SHORT COMMUNICATION

Dehydroepiandrosterone sulfate does not prevent spontaneous and iodine-induced lymphocytic thyroiditis and diabetes mellitus in the BB/Wor rat

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Abstract

Chronic feeding of dehydroepiandrosterone (DHEA) and its sulfated metabolite, dehydroepiandrosterone sulfate (DHEAS), has previously been reported to decrease hyperglycemia, obesity, cancer, and autoantibody generation in a number of animal models and to increase muscle mass and physiological and psychological well-being in elderly humans, although these latter studies remain controversial. The present study was carried out to determine whether large amounts of DHEAS given orally would prevent the occurrence of spontaneous and iodine-induced autoimmune lymphocytic thyroiditis (LT) and/or spontaneous insulin-dependent diabetes mellitus (DM) in male and female BB/Wor rats. DHEAS was administered by gavage (44 mg/rat/day) or in the chow (133 mg/rat/day) to LT- and DM-prone rats from 30 to 120 days of life; some of these rats also received iodine in the drinking water to enhance the incidence and intensity of LT. Onset of DM requiring protamine zinc insulin and its maintenance dose were assessed. Rats were killed at 90 or 120 days of age and blood, thyroid, adrenals, pancreases, testes, and ovaries were removed. Serum glucose, DHEA, DHEAS, thyroxine (T₄), tri-iodothyronine (T₃) and thyrotropin (TSH) concentrations were measured in all rats in both experiments. Serum DHEAS concentrations were 10-fold higher in the rats given the steroid by gavage or in the diet compared with levels in control rats. DHEAS administered over a prolonged period of time had no significant effect on body weight, incidence and severity of DM, incidence and intensity of spontaneous and iodine-induced LT, and thyroid, pancreas and testes weights but did significantly decrease adrenal and ovarian weights. Serum T₄, T₃, and TSH concentrations were similar in control and DHEAS-treated rats. In conclusion, DHEAS did not prevent the occurrence of iodine-induced or spontaneous autoimmune LT or spontaneous DM in the BB/Wor rat, at variance with its reported immunosuppressive effects in other animal models.

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Introduction

It has been suggested that 3-hydroxyandrost-5-ene 17-one (DHEA) and its sulfate ester (DHEAS) are useful in the treatment and prevention of cardiovascular disease (1), high cholesterol (2), obesity (3, 4), cancer (5), Alzheimer's disease (6), insulin-dependent diabetes mellitus (DM) (7–11), and other immune modulated diseases (12–14), and in slowing the aging process and improving memory (15–20). The efficacy of these claims is debatable.

DHEA arises, in part, from the adrenal gland, but mostly as a result of conversion from DHEAS in peripheral tissues. The serum concentrations of DHEA

and DHEAS are low in prepubescent children, rise to peak levels in adults in their 20s, and decrease thereafter as the individual ages (21, 22). The levels of DHEAS in the blood are the highest of any steroid and are three orders of magnitude greater than those of DHEA (23). DHEA and DHEAS have no designated biological function, but are converted to active substances that serve as important regulators of cell and tissue physiology, acting at the cell nucleus to promote synthesis of RNA and proteins (24).

DHEA, when fed chronically to animal models of human diseases, has been reported to have therapeutic value with few adverse effects in animals exposed to large doses even when given over a lifetime (4, 6, 14,

25, 26). However, liver tumors, induction of polycystic ovaries and uterotrophic estrogen effects have been reported (24, 26). Disorders in animals in which beneficial effects have been demonstrated include obesity (4, 27), perhaps by inhibiting glucose-6-phosphate dehydrogenase (28), atherosclerosis (2), cancer (5, 29–32), lupus (33), and DM (8–10, 34). Anti-glucocorticoid actions have also been noted (35).

Studies by Coleman *et al.* demonstrated that three major metabolic products of DHEA, i.e. DHEAS, α -hydroxyetiocholanolone, and β -hydroxyetiocholanolone, and one putative product, 17 β -estradiol, prevented the development of severe DM in genetically diabetic mice (9), suggesting that some of the beneficial effects of DHEA may be mediated by these metabolites. In most studies, DHEA or DHEAS administration exhibits beneficial effects only when fed orally, suggesting that the conversion to active substances occurs within the splanchnic system.

