

Dehydroepiandrosterone-Dependent Induction of Peroxisomal Proliferation Can Be Reduced by Aspartyl Esterification without Attenuation of Inhibitory Bone Loss in Ovariectomy Animal Model

The purpose of this study was to determine whether esterification of dehydroepiandrosterone with aspartate (DHEA-aspartate) could reduce peroxisomal proliferation induced by DHEA itself, without loss of antiosteoporotic activity. Female Sprague-Dawley rats were ovariectomized, then DHEA or DHEA-aspartate was administered intraperitoneally at 0.34 mmol/kg BW 3 times a week for 8 weeks. DHEA-aspartate treatment in ovariectomized rats significantly increased trabeculae area in tibia as much as DHEA treatment. Urinary Ca excretion was not significantly increased by DHEA or DHEA-aspartate treatment in ovariectomized rats, while it was significantly increased by ovariectomy. Osteocalcin concentration and alkaline phosphatase activity in serum and cross linked N-telopeptide type I collagen level in urine were not significantly different between DHEA-aspartate and DHEA treated groups. DHEA-aspartate treatment significantly reduced liver weight and hepatic palmitoyl-coA oxidase activity compared to DHEA treatment. DHEA-aspartate treatment maintained a nearly normal morphology of peroxisomes, while DHEA treatment increased the number and size of peroxisomes in the liver. According to these results, it is concluded that DHEA-aspartate ester has an inhibitory effect on bone loss in ovariectomized rats with a marked reduction of hepatomegaly and peroxisomal proliferation compared to DHEA.

Key Words: Dehydroepiandrosterone; Aspartic Acid; Osteoporosis; Peroxisomal Proliferation; Ovariectomy

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INTRODUCTION

Human and some primates are unique as their adrenals secrete large amounts of dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S), which are converted into androstenedione and then into potent androgens and estrogens in peripheral tissues. The marked reduction in the formation of DHEA-S by the adrenals during aging results in a dramatic fall in the formation of androgens and estrogens in peripheral target tissue, a situation that is thought to be associated with age-related diseases (1, 2).

Recently, it was reported that DHEA increased bone formation and decreased bone resorption (3, 4). Labrie et al. (5) reported medically important beneficial effects of DHEA administration on post-menopausal women. The bone mineral density and the level of plasma osteocalcin, a marker of bone formation, were significantly increased, and the level of urinary hydroxyproline excretion, a

marker of bone resorption, was decreased in DHEA administered group. Furthermore, the inhibitory effect of DHEA on growth of breast cancer xenografts in nude mice supports the beneficial use of DHEA as a hormone replacement therapy for women. Kim (6) revealed that DHEA treatment in ovariectomized rats significantly increased the reduced trabeculae area in proximal tibia metaphysis up to the normal level.

However, it was also reported that DHEA increased lipid peroxidation, especially in liver, and induced hepatomegaly with peroxisomal proliferation, which accompanies high increases of peroxisomal fatty acid β -oxidation and hepatic microsomal P450 system in some rodents (7, 8). Some researchers (9, 10) reported that DHEA sulfate is good inducer of cytochrome P450 4A1 and peroxisomal β -oxidation in rat liver and these inductions revealed a preference for 3β -sulfate from DHEA. Yamada et al. (11) also found that inhibiting cellular sul-

fate conjugation almost completely suppress the induction effect of DHEA on peroxisomal β -oxidation in cultured hepatocytes, giving additional evidence that DHEA-sulfate is an active metabolite responsible for enzyme induction by DHEA. Previously, we have observed that the cellular redox potential modulation by combination of aspartate with DHEA reduced palmitoyl-coA oxidase activity (12). Though its mechanism was not clear, it is presumed that enforcing of NAD^+ generation through activation of aspartate-malate shuttle by aspartate could affect the peroxisomal proliferation. Therefore, we assumed that modification of DHEA with aspartate by esterification might reduce peroxisomal proliferation in rat liver compared to DHEA by inhibition of 3-sulfation of DHEA and redox potential modulation. The aim of this study is to determine whether DHEA-aspartate ester is more effective and safe antiosteoporotic agent with low side effect on liver than DHEA.

MATERIALS AND METHODS

Chemicals

Dehydroepiandrosterone (3 β -hydroxy-5-androsten-17-one) and L-aspartate were purchased from Sigma (St. Louise, MO, U.S.A.). DHEA-aspartate ester (DHEA-asp, (3R)-3-amino-4-[(3R, 10S, 13R)-10,13-dimethyl-17-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl]oxy}-4-oxobutanoic acid) was synthesized and provided by R&D center of Daesang Co. Ltd. Korea. The structures of these chemicals are shown in Fig. 1.

