

ORIGINAL ARTICLE

Combination of glucosamine and low-dose cyclosporine for atopic dermatitis treatment: A randomized, placebo-controlled, double-blind, parallel clinical trial

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ABSTRACT: Our recent pilot study showed better outcomes using a combination of low-dose cyclosporine and glucosamine than cyclosporine alone in the treatment of atopic dermatitis (AD). Here, a randomized, placebo-controlled, double-blind, parallel-designed study was planned to compare the efficacy and safety of low-dose cyclosporine and glucosamine combination to low-dose cyclosporine alone for the treatment of patients with moderate to severe AD. AD patients with a Severity Scoring of Atopic Dermatitis (SCORAD) index ≥ 30 were randomly assigned in a 1:1 ratio to receive either cyclosporine 2 mg/kg and glucosamine 25 mg/kg (group A) or cyclosporine and placebo (group B) for 8 weeks. SCORAD indices, serum levels of chemokine ligand 17 and interleukin-31, eosinophil counts, and blood cyclosporine levels were examined before and after treatment. The SCORAD indices for group A ($n = 19$) were significantly reduced after the treatment and a significant correlation between the changes in the SCORAD indices and changes in the serum levels of chemokine ligand 17, but not interleukin-31, was detected. Glucosamine combined with cyclosporine did not increase adverse events and serum cyclosporine levels compared with cyclosporine alone. Therefore, combination of low-dose cyclosporine and glucosamine may be useful to allow the long-term use of cyclosporine in the treatment of patients with moderate to severe AD.

KEYWORDS: atopic dermatitis, CCL17, combination, glucosamine, low-dose cyclosporine, SCORAD

Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterized by pruritus with typical distribution and morphology of the lesions, and various clinical symptoms. While mild

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symptoms can generally be controlled by topical agents, moderate to severe symptoms often cannot. Cyclosporine has been demonstrated to consistently decrease the severity of AD (1) and is recommended as the first-line treatment for AD patients requiring systemic treatment (2–4). Cyclosporine is recommended for use for no more than 1 year, although common adverse events are usually reversed after discontinuation (5). Moreover, discontinuation usually results in a relapse of AD or in rebound phenomenon in more severe cases (6). Low-dose cyclosporine (7,8) or intermittent treatment regimens (9) have been suggested to be ideal for the long-term use of cyclosporine.

Glucosamine, a common constituent of glycosaminoglycans in the cartilage matrix and synovial fluid, is popular for the treatment of osteoarthritis. Glucosamine has a transglutaminase (TGase) 2 inhibitory effect (10). Anti-inflammatory activity of glucosamine or other TGase inhibitors plays a role in their therapeutic effects and complementary properties, which may allow combination therapy, in osteoarthritis (11,12), inflammatory bowel disease (13), and some type of cancers (14). Inhibition of nuclear factor-kappa B (NF- κ B) activation has been suggested as a mechanism of glucosamine to be an effective chemosensitizer (10). In previous studies, we also identified that glucosamine enhances apoptosis of activated T cells from human and NC/Nga mice with *Dermatophagoides farinae*-induced AD-like lesions by inhibition of NF- κ B activation (15), along with inhibition of Th2 immune response (16). Cyclosporine inhibits nuclear factor of activated T-cell (NFAT) activation. Because both NFAT and NF- κ B are key transcription factors for cytokine gene expression and T-cell function (17,18), a synergistic effect from combination of cyclosporine and glucosamine was expected. Almost devoid of toxicity in glucosamine sulfate also allows a long-term therapeutic use. In fact, the combination of glucosamine with low-dose cyclosporine significantly reduced the clinical scores, with corresponding immune parameter changes, compared with low-dose cyclosporine alone in an AD mouse model (19). Additionally, our pilot study, which compared the therapeutic effects of a combination of low-dose cyclosporine and glucosamine to those of low-dose cyclosporine alone in AD patients, reported better clinical outcomes with combination treatment (20). However, the study was performed as an open, repeated, crossover study of single and combination regimens without a washout period.

