BRIEF REPORT

A DOUBLE-BLIND RANDOMIZED CONTROLLED TRIAL OF KETOTIFEN VERSUS PLACEBO IN EARLY DIFFUSE SCLERODERMA

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To determine the efficacy of the mast cell–stabilizing drug ketotifen in scleroderma, we conducted a 6-month, randomized, prospective, double-blind, placebo-controlled trial in 24 patients. No significant improvement in the clinical paramenters, pulmonary function, global assessments, and mast cell releasability was noted. Pruritus tended to improve in the group taking the active drug. Six months of treatment with ketotifen (6 mg/day), therefore, produced no apparent benefit in patients with early scleroderma. We were unable to address the role of mast cells in scleroderma since mast cell suppression was not achieved.

Ketotifen is a drug which reportedly both inhibits its mediator release from mast cells and acts as a histamine antagonist (1,2). We have previously reported beneficial effects of ketotifen in 2 patients whose progressive systemic sclerosis was recalcitrant to D-penicillamine therapy (3). In addition, this drug has recently been shown to effectively inhibit the development of fibrosis in the tight-skin mouse model of scleroderma (4).

The mast cell has been emphasized recently as a potential stimulus for the activation of fibroblasts in scleroderma (5,6). The early lesion of scleroderma encompasses an inflammatory phase in which activated fibroblasts are observed near numerous infiltrating mast cells. The involved skin contains a significantly higher number of mast cells (7–9). Furthermore, mast cell–derived mediators are capable of promoting fibroblast proliferation and collagen synthesis in vitro (5). Based on these observations, and in an effort to extend our clinical experience with ketotifen, we performed a randomized controlled trial of ketotifen versus placebo in the treatment of early diffuse scleroderma.

PATIENTS AND METHODS

Patients and clinical assessment. Twenty-four patients, most of whom were referred to us for this study, were chosen for inclusion in our trial. These patients had diffuse (generalized) systemic sclerosis, according to the American Rheumatism Association preliminary criteria (10), the onset of which had occurred less than 3 years after the first non-Raynaud's symptom. Criteria for exclusion included age <18, disease duration >3 years, use of remittive agents, immunosuppressive agents, or corticosteroids (>5 mg/day of prednisone equivalent) within the preceding 3 months, and concomitant metabolic or neoplastic disease. Subjects with renal insufficiency, pulmonary disease, or evidence of cardiac involvement were not excluded if the disorder was deemed secondary to the systemic sclerosis.

Protocol. After giving their informed consent, 24 patients were randomized to receive ketotifen or placebo for 6 months, in a prospective, double-blind study with a parallel design. A computer-generated randomization code was provided by Sandoz Pharmaceuticals at our request. Patients were assigned to 1 of

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2 groups, to receive either 3 mg of ketotifen twice daily (Zaditen; Sandoz Pharmaceuticals, Basel, Switzerland) or identical placebo tablets twice daily. Patients were instructed not to take any antihistamines during the study period.

Clinical evaluation. At the start of the trial, a complete medical history and physical examination were performed. The presence and severity of pruritus was scored using a 0–3 scale, where 0 = none, 1 = mild, 2 = moderate, and 3 = severe. Skin involvement was assessed by determining a total skin score (using a 0–3 scale [11]) over 19 separate areas covering the entire dermal surface. A 4-mm punch biopsy was performed, the wet weight after removing subcutaneous fat was measured, and the sample was dried for use in determining the hydroxyproline content (12). The hydroxyproline analysis was kindly performed by Joseph N. Harris in the laboratory of Dr. James R. Seibold (Robert Wood Johnson Medical School, New Brunswick, NJ). Further baseline evaluations included complete pulmonary function testing, intradermal skin testing for mast cell releasability, using a panel of secretagogues (morphine, compound 48/80, polymyxin B, histamine, and saline) and laboratory analysis.

The skin testing was performed and recorded independently by a physician's assistant, who was blinded to the protocol. The results of these skin tests were not revealed to the investigators until the termination of the study. The laboratory evaluation included, at each visit, a complete blood cell count, multichannel automated serum chemistry profile, and urinalysis. Patients were seen every 6 weeks for a history, functional assessment, determination of pruritus score, physician global assessment, physical examination, determination of skin scores, and skin testing. Repeat pulmonary function testing was performed at the 3-month and 6-month visits. At the last visit, a second skin biopsy was performed, and the sample was processed as described above.

Statistical analysis. Statistical comparisons of the clinical and laboratory values between treatment groups were made using Snedecor's F test and unpaired *t*-test to determine the equality of variances and significance of differences at each time point during the study with respect to the baseline values.