Animals and experimental design

Female Sprague-Dawley rats, 8 weeks of age, were maintained under standard conditions (22-25°C, 12 hr light/dark cycle). All animals were provided with rat diet chow (Samyang, Korea) and water ad libitum. They were acclimatized for 1 week and randomly allocated to the following groups (1) sham control (SC; n=4) (2) ovariec-

tomy control (OC; n=7) (3) ovariectomy and DHEA treatment (ODH; n=7) (4) ovariectomy and DHEA-aspartate ester treatment (ODA; n=7) group. The animals of experimental groups were ovariectomized and control rats were received sham operations. DHEA and DHEA-asp dissolved in propylene glycol were administered to experimental groups intraperitoneally at 0.34 mmol (as much as 100 mg DHEA)/kg body weight (BW) 3 times a week for 8 weeks from postoperative 3 weeks, while the control rats were treated only with vehicle.

Sample preparation

As soon as 24-hr urine of all rats was collected on the day before sacrifice, urine volume was recorded and centrifuged, then stored at -70°C in aliquots until analysis. All animals were sacrificed by decapitation. Serum was prepared by centrifugation at 1,000 g for 20 min and stored at -70°C in aliquots until analysis. Liver was removed and weighed rapidly. The fresh liver tissue was sectioned approximately by 2 \times 2 \times 2 mm and washed with Tris buffered saline (TBS, 0.05 M, pH 7.6) for immunocytochemistry of peroxisomal proteins and the remains were frozen rapidly in liquid nitrogen for biochemical analysis. Tibia was fixed in 4% formalin solution for histomorphometry.

Bone histomorphometry

Bone histomorphometry was carried out after Kim's method (13) as follows: after fixation of tibia, 30 μm cross-section of tibia was made and decalcified in 10% nitric acid for 6 hr, then they were dehydrated with alcohol and embedded in xylene and paraffin, and cut into 4 μm thick section. Each section was stained with Hematoxylin-Eosin. The trabeculae area in cancellous bone of proximal tibia metaphysis was measured by quantitative image analysis system (Wild Leitz, Germany).

Electron microscopic immunocytochemistry

The fresh liver tissue was sectioned by 2 \times 2 \times 2 mm

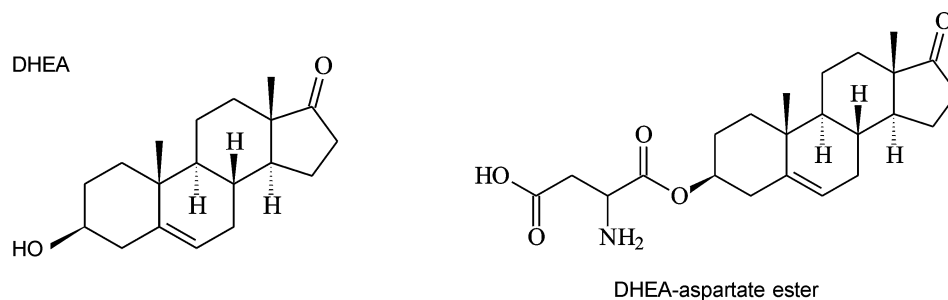


Fig. 1. The chemical structures of DHEA and DHEA-aspartate ester.

and then washed with TBS (0.05 M, PH 7.6). Sections were preincubated with 50 mg/mL of 3,3-diaminobenzidine tetrahydrochloride (DAB) solution without H₂O₂ for 1 hr, then followed by 1 hr incubation in the complete medium at room temperature (25°C). Complete incubation medium contained DAB in the TBS buffer, pH 10.5 and 2% H₂O₂ for cytochemical visualization of catalase. The stained tissues were fixed with 2.5% glutaraldehyde followed by routine electromicroscopic tissue preparation and embedded in Epon 812. After thin section by Sorvall 5000 ultramicrotome, it was lightly counterstained with lead citrate and was analyzed by ZEOL 200 CX electron microscope.

Preparation of subcellular fractions

Liver was homogenized in 9 volumes of ice-cold 11.5% KCl with 0.2 mM phenylmethylsulfonylfluoride (PMSF) and 1 mM dithiothreitol (DTT). Homogenates were centrifuged at 800 *g* for 10 min to remove cell debris and nuclei. The supernatant (10% liver homogenate) was collected for biochemical analysis.

Biochemical analysis

The levels or activities of serum protein, albumin, lactate dehydrogenase (LDH), blood urea nitrogen (BUN) aspartate aminotransferase (AST), alkaline phosphatase activity (ALP), total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol and total triglyceride were measured with Technicon autoanalyzer (Technicon Inc., U.S.A.). The level of osteocalcin (bone gla-protein) was determined by a competitive radioimmunoassay using a commercial kit (Brahams, Germany). The osteocalcin molecules in serum competed with radioactive-labeled osteocalcin in the tracer (38-49 ¹²⁵I) for binding sites on the highly specific antibody (polyclonal, sheep) bound to the tubes. After the samples were incubated, non-bound tracer components were decanted and subsequently removed by washing. The residual radioactivity in the coated tubes was then measured using a γ -counter. Concentrations of calcium in serum and urine were measured using a kit (Youngdong Pharm. Co., Korea). Cross-linked N-telopeptide of type I collagen (NTx) in urine was determined by ELISA method using a commercial kit (Brahams, Germany). Creatinine concentration in urine was determined by modified Jaffé method using a commercial kit (Youngdong Pharm. Co., Korea).

