   | -2.0 $\pm$ 1.6   |
| <i>P</i> value  |  | 0.101   | >0.2   |
| Left hand extension, <i>mm</i>  | -3.7 $\pm$ 2.5                         | 1.1 $\pm$ 2.9   | -2.6 $\pm$ 1.8   |
| <i>P</i> value  |  | 0.179   | >0.2   |
| Total musculoskeletal assessment (synovitis) score <sup>‡</sup>                                     | 0.5 $\pm$ 0.8                          | -0.9 $\pm$ 0.6  | 0.2 $\pm$ 0.5  |
| <i>P</i> value  |  | 0.133   | >0.2   |
| Physician global assessment of disease severity, <i>mm</i> <sup>§</sup>                             | -6.3 $\pm$ 3.1                         | -11.5 $\pm$ 4.0   | -5.3 $\pm$ 3.2   |
| <i>P</i> value  |  | >0.21   | >0.2   |
| Patient assessment of Raynaud attacks on daily activities, <i>mm</i> <sup>  </sup>                  | 0.7 $\pm$ 5.4                          | 4.2 $\pm$ 7.9   | 0.3 $\pm$ 5.6  |
| <i>P</i> value  |  | >0.2  | >0.2   |
| Patient global assessment of corrected amount of predicted disease severity, <i>mm</i> <sup>¶</sup> | -3.8 $\pm$ 5.5                         | -6.0 $\pm$ 5.9  | 0.4 $\pm$ 4.4  |
| <i>P</i> value  |  | >0.2  | >0.2   |
| Corrected amount of predicted lung diffusion capacity, %  | 3.1 $\pm$ 3.0                          | 2.3 $\pm$ 2.0   | -0.3 $\pm$ 1.7   |
| <i>P</i> value  |  | >0.2  | >0.2   |
| Amount of predicted forced vital capacity, %  | -3.8 $\pm$ 1.9                         | -1.0 $\pm$ 1.2  | -4.7 $\pm$ 1.3   |
| <i>P</i> value  |  | 0.153   | >0.2   |
| Total cutaneous ulcers, <i>n</i>  | 0.7 $\pm$ 0.5                          | -1.3 $\pm$ 1.4  | 0.3 $\pm$ 0.3  |
| <i>P</i> value  |  | 0.065   | >0.2   |
| Score on the HAQ Disability Index**   | 0.053 $\pm$ 0.11                       | -0.125 $\pm$ 0.10   | 0.036 $\pm$ 0.06   |
| <i>P</i> value  |  | 0.154   | >0.2   |
| Score on HAQ component 3 (eating)   | 0.105 $\pm$ 0.13                       | -0.429 $\pm$ 0.19   | -0.042 $\pm$ 0.09  |
| <i>P</i> value  |  | 0.024   | >0.2   |
| Score on HAQ component 5 (hygiene)  | -0.053 $\pm$ 0.22                      | -0.238 $\pm$ 0.22   | 0.167 $\pm$ 0.13   |
| <i>P</i> value  |  | >0.2  | >0.2   |
| Score on HAQ component 6 (reaching)   | 0.105 $\pm$ 0.13                       | -0.190 $\pm$ 0.16   | 0.000 $\pm$ 0.13   |
| <i>P</i> value  |  | 0.167   | >0.2   |
| Score on HAQ component 7 (gripping)   | 0.053 $\pm$ 0.12                       | -0.190 $\pm$ 0.15   | 0.042 $\pm$ 0.07   |
| <i>P</i> value  |  | >0.2  | >0.2   |

\* Data are presented as the mean  $\pm$  SE. *P* values were computed by comparing treatment groups to the placebo group with analysis of covariance adjusting for value at week 0. HAQ = Health Assessment Questionnaire.

<sup>†</sup> Equals vertical interlabial distance.

<sup>‡</sup> The sum of the swelling and tenderness scores of the metacarpophalangeal joints (as a unit), wrists, and knees.

<sup>§</sup> Made on a visual analogue scale of 0 mm (no effect) to 100 mm (worst possible effect).

