EXTENDED REPORT

Dehydroepiandrosterone suppresses interleukin 10 synthesis in women with systemic lupus erythematosus

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Objective: To study the effects of dehydroepiandrosterone (prasterone, DHEA) 200 mg/day on cytokine profiles in adult women with active systemic lupus erythematosus (SLE).

Methods: In a double blind, randomised, placebo controlled study conducted as part of a larger multicentre study, 30 adult women with active SLE received oral DHEA 200 mg/day or placebo for 24 weeks. Baseline prednisone (<10 mg/day) and other concomitant SLE medications were to remain constant. The levels of cytokines including interleukin (IL) 1, IL2, interferon γ , IL4, and IL10 were determined by ELISA. The mean change from baseline to 24 weeks of therapy was analysed.

Results: The two groups (DHEA $n=15$; placebo $n=15$) were well balanced for baseline characteristics. Only IL1 β and IL10 could be detected in the serum of lupus patients; however, there was no significant mean (SD) difference in serum IL1 β before and after treatment (9.94 (8.92) v 9.20 (6.49) pg/ml). IL10 demonstrated a greater and significant reduction from baseline (9.21 (9.66) to 1.89 (1.47) pg/ml in the DHEA treatment group).

Conclusions: In a 24 week study of adult Chinese women with mild to moderate SLE, treatment with DHEA 200 mg once daily resulted in significant reduction of serum levels of IL10. This finding may suggest why DHEA could significantly reduce lupus flares.

Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease characterised by alterations in T cell, B cell, and accessory cell function, which facilitates polyclonal B cell activation, autoantibody production, and an inflammatory response in various organs.¹

Although the aetiology of SLE is unknown, hormonal influences may play a key role in disease development and progression. Several studies have noted alterations in oestrogen and androgen metabolism in patients with SLE, including decreased levels of androgens (androstenedione, dehydroepiandrosterone, dehydroepiandrosterone sulphate, and testosterone) in female patients with SLE, especially in those with active disease.^{2–3}

Dehydroepiandrosterone, an adrenal steroid, is secreted primarily as its sulphated metabolite, DHEA sulphate (DHEA-S).⁴ DHEA is believed to have mild intrinsic androgenic properties, and in peripheral tissues, both it and DHEA-S can be converted to various other androgens, including androstenedione and testosterone.^{5–7}

Two clinical studies conducted at Stanford University, one an open label study and the other a double blind, placebo controlled study, suggested that DHEA administered orally as daily doses of 200 mg is well tolerated and may have steroid sparing effects and reduce flares in patients with mild to moderate SLE.^{8–9} Furthermore, in a subsequent multicentre phase II/III double blind, randomised, placebo controlled study for women with active SLE disease activity, DHEA 200 mg/day enabled prednisone reduction to physiological levels in a significantly greater proportion of patients than did placebo, while maintaining or improving overall SLE disease activity.¹⁰ In another study in which 381 women with mild/moderate SLE were double blind treated with DHEA 200 mg/day or placebo for 12 months, it was demonstrated that DHEA improved or stabilised SLE disease and its symptoms without clinical deterioration, and prevented loss of bone mineral density in a significantly greater proportion of patients than did placebo.^{11–12} Our recent studies¹³ also showed that DHEA was well tolerated and significantly

reduced flares and improved patient global assessments in women with SLE. However, the mechanisms of action of DHEA for SLE control are not yet well known.

Many investigators have presented data demonstrating that T cells and T cell derived cytokines play a critical role in driving B cell differentiation and autoantibody production in SLE.^{14–15} In addition, increasing evidence suggests that proinflammatory cytokines, such as interleukin (IL) 1 and tumour necrosis factor (TNF) α , play an important role in promoting tissue damage in SLE.¹⁶

The aim of the current study was to evaluate the role of cytokines in successful treatment of SLE with DHEA.

PATIENTS AND METHODS

Study design

This was a randomised, double blind, placebo controlled study conducted as a substudy within a larger multicentre study.¹³ Patients enrolled were adult women with SLE according to American College of Rheumatology criteria,¹⁷ who were receiving prednisone dose (or its corticosteroid equivalent) at study entry of 0 to 10 mg/day. Patients had to have active SLE, defined as a Systemic Lupus Activity Measure 18 score of ≥ 7 and baseline Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score >2 .¹⁹

Patients treated with hydroxychloroquine, azathioprine, methotrexate, or cyclophosphamide, or a combination of these had to have been on a stable regimen with no change in dose and drug combination for at least 6 weeks prior to study entry. Patients who were receiving androgens, immunoglobulins, ciclosporin A, or other immunosuppressive agents except those noted were excluded. After a 10 day screening and qualifying baseline period, patients were assigned by predetermined randomisation code to receive DHEA

Abbreviations: DHEA-S, dehydroepiandrosterone sulphate; IL, interleukin; PBMC, peripheral blood mononuclear cell; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; TNF, tumour necrosis factor

200 mg/day (provided by Genelabs Biotechnology Co Ltd) or placebo for 24 weeks.

