

ORIGINAL ARTICLE

# Combination of glucosamine improved therapeutic effect of low-dose cyclosporin A in patients with atopic dermatitis: A pilot study

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## ABSTRACT

Both glucosamine and cyclosporin have been reported to show immunomodulatory effect with inhibition of each different key transcription factor for cytokine gene expression and T-cell function. The overall purpose of this pilot study was to assess the feasibility of the combination of cyclosporin with glucosamine for the treatment of patients with atopic dermatitis. Twelve patients more than 12 years old who required systemic cyclosporin were included in the study. Two of them dropped out due to violation of medication schedule. The single (S) and combination (C) regimens were crossed over every 2 weeks without a washout period between the cross-over for 6 months. Five patients were randomly assigned to the S regimen first (SC sequence), whereas the other five were given the C first (CS sequence). The change of SCORAD index was analyzed as the primary efficacy end-point by general linear model and piecewise linear mixed model. The SCORAD index was reduced with both SC and CS sequence regimens. In particular, index reduction with the C was more than that associated with S regimen; this difference increased as time lapsed. The glucosamine combination was predicted to cause an additive decrease in the mean percent change of the SCORAD index (~6%), with decreasing interleukin (IL)-4 and IL-5 cytokine levels but without increasing treatment-related adverse events. This study suggests that the C would produce better clinical outcomes than the S regimen in patients with atopic dermatitis, although confirmatory clinical trials are warranted to determine the effect of combination.

**Key words:** atopic dermatitis, combination, cyclosporin, glucosamine, SCORAD.

## INTRODUCTION

Atopic dermatitis (AD) is associated with a wide range of clinical symptoms.<sup>1</sup> Because systemic steroids are recommended for limited cases of acute flare-ups as short therapeutic courses, the steroid-sparing effect of cyclosporin has been considered to be a significant advantage in AD.<sup>2</sup> However, it is recommended that cyclosporin be continually used for no more than 1 year. There are few studies which have assessed the long-term effectiveness and safety of cyclosporin for treating AD. Discontinuation has usually resulted in a relapse of AD or rebound phenomenon in worse cases.<sup>3</sup> Therefore, developing a new method to reduce these effects would be worthwhile, although common adverse events including hypertension, renal dysfunction, hypertrichosis and gastrointestinal effects are usually reversed after discontinuation.<sup>4</sup> In fact, dosage reduction of cyclosporin has been found to overcome adverse events.<sup>2,5</sup>

Glucosamine has been used for patients with osteoarthritis.<sup>6,7</sup> The suppression of inflammatory activity may result from the potential immunoregulatory capability of glucosamine.<sup>8–10</sup> Moreover, glucosamine has inhibited transglutaminase 2-mediated nuclear factor (NF)-κB activation,<sup>11</sup> whereas cyclosporin inhibits nuclear factor of activated T-cell (NFAT) activation. Both NFAT and NF-κB are key transcription factors for cytokine gene expression and T-cell function.<sup>12,13</sup> Because glucosamine reduces T-helper (Th)2 cytokines more than Th1 not only in *Der-matophagoides farinae*-induced AD-like lesions of NC/Nga mice but also in peripheral blood leukocytes from patients with AD,<sup>14</sup> the combination of these two reagents may have a stronger effect on Th2 immunosuppression compared to cyclosporin alone. In addition, oral glucosamine administration does not exert any adverse effects on blood, urine or fecal parameters.<sup>15</sup> Therefore, we compared the effect of a combined cyclosporin and glucosamine regimen to that of cyclosporin alone for treating AD recalcitrant to topical therapy.

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## METHODS

### Inclusion and exclusion criteria

Male and female patients more than 12 years old who had AD recalcitrant to topical therapy were included in this study. Patients who had taken any immunosuppressants including cyclosporin within 1 month prior to the study were excluded. Women who were pregnant or breast-feeding, patients with malignant tumors or psychotic disorders, and individuals with diseases or who were receiving concurrent therapies that could interfere with the evaluation were excluded. Patients were required to agree with regular visits at several times according to the experiment schedule. The Institutional Review Board of Dongguk University Ilsan Hospital approved this study. The study was conducted after obtaining informed written consent or assent according to the Declaration of Helsinki Principles.