The activities of palmitoyl-coA oxidase and catalase in liver were measured by the methods of Small et al. (14) and Aebi (15), respectively. Protein in tissue was determined by Lowry's method (16) with bovine serum

albumin as a standard.

Statistical analysis

Data were expressed as means \pm SD. Statistical differences between two groups were determined by t-test in SAS.

RESULTS

Tibial cancellous bone histomorphometry

Fig. 2 shows the cancellous bone in proximal tibia metaphysis stained by hematoxylin-eosin. The changes of trabeculae area in cancellous bone of proximal tibia metaphysis are summarized in Table 1. Ovariectomy caused the marked reduction ($p < 0.0001$) of trabeculae area in tibial metaphysis to 38% level of sham control group. DHEA or DHEA-aspartate treatment to ovariectomized rats, however, significantly ($p < 0.05$) increased it up to 59.2% and 52.1% level of sham control group, respectively (Table 1), which suggest that both DHEA and DHEA-aspartate were equally effective on inhibition of bone loss.

Serum calcium level and biological markers of bone turnover rate

Serum calcium level was significantly ($p < 0.05$) reduced and urinary calcium excretion was significantly ($p < 0.05$) increased by ovariectomy. DHEA or DHEA-aspartate treatment in ovariectomized rats did not affect the serum calcium level and urinary calcium excretion. Osteocalcin level, total alkaline phosphatase activity in serum and NTx (Cross-linked N-telopeptide of type I

Table 1. Trabeculae area in cancellous bone of tibia

Group	Trabeculae area* (mm ²)
SC	0.71 \pm 0.09
OC	0.27 \pm 0.05 [†]
ODH	0.42 \pm 0.18 ^{†,‡}
ODA	0.37 \pm 0.06 ^{†,‡}

Values are expressed as mean \pm SD.

*Trabeculae area within the reference area of 2 mm² in proximal tibial metaphysis was calculated by a computer system attached to image analyzer.

SC, sham control; OC, ovariectomized control; ODH, ovariectomy and DHEA treatment; ODA, ovariectomy and DHEA-asp treatment