Clinical symptoms are the most important outcome to be assessed in AD trials, and validated

instruments should be used to assess the clinical symptoms. Although the Severity Score of Atopic Dermatitis (SCORAD) is considered the best instrument (21,22), additional uniform, valid, and reliable diagnostic criteria must be established (23). Chemokine ligand 17 (CCL17), which is also known as thymus and activation-regulated chemokine TARC, is a Th2-specific chemokine attracting CC chemokine receptor 4-positive (CCR4+) or CCR8+ cells. A role of CCL17 in the pathogenesis of AD has been suggested based on the correlation between serum CCL17 levels and disease activity (24–27). Interleukin-31 (IL-31) has been identified as a pruritogen of AD (28), and in addition to AD, increased IL-31 expression has been reported in other pruritic skin disorders, including allergic contact dermatitis (29,30). IL-31 is expressed primarily in skin-homing cutaneous lymphocyte-associated antigen-positive Th2 cells (31,32), and serum IL-31 levels are preferentially increased in AD, suggesting that IL-31 may represent an objective marker of AD severity (33–35).

Based on these previous results and on our own pilot study, we planned a randomized, placebo-controlled, double-blind study as a parallel design to compare the efficacy and safety of the aforementioned combination and cyclosporine alone for treating AD refractory to topical therapy using clinical and immune parameters.

Methods

Patients

Patients aged ≥ 7 years of both sexes with moderate to severe AD (SCORAD index ≥ 30) refractory to topical therapy were included in this study. Diagnosis of AD was based on previously described criteria (36). Exclusion criteria included any prior systemic treatments, including systemic steroids, cyclosporine, and phototherapy within 1 month prior to the study; sensitivity to the study drugs; malignancy or lymphoproliferative disease within the past 5 years; history of psychotic disorders; history of substance abuse; pregnancy, intention to become pregnant during the study period, or lactation; and diseases or concurrent therapies that could interfere with the evaluation of the study. Patients were required to agree to a number of regular visits, according to the experiment schedule.

Study design

This study was designed as a single-center, prospective, randomized, placebo-controlled,

double-blind, parallel clinical trial aimed at evaluating the efficacy and safety of low-dose cyclosporine and glucosamine combination in patients with moderate to severe AD. The study protocol was approved by the institutional review board of Dongguk University Ilsan Hospital, and the study was conducted after obtaining informed written consent from all participants, according to the Declaration of Helsinki principles. Each patient received a unique identification number and randomized assignment from an Interactive Web Response System. Patients and clinicians were blinded throughout the study concerning treatment assignments.

Intervention

Patients were randomly assigned in a 1:1 ratio to receive either cyclosporine (25 mg/capsule; Cipol-N®, Chongkundang, Seoul, South Korea) and glucosamine sulfate (312.5 mg/capsule; group A) or cyclosporine and placebo (group B) according to a randomization schedule prepared by the Biomedical Research Institute in Seoul National University Hospital (Seoul, South Korea). Glucosamine and the placebo were produced by Dong-A Pharmaceutical Co. (Seoul, South Korea) as capsules identical in appearance. The daily doses of cyclosporine and glucosamine were 2 and 25 mg/kg body weight, respectively. The dose was determined by the body weights of each patient at the time of enrollment, with rounding up or down to adjust to the nearest appropriate dose as needed. Readjustment of the dose caused by changes in body weight during the study was not needed for any patient. The assigned drugs, which were given to each patient in a prepacked envelope every 2 weeks, were administered orally as twice-a-day-divided doses for 8 weeks. No other topical or systemic treatment was allowed during the study. Patients were provided the same kind of emollient to apply to the whole body twice daily, and received instructions for its use.

Clinical efficacy assessment

The treatment outcomes were assessed using the SCORAD index (37) as the efficacy endpoint. The SCORAD was measured every 2 weeks just before administration of each treatment by two independent dermatologists who were blinded to the treatment assignment (SY Jin, WS Lim). Clinical efficacy was evaluated by the percent change of the SCORAD index for each period.