### Drug administration schedule

The treatment with cyclosporin alone (S) and the combination of cyclosporin with glucosamine (C) were crossed over approximately every 2 weeks for 6 months for each patient. If the S regime was administrated first, C administration followed (SC sequence), whereas patients receiving the C regime subsequently received S (CS sequence). Patients were randomly assigned to receive either sequence regimen. No washout period was included between the cross-over because relapse or rebound exacerbation of AD with abrupt discontinuation of the administrated drugs was of concern. Less than 3 mg/kg per day of cyclosporin was administrated to the patients throughout the period, with or without 1000 mg/day of glucosamine sulfate. Blood levels of cyclosporin were measured in five patients both with and without the glucosamine combination by radioimmunoassay. All patients were allowed to apply the prescribed low-potency corticosteroid ointment.

### Outcome assessment

The treatment outcomes were assessed with the SCORAD index<sup>16</sup> as the primary efficacy end-point. The SCORAD was measured every 2 weeks just before the administration of each treatment by two independent dermatologists who were blinded to the treatment assignment. The percent change of the SCORAD index for each period was calculated as follows:

$$\frac{\% \text{Change of SCORAD index for each period} = \frac{\text{SCORAD index at period end} - \text{baseline SCORAD index}}{\text{baseline SCORAD index}} \times 100$$

Levels of the cytokines interleukin (IL)-4, IL-5 and interferon (IFN)- $\gamma$ , which were measured in peripheral blood mononuclear cells of patients using real-time polymerase chain reaction, were included as secondary efficacy variables for the assessment. The percent change in the relative amounts of mRNA and the ratios of each cytokine to the amount of glyceraldehyde 3-phosphate dehydrogenase for each period was calculated.

Adverse drug reactions were examined at every visit in all patients.

### Statistical analysis

Demographic findings and characteristics are expressed as either the median and the range or count, and compared using the Mann–Whitney *U*-test or Fisher's exact test according to the treatment groups.

The percent changes of the SCORAD index for the two regimens in terms of the period, sequence and subject nested in the sequence were compared to those calculated with the general linear model approach using SPSS ver. 12. Changes in the SCORAD index associated with the different regimens throughout the treatment period were analyzed with a piecewise linear mixed effect model using NONMEN ver. 6. Based on pharmacokinetics,<sup>17,18</sup> it was assumed that no carry-over effects of glucosamine would be observed when the regimen was changed from C to S. All statistical tests were two-sided and  $P < 0.05$  was regarded as statistically significant.

The cytokine ratio changes were analyzed by the Mann–Whitney test.

## RESULTS

### Demographic and clinical characteristics

Ten patients completed the present study. Eight were male and two were female. Ages ranged 13–41 years with a median of 16.5 years. The lesions were widely distributed with a SCORAD index of  $61.06 \pm 10.83$  (range, 39.5–74.2). The daily dose of cyclosporin administrated was  $2.41 \pm 0.30$  mg/kg (range, 2.0–2.9). The blood concentrations of cyclosporin in the presence or absence of glucosamine were  $31.6 \pm 12.6$  ng/mL (range, 15.5–46.6) and  $31.5 \pm 14.4$  ng/mL (range, 11.7–46.9), respectively. There were no differences in the baseline demographic and clinical characteristics between the five SC sequences and the other five CS sequences (Table 1).

### SCORAD index change between C and S regimen groups

There were no statistically significant differences in terms of sequence, period or subject effects during the total treatment period. However, the index reduction by the C regimen was greater than that by the S; this difference increased over time (Table 2), although no significant association existed between the drug administrated and treatment period. A piecewise linear SCORAD index decline was preferred over a single linear decline to reflect the early rapid and late slow changes of the SCORAD index over time (Fig. 1). The mean percent change in the SCORAD index with the S or C regimen, which was predicted by the piecewise linear model, showed that the glucosamine combination resulted in an additive decrease in SCORAD of approximately 6% (Fig. 2).

Both the S and C regimens were generally tolerable, and no serious adverse events were observed in any of the participants.

