Prevention of Orotic-Acid-Induced Fatty Liver in Male Rats by Dehydroepiandrosterone and/or Phenobarbital

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ABSTRACT. Dehydroepiandrosterone (DHEA) is a steroid hormone which induces the peroxisome proliferation in rodents. The fatty liver induced by orotic acid and a high sucrose diet in male rats was prevented by the administration of DHEA and/or phenobarbital (PB). A significant increase in the liver weight was induced in the DHEA group (relative weight) and the DHEA + PB group (absolute and relative weight). The liver weight increased more conspicuously in the DHEA + PB group than in the DHEA group. The increase in the liver weight was caused by an increase in the cell size and peroxisome number. In addition, the administration of DHEA alone and the combination of DHEA and PB prevented the lipid droplet accumulation in hepatocytes. The administration of PB alone also prevented the accumulation of lipid droplets without any increase in the liver weight. — KEY WORDS: dehydroepiandrosterone, fatty liver, orotic acid, peroxisome proliferator, phenobarbital.

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Dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one, DHEA) is a metabolic intermediate in the testosterone, estrone, and estradiol synthesis pathway mainly in the human adrenal cortex and rodent gonads [1, 28]. The actions of this hormone reported thus far included a possible antiobesity effect [2, 16], preventive action on the development of atherosclerosis [17], and preventive action on hyperglycemia in the experimental diabetes [5]. It is interesting that this hormone is a peroxisome proliferator [12, 15, 24, 25, 31]. Most peroxisome proliferators including clofibrate [9, 27] and bezafibrate [13, 22, 23] are hypolipidemic agents, and decrease serum triglycerides and cholesterol. Some of these peroxisome proliferators were reported to be capable of metabolizing and diminishing intracellular lipid droplets [6, 7]. In this experiment, therefore, the effect of DHEA, a different type of peroxisome proliferator, was examined in the fatty liver experimentally induced by orotic acid and a high sucrose diet. In addition, the actions of phenobarbital (PB), another standard hepatomegaly inducer, were compared with those of DHEA when used alone and in combination with the latter.

MATERIALS AND METHODS

Animals and treatment: A total of 24 6-week-old male Sprague-Dawley strain rats weighing 166 to 196 g were obtained from Charles River Japan, Inc., and randomly assigned to 4 groups to obtain a similar mean body weight at the start of the experiments. The rats were individually housed in stainless steel wire-mesh cages, and given free access to water and standard food (a mixture of powdered laboratory chow, Oriental Yeast Co., Ltd., Japan, containing 50% sucrose and 1% orotic acid). The animal room was maintained at 22°C to 24°C with a relative humidity of 45% to 60%, a ventilation frequency of 13 to 15 air exchanges/hr, and a 12-hr light/12-hr dark cycle (lights on: 07:00 to 19:00 hr). Control animals were fed the standard food for 2 weeks, and DHEA group rats were fed the standard food with 0.5% DHEA (Sigma Chemical Co.). Animals in the PB group were fed the standard food and intraperitoneally injected with 6% PB (Wako Pure Chemicals Co.) at 60 mg/kg, once a day, 5 days a week, for 2 weeks. The DHEA + PB group were fed standard food with concomitant treatment with DHEA and PB. Body weight and food consumption were measured every 7 days. The final body weight was measured on the day of necropsy to calculate the relative organ weight. All animals were euthanized by exsanguination from abdominal veins and arteries following ether anesthesia and laparotomy. They were then examined for gross findings, and the absolute of the liver and organ-to-body weight ratio (relative weight) were determined. The liver of all animals was fixed in 10% neutral formalin, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin. For peroxisome visualization in the control and DHEA + PB groups, 45-μm-thick tissue slices fixed in 2.5% glutaraldehyde were reacted for 60 min at 37°C in alkaline 3,3'-diaminobenzidine medium [20] and postfixed in 1% OsO_4. They were then embedded in epoxy resin, and 1 μm semi-thin sections were stained with toluidine blue.

Statistical analysis: Body weight gain, food consumption, and organ weight were analyzed by one-way analysis of variance. When one-way analysis yielded a significant difference ($P<0.05$), the values were analyzed by Dunnett's test.

RESULTS

In comparison with the control group, significant

Table 1. Changes of body weight gain and food consumption

	Body weight gain		Food consumption	
	Days 1–8 (g)	Days 1–15 (g)	Days 1–8 (g/day)	Days 8–15 (g/day)
Control	44.2 ± 6.8	94.3 ± 11.4	22.2 ± 1.6	22.3 ± 1.5
DHEA	6.7 ± 7.1**	47.3 ± 7.1**	14.3 ± 1.4**	18.2 ± 1.7**
PB	45.8 ± 6.8	92.2 ± 11.5	23.3 ± 2.0	22.5 ± 2.3
DHEA + PB	10.8 ± 6.2**	47.5 ± 5.6**	16.3 ± 1.6**	18.0 ± 1.7**

Each value represents the mean ± S.D. for 6 rats in each group. Body weight gain means the change of the body weight from the first day of treatment (Day 1). Food consumption is calculated from the means of each interval. Significantly different from each matched control; * P<0.05, ** P<0.01.

