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Prevention of diabetes, hepatic injury, and colon cancer with dehydroepiandrosterone☆

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Abstract

The levels of dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) peak in human in their twenties, then decrease gradually with age. The physiological importance of DHEA was not clear until recent research reports showing that DHEA has beneficial effects on preventing diabetes, malignancy, inflammation, osteoporosis, and collagen disease. We summarize our results concerning diabetes, hepatitis, and colon cancer.

In 1982, Coleman et al. [Diabetes 31 (1982) 830] reported that DHEA decreased hyperglycemia in diabetic *db/db* mice, which become insulin resistant. We measured hepatic gluconeogenic enzymes in an attempt to elucidate the mechanical mechanism of DHEA action. The activity and gene expression of hepatic gluconeogenic enzyme such as glucose-6-phosphatase (G6Pase) was increased in *db/db* mice despite hyperinsulinemia compared to control *db/+m* mice. DHEA, like troglitazone, decreased these levels in *db/db* mice. We also showed that DHEA improved the insulin resistance caused by aging or obesity using the glucose clamp technique in another animal model. In humans, the serum DHEA concentration was shown to be associated with hyperinsulinemia in diabetes. It also became clear that DHEA increased insulin secretion in old-aged *db/db* mice. DHEA increases not only insulin sensitivity due to the effects in the liver and muscle, but also insulin secretion.

As an effect of DHEA on T-cell mediated hepatitis induced by concanavalin A (ConA), DHEA reduced hepatic injury by inhibiting several inflammatory mediators and apoptosis. As an effect of DHEA on carcinogenesis, DHEA would be a potential chemopreventative agent against colon cancer because it decreases the number of azoxymethane (AOM) induced aberrant crypt foci, which is a possible precursor to adenoma and cancer in a murine model.

Thus, since DHEA has many beneficial effects experimentally, we should consider administration of DHEA in the future, and common mechanisms among these actions of DHEA should be elucidated in further studies.

Keywords: Dehydroepiandrosterone (DHEA); Diabetes; Troglitazone

1. Introduction

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEA-S) are the most abundant circulating adrenal steroids in humans. The serum concentration of DHEA-S in human plasma is approximately 300-fold higher than that of DHEA and at least 20-fold higher than that of any other steroid hormone. DHEA is considered to be a weak androgen. Peak levels of DHEA and DHEA-S occur around the age of 20 and decrease gradually to 5% of these peak values by age 90. DHEA is synthesized from pregnenolone, and more than 90% of the synthesis in the entire body is carried out in the adrenal gland. DHEA is metabolized to androstenedione, testosterone, and estrogens.

The physiological importance of DHEA was not clear until recent research reports that DHEA has beneficial effects with regard to preventing diabetes, malignancy, inflammation, osteoporosis, and collagen disease.

Here, we describe the effects of administered DHEA on diabetes, hepatitis, and colon cancer as conducted in our laboratory.

2. Effect of DHEA on diabetes mellitus

The administration of DHEA has been reported to have beneficial effects on diabetes. Non-insulin dependent diabetes mellitus (NIDDM) is characterized by impaired

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Table 1
Comparison of blood glucose levels and hepatic G6Pase activity

	db/+*m*	*db*/*db*	*db*/*db* + DHEA	*db*/*db* + TGZ
BG (mg/dl)	157 ± 5	538 ± 46*	365 ± 43#	310 ± 55#
G6Pase (nmol/mg protein/mm)	69 ± 8	172 ± 5*	128 ± 7#	105 ± 12#

TGZ represents troglitazone. Each value represents mean + S.E. From [12].
* $P < 0.05$ vs. control *db*/+*m* mice.
# $P < 0.05$ vs. control *db*/*db* mice.

capacity to secrete insulin, insulin resistance, or both. Insulin resistance is considered as the result of increased glucose output in the liver and decreased glucose intake in muscle and adipose tissue. The mouse model C57BL/KsJ-*db*/*db*, which becomes obese, hyperglycemic, and hyperinsulinemic [1,2], represents a typical NIDDM model. Coleman et al. [3,4] reported that dietary administration of DHEA to *db*/*db* mice induced remission of hyperglycemia and increased insulin sensitivity. Many other studies suggest that DHEA increases the insulin sensitivity [5–8]. Recently, Ishizawa et al. [9] reported that treatment with DHEA for one year ameliorates insulin sensitivity by 30% in post-menopausal women.