### Cytokine ratio change between C and S regimen groups

Cytokine mRNA levels were examined at every visit for the first 3–4 months. The levels of IL-4 and IFN- $\gamma$  could be measured in

**Table 1.** Demographic and clinical characteristics of the patients

|                                  | SEQ     | <i>n</i> | Median   | SD         | Range       | <i>P</i> -value |
|----------------------------------|---------|----------|----------|------------|-------------|-----------------|
| Baseline SCORAD                  | SC      | 5        | 61.06    | 12.96      | (39.5–74.2) | 1.000           |
|                                  | CS      | 5        | 61.06    | 9.79       | (50.4–73.0) |                 |
|                                  | SC + CS | 10       | 61.06    | 10.83      | (39.5–74.2) |                 |
| Age (years)                      | SC      | 5        | 19.20    | 6.91       | (13–28)     | 0.873           |
|                                  | CS      | 5        | 20.20    | 11.71      | (13–41)     |                 |
|                                  | SC + CS | 10       | 19.70    | 9.08       | (13–41)     |                 |
| Cyclosporin dose (mg/kg per day) | SC      | 5        | 2.42     | 0.34       | (2.0–2.8)   | 0.923           |
|                                  | CS      | 5        | 2.40     | 0.30       | (2.1–2.9)   |                 |
|                                  | SC + CS | 10       | 2.41     | 0.30       | (2.0–2.9)   |                 |
| Sex                              | SC      | 5        | Male (4) | Female (1) |             | 1.000           |
|                                  | CS      | 5        | Male (4) | Female (1) |             |                 |
|                                  | SC + CS | 10       | Male (8) | Female (2) |             |                 |

CS, combination–single; SC, single–combination; SEQ, sequence; SD, standard deviation.

**Table 2.** General linear model results for the percent change of SCORAD index during treatment periods

| Effect | <i>P</i> -values according to period (month) |        |         |          |          |          |          |          |          |            |            |            |
|--------|----------------------------------------------|--------|---------|----------|----------|----------|----------|----------|----------|------------|------------|------------|
|        | 1 (0.5)                                      | 2 (1)  | 3 (1.5) | 4 (2)    | 5 (2.5)  | 6 (3)    | 7 (3.5)  | 8 (4)    | 9 (4.5)  | 10 (5)     | 11 (5.5)   | 12 (6)     |
| DRUG   | 0.4119                                       | 0.6643 | 0.0272* | 0.0099** | 0.0027** | 0.0011** | 0.0031** | 0.0005** | 0.0002** | <0.0001*** | <0.0001*** | <0.0001*** |
| SEQ    | NA                                           | 0.3222 | 0.8183  | 0.7377   | 0.3337   | 0.1548   | 0.2692   | 0.6618   | 0.8016   | 0.8445     | 0.1288     | 0.7888     |
| PER    | NA                                           | 0.0882 | 0.0824  | 0.1617   | 0.2529   | 0.0353   | 0.0556   | 0.0927   | 0.0991   | 0.1342     | 0.1985     | 0.1972     |
| SUB    | NA                                           | 0.9867 | 0.9793  | 0.9053   | 0.9872   | 0.9394   | 0.9802   | 0.9722   | 0.9423   | 0.8683     | 0.9811     | 0.9806     |

\* $P < 0.05$ ; \*\* $P < 0.01$ , \*\*\* $P < 0.0001$ . DRUG, single versus combination regimen effect; NA, not available; SEQ, combination–single versus single–combination sequence effect; PER, period effect; SUB, subject effect.

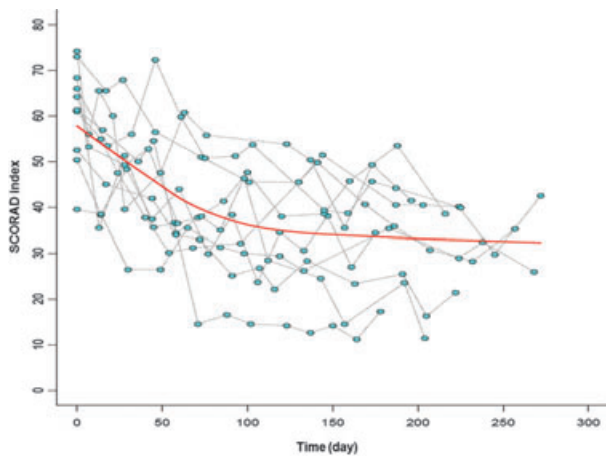


**Figure 1.** Plot for SCORAD versus time data; lines to connect all data points in each patient are shown. When the SCORAD versus time data were graphically expressed, a piecewise-linear decline of SCORAD was preferable to a linear decline. Moreover, the declines of SCORAD fluctuated according to either S (cyclosporin alone) or C (combination of cyclosporin and glucosamine) treatment regimens. Red solid line indicates the less smooth regression line for SCORAD data.