[†]Significantly different from SC group at $p < 0.05$ at least by t-test

[‡]Significantly different from OC group at $p < 0.05$ at least by t-test

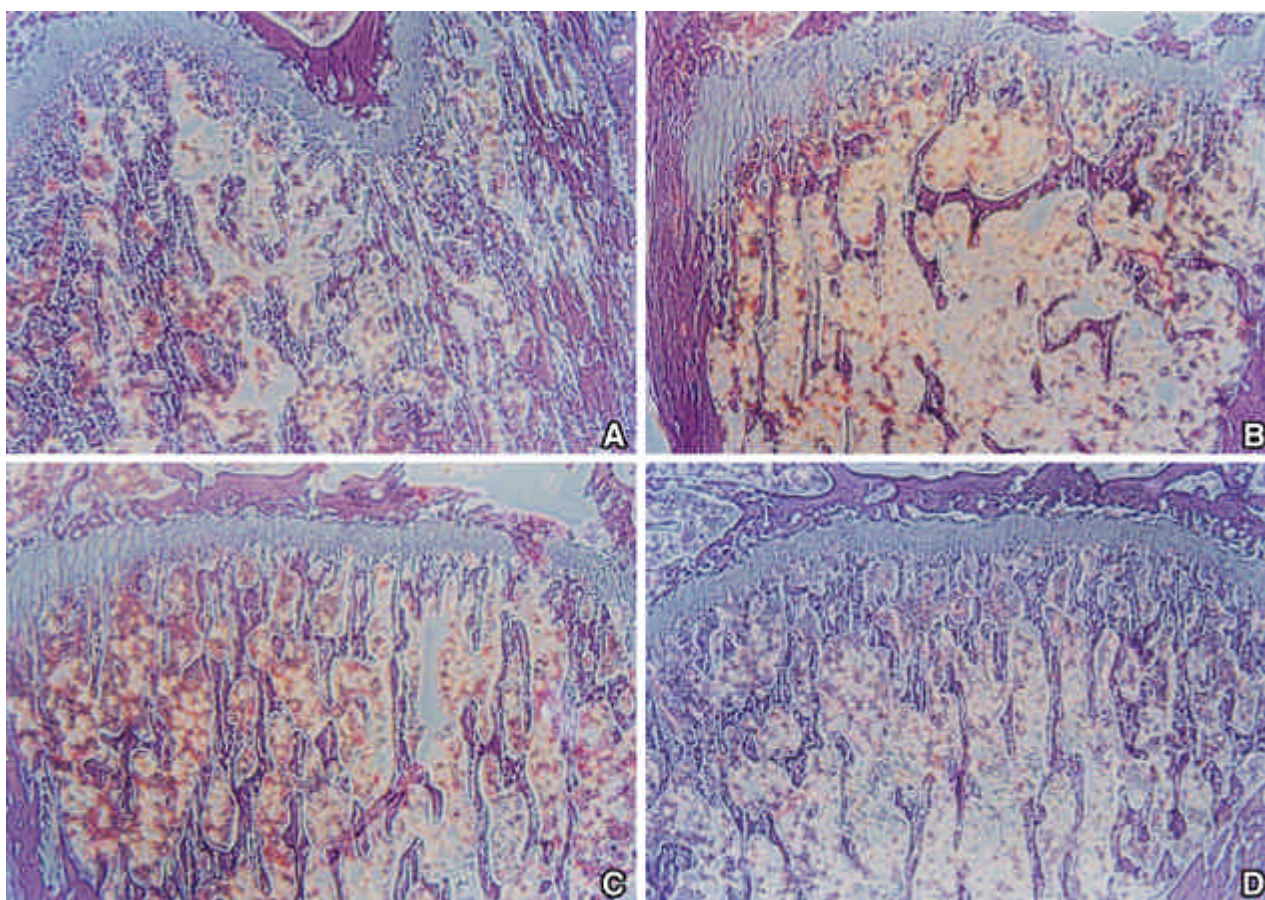


Fig. 2. Photographs of longitudinal sections of the proximal tibia. Tibia were removed and fixed in 4% formalin after 8 weeks of treatment with DHEA, DHEA-asp or vehicle, ip. at 0.34 mmol/kg BW from 3 weeks after ovariectomy. Fixed tibia were embedded in paraffin, sectioned, mounted and stained with Hematoxylin-eosin. The trabecular bone in proximal tibia metaphysis was observed by microscope ($\times 20$). (A) Sham control with vehicle. (B) Ovariectomy and treatment with vehicle: shows marked resorption of trabeculae. (C) Ovariectomy and treatment with DHEA: shows marked increase of the trabeculae area compared to B. (D) Ovariectomy and treatment with DHEA-aspartate: shows also increase of the trabeculae area compared to B.

Table 2. Serum calcium level and biological markers of bone turnover rate

Group	Ca in serum (mg/dL)	Urinary Ca excretion (mg/day)	Osteocalcin in serum (ng/mL)	Alkaline phosphatase activity in serum (U/L)	NTx/creatinine* in urine (nmol/mmol)
SC	12.67 \pm 0.09	1.36 \pm 0.36	2.36 \pm 0.20	199.0 \pm 41.7	40.8 \pm 30.4
OC	10.77 \pm 0.74 [†]	2.76 \pm 1.25 [†]	2.36 \pm 0.59	182.0 \pm 23.7	71.3 \pm 15.2
ODH	11.04 \pm 0.95 [†]	2.12 \pm 1.25	2.29 \pm 0.28	190.7 \pm 66.0	64.1 \pm 29.3
ODA	10.99 \pm 0.73 [†]	1.43 \pm 0.81	2.73 \pm 0.47	202.7 \pm 73.0	67.3 \pm 31.6

Values are expressed as mean \pm SD.

*Cross-linked N-telopeptide of type I collagen/creatinine

SC, sham control; OC, ovariectomized control; ODH, ovariectomy and DHEA treatment; ODA, ovariectomy and DHEA-asp treatment

[†]Significantly different from SC group at $p < 0.05$ at least by t-test.

collagen)/creatinine in urine were not affected by ovariectomy or subsequent treatment with DHEA or DHEA-aspartate (Table 2).

Body and liver weights

Ovariectomy significantly increased body weight in

rats. Body weight gain in ovariectomized rats during the experiment was significantly higher ($p < 0.0001$) than that in sham control rats. However, DHEA or DHEA-aspartate treatment in ovariectomized rats significantly reduced ($p < 0.001$) body weight gain (Table 3). On the other hand, DHEA or DHEA-aspartate treatment in ovariectomized rats significantly increased liver weight

Table 3. Body and liver weights

Group	Initial body weight (g)	Final body weight (g)	Body weight gain* (g)	Liver weight (g)	Liver/BW (%)
SC	184.5±8.3	255.5±9.3	71.0±7.5	7.12±0.44	2.79±0.09
OC	189.0±5.9	335.3±13.5 [†]	146.3±9.4 [†]	8.13±0.74 [†]	2.48±0.15 [†]
ODH	191.9±6.9	292.6±22.1 ^{†,†}	100.7±18.5 ^{†,†}	11.27±1.35 ^{†,†}	3.86±0.46 ^{†,†}
ODA	182.5±7.3	277.7±32.6 [†]	95.2±27.7 [†]	9.60±1.25 ^{†,†,§}	3.48±0.45 ^{†,†}

Values are expressed as mean±SD.