2.1. Gluconeogenesis

Many studies do not fully state the mechanism of increasing insulin sensitivity. Therefore, we evaluated glucose metabolizing enzyme activities in the liver and muscle in *db*/*db* mice. The plasma insulin level of *db*/*db* mice was higher than that of *db*/+*m* mice. Despite hyperinsulinemia, the activity and mRNA level of hepatic glucose-6-phosphatase (G6Pase), which is a gluconeogenic enzyme and is normally suppressed by the action of insulin [10], were increased in *db*/*db* mice compared to *db*/+*m* mice as shown in Table 1 and Fig. 1. Dietary administration of DHEA significantly decreased blood glucose in *db*/*db* mice and hepatic G6Pase activity and gene expression in *db*/*db* mice (Table 1 and Fig. 1) [11,12]. Troglitazone, which was the first drug to be introduced in clinical medicine to improve insulin sensitivity, also decreased blood glucose and hepatic G6Pase activities and gene expression in *db*/*db* mice.

Recently, we measured the glucose production in *db*/*db* mice, using ^{14}C labeled sodium bicarbonate. In *db*/*db* mice, hepatic glucose production was increased compared to *db*/+*m* mice and administration of DHEA decreased this glucose production in *db*/*db* mice (unpublished data). McIntosh and Berdanier [13] also reported that administration of DHEA decreased hepatic glucose production in isolated hepatocytes from prediabetic male BHE/cdb rats. Base on these results, DHEA may suppress hepatic glucose output by decreasing G6Pase activity and gene expression in *db*/*db* mice. 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 for the hypoglycemic effects of DHEA as an insulin-sensitizing agent in *db*/*db* mice.

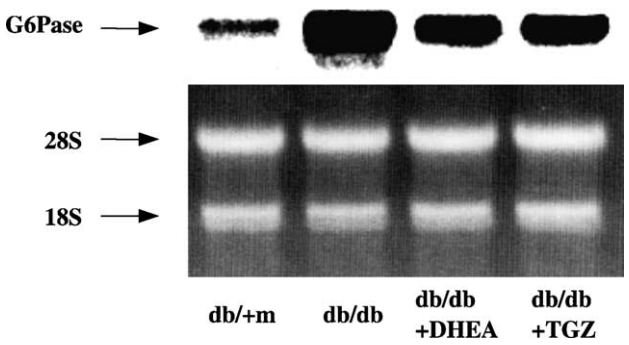

Fig. 1. Comparison of G6Pase mRNA in four groups of mice. A representative result is shown for Northern blot analysis. From [12].

In contrast, there are no significant positive relationships between muscle enzyme activities and blood glucose levels [1].

Since androstenedione, a DHEA metabolite, exhibited almost no effect on either of these enzyme activities or blood glucose in *db*/*db* mice, these actions of DHEA, which are similar to troglitazone, are presumed to be caused by DHEA itself.

DHEA is considered to increase the insulin sensitivity in the same way troglitazone does in the liver; however, the effects are different for DHEA and troglitazone. Administration of troglitazone increased body weight in *db*/*db* mice; however, DHEA did not change the body weight. In another study [8], administration of DHEA decreased the body weight of diabetic rats. DHEA also has an effect on insulin secretion in *db*/*db* mice that is different from that of troglitazone. First, DHEA was administered to 8-week-old *db*/*db* mice and did not increase the plasma insulin level in *db*/*db* mice. When we administered DHEA to 15-week-old *db*/*db* mice, however, DHEA significantly increased the plasma insulin level in *db*/*db* mice. The plasma insulin level in *db*/*db* mice gradually declined from about 12 weeks of age, and DHEA is considered to slow this decline by preventing atrophy of pancreatic β cells as Coleman reported in 1982 [3,4]. As to the effect on insulin secretion, Dillon et al. [14] reported that DHEA-S enhanced glucose-stimulated insulin secretion when administered in vivo to rats or in vitro to β cell lines, without changing the cellular insulin content.