Physicians and patients were instructed to make all efforts to keep doses of prednisone and other SLE medications constant during the study. Prednisone dosage was permitted to increase up to 10 mg/day over baseline dosage for up to 2 weeks, but only if medically required. Changes in non-steroidal anti-inflammatory drugs were allowed, but only if medically required and approved by the treating physician.

The protocol was approved by the institutional review board. All patients gave written informed consent.

Patient grouping

A total of 32 patients was randomised into the study; 17 patients received DHEA 200 mg/day and 15 patients received placebo. Fifteen (88%) of the patients receiving DHEA and 15 (100%) of the patients in the placebo group completed the study.

Hormone and cytokine determination

Routine laboratory and hormone assays were conducted by Mithra Bioindustry Company Ltd, Taipei, Taiwan. Various serum cytokines including IL1 β , IL2, IFN γ , IL4, and IL10 in SLE patients were measured using ELISA kits (R&D, Minneapolis, MN, USA) according to the manufacturer's instructions. The lowest detection levels were: IL1 β , 0.5 pg/ml; IL2, 1.6 pg/ml; IFN γ , 15.6 pg/ml; IL4, 0.25 pg/ml; and IL10, 0.781 pg/ml. Serum testosterone, oestradiol, and cytokine profiles were measured at baseline and last visit.

Statistical analysis

Wilcoxon matched paired signed ranks test was used, and level of significance was set at 0.05.

RESULTS

Baseline characteristics demographics

The patient population consisted of Chinese women. There were only two patients (one in each group) with a baseline SLEDAI score of <3.

The two treatment groups were well matched for age, baseline prednisone dose/use, menopausal status, cytotoxic use, and antimalarial use. The groups were also well matched with regard to values at baseline for scoring instruments (data not shown). The prednisone dose at study completion in DHEA and placebo group was 7.2 (2.8) *v* 6.3 (3.9) mg/day. There was no statistical difference (*p* = 0.4175) compared with the dose at study entry (table 1).

Hormone determination

Oestradiol decreased in both groups. The mean decrease was slightly larger for the DHEA group (48 *v* 24 pg/ml). As the study population comprised mainly premenopausal women, and measurements were not timed in relation to menses,

Figure 1 Hormone determination from baseline to last visit. (A) Change in oestradiol (B) change of testosterone. Data are means. The *p* value of the change in testosterone between groups was <0.05 (one way analysis of variance).

changes in mean and median oestradiol levels were probably related to variability in these data and were not of clinical relevance. Mean testosterone levels increased in the DHEA group and decreased in the placebo group (470 ng/l compared with -70 ng/l, respectively) (fig 1). Most patients in both treatment groups had DHEA-S levels of 0–2 g/l at baseline. At the post-baseline visits, approximately 60% of patients in the DHEA treatment group had levels of >10 mg/l. The levels for the remaining 40% were distributed over each of the lower 2 g/l incremental ranges (data not shown). There was no evidence of elevated DHEA-S levels in the placebo group at baseline or on subsequent visits.

Cytokine determination

IL10 was significantly higher at the last visit in the placebo treated group compared with the DHEA treated group (placebo 9.06 (7.46) *v* DHEA 1.89 (1.47) pg/ml; *p* = 0.045) (fig 2). In addition, the reduction in IL10 from baseline to the last visit was significant within the DHEA treated group, with mean baseline concentration of 9.21 (9.66) and mean last visit concentration of 1.89 (1.47) pg/ml (*p* = 0.005) (fig 2).

Table 1 Demographic summary by treatment group

	DHEA (n = 15)	Placebo (n = 15)	p
Age (years)			
Mean/median (SD)	33.3/32.0 (10.2)	32.0/32.0 (9.6)	0.672
Range	18.0 to 62.0	18.0 to 59.0	
Prednisone dose (mg/day)			
Mean/median (SD)	7.0/7.5 (3.1)	6.7/5.0 (3.1)	0.732
Range	0.0 to 10.0	0.0 to 10.0	
Pre-menopausal	14 (93.3%)	14 (93.3%)	1.000
Cytotoxic use at baseline	6 (40.0%)	6 (40.7%)	0.940
Prednisone use at baseline	14 (93.3%)	14 (93.3%)	1.000
Antimalarial use at baseline	9 (65.0%)	10 (66.6%)	0.602

*The *p* values for continuous variables were obtained with one way analysis of variance, and *p* values for categorical variables from a χ^2 test.