*Body weight gains for 12 weeks from 8 wk-age to 20 wk-age

SC, sham control; OC, ovariectomized control; ODH, ovariectomy and DHEA treatment; ODA, ovariectomy and DHEA-asp treatment

[†]Significantly different from SC group at $p<0.05$ at least by t-test

^{††}Significantly different from OC group at $p<0.05$ at least by t-test

[§]Significantly different between ODH and ODA groups at $p<0.05$ at least by t-test

and the ratio of liver weight to body weight ($p<0.05$), but liver weight was less increased in DHEA-aspartate treated rats than in DHEA treated rats (Table 3).

Peroxisomal changes

DHEA treatment increased the number and the size of peroxisomes in the sections of rat livers stained with alkaline diaminobenzidine, indicative of catalase activity. But the rat liver treated with DHEA-aspartate showed

normal range of peroxisomal morphology with a few enlarged peroxisomes (Fig. 3).

Palmitoyl-coA oxidase activity in liver was significantly elevated ($p<0.0001$) by DHEA or DHEA-aspartate treatment in ovariectomized rats. But, DHEA-aspartate treatment increased it by about three fold of control level, while DHEA itself increased it six fold (Fig. 4). In contrast, catalase activity showed no significant changes despite of DHEA or DHEA-aspartate treatment in ovariectomized rats. As it is similar to palmitoyl-coA

