

50 mg DHEA was orally administered for 2 weeks. DHEA (25 mg or 50 mg) administration for 1 week or 2 weeks resulted in several fold increase in the serum concentrations of DHEA, DHEA-S and estradiol, while the same treatment did not result in a significant increase in that of testosterone. In addition, somatomedin C, a possible cause of somatopause with aging was slightly increased by 50 mg DHEA administration, suggesting that DHEA may also be effective in the improvement of somatopause (Fig. 2). No significant side effects were observed in respect to symptoms and blood chemistry. Based on these data, we are now planning a long-term trial of DHEA replacement in normal elderly individuals.

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Supplement. Anti-apoptotic Effect of Dehydroepiandrosterone and Its Role in an Aging Society

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Introduction

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are weak androgens produced primarily by the adrenal gland. Although their plasma concentrations exceed by

far those of any other adrenal product, their physiological roles have not yet been determined. Serum levels of DHEA and DHEAS increase until approximately age 25, and then decrease progressively. The decline of DHEAS concentrations with aging has led to the suggestion that DHEAS could itself play a physiological role and be implicated in longevity (1). Epidemiological evidence has shown that adult men with high plasma DHEAS levels are less likely to die of cardiovascular disease (2). Moreover, the pharmacological effects

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in animals such as rodents have demonstrated many beneficial effects, for example increased immune function (3, 4), the prevention of atherosclerosis (5), osteoporosis (6), diabetes, and obesity (7). Studies on the efficacy and safety of DHEA supplementation as a hormone replacement therapy have been reported in some foreign countries (8–10). However, such an evaluation has not yet been conducted in Japan.

DHEA and experimental hepatitis

The immunomodulating effects of DHEA were reported in some studies. Yu et al (3) showed that DHEA administration suppressed allergic airway inflammation in *Dermatophagoides farinae*-sensitized mice. In this dust-mite-induced asthma model administered with DHEA, serum interleukin-4, interleukin-5, and interferon-gamma levels were decreased compared to the control mice. Du et al (4) showed that administration of DHEA suppressed experimental allergic encephalomyelitis in SJL/J mice. In this model, DHEA decreased demyelination/inflammation and the expression of inflammatory cytokines in the central nervous system, which were mediated by the inhibition of NF-kappaB activation.

We evaluated the effect of DHEA in a mice model of experimental hepatitis established by Concanavalin A (ConA). Mice were fed 0.4% DHEA-added food for a week before ConA injection. Administration of DHEA significantly suppressed the elevation of serum alanine aminotransferase (ALT) activity, and tissue damage of the liver was obviously reduced on pathological examination. Gene expression of inflammatory cytokines, such as tumor necrosis factor (TNF)- α , inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, and macrophage migration inhibitory factor (MIF), was significantly reduced. Apoptosis, which was ascertained by DNA laddering on electrophoresis and transferase-mediated dUTP nick end labeling (TUNEL) staining, was also significantly suppressed. This anti-apoptotic effect was mediated by suppression of Fas expression. It was concluded that DHEA could repress ConA-induced experimental hepatitis by means of either an anti-inflammatory effect or anti-apoptotic effect. These effects of DHEA are confirmed *in vitro* using cultured rat hepatocytes.

We evaluated whether DHEA has an anti-apoptotic effect on human peripheral blood cells. Peripheral blood lymphocytes obtained from healthy volunteers were stimulated by Staurosporine and pre-incubated in the presence or absence of DHEA. The apoptotic cell count measured by flow cytometry was significantly reduced by pre-incubated DHEA. The anti-apoptotic effect of DHEA for human peripheral blood lymphocytes was shown in this study.

Anti-diabetic effect of DHEA in animal models

In 1982, Coleman et al (7) revealed that dietary administration of DHEA to insulin-resistant diabetic db/db mice induced remission of hyperglycemia and increased insulin sensitivity. There are some other reports suggesting that DHEA administration to diabetic rodent models increases

insulin sensitivity (11, 12). We evaluated the effect of DHEA on glucose metabolism in diabetic db/db mice under the same conditions as those of Coleman's experiments and compared the results with those of troglitazone administration. Administration of 0.4% DHEA-added food decreased the blood glucose level to the same level as 0.2% troglitazone-added food. Although the serum insulin level was decreased in the troglitazone-treated group, it was increased in the DHEA-treated group.