<sup>||</sup> Made on a visual analogue scale of 0 mm (no limitation of activities) to 100 mm (very severe limitation of activities).

<sup>¶</sup> Made on a visual analogue scale of 0 mm (no disease) to 100 mm (very severe disease).

\*\* The sum of the scores of the eight components that make up the HAQ. The higher the disability index, the worse the disability. Each component was scored on a scale of 1 (able to do without any difficulty) to 4 (unable to do).

## Safety

All randomly assigned and treated patients (*n* = 68) were included in the safety analysis. Menorrhagia and metrorrhagia were more common in the relaxin groups than in the placebo group. Menorrhagia was observed in 11% of patients receiving placebo, 35% of patients receiving 25  $\mu\text{g}/\text{kg}$  of relaxin per day, and 19% of patients receiving 100  $\mu\text{g}/\text{kg}$  of relaxin per day. Metrorrhagia was observed in 5% of patients receiving placebo, 9% of patients receiving 25  $\mu\text{g}/\text{kg}$  of relaxin per day, and 27% of patients receiving 100  $\mu\text{g}/\text{kg}$  of relaxin per day. Although the differences did not reach statistical significance, these events have been observed in other clinical studies of relaxin and can be related to known biological effects of relaxin on the endometrium (23).

Local skin reactions, including pain, inflammation, and rash, were common at infusion sites but were approximately balanced among groups. Local infections at infusion sites, which occurred in 10% of patients during the study, were also approximately balanced among groups.

Seven patients withdrew from the study because of adverse events (1 in the placebo group, 3 in the 25- $\mu\text{g}/\text{kg}$  group, and 3 in the 100- $\mu\text{g}/\text{kg}$  group). Three of these patients (1 in the 25- $\mu\text{g}/\text{kg}$  group and 2 in the 100- $\mu\text{g}/\text{kg}$  group) withdrew because of local skin reactions or cellulitis at the infusion site. The remaining events that resulted in early withdrawal (scleroderma renal crisis [1 patient in the placebo group], pseudomembranous colitis [1 patient in the 25- $\mu\text{g}/\text{kg}$  group], anxiety [1 patient in the 25- $\mu\text{g}/\text{kg}$  group], and tachyarrhythmia [1 patient in the 100- $\mu\text{g}/\text{kg}$  group]) were due to primary disease or to intercurrent illness. No patients died during the study. One patient in the 25- $\mu\text{g}/\text{kg}$  group who had preexisting cardiac disease died of sudden cardiac arrest 17 days after completing the last day of dosing.

As was seen in earlier clinical studies (11, 12), administration of relaxin resulted in a dose-dependent decrease in hemoglobin levels. Mean decreases from day 0 to week 24 were approximately 17 g/L in the 100- $\mu\text{g}/\text{kg}$  group, 11 g/L in the 25- $\mu\text{g}/\text{kg}$  group, and 6 g/L in the placebo group. No evidence of

erythrocyte destruction or underproduction was seen. We observed no other pattern of abnormal laboratory results that could have been attributed to the study drug.

## Discussion

Many advances have been made in the management of systemic sclerosis and its complications, including effective palliative agents (proton-pump inhibitors for esophageal reflux, calcium-channel blockers for the Raynaud phenomenon) and focused agents for specific organ complications (angiotensin-converting enzyme inhibitors for the hypertension of renal involvement, cyclophosphamide for alveolitis) (8, 24). However, no proven effective disease-modifying therapies are available (9, 25). There are many impediments to the development of such therapies, including a lack of consensus about definitions of "disease modification" (9, 25–27), a dearth of robustly designed prospective trials, and a lack of established guidelines for approval of therapies by regulatory agencies. Against this background, much progress has recently been made in the clinical science of outcome assessment in scleroderma, and rational proposals for trial design have been developed (17, 25).

Our placebo-controlled trial shows that an agent is superior in the therapy for basic fibrotic disease features. We demonstrated that recombinant human relaxin, 25  $\mu\text{g}/\text{kg}$  per day, is associated with statistically significant reductions in the extent and severity of scleroderma-related skin thickening. Effect was seen as early as 4 weeks and was sustained through 24 weeks of treatment.