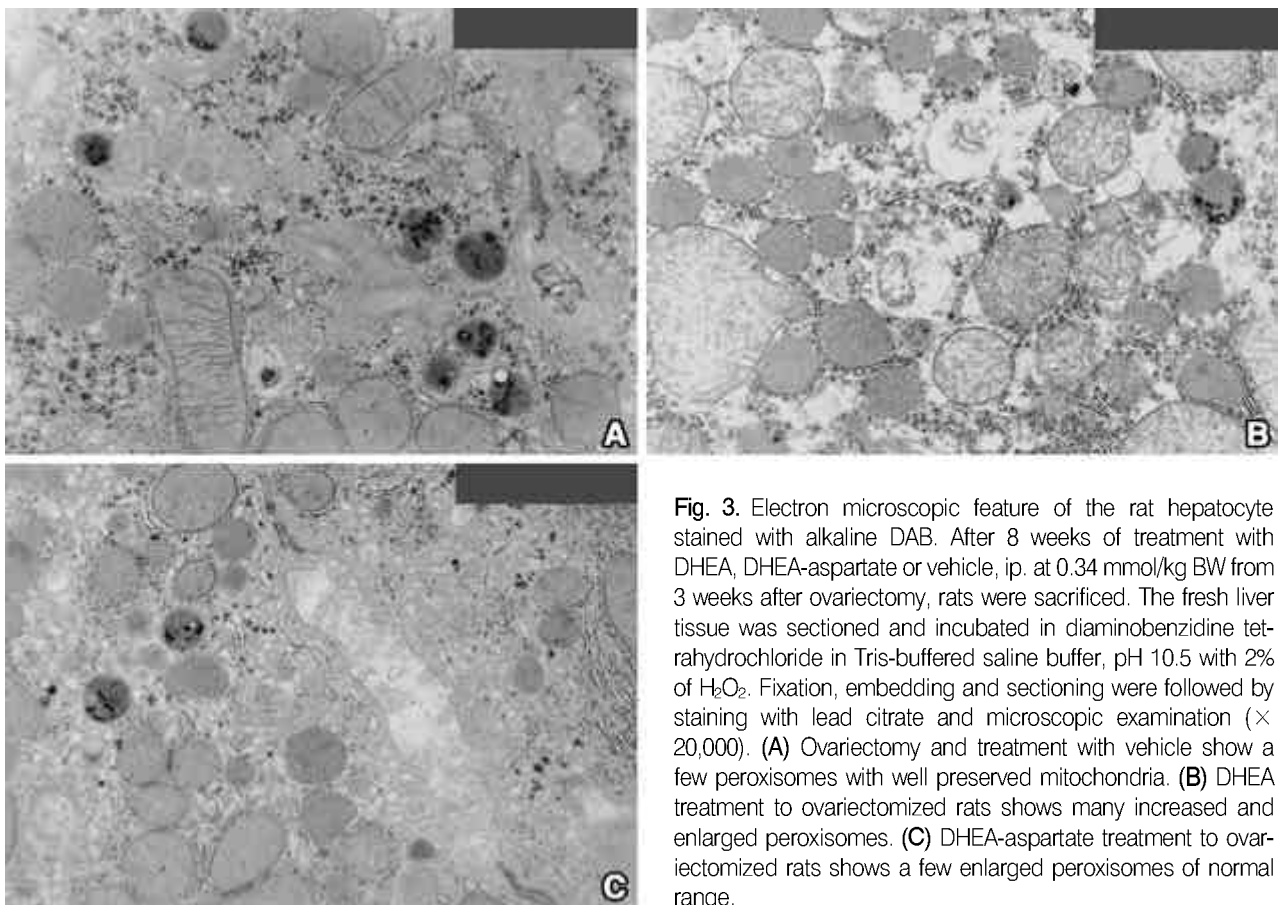


Fig. 3. Electron microscopic feature of the rat hepatocyte stained with alkaline DAB. After 8 weeks of treatment with DHEA, DHEA-aspartate or vehicle, ip. at 0.34 mmol/kg BW from 3 weeks after ovariectomy, rats were sacrificed. The fresh liver tissue was sectioned and incubated in diaminobenzidine tetrahydrochloride in Tris-buffered saline buffer, pH 10.5 with 2% of H_2O_2 . Fixation, embedding and sectioning were followed by staining with lead citrate and microscopic examination ($\times 20,000$). (A) Ovariectomy and treatment with vehicle show a few peroxisomes with well preserved mitochondria. (B) DHEA treatment to ovariectomized rats shows many increased and enlarged peroxisomes. (C) DHEA-aspartate treatment to ovariectomized rats shows a few enlarged peroxisomes of normal range.

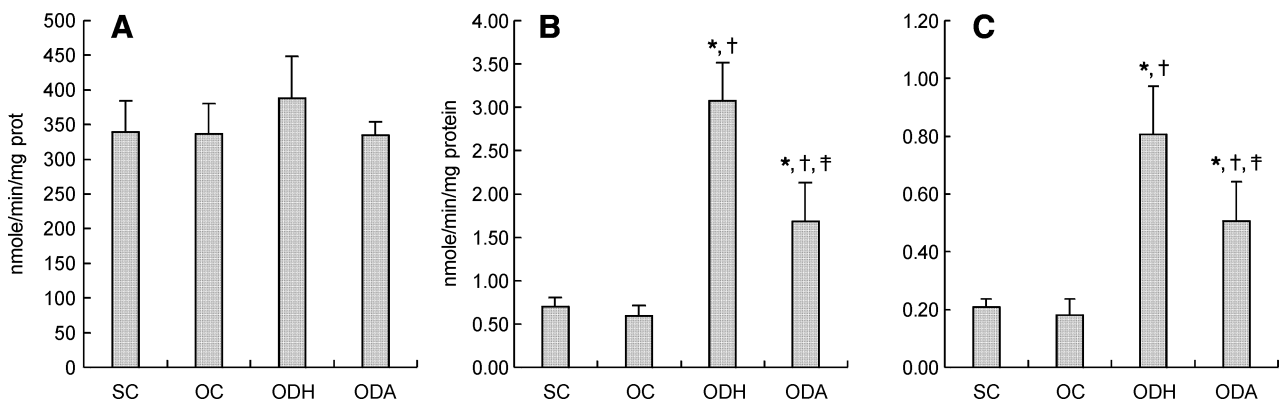


Fig. 4. Activities of peroxisomal enzymes in rat liver. (A) Catalase activity (B) palmitoyl-coA oxidase activity (C) the ratio of palmitoyl-coA oxidase activity to catalase activity. SC, sham control; OC, ovariectomized control; ODH, ovariectomy and DHEA treatment; ODA, ovariectomy and DHEA-asp treatment. *Significantly different from SC group at $p < 0.05$ at least by t-test. †Significantly different from OC group at $p < 0.05$ at least by t-test. ‡Significantly different between ODH and ODA at $p < 0.05$ at least by t-test.

oxidase activity, the ratio of palmitoyl-coA oxidase activity to catalase activity in DHEA-aspartate treated rats was significantly lower than that in DHEA treated rats (Fig. 4).

Serum lipid profile and chemistry

Ovariectomy induced some changes in serum lipid profile (Table 4). Ovariectomy reduced serum triglyceride level and elevated LDL-cholesterol level significantly ($p < 0.05$), while it did not affect significantly on levels of total cholesterol and HDL-cholesterol. DHEA treatment in ovariectomized rats increased serum triglyceride and reduced serum LDL-cholesterol level to the levels of sham control rats. DHEA-aspartate treatment in ovariectomized rats did not increase serum triglyceride level unlike DHEA treatment, but it reduced serum LDL-cholesterol level significantly ($p < 0.05$) as much as DHEA treatment. DHEA-aspartate treatment in ovariectomized rats also reduced serum total cholesterol, while DHEA treatment did not affect it (Table 4). Ovariectomy reduced serum protein and albumin levels, and DHEA or DHEA-aspartate treatment in the ovariectomized rats also reduced serum protein level. Lactate

dehydrogenase activity in serum was increased by DHEA or DHEA-aspartate treatment compared to ovariectomized control. BUN, AST and ALT in serum were not significantly affected by DHEA or DHEA-aspartate treatment (Table 5).