Quantitation of CCL17 and IL-31

Peripheral blood from patients was drawn into K2EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and quickly mixed by inversion. The plasma was separated from the blood by centrifugation for 10 minutes at 3000 rpm and stored at -80°C before analysis. A commercial enzyme-linked immunosorbent assay kit (R&D systems, Minneapolis, MN, USA) was used for measurement of the CCL17 and IL-31 levels.

Safety assessment

Adverse events and vital signs were recorded at each visit, and it was determined whether the event was directly related to the drug administration. Laboratory examinations, such as complete blood count with eosinophils and blood creatinine levels, were performed before and at the end of the treatment period. The serum cyclosporine levels were measured immediately after completion of the treatment.

Statistical analyses

Demographic findings and characteristics are expressed as either mean $\pm$ standard deviation or absolute counts. Comparisons of categorical data between the two groups were assessed by the chi-square test or Fisher exact test. Comparisons of continuous variables between the two groups were performed using the Student's *t*-test or Mann-Whitney *U*-test. Efficacy and safety analyses included patients who completed the study and all randomized patients (intent-to-treat analysis), respectively. Comparisons of the percent changes of SCORAD and CCL17 and IL-31 levels were performed using the Student's *t*-test. The correlations between the percent changes of SCORAD and immunological parameters were analyzed using Pearson correlation and single linear regression analyses. Last observation carried forward imputation was used for missing values. Statistical analysis of the clinical and laboratory data was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). All comparisons were two-tailed, and *p*-values <0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of patients

Sixty-four patients were screened between November 2012 and June 2013. A total of 43 patients were

enrolled in the present study. Twenty-two patients were randomly allocated to group A, and 21 to group B. Three patients in group A and two in group B were lost to follow-up without starting treatment. A total of 33 patients (16 in group A and 17 in group B) out of the 38 who started the assigned treatment completed the study.

The demographic and clinical characteristics of the 33 patients who completed the study are summarized in Table 1. Ten patients were men and six were women in group A, whereas nine patients were men and eight were women in group B. The patient ages in group A ranged from 12 to 46 years with a mean age of 21 years, and those in group B ranged from 9 to 49 years with a mean of 19 years. Lesions were widely distributed, with a mean SCORAD index of 59.9 ± 10.6 (range, 44.2–75.0) in group A and 56.1 ± 9.0 (range, 38.2–72.8) in group B. No differences in the other baseline demographic and clinical characteristics, including body weight, height, vital signs, and other atopic disease history, were observed between the groups.

SCORAD index change

In group A, 15 (93.8%) of 16 patients experienced clinical improvement, with >50% reduction of the SCORAD index observed in four (25.0%) patients. The mean SCORAD index after 8-week treatment was 40.1 ± 14.6 , with a mean change of $33.7 \pm 18.6\%$. In group B, clinical improvement was noted in 10 (58.8%) of 17 patients, with >50% reduction of the SCORAD index noted in only one (5.9%) patient. The mean SCORAD index after 8-week treatment was 47.9 ± 14.9 , with a mean change of $14.5 \pm 23.9\%$. The percent changes of the SCORAD index between the two groups showed a significant ($p < 0.05$) difference at 6 and 8 weeks (FIG. 1).

CCL17 and IL-31 levels

Blood sampling was performed in 15 patients from group A and 16 patients from group B because of refusal of two patients. Serum levels of CCL17 before and after the treatment were 1033.5 ± 1459.0 and 943.2 ± 1641.8 pg/mL, respectively. Percent changes of CCL17 levels paralleled with those of SCORAD in 14 of 23 patients with clinical improvement and in seven of eight patients without clinical improvement, showing a correlation coefficient (r) of 0.52 and a significant ($p < 0.05$) correlation between clinical efficacy and CCL17 level changes (FIG. 2a). Serum levels of IL-31 before and after the treatment were 5.6 ± 6.4 and 7.1 ± 8.0 pg/mL, respectively. Correlation in IL-31 changes was noted in only seven of 23 patients with clinical improvement, and in four of eight patients without clinical improvement. No significant correlation between clinical efficacy and IL-31 level changes was observed, with a correlation coefficient (r) of 0.29 (FIG. 2b).