In addition to serving as the primary basis for disease classification, skin thickening in systemic sclerosis is viewed as a clinical surrogate of disease progression (8, 26, 28). Skin involvement alone has important symptomatic and functional consequences and can be viewed as an independent outcome variable (29). Our study confirms the clinical meaningfulness of reduced skin thickening by demonstrating parallel trends in patient functional status, patient global assessments, and other measures of fibrosis, including hand extension and oral aperture.

Another potential clinical application of our results involves the effect of recombinant human relaxin on lung function. Patients with scleroderma experience a progressive loss of forced vital capacity (16, 30, 31). In our study, mean forced vital capacity decreased over 24 weeks by approximately 1.0% of that predicted in patients receiving 25  $\mu\text{g}/\text{kg}$  of recombinant human relaxin per day and by approximately 3.8% of that predicted in those receiving placebo ( $P = 0.153$ ). These differences are of uncer-

tain clinical importance; however, if this effect is confirmed and could be shown to persist over longer courses of therapy, it would be expected to result in clinical benefit and perhaps improved long-term survival (9, 17, 25).

The natural history of systemic sclerosis with diffuse scleroderma varies. Early disease is characterized by progression of skin thickening and initial accrual of visceral involvement. Many patients stabilize, and skin thickening can spontaneously improve in later disease, although visceral involvement continues to worsen (8, 17, 30). We sought to pursue a reversal paradigm by selecting patients in whom skin involvement was moderate to severe but also clinically stable (neither worsening nor spontaneously improving). This design facilitated expeditious identification of a homogeneous group of patients for study. As expected, we documented a tendency for slight improvements in skin thickening in patients who received placebo, as well as a tendency for slow loss of pulmonary function. The design and results of our study demonstrate the need for inclusion of placebo controls in clinical trials related to scleroderma, particularly those that focus on skin involvement. On the basis of *in vitro* experiments that demonstrate attenuation of profibrotic cytokine effects (5), recombinant human relaxin should have clinical value as a preventive agent. Skin score did not worsen in any of the patients receiving 25  $\mu\text{g}/\text{kg}$  of relaxin per day. Although this might suggest a preventive effect, our study sample does not permit this conclusion.

A higher dose of recombinant human relaxin (100  $\mu\text{g}/\text{kg}$  per day) did not result in clinical benefit. Our data do not offer a complete explanation for this finding. *In vitro* studies with human dermal fibroblasts demonstrate a dose-dependent down-regulatory effect on secretion of interstitial collagens as well as a dose-dependent decrease in transforming growth factor- $\beta$ -induced secretion of type I and type III collagen (5). Relaxin also stimulates procollagenase expression at the transcription level but elicits the maximum response at concentrations of 0.1 to 10  $\text{ng}/\text{mL}$  (5). The same biphasic response of collagenase has also been noted in studies of murine and human lung fibroblasts (6).

During our trial, mean serum relaxin levels for patients receiving 100  $\mu\text{g}/\text{kg}$  of relaxin per day usually exceeded 30  $\text{ng}/\text{mL}$ . *In vivo* and *in vitro* effects may not be directly comparable. Pilot tissue assays of collagenase expression performed during our study were not revealing. It is possible that higher doses of relaxin fail to stimulate collagenase secretion and thus blunt clinical effects that reflect reversal of fibrosis. A parallel trend suggesting optimal clinical effect of relaxin at 25  $\mu\text{g}/\text{kg}$  per day was

noted in an open-label safety study that was 24 to 48 weeks in duration (12).

In summary, 24 weeks of recombinant human relaxin, 25 µg/kg per day, administered by continuous subcutaneous infusion resulted in statistically significant reductions in the extent and severity of skin thickening in patients with stable, moderate to severe diffuse scleroderma. Improvement in skin thickening was accompanied by small positive trends in other measures of fibrosis, including forced vital capacity, oral aperture, and ability to extend the hand, as well as in patient functional status and global assessments. Because our results suggest that it is possible to reverse features of scleroderma, they have broad implications for various fibrosing diseases. Confirmation is required in larger controlled studies, one of which is in progress.

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