DISCUSSION

Reduction in skeletal mass caused by an imbalance between bone resorption and bone formation is the hallmark of osteoporosis. Loss of gonadal function and aging are the two most important factors contributing to the development of this condition. Starting at around the fourth or fifth decade of life, both women and men lose bone at a rate of 0.3 to 0.5 percent per year. After menopause, the rate of bone loss increases as much as 10 fold (17, 18). In humans, other primates and rodents, the loss of bone mass follows loss of ovarian function is associated with increases in the rates of bone resorption and formation, but with the former exceeding the latter (19, 20).

Ovariectomy causes osteopenia in rats and has been used as a study model for postmenopausal bone loss (21-

Table 4. Lipid profile in serum

	Triglyceride level (mg/dL)	Total cholesterol level (mg/dL)	HDL-cholesterol level (mg/dL)	LDL-cholesterol level (mg/dL)
SC	136.0 ± 30.1	90.0 ± 13.3	39.0 ± 3.7	23.8 ± 6.6
OC	69.7 ± 9.5 [†]	107.1 ± 14.4	45.9 ± 6.2	47.3 ± 9.0 [†]
ODH	141.7 ± 52.9 [†]	97.1 ± 27.8	38.4 ± 13.4	30.4 ± 18.5 [†]
ODA	90.1 ± 33.5	87.5 ± 16.1 [†]	36.8 ± 6.7	32.5 ± 11.3 [†]

Values are expressed as mean ± SD.

SC, sham control; OC, ovariectomized control; ODH, ovariectomy and DHEA treatment; ODA, ovariectomy and DHEA-asp treatment

[†]Significantly different from SC group at $p < 0.05$ at least by t-test

[‡]Significantly different from OC group at $p < 0.05$ at least by t-test

Table 5. Clinical chemistry in serum

	Protein (mg/dL)	Albumin (mg/dL)	BUN (mg/dL)	LDH (U/L)	AST (U/L)	ALT (U/L)
SC	7.7±0.5	4.9±0.3	28±4.9	3,828±686	302±66	64.3±8.4
OC	7.1±0.1 [†]	4.4±0.2 [†]	25±2.9	3,004±632	282±40	62.6±5.1
ODH	6.4±0.3 ^{†,‡}	4.4±0.2 [†]	25±5.7	4,027±874 [†]	284±45	64.3±15.7
ODA	6.4±0.5 ^{†,‡}	4.2±0.3 [†]	26±5.7	4,298±848 [†]	296±39	73.7±14.5

Values are expressed as mean±SD.

BUN, blood urea nitrogen; LDH, lactate dehydrogenase activity; AST, aspartate aminotransferase activity; ALT, alanine aminotransferase activity; SC, sham control; OC, ovariectomized control; ODH, ovariectomy and DHEA treatment; ODA, ovariectomy and DHEA-aspartate treatment

[†]Significantly different from SC group at $p < 0.05$ at least by t-test.

[‡]Significantly different from OC group at $p < 0.05$ at least by t-test.

23). Histomorphometry of cancellous bone is useful to estimate changes in bone remodeling because approximately 25 percent of cancellous bone is resorbed and replaced every year in adults, as compared with only 3 percent of cortical bone (24). Turner et al. (25) reported that trabeculae volume in tibial cancellous bone of ovariectomized rats was reduced by more than 60% at 28 days after ovariectomy. Takano-Yamamoto and Rodan (26) reported that ovariectomy for 22 days caused a 50% loss in trabeculae volume in cancellous bone of rat femur, a two fold increase in osteoclast number, three fold increase in osteoblast number, and eight fold increase in the relative osteoid surface. The mechanism for the loss of trabeculae in ovariectomized rats is unclear, but the increase in bone resorption and osteoclastic activity might be larger than the increase in osteoblastic activity and bone formation to account for the net loss of bone. In the present experiment, trabeculae area in tibial cancellous bone was also reduced by 60% at 11 weeks after ovariectomy in the limit of the small number of sham control rats (Table 1).

Decrease of serum ionized calcium level in ovariectomized rats in spite of increased bone resorption might be caused by increase of urinary calcium excretion and intestinal calcium secretion by estrogen deficiency, which were supported by other studies (27-30). Osteocalcin concentration in serum is known to be correlated with histological and biochemical indices of bone formation (30, 31). Pyridinoline and deoxypyridinoline levels in urine are known to be promising markers for bone resorption because both of them are non-reducible cross-links that stabilize the collagen chains within the extracellular matrix in bone tissue. About 40% of these cross-links appear free in urine and the remainders are in peptide form such as N-telopeptide (NTx) (32). It is known that menopause raises both bone resorption and bone formation as shown by increase in serum osteocalcin and bone specific alkaline phosphatase and urinary pyridinoline and NTx in postmenopausal women (33,

34). However, Kippo et al. (35) reported that ovariectomy did not change significantly serum osteocalcin level in rat and increased urinary hydroxyproline/creatinine, and estrogen treatment in ovariectomized rats decreased both serum osteocalcin and urinary hydroxyproline/creatinine levels. Since bone formation or resorption rate was increased rapidly and maximized at one month after ovariectomy and declined toward control level (20), it can be suggested that minimal changes in the levels of osteocalcin and NTx/creatinine after ovariectomy in the present study might be due to time lag of the determination, analyzed 11 weeks after ovariectomy (Table 2).