2.2. Insulin sensitivity in older rat and serum tumor necrosis factor-α

Fink et al. [15] previously reported that glucose sensitivity (tolerance) decreases with age and glucose intolerance

develops as a part of the aging process. Therefore, we evaluated the effects of exogenous DHEA on tissue sensitivity of insulin that are associated with the aging in rats using the hyperinsulinemic euglycemic clamp technique. The glucose metabolic clearance rate (MCR) of control rats showed a gradual decline with advancing age. The glucose MCR of DHEA-treated rats also exhibited a gradual decline with the aging process. However, the MCR of DHEA-treated rats was significantly higher than that of the control rats. Since glucose MCR is a parameter indicating the insulin sensitivity especially in muscles and the body composition was not changed after the injection of DHEA, DHEA is considered to work on muscles to increase insulin sensitivity.

When DHEA was administered to obese Zucker rats, the MCR, which was normally reduced in obese rats, was increased significantly compared with the obese control rats. It is known that administration of DHEA to rats decreases their food intake. The pair-fed obese rats also showed levels of weight reduction similar to those of DHEA-treated rats. The increase in MCR of DHEA-treated rats was significantly greater than that in the pair-fed rats, suggesting a direct ameliorating effect of DHEA on the insulin sensitivity of obese rats. Tumor necrosis factor-α (TNF-α) is a cytokine that is secreted from adipose tissue and causes insulin resistance. Serum TNF-α levels were measured by bioassay. The serum TNF-α level was also reduced significantly in DHEA-treated mice; however, it was not reduced in pair-fed obese rats as indicated. These results suggest that DHEA treatment reduces body weight and serum TNF-α independently, and that both may ameliorate insulin resistance in this rat model.

2.3. Serum DHEA levels in diabetes with hyperinsulinemia

Nestler et al. [16] reported a progressive decline of serum DHEA-S levels during hyperinsulinemic-euglycemic clamp in normal men and women without affecting serum testosterone, progesterone, or cortisol. Furthermore, they demonstrated that a reduction in serum insulin concentration is associated with an increase in serum DHEA and DHEA-S [17]. To elucidate further the interaction between insulin and DHEA concentrations, the serum DHEA and DHEA-S levels in diabetic patients with hyperinsulinemia, whose fasting serum insulin concentrations were greater than 10 mU/ml (71.8 pmol/l), were evaluated [18]. Diabetic patients with hyperinsulinemia produced significantly lower levels of serum DHEA and DHEA-S than did the controls. These studies suggest that in diabetic patients with hyperinsulinemia, the baseline DHEA level is suppressed compared to that of the control subjects or non-hyperinsulinemic diabetic patients.

3. Effect of DHEA on hepatic injury and cancer [19,20]

It is known that DHEA is a potential immunological regulator. In a recent study, Yoshida et al. [19] in our laboratory examined the effect of DHEA on hepatitis and the modulation of apoptosis induced by concanavalin A (ConA) which is an established experimental T-cell mediated hepatitis model. Mice were treated with DHEA and injected with ConA. The expression of TNF-α, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and macrophage migration inhibitory factor (MIF) were measured by quantitative RT-PCR in the liver. Apoptosis was detected by the TUNEL method and DNA fragmentation test. In DHEA-treated mice, the serum ALT levels and expression of these mediators were significantly decreased. Few hepatocytes were shown to have TUNEL positive nuclei in DHEA-treated mice compared to control mice. DNA fragmentation was observed in the control mice, whereas DNA fragmentation was significantly reduced in DHEA-treated mice. The results strongly suggest that DHEA can reduce T-cell mediated liver injury by inhibiting the expression of several cytokines and apoptosis. Thus, DHEA may be a candidate for a novel therapy for liver injury.