Most of these studies were carried out in mice, with few data in rats. The BioBreeding/Worcester (BB/Wor) rat model has been used extensively to study the immunopathology of autoimmune DM and lymphocytic thyroiditis (LT), similar to Hashimoto's thyroiditis in humans. This rat develops spontaneous DM and LT from approximately 90 to 120 days of age (36–39), with an incidence of approximately 60% for DM and 40% in some LT-prone lines (40). Neither of these spontaneously occurring diseases exhibit a gender-specific incidence (37, 39–41). We have demonstrated that excess dietary iodine doubles the incidence, increases the severity and results in an earlier onset of LT in this model (41), as occurs in the obese strain chicken (42), buffalo rat (43), and non-obese diabetic (NOD) mouse (44).

The present studies were undertaken to determine the effect of DHEAS administration on the development of both DM and LT in the BB/Wor rat.

Materials and methods

Animals

Male and female type I DM-prone BB/Wor rats from the NB subline, 25–30 days of age, were obtained from the colony maintained at the University of Massachusetts Medical Center, Worcester, MA, USA. This study was approved by the Animal Use Committee. Animals in all experiments were weighed and tested for glycosuria (Tes-Tape, Eli Lilly Co., Indianapolis, IN, USA) three times per week beginning at 50 days of age. Criteria for the diagnosis of DM were the combination of glycosuria and weight loss. Rats that developed DM were treated daily with protamine zinc insulin (PZI) 0.2–2.0 units/rat s.c. The dose was adjusted to maintain weight gain and prevent ketosis. To avoid hypoglycemia, trace and 1+ glycosuria were allowed. In addition, rats with ketonuria were treated parenterally

with 6–8 ml 50 meq sodium bicarbonate and Ringer's lactate solutions. Rats were killed and thyroids removed, weighed and placed in Bouin's solution for histological analysis. Blood was collected from the trunk and sera stored frozen at -20°C until assayed. Some groups of rats received 0.05% iodine in their drinking water to enhance the development of LT. Adrenal, pancreas, testes and ovary weights were obtained where reported. DHEAS was purchased from Sigma Chemical Company (St Louis, MO, USA).

Experiment I

Thirty-two male and female BB/Wor rats were divided into two treatment groups as follows: Group I received 500 μl vehicle by gavage daily and water and Purina chow were available *ad libitum*. Group II received 20 mg DHEAS/100 g body weight/day as a suspension in water by gavage and water and Purina chow were available *ad libitum*. This treatment resulted in an average daily dose of 44 mg DHEAS/rat. All rats were killed on day 120.

Experiment II

Seventy-eight male and female BB/Wor rats were randomized into four treatment groups as follows: Group I rats were fed Purina chow and water which were available *ad libitum*; Group II received 0.5% DHEAS in powdered chow from 30 to 120 days of age. This resulted in an average daily dose of 133 mg DHEAS/rat. Groups I and II were killed at 120 days of age. Group III received iodine (0.05%) in the drinking water from 30–90 days of age. Group IV received 0.5% DHEAS in powdered chow and 0.05% iodine in the drinking water from 30 to 90 days of age. This resulted in an average daily dose of 133 mg DHEAS/rat. Groups III and IV were killed at 90 days of age.

Histology

Thyroids were embedded in paraffin wax and multiple sections of 4 μm were obtained from the equatorial portion of each thyroid and stained with hematoxylin and eosin. LT was subjectively graded and interpreted without knowledge of treatment.

Assays

All sera from an individual experiment were assayed in the same assay, in random order and in duplicate. Serum tri-iodothyronine (T_3), thyroxine (T_4), and thyrotropin (TSH) concentrations were determined by species-adapted specific RIAs. Serum DHEA and DHEAS were measured in duplicate by RIA kits. Serum glucose was determined by the glucose oxidase method. Serum anti-thyroglobulin antibody (anti-Tg Ab) was measured by an enzyme-linked immunosorbent assay.