Labrie et al. (5) reported increase in the bone mineral density at the hip with a 2.1 fold increase in plasma osteocalcin by 12 months of DHEA administration to postmenopausal women. Also, 20% decrease in plasma bone alkaline phosphatase and 28% decrease in urinary hydroxyproline/creatinine ratio were concomitantly observed. The stimulatory effects of DHEA on bone mineral density accompanied by an increase in serum osteocalcin might be the result of bone formation stimulation by DHEA androgenic action, not by its estrogenic action (4, 37, 38). Martel et al. (4) revealed that the potent stimulatory effect of DHEA on bone formation in ovariectomized rats would be mainly due to the local formation of androgens in bone cells and their intracrine action in osteoblasts, because it could be inhibited by antiandrogen flutamide treatment but not by antiestrogen EM-800 treatment. In the present experiment, we found that DHEA-aspartate has the comparable effect as DHEA on inhibition of bone loss in ovariectomized rats (Table 1, Fig. 2).

In spite of the beneficial effect of DHEA on osteoporosis and aging, the use of DHEA is not strongly recommended, since DHEA would induce several side effects such as induction of hepatomegaly, peroxisomal proliferation and cytochrome P450 4A1 induction, illustrated in rodents. In our previous study (12), we observed that simultaneous administration of aspartate with

DHEA could reduce DHEA-related peroxisomal fatty acid oxidation. Therefore, we assumed that conjugation of aspartate to DHEA might be an effective method to reduce DHEA-related adverse effects. The induction of peroxisomal proliferation by DHEA treatment was found to be related with the level of DHEA sulfate, major DHEA metabolite, rather than DHEA itself (9, 10). It can be assumed that the blocking C₃ hydroxyl group with aspartyl moiety may intervene the DHEA sulfate formation, resulting in less induction of peroxisomes. Moreover, in our previous study, we have reported that the redox potential adjustment with aspartate might modulate the DHEA-inducibility of peroxisome probably through aspartate-malate shuttle (12). Therefore, we modified the C₃ hydroxyl group with aspartyl moiety. In particular, we designed that the conjugate could be dissociated readily in the cell by abundant cellular esterase activities. Thus, esterification of aspartate to DHEA was conducted, and the conjugated compounds were subjected to test for its efficacy and safety (Fig. 1). DHEA treatment in ovariectomized rats increased the number and size of peroxisomes in rat liver with a six-fold increase in palmitoyl-coA oxidase activity over controls. But the rat liver treated with DHEA-aspartate showed normal range of peroxisomal morphology with only a three fold increase in palmitoyl-coA oxidase activity over controls (Fig. 3, 4). Since the reduction in activity of peroxisomal enzyme and number of peroxisomes might be directly related with the decrease of hepatomegaly (8), it can be suggested that aspartyl esterification of DHEA could decrease the side effect of DHEA, especially hepatomegaly.

The biochemical data on the peroxisomal enzyme activities are closely correlated with the changes in liver weight. As shown in Table 3, DHEA treatment significantly increased liver weight and its ratio to body weight, but DHEA-aspartate treatment showed the relatively less increase of liver weight than DHEA itself.

Moreover, since DHEA-aspartate treatment to ovariectomized rats reduced effectively the levels of serum total cholesterol and LDL-cholesterol without increase of serum triglyceride level (Table 4), DHEA-aspartate can be suggested to be a better agent than DHEA for the control blood lipid profile in postmenopausal women. Recent studies showed that DHEA has apparently no deleterious effect on the serum lipid profile (5, 39). The detailed mechanism for the differences in the serum lipid profile change by DHEA and DHEA aspartate remains to be illustrated.

Serum protein and albumin were reduced by ovariectomy in the present study and other study (40). But the mechanism is not clear yet. Increased serum LDH by DHEA or DHEA-aspartate treatment may be related

with increased liver weight, and it can not be excluded the possibility of liver damage.

Taken together, it can be suggested DHEA-aspartate would be better candidate for an agent to prevent postmenopausal osteoporosis because DHEA-aspartate had a comparable effect with DHEA on inhibition of bone loss in ovariectomized rats, and reduced markedly peroxisomal proliferation and peroxisomal fatty acid β -oxidation compared to DHEA. Since the only trabecular bone area in tibia was measured in this study, it can not be insisted that DHEA and DHEA-aspartate have strong effects on prevention of postmenopausal osteoporosis. Further metabolic and pharmacological studies of DHEA-aspartate ester are needed.

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