Adverse events

Among the 19 intention-to-treat patients in each group, three from group A and four from group B experienced adverse events, with abdominal pain being the most common adverse event ($n = 3$). The pain was too severe to continue medication in one patient in group A and one patient in group B, who were sisters. On the other hand, the pain in another patient treated with cyclosporine was not severe enough to discontinue medication. Mild headache and hypertrichosis developed in one patient per event from group B. Upper respiratory tract infection and varicella developed in one patient per event from group A, resulting in discontinuation of the medication.

Table 1. Baseline demographic and disease characteristics

	Group A Cs + glucosamine ($n = 16$)	Group B Cs + placebo ($n = 17$)	p -value
Sex			
Number of male patients	10	9	
Number of female patients	6	8	
Age, years (mean $\pm$ SD)	12–46 (20.6 ± 9.5)	9–49 (19.5 ± 9.8)	0.63
Body weight, kg (mean $\pm$ SD)	51.0–120.1 (69.1 ± 19.6)	34.2–112.2 (64.3 ± 18.1)	0.56
Body height, cm (mean $\pm$ SD)	145.7–174.6 (163.4 ± 9.2)	140.0–181.6 (160.2 ± 13.3)	0.44
SCORAD index (mean $\pm$ SD)	43.9–75.0 (59.9 ± 10.6)	38.2–72.8 (56.1 ± 9.0)	0.28
History of other allergic diseases			
Asthma	2	3	0.68
Allergic rhinitis	5	9	0.21

Cs, cyclosporine; SCORAD, Scoring of Atopic Dermatitis; SD, standard deviation.

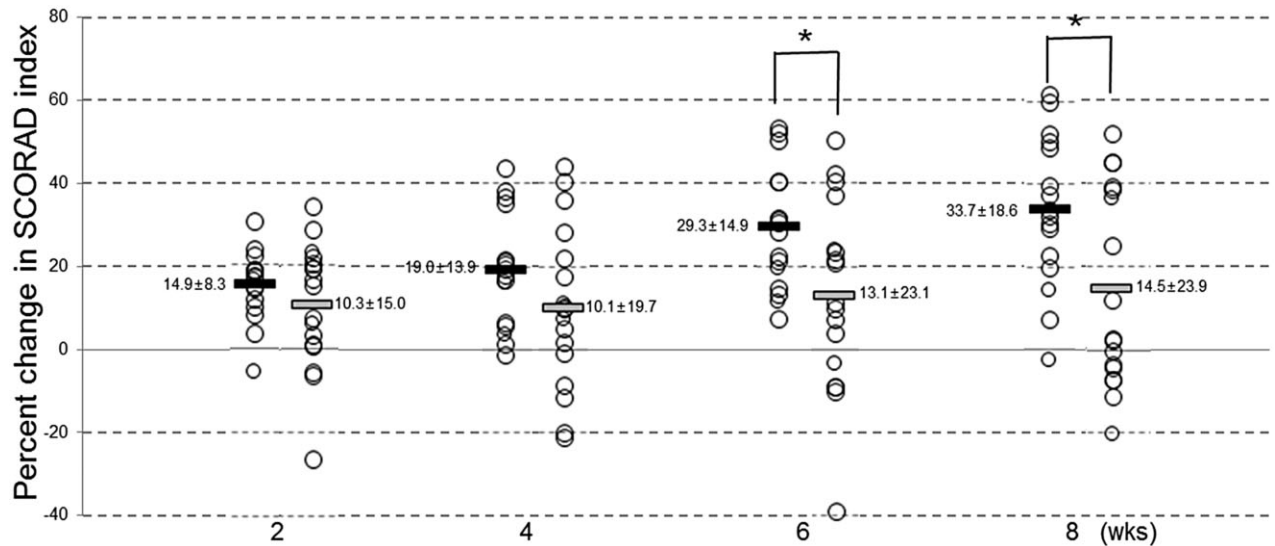


FIG. 1. Efficacy of cyclosporine and glucosamine sulfate combination treatment for moderate to severe atopic dermatitis. Percent improvement in the SCORAD index by cyclosporine and glucosamine (group A) and cyclosporine and placebo (group B) every 2 weeks for 8 weeks of treatment. Black and gray bars represent the mean of each treatment period in group a and group b, respectively. Values nearby each bar are presented as mean $\pm$ standard deviation. * $p < 0.05$ versus group B.