Figure 2 Comparison of cytokine profiles. (a) Change in IL1 β after treatment for DHEA and placebo groups. Patients treated with DHEA showed an average decrease of 0.74 compared with patients with placebo, who showed an average increase of 1.8. (b) Change in IL10 in the two groups. Patients treated with DHEA revealed a statistically significant decrease ($p < 0.01$) compared with their own baseline. The changes from baseline between the DHEA and placebo groups also reached statistical significance ($p < 0.05$).

DISCUSSION

The potential benefit of using DHEA in the treatment of SLE was suggested by several reports, including the notion that androgens might favourably affect the clinical expression of SLE^{1 20 21} and the observation of low circulating levels of DHEA and DHEA-S in patients with active SLE disease.^{2 3} Furthermore, in the NZB/NZW mouse model of SLE,²² DHEA is associated with significantly delayed onset of disease and a reduction in mortality although the mechanism for the protective effects is not entirely clear.²³ In addition, DHEA may also have immunomodulatory effects, including a shift from Th2 to Th1, suggesting a potential benefit in SLE.^{24 25} Previously, we found significant reductions in the proportion of patients with flare and serious SLE related adverse events, and improvement in patient global assessments in patients who received DHEA compared with those who received placebo.¹³ Multiple mechanisms could be mediating these effects, including favourable changes in the androgen/oestrogen ratio. In addition, Th2/Th1 balance and suppression in inflammatory cytokines could be reasonable mechanisms for DHEA effects.

Investigations showed that DHEA may help to regulate immune function in aged female C57BL/6 mice by significantly increasing Th1 cytokines (IL2 and IFN γ) or significantly decreasing Th2 cytokines (IL6 and IL10), thus regulating cytokine production.²⁶ DHEA also suppressed the production of cytokines IL6 and TNF α by Th2 cells that were otherwise stimulated by retrovirus infection.²⁷ Furthermore, preincubation of peripheral blood mononuclear cells (PBMCs) from adult male SLE patients with DHEA reduced IL4 production stimulated by concanavalin A.²⁸

In this double blind study conducted to evaluate the effects of DHEA on cytokine profiles in SLE patients, we studied the balance of Th1/Th2 using IL2, IFN γ , IL4, and IL10, and

found a significant reduction of IL10 in patients' blood after DHEA treatment for 24 weeks. IL6 was not originally included in this study, which in hindsight was possibly not the correct decision.

IL10 is a B cell stimulatory cytokine that also inhibits type 1 cytokine response.^{29 30} Several lines of evidence suggest that IL10 plays a critical role in the immunopathogenesis of SLE. Lupus patients have increased serum levels of IL10, which may correlate with disease activity.³¹ PBMCs from patients with SLE exhibit increased IL10 mRNA expression and increased spontaneous IL10 production.^{32 33} In addition, the peripheral blood of SLE patients contains a significantly increased number of IL10 secreting cells.³⁴ Disease activity in SLE patients correlates with the ratio of IL10 to IFN γ secreting cells.³⁴

Lymphocytes exposed to DHEA were noted to produce increased quantities of IFN γ and IL2, and lesser amounts of other interleukins such as IL4.³⁵ Suzuki *et al* suggested a causal link between low serum DHEA levels and diminished IL2 secretion by activated peripheral blood T lymphocytes.³⁶ The addition of exogenous DHEA to T cells in vitro resulted in a significant restoration of IL2 secretion by such cells, suggesting that the defect in the production of this cytokines was not related to exhaustion but rather to a relative shortage of the androgen. These investigators suggested that the relative abundance of the enzyme DHEA sulphatase, which converts the sulphate ester to the biologically active form in various lymphoid tissues, could determine how much of this immunomodulatory effect occurs and which T cell phenotype would predominate at the level of the local immunological microenvironment.³⁷ This investigation may suggest some mechanistic insights into how DHEA affects IL10 secretion.

There is evidence that PBMCs from SLE patients produce up to 30 times more IL10 than normal PBMCs in vitro, with most of this IL10 being derived from monocytes and B cells. However, the current study did not investigate the effect of DHEA or testosterone on IL10 secretion from B cells or monocytes, and to our knowledge, it has not been described in the literature. The reduction in IL10 levels was caused by increased catabolism or decreased production; which cell type is involved should be investigated further. Park *et al* showed that mean (SE) IL10 levels in SLE patients were significantly higher than those of controls (29.2 (6.8) v 3.5 (0.6) pg/ml). Elevated IL10 levels correlated well with the SLEDAI in SLE patients. The changes in serum IL10 levels also correlated with the changes in the SLEDAI score during the patients' disease course.

Our finding of significant IL10 suppression by DHEA 200 mg daily for 24 weeks in adult Chinese female patients with mild to moderate SLE may add new knowledge to the understanding of how DHEA offers meaningful benefit to SLE patients.

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