all patients, whereas those of IL-5 were detectable in only five. Despite the smaller number of detectable IL-5 levels, both IL-4 and IL-5 levels in the C regimen decreased significantly more

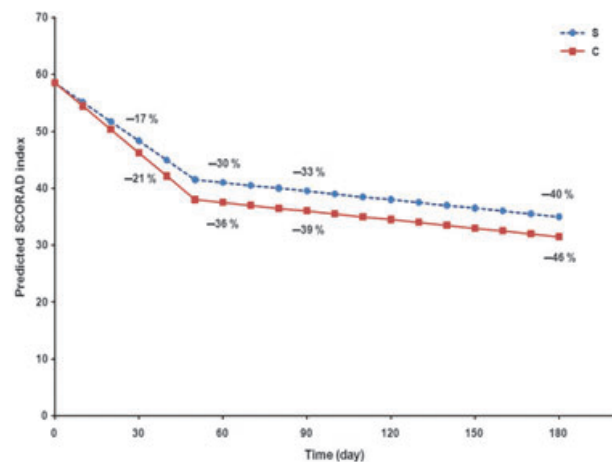


**Figure 2.** Plot for mean predicted SCORAD indices and percent changes from baseline SCORAD indices at 1, 2, 3 and 6 months after S (cyclosporin alone) and C (combination of cyclosporin and glucosamine) administration in patients with severe atopic dermatitis.

than those in the S regimen ( $P = 0.028$  and  $P = 0.01$ , respectively). In contrast, IFN levels were not significantly different between the S and C regimens.

## DISCUSSION

Dosage reduction of cyclosporin has been proposed to overcome concerns about the long-term safety of this drug<sup>4</sup> along with AD relapse.<sup>3</sup> In our study, approximately half of the recommended dose ( $2.41 \pm 0.30$  mg/kg), a little less than the so-called low-dose (3 mg/kg),<sup>19</sup> was used. In fact, the blood levels of cyclosporin were much less than 200 ng/mL, which has been recommended to minimize renal toxicity for long-term therapy.<sup>20</sup> Although the doses we administered for our study reduced the SCORAD index to a lesser degree than the low-dose regimen used in a systemic review and meta-analysis (~30% in 2 months),<sup>19</sup> the glucosamine combination reduced the SCORAD significantly more than cyclosporin alone. Moreover, the combination could discontinue cyclosporin without abrupt exacerbation of AD in half of the patients (data not shown). The significant reduction in both IL-4 and IL-5 levels, but not IFN- $\gamma$ , supported the efficacy of the glucosamine combination in patients with AD.

Glucosamine is one of the most popular dietary supplements and is widely used to treat osteoarthritis. Despite controversial clinical trial results, uncommon and minor adverse effects during long-term treatment<sup>21,22</sup> have led to several clinical disorders, such as poor wound healing, skin wrinkles and skin hyperpigmentation.<sup>23</sup> No adverse reactions were experienced in this study. Although a potential glucosamine-warfarin interaction has been reported,<sup>24</sup> glucosamine has no significant effect on cytochrome P450 (CYP) activity.<sup>25</sup> Therefore, no drug-drug interactions are expected between glucosamine and cyclosporin, which is metabolized by CYP3A.<sup>26</sup> In fact, the blood concentrations of cyclosporin did not change significantly by the glucosamine combination used in this study.

This pilot trial was performed to prepare a full-scale comparative study, because no clinical trial data of glucosamine was available in the patients with AD. Feasibility of the crossover between the two regimens was confirmed from this pilot study. Additionally, analysis of data from the repeated crossover suggested clinical benefit of combining glucosamine with low-dose cyclosporin. Although no washout period was included to prevent relapse or rebound, the 3-day period was sufficient to eliminate glucosamine from the body to analyze and compare the effect between treatments with S and C regimens. Taken together, this study indicated that a simultaneous administration of glucosamine could be another way to reduce the effective dose of cyclosporin. Therefore, the efficacy and safety of the combination regimen is warranted to be assessed in a full-scale clinical trial.

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