Statistical analyses

All parametric data are expressed as the means $\pm$ S.E.M. Analysis of variance was performed using ANOVA with Student Newman Keuls multiple comparison tests. Non-parametric data sets were analyzed using the Fisher exact test (2×2 tables).

Results

Experiment I

DHEAS administration by gavage did not affect the incidence of spontaneous LT or DM at 120 days of age even though serum DHEA and DHEAS concentrations were markedly and significantly elevated. There were no significant differences in serum T_4 , T_3 , TSH, or anti-Tg Ab concentrations, or thyroid weights. Serum glucose, determined at death, was significantly decreased in the DHEAS-treated rats (292 ± 28 mg/dl vs 392 ± 34 mg/dl, $P < 0.02$) although there was no difference in the incidence of DM. There were no sex-related differences in the incidence of either DM or LT in the two groups.

DHEAS administration did not affect the rate of weight gain, the absolute weight gain or the adrenal or pancreas weights.

Experiment II (Tables 1 and 2)

In this experiment, DHEAS was added to powdered Purina chow. Serum DHEA and DHEAS concentrations were significantly increased in the rats receiving DHEAS. Thyroid weights and serum TSH concentrations were significantly increased in the iodine-treated rats compared with rats not receiving iodine but DHEAS administration did not affect these increases. DHEAS administration did not affect serum T_4 and T_3 concentrations, except for a small but significant

Table 2 Effect of DHEAS administration (0.5% in chow or approximately 133 mg/rat/day) from 30 days of age to death at 90 days on the incidence of iodine-induced LT and DM, and on the serum concentrations of DHEA, DHEAS, T_4 , T_3 , TSH, anti-Tg Ab, and glucose in the BB/Wor rat. Iodine was administered at a concentration of 0.05% in drinking water. Values are means $\pm$ S.E.M.

	Treatment	
	Iodine	Iodine + DHEAS
Number of rats	18	20
% LT	61	47
% DM	21	47
TSH (μ U/ml)	191 ± 66	295 ± 42
T_4 (μ g/dl)	5.6 ± 0.5	3.8 ± 0.6
T_3 (ng/dl)	77 ± 3.6	75 ± 6.6
Anti-Tg Ab (OD)	0.32 ± 0.02	0.24 ± 0.03
Glucose (mg/dl)	226 ± 29	295 ± 42
DHEA (μ g/ml)	0.19 ± 0.04	2.7 ± 0.5^a
DHEAS (μ g/ml)	1.2 ± 0.1	22.2 ± 1.3^a

^a $P < 0.001$ vs iodine.

decrease in serum T_4 concentrations in the rats receiving iodine and DHEAS compared with the iodine-treated rats, and a small decrease in serum T_3 values in rats receiving DHEAS compared with control rats.

DHEAS administration did not affect the incidence of spontaneous LT or iodine-induced LT. Both the incidence and severity of the LT was higher in the iodine-treated rats. There was no change in serum anti-Tg Ab in the rats receiving DHEAS. These antibodies were increased in the rats with LT compared with those without LT, but no significant overall change in optical density was observed when all iodine-treated rats were compared with all rats not receiving iodine. There were no sex-related differences in the incidence of DM or LT in the four groups.

DHEAS did not affect serum glucose concentrations at death or the incidence of DM, but significantly decreased the weights of the adrenals (15.9 ± 1.6 mg vs 27.2 ± 3.6 mg, $P < 0.02$) and ovaries (36.6 ± 2.5 mg vs 66.5 ± 4.2 mg, $P < 0.02$).

Discussion

Studies in the past have shown that the administration of DHEA had a beneficial effect on the immune system (12–15, 33, 45, 46). Because DHEA and DHEAS interconvert, the administration of either can lead to high circulating levels of both steroids. Because of its greater water solubility, we chose to administer DHEAS in our studies although, as noted, this resulted in elevated circulating levels of both steroids.