RESULTS

Patient characteristics and demographics. The pertinent baseline characteristics of all 24 study patients are shown in Table 1. Two patients had late disease (>10 years) but were entered into the study because of a recent disease flare and because, in the investigator's judgment, their inclusion would provide important information. Two patients in the ketotifen treatment group withdrew from the study at month 5 because of worsening disease, and 1 patient in the placebo treatment group withdrew at month 3 because of persistent somnolence. The 2 groups were otherwise well matched in terms of age, sex, severity of pruritus, skin test reactivity, and disease activity at baseline, as reflected by the skin scores, presence of pulmonary disease, and maximum oral aperture (Table 1).

Table 1. Demographic and clinical characteristics of the 24 scleroderma patients studied*

Characteristic	Ketotifen-treated group	Placebo-treated group
No. of females/males	7/5	9/3
Age, mean ± SD years	47 ± 16	46 ± 15
Duration of disease, mean ± SEM months	28 ± 5	50 ± 6.7 (34 ± 5)†
Skin involvement, mean ± SEM total skin score	31 ± 3.4	31 ± 3.4
Maximum oral aperture, mean ± SD cm	3.8 ± 1	3.7 ± .78
Skin reactivity, mean ± SD skin test score	2.3 ± 1.3	2.5 ± 0.5
Severity of pruritus, mean ± SD	2.8 ± 1.1	1.7 ± 1.7
No. with pulmonary disease/no. tested	9/11	10/12

* Skin involvement was assessed at 19 sites, and a total skin score (0–3 scale) was derived, as described elsewhere (11). Skin reactivity to specific mast cell secretagogues, according to degree of wheal and flare reactions, was scored on a scale of 0–3, where 0 = no reaction and 3 = severe reaction. Severity of pruritus was scored on a scale of 0–3, where 0 = none and 3 = severe. One patient in the ketotifen treatment group was not able to perform the pulmonary function tests.

† Value in parentheses is the mean ± SEM without the data for the 2 patients with late disease (see Patients and Methods for details).

Efficacy outcome. Twenty-three patients completed at least 5 months of the trial, and their data were used for statistical analysis. A marginal, statistically significant, improvement in the total skin score was seen in the placebo-treated group, compared with the ketotifen-treated group, at week 12 ($P < 0.05$), but not at week 24 (Table 2). No other statistically significant difference between the 2 patient groups was identified. A somewhat unexpected result was the lack of efficacy of ketotifen in reversing skin test reactivity to mast cell stimuli.

Global assessments by the patient and physician are shown in Table 3. No significant difference between the ketotifen-treated group and the placebo-

Table 2. Clinical variables at baseline and at 6-week intervals during the 6 months of the trial*

Variable, treatment group	Baseline (week 0)	Week 6	Week 12	Week 18	Week 24
Total skin score					
Ketotifen	31 ± 11	32 ± 7.8	35 ± 10	34 ± 13	31 ± 16
Placebo	31 ± 11	26 ± 9.4	26 ± 11	25 ± 12	26 ± 9.3
Severity of pruritus					
Ketotifen	2.8 ± 1.1	1.9 ± 1.5	1.5 ± 1.5	1.4 ± 1.6	1.5 ± 1.5
Placebo	1.9 ± 1.4	2.3 ± 1.2	2.5 ± 1.7	1.6 ± 1.5	1.3 ± 1.8
Maximum oral aperture (cm)					
Ketotifen	3.8 ± 1.0	4.1 ± 1.2	4.0 ± 8.2	4.3 ± 1.1	4.7 ± 0.94
Placebo	3.7 ± 0.78	3.8 ± 0.93	4.2 ± 0.97	4.1 ± 1.1	3.8 ± 0.72
Skin test score					
Ketotifen	1.9 ± 0.87	2.0 ± 1.1	1.6 ± 0.89	1.7 ± 1.1	1.9 ± 1.0
Placebo	2.0 ± 0.47	2.4 ± 0.62	2.0 ± 0.87	2.3 ± 0.75	2.0 ± 0.57
Hydroxyproline (μg/mg dry tissue)					
Ketotifen	130 ± 17	–	–	–	120 ± 22
Placebo	140 ± 25	–	–	–	120 ± 29
Total lung capacity (liters)					
Ketotifen	5.5 ± 2.3	–	5.2 ± 2.2	–	5.7 ± 2.2
Placebo	4.9 ± 2.0	–	4.3 ± 0.92	–	4.2 ± 0.91
Forced vital capacity (liters)					
Ketotifen	3.7 ± 1.6	–	3.5 ± 1.6	–	3.9 ± 1.7
Placebo	3.0 ± 0.8	–	2.9 ± 0.84	–	2.9 ± 0.71
Carbon monoxide diffusing capacity (ml/minute/mm Hg)					
Ketotifen	8.8 ± 7.3	–	7.2 ± 5.9	–	6.8 ± 5.2
Placebo	8.8 ± 7.9	–	6.9 ± 6.9	–	8.5 ± 8.3

* Values are the mean ± SD (see Table 1 for details of scoring systems used). The placebo-treated group had significantly lower total skin scores at week 12 compared with those in the ketotifen-treated group at the same examination ($P < 0.05$). All other differences were not statistically significant.

treated group was noted for either of these categories. More patients taking ketotifen were noted to have improved, both from the patient's and the physician's perception; however, the numbers were too small to reach statistical significance. No patient developed side effects from ketotifen therapy. Several patients commented about increased hair growth over involved extremities while taking ketotifen, although this was not rigorously quantitated.