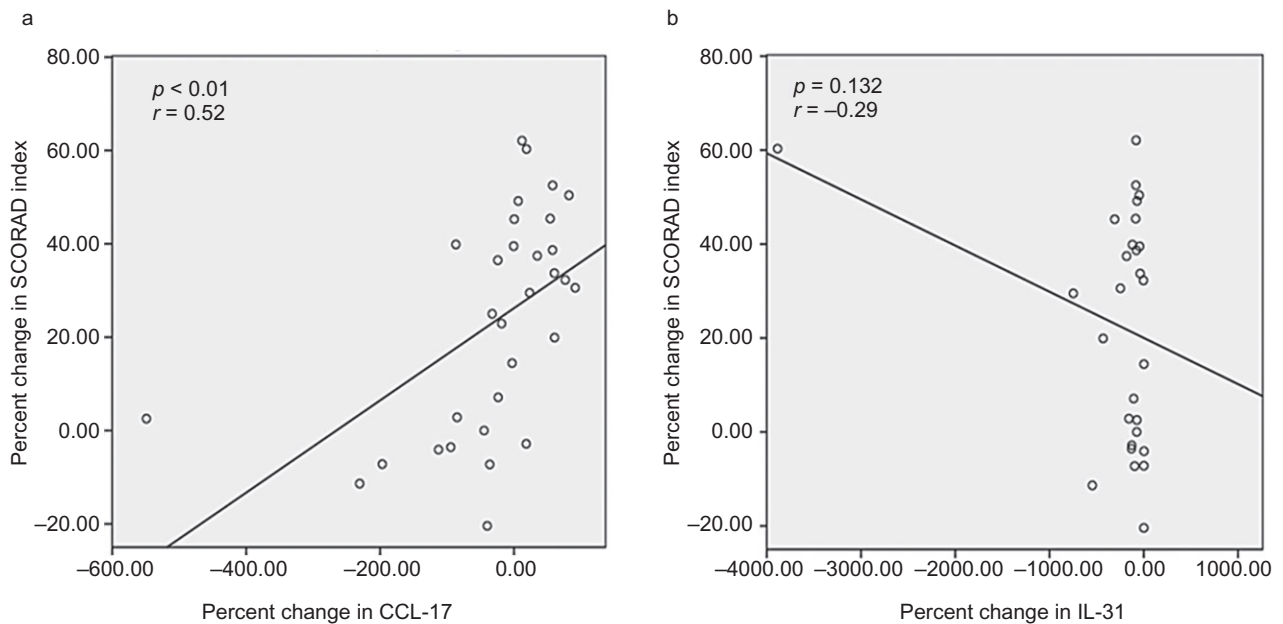


FIG. 2. Correlation between percent changes of SCORAD index and CCL17 and IL-31 levels. Each circle represents the percent changes in SCORAD and CCL17 (a) or SCORAD and IL-31 (b) of each individual patient. A correlation coefficient (r) over 0.5 was considered as a positive correlation.

No participant showed hypertension or abnormal laboratory examination results, including serum creatinine. The levels of serum cyclosporine were 15.5–143.8 ng/mL (mean, 51.3 ± 34.5 ng/mL) in group A and 10.2–380.1 ng/mL (mean, 88.2 ± 128.4 ng/mL) in group B. One patient in group A had serum cyclosporine levels between 100 and 200 ng/mL and three patients in group B had levels >200 ng/mL.