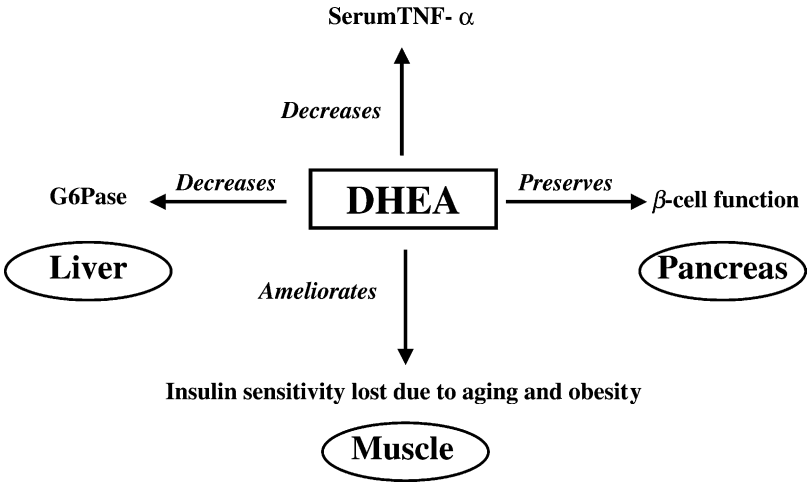

Fig. 2. Summary of effects of DHEA on diabetes in vivo.

Nyce et al. [21] reported that administration of DHEA to mice significantly inhibits the rate of appearance of 1,2-dimethylhydrazine (DMH)-induced macroscopic colon cancer. Osawa et al. [20] in our laboratory investigated the effect of DHEA on precursors to colon cancer. Aberrant crypt foci (ACF) of colon cancer are considered to be possible precursors of adenoma and cancer. The chemopreventive effect of DHEA was evaluated in azoxymethane (AOM)-induced ACF of female BALB/c mice. The numbers of ACF and aberrant crypt, which contain ACF, were significantly decreased in DHEA-treated mice. Based on the results, DHEA is considered to be a chemopreventive agent against colon cancer.

4. Conclusion

Our view, as shown in Fig. 2, is that DHEA is considered to increase not only insulin sensitivity due to the effects in the liver and muscle, but also insulin secretion in animal models. In humans, the serum DHEA concentration is associated with hyperinsulinemia in diabetes. DHEA reduces T-cell mediated liver injury and has a chemopreventitive effect on the precursors to colon cancer.

Its role and side effects in the human body have yet to be elucidated. We think that exogenous DHEA administration is not currently warranted in patients. However, as DHEA has many beneficial effects, we should consider the administration of DHEA in the future and further research should reveal a common mechanism of DHEA actions.