Activities of both hepatic gluconeogenic enzymes, glucose-6-phosphatase (G6Pase) and fructose-1, 6-bisphosphatase (FBPase), were higher in db/db than in heterozygote littermate db/+m mice. Dietary administration of DHEA or troglitazone for 15 days significantly decreased hepatic G6Pase and FBPase activities in both db/db and db/+m mice. Hepatic G6Pase and FBPase activities showed a linear relationship with blood glucose in all groups of mice, suggesting that the activities of G6Pase and FBPase are closely related to blood glucose levels (13). Expression of the G6Pase gene in db/db mice was significantly elevated compared to that in db/+m mice. In contrast, mRNA level of FBPase was not elevated in db/db mice. Administration of DHEA significantly decreased the elevated mRNA level of G6Pase in db/db mice. No significant change was observed in the mRNA level of FBPase after the administration of DHEA. Administration of troglitazone also decreased the mRNA level of G6Pase in db/db mice although no change was observed in the mRNA level of FBPase. These results suggest that the elevation of G6Pase mRNA is important in elucidating the cause of insulin resistance, and that the G6Pase gene is at least one target molecule of DHEA that functions as an insulin-sensitizing agent in db/db mice (14).

Clinical trial of DHEA supplementation

In some foreign countries, various clinical trials of DHEA supplementation have been reported, e.g., post menopausal women (15–17), elderly men and women (8, 18), and patients with Addison's disease (10, 19, 20). In Japan, clinical evaluation of DHEA supplementation has not yet taken place. We evaluated the efficacy and safety of oral administration of DHEA to healthy volunteers. Seventeen subjects (age 27–62 years, mean 40.6 \pm 9.6 years) were recruited from our department. All subjects were given a single dose of 25 mg DHEA every morning for two weeks. Blood count, biochemical parameters including hepatic and renal function, electrolytes, lipid, uric acid and glucose, and a 2-hour 75 g oral glucose tolerance test (75 g OGTT) were performed before and after the two weeks of DHEA supplementation.

Serum levels of DHEA, DHEAS, estradiol (E2), androstenedione, and free testosterone were appropriate for the age of the volunteers when measured at the baseline examination showing the gradual decrease with age. After administration of 25 mg of DHEA for two weeks, serum hormone levels of not only DHEA, but also DHEAS and androstenedione increased. Moreover, in older subjects who were more than 40 years of age, the serum E2 level also increased after DHEA

supplementation. DHEA supplementation in the older subjects could lead to restoration as high as younger subjects' serum levels of DHEA, DHEAS, androstenedione, and E2. No one experienced any adverse event in their physical condition and mental status. Blood count, hepatic and renal functions, electrolytes, and uric acid showed no significant changes. The serum triglyceride level decreased significantly after DHEA supplementation; however, the serum cholesterol level did not change. The 75g OGTT, in which blood glucose and insulin were measured at 30, 60, 90, and 120 minutes after glucose loading, was performed before and after DHEA supplementation. The blood glucose and insulin measurements taken after DHEA supplementation exhibited no significant change compared to those before DHEA supplementation. However, a negative correlation was observed between the serum DHEA level and homeostasis model assessment (HOMA-R) score. This correlation was significant both before and after DHEA supplementation. Therefore, these observations suggested that lower serum DHEA levels may predict insulin resistance. The correlation against HOMA-R was not observed in serum DHEAS, androstenedione, or E2 levels.

Conclusions

Physiological functions of adrenal androgens are not as well known as those of glucocorticoids or mineralcorticoids. However, there are some reports suggesting that DHEA has an anti-inflammatory effect and anti-diabetic effect on experimental animals. Our clinical trial of DHEA supplementation supported these findings. Insulin resistance could play a central role in the pathogenesis of metabolic syndromes and their control would lead to a reduction in the risk of coronary heart disease and cerebrovascular disease. Maintaining youthful levels of serum DHEA in aged people has been expected to prevent such diseases in an aging society. As the balance of population in our society progressively shifts to favor seniors, such preventative supplementation has been predicted to increase in significance.

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