Table 3. Patient and physician global assessments at week 24*

	Improved	Worsened	Unchanged
Patient assessment			
Ketotifen-treated group	4	4	4
Placebo-treated group	2	6	3
Physician assessment			
Ketotifen-treated group	2	7	3
Placebo-treated group	0	9	2

* Patients were evaluated at week 24, except for the 2 patients in the ketotifen-treated group who were evaluated at week 18, prior to withdrawing from the trial. Only 11 patients in the placebo-treated group were available for long-term followup.

DISCUSSION

In the pathogenesis of scleroderma, the most critical event appears to be the overproduction of collagen, glycosaminoglycans, and fibronectin by fibroblasts. It is likely that cytokines elaborated by infiltrating cells, such as mononuclear cells and/or mast cells, provide stimulatory signals to enhance expression of matrix production by scleroderma fibroblasts. An increased number of mast cells has been observed in the early and active phase of scleroderma (7–9). Nevertheless, the contribution of mast cells and their products to the characteristic pathology of systemic sclerosis remains speculative.

Mast cells may influence the turnover of extracellular matrix components and enhance connective tissue cell activation, as available in vitro data suggest (5,6,13). Most of the evidence to date promoting mast cell involvement in scleroderma is primarily based on the enhanced local accumulation of mast cells, al-

though ultrastructure analysis may reflect degranulation and secretion of mast cell products (14). Results of animal model experiments suggest that there is activation and degranulation of mast cells immediately prior to the development of cutaneous fibrosis, as indicated in the graft-versus-host disease (GVHD) model of scleroderma (5,15). These highly degranulated mast cells are found not only in animal models of GVHD, but also in skin biopsy specimens from patients with scleroderma (16). In addition, administration of the mast cell–stabilizing agents cromolyn or ketotifen to the tight-skin mouse model of scleroderma resulted in prevention of dermal sclerosis (4).

Many of the mediators released by the mast cell, including histamine, heparin, and the granules themselves, have pro-fibrotic effects on fibroblasts in vitro, as has been reviewed recently by Claman (5). Recent evidence suggests that patients with scleroderma have elevated levels of circulating histamine (17). Mast cells derived from rodents have been found to contain an array of cytokines, including the pro-fibrotic factor transforming growth factor β (18). On the other hand, our laboratory has focused on the ability of mast cell products to promote the breakdown of collagen. Specifically, the neutral protease tryptase, a major specific mast cell–derived product, can activate latent metalloenzymes which degrade collagen and other matrix components (19). Therefore, the release of certain mast cell constituents may potentiate the removal of fibroblast products and, hence, be beneficial in the setting of fibrosis.

It is not known whether the activated mast cell in scleroderma has a role in increasing collagen deposition or enhancing its removal or whether it is merely an innocent bystander (6). In an attempt to gain insight into this question, we performed a double-blind placebo-controlled trial utilizing an oral mast cell–stabilizing agent in patients with early scleroderma. Ketotifen has been shown in previous studies to inhibit dermal mast cell histamine release in individuals with cold-induced urticaria (2) and to inhibit basophil release in vitro (1). Dosages used to treat atopic conditions range from 2 mg/day to 12 mg/day; the side effect of somnolence limits the use of higher dosages (1,2). However, in the present study, dermal mast cell releasability following a number of well-characterized stimuli was not inhibited by this drug at the dosages employed. The reasons for this are not clear. Possibilities include a lack of absorption through the gut in patients with systemic sclerosis or an altered metabolism of the drug in these individuals (data on blood levels were not available). It is also conceivable that the mast cells populating the skin of patients with scleroderma are different and are less responsive to this agent. In fact, preliminary studies analyzing the protease content of mast cells within the skin of scleroderma patients suggest an unusually high percentage of tryptase-positive, chymase-negative mast cells compared with controls (20). Finally, it is possible that we administered too low a dosage to stabilize mast cells in the presence of the relatively high doses of secretagogues used for skin testing.

The original intent of this study, which was to test the hypothesis that mast cells contribute to the pathogenesis of systemic sclerosis, may not have been satisfied despite the negative results of this trial. Since we were unable to obtain evidence for mast cell suppression, this, unfortunately, remains an open issue. Nevertheless, we can conclude from this study that ketotifen administered for 6 months at a dosage of 6 mg/day does not appear to be superior to placebo in the treatment of patients with early systemic sclerosis. Trials using higher dosages for more prolonged periods of time may be worthwhile, considering the safety profile of this drug. Alternatively, we look forward to the development of more potent mast cell–stabilizing agents in the near future.

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