Discussion

Reduction of cyclosporine doses has been suggested as an ideal way to allow long-term cyclosporine use with fewer adverse effects (7,9). Although low-dose cyclosporine usually indicates a dose of 3.0 mg/kg/day, 2.5 mg/kg/day has also been tried, with or without dose adjustment (1). We here examined whether cyclosporine at a dose of

2.0 mg/kg/day was effective for the treatment of AD based on the results of our previous pilot study (20). Our results showed that the efficacy of cyclosporine alone was not encouraging, with no significant symptomatic improvement and percent changes of approximately 14% (FIG. 1). On the other hand, combination of cyclosporine 2.0 mg/kg/day with the maximum allowed therapeutic dose (25 mg/kg/day) of glucosamine significantly improved the effects of cyclosporine on the clinical symptoms of AD (percent change, 34%; FIG. 1). Among 15 clinical trials described in a recent meta-analysis (1), a randomized controlled trial examining the use of low-dose (3 mg/kg) cyclosporine for 8 weeks found that the treatment resulted in a 48% reduction in mean severity, as measured by total body severity assessment (38). An open-label uncontrolled study investigating the use of 2.5 mg/kg cyclosporine for 48 weeks without dose adjustments for the first 8 weeks reported a 39% reduction in mean Six Area Six Sign Atopic Dermatitis (39). Considering the concurrent use of topical steroids in these trials, but not in our study, the efficacy of the glucosamine combination in this study may not be lower than that in these other trials.

Serum IL-31 changes did not show any correlation with clinical improvement in this study (FIG. 2b), although IL-31 levels have been previously related with Th2 cells and suggested as a marker of AD severity (33–35). However, similar to the results of previous studies (24–27), changes in serum CCL17 levels here showed a significant correlation with clinical efficacy (FIG. 2a), supporting the notion of an important role of Th2-mediated immune responses in AD.

Adverse effects were noted in seven of the 38 intention-to-treat patients. Abdominal discomfort was reported by three patients, and two of these received cyclosporine alone. Moreover, headache and hypertrichosis developed in two patients treated with cyclosporine alone. Because these symptoms disappeared after discontinuation of the medication in all patients, they were considered to be caused by cyclosporine. Whereas gastrointestinal symptoms are common adverse events of cyclosporine, along with hypertension, renal dysfunction, and hypertrichosis (5), the causality relations were conversely unclear in two patients with upper respiratory tract infection and varicella treated with the drug combination.

The trough levels of serum cyclosporine, which are supposed to be examined after 8–12 h of fasting, have been reported to be 75.3 ng/mL, 66.4 ng/mL, and 96.5 ng/mL after 100 mg/day,

150 mg/day, and 200 mg/day cyclosporine administration, respectively (40). Here, we found that the mean serum concentrations did not increase after 100 mg/day cyclosporine administration (51.3 ng/mL in group A and 88.2 ng/mL in group B). Blood drawn immediately or a few hours after a meal may have caused the abnormal serum levels (40) observed in three patients treated with cyclosporine alone and in one patient treated with the combination regimen. Although further studies remain to be clarified, this result suggested that glucosamine did not change serum cyclosporine levels and that the better efficacy of the combination treatment compared with that of cyclosporine alone was likely not related to glucosamine-induced increases in serum cyclosporine levels. Drug–drug interaction between cyclosporine and glucosamine has also not been reported.

This study was performed as a randomized, double-blind, placebo-controlled, parallel design, although the number of participants was relatively small. Unlike in previous reported trials, including our own open, crossover pilot study (20), all participants were recommended to apply the same emollient, but not topical steroids. These different conditions may have eliminated bias from the repeated crossover and the effects of topical steroids. Therefore, it became more obvious that the combination involving glucosamine significantly improved the efficacy of low-dose cyclosporine in AD patients. In addition, changes in serum levels of CCL17, a potential biomarker for AD, were found to significantly correlate with clinical improvement. Overall, the reliable evidence for combination efficacy suggested that the glucosamine and low-dose cyclosporine combination may be a useful treatment strategy for moderate to severe AD.

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Conflict of interest

None declared.

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