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References

[1] K. Aoki, M. Homma, T. Hirano, K. Oka, S. Satoh, K. Mukasa, S. Ito, H. Sekihara, mRNA and enzymatic activity of hepatic 11 beta-hydroxysteroid dehydrogenase type 1 are elevated in C57BL/KsJ-*db*/*db* mice, Life Sci. 69 (2001) 2543–2549.
[2] R. Gunnarsson, Function of the pancreatic B-cell during the development of hyperglycemia in mice homozygous for the mutations “diabetes” (*db*) and “misty” (m), Diabetologia 11 (1975) 431–438.
[3] D.L. Coleman, E.H. Leiter, R.W. Schwizer, Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice, Diabetes 31 (1982) 830–833.
[4] D.L. Coleman, R.W. Schwizer, E.H. Leiter, Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutants and aged normal mice, Diabetes 33 (1984) 26–32.
[5] L. Ladriere, A. Laghmich, F. Malaisse-Lagae, W.J. Malaisse, Effect of dehydroepiandrosterone in hereditarily diabetic rats, Cell Biochem. Funct. 15 (1997) 287–292.
[6] M. Kimura, S. Tanaka, Y. Yamada, Y. Kikuchi, T. Yamakawa, H. Sekihara, Dehydroepiandrosterone decreases serum TNF α and restores insulin sensitivity: independent effect from secondary weight reduction in genetically obese Zucker fatty rats, Endocrinology 139 (1998) 3249–3253.
[7] K. Mukasa, M. Kaneshiro, K. Aoki, J. Okamura, T. Saito, S. Satoh, H. Sekihara, Dehydroepiandrosterone (DHEA) ameliorates the insulin sensitivity in older rats, J. Steroid Biochem. Mol. Biol. 67 (1998) 355–358.
[8] A. Lasco, N. Frisina, N. Morabito, A. Gaudio, E. Morini, A. Trifiletti, G. Basile, V. Nicita-Mauro, D. Cucinotta, Metabolic effects of dehydroepiandrosterone replacement therapy in postmenopausal women, Eur. J. Endocrinol. 145 (2001) 457–461.
[9] M. Ishizawa, T. Ishizuka, K. Kajita, A. Miura, Y. Kanoh, M. Kimura, K. Yasuda, Dehydroepiandrosterone (DHEA) stimulates glucose uptake in rat adipocytes: activation of phospholipase D, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 130 (2001) 359–364.
[10] S.J. Pilkis, D.K. Granner, Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis, Annu. Rev. Physiol. 54 (1992) 885–909.
[11] K. Aoki, T. Saito, K. Mukasa, M. Kaneshiro, S. Kawasaki, A. Okamura, H. Sekihara, Dehydroepiandrosterone suppresses the elevated hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase activities in C57BL/KsJ-*db*/*db* mice: comparison with troglitazone, Diabetes 48 (1999) 1579–1585.
[12] K. Aoki, T. Kikuchi, K. Mukasa, S. Itoh, A. Nakajima, S. Satoh, A. Okamura, H. Sekihara, Dehydroepiandrosterone suppresses elevated hepatic glucose-6-phosphatase mRNA level in C57BL/KsJ-*db*/*db* mice: comparison with troglitazone, Endocrinol. J. 47 (2000) 799–804.
[13] M.K. McIntosh, C.D. Berdanier, Antiobesity effects of dehydroepiandrosterone are mediated by futile substrate cycling in hepatocytes of BHE/cbd Rats, J. Nutr. 121 (1991) 2037–2043.
[14] J.S. Dillon, G.C. Yaney, Y. Zhou, N. Voilley, S. Bowen, S. Chipkin, C.R. Bliss, V. Schultz, F.C. Schuit, M. Prentki, D.J. Waxman, B.E. Corkey, Dehydroepiandrosterone sulfate and beta-cell function: enhanced glucose-induced insulin secretion and altered gene expression in rodent pancreatic beta-cells, Diabetes 49 (2000) 2012–2020.
[15] R.I. Fink, O.G. Kolterman, J. Griffin, J.M. Olefsky, Mechanisms of insulin resistance in aging, J. Clin. Invest. 71 (1983) 1523–1535.
[16] J.E. Nestler, J.N. Clore, J.F. Strauss, W.G. Blackard, The effects of hyperinsulinemia on serum testosterone, progesterone, dehydroepiandrosterone sulfate, and cortisol levels in normal women and in a woman with hyperandrogenism, insulin resistance, and acanthosis nigricans, J. Clin. Endocrinol. Metab. 64 (1987) 180–184.
[17] J.E. Nestler, N.A. Beer, D.J. Jakubowicz, C. Colombo, R.M. Beer, Effects of insulin reduction with benfluorex on serum dehydroepiandrosterone (DHEA), DHEA sulfate, and blood pressure in hypertensive middle-aged and elderly men, J. Clin. Endocrinol. Metab. 80 (1995) 700–706.
[18] Y. Yamaguchi, S. Tanaka, T. Yamakawa, M. Kimura, K. Ukawa, Y. Yamada, M. Ishihara, H. Sekihara, Reduced serum dehydroepiandrosterone levels in diabetic patients with hyperinsulinemia, Clin. Endocrinol. (Oxford) 49 (1998) 377–383.
[19] S. Yoshida, A. Nakajima, K. Mukasa, M. Omura, K. Nagashima, K. Wada, H. Sekihara, Dehydroepiandrosterone (DHEA) as a novel therapeutic agent for acute hepatic injury, In: Proceedings of the Endocrine Society’s 83rd Annual Meeting Program and Abstracts, 2001, p. 422.
[20] E. Osawa, A. Nakajima, S. Yoshida, M. Omura, H. Nagase, N. Ueno, K. Wada, N. Matsuhashi, M. Ochiai, H. Nakagama, H. Sekihara, Chemoprevention of precursors to colon cancer by dehydroepiandrosterone (DHEA), Life Sci. 19 (70) (2002) 2623–2630.
[21] J.W. Nyce, P.N. Magee, G.C. Hard, A.G. Schwartz, Inhibition of 1,2-dimethylhydrazine-induced colon tumorigenesis in Balb/c mice by dehydroepiandrosterone, Carcinogenesis 5 (1984) 57–62.