Our interest in DHEA was stimulated by the beneficial effects of DHEA in reducing the incidence of DM in both genetically predisposed C57BL/KsJ mice or in streptocytosin-induced DM. Improvement was observed with 0.4% DHEA in the diet, including less hyperglycemia and inhibition of beta-cell destruction and islet

Table 1 Effect of DHEAS administration (0.5% in chow or approximately 133 mg/rat/day) from 30 days of age to death at 120 days on the incidence of spontaneous LT and DM, and on the serum concentrations of DHEA, DHEAS, T_4 , T_3 , TSH, anti-Tg Ab, and glucose in the BB/Wor rat. Control rats received no treatment. Values are means $\pm$ S.E.M.

	Control	DHEAS treated
Number of rats	22	20
% LT	24	25
% DM	91	80
TSH (μ U/ml)	50 ± 3.2	58 ± 3.6
T_4 (μ g/dl)	4.2 ± 0.7	3.2 ± 0.3
T_3 (ng/dl)	54 ± 4.6	69 ± 5.0^a
Anti-Tg Ab (OD)	0.28 ± 0.05	0.20 ± 0.05
Glucose (mg/dl)	316 ± 34	239 ± 34
DHEA (μ g/ml)	0.21 ± 0.07	1.89 ± 0.38^b
DHEAS (μ g/ml)	5.7 ± 1.2	44.0 ± 5.1^b

^a $P < 0.04$, ^b $P < 0.001$ vs control.

atrophy (8). DHEAS was shown to be as effective as DHEA at a dose of 0.1% in the diet.

In the present study, DHEAS had no consistent effect on the serum concentrations of TSH, T₄ and T₃ in the LT- and DM-prone BB/Wor rats, suggesting that it has no adverse effects on thyroid function. Furthermore, DHEAS administration did not decrease the incidence of spontaneous or iodine-induced LT or serum anti-Tg Ab levels. Similarly, no significant differences in the incidence of DM, as determined by glycosuria and insulin requirements, were observed. The doses of DHEAS were higher than those employed in the mouse experiments discussed above.

The pathophysiology of DM and LT, although not completely defined, is probably due to a combination of cell-mediated and humoral factors in genetically susceptible hosts. The mechanism of action of DHEA is unclear and the diseases in which DHEA has been shown to be helpful vary in their immune abnormalities, suggesting multiple and varying mechanisms for each model. DHEA has been shown to enhance immune function and to oppose the action of glucocorticoids by its conversion to androgens or estrogens (10, 47, 48). A possible mechanism for the immunomodulatory effects of DHEA on DM previously observed in mice may be derived from its conversion to androgen or estrogen metabolites. The estrogenic effect of DHEA has been demonstrated since its administration results in increased intrauterine weight (24, 26). Estrogens affect the quantity and/or quality of lymphocytes and monocytes resulting in altered immune function. The mechanisms of action of estrogens on the immune system include direct chemical interactions, membrane reactive metabolites with mononuclear target cells, and induced soluble immunoregulatory factors produced by lymphoid tissue (11, 47, 49, 50).

Gender is a major determinant of the incidence and severity of DM in the CBA/LT-db/db mouse; males develop lethal DM whereas females are resistant (9). Ovariectomy increases the incidence and severity of DM and a replacement dose of estrogen given for two weeks prevents increases in blood glucose and circulating insulin levels (49). Furthermore, 5 α -dihydrotestosterone (5 α DHT) administration to the NOD mouse prevented the development of insulinitis (51). However, spontaneous DM in the C57BL/KsJ mouse does not have a gender-specific preference, yet DHEA administration markedly decreased the occurrence of DM (9). We have observed that orchidectomy or ovariectomy or the chronic administration of testosterone or DHT had no effect on the incidence of DM or LT in the BB/Wor rats (S Alex, C Longcope & L E Braverman, unpublished observations). Thus, our findings that DHEAS treatment failed to elicit an improvement in either DM or LT in the BB/Wor rat may be due to the fact that this model exhibits no gender-specific incidence of either of these diseases.

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