Table 2. Final body weight and liver weight

	Final body weight (g)	Liver weight	
		Absolute (g)	Relative (%)
Control	246.7 ± 13.9	11.70 ± 1.43	4.75 ± 0.44
DHEA	206.8 ± 8.3**	11.82 ± 0.83	5.70 ± 0.26**
PB	254.2 ± 15.1	13.05 ± 0.85	5.13 ± 0.23
DHEA + PB	213.5 ± 8.3**	13.52 ± 0.78*	6.33 ± 0.23**

Each value represents the mean ± S.D. for 6 rats in each group. Relative liver weight means the percent of the liver weight in the body weight. Significantly different from each matched control; * P<0.05, ** P<0.01.

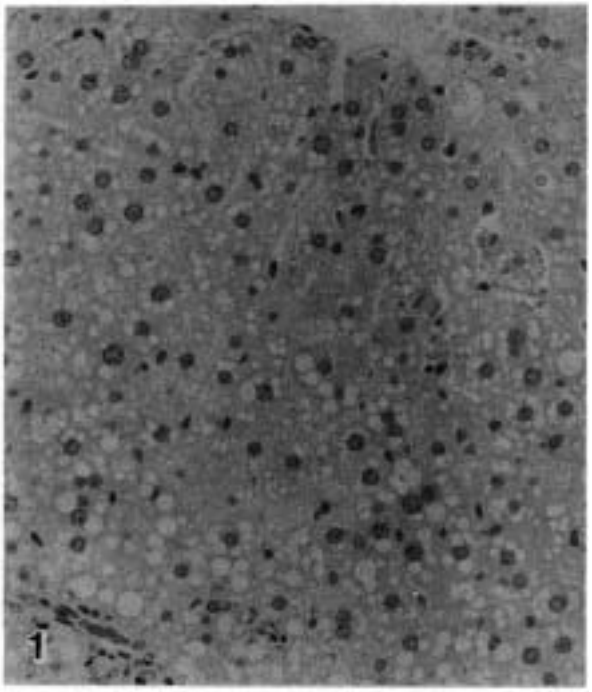

Fig. 1. Fatty liver in the control group, fed a high-sucrose diet containing 1% orotic acid for 2 weeks. The hepatocytes contain a various size of lipid droplets. H. E. stain. × 80.

decreases in body weight gain and food consumption were observed in the DHEA group and the DHEA + PB group on days 8 and 15 (Table 1) from the beginning of experiment. No significant differences in body weight gain or food consumption were observed between the PB group and the control group, or between the DHEA group and the DHEA + PB group. The liver was enlarged but of normal color in all the experimental groups, although it was discolored in the control group. In comparison with the liver weight in the control group, the absolute liver weight was significantly increased in the DHEA + PB group, and the relative liver weight was also increased in all the experimental groups (Table 2). The hepatocytes varied in size in the control fatty liver and contained numerous lipid droplets (Fig. 1). A small number of lipid droplets were observed in the hepatocytes of the rats in the PB group, mainly in the periportal areas (Fig. 2). There were few lipid droplets in

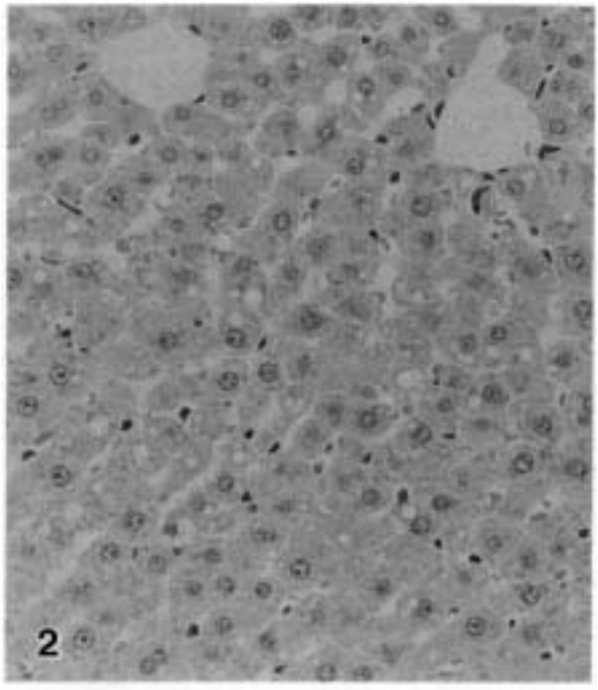

Fig. 2. Hepatocytes in the PB group. A small number of lipid droplets are observed, mainly in the periportal areas. H.E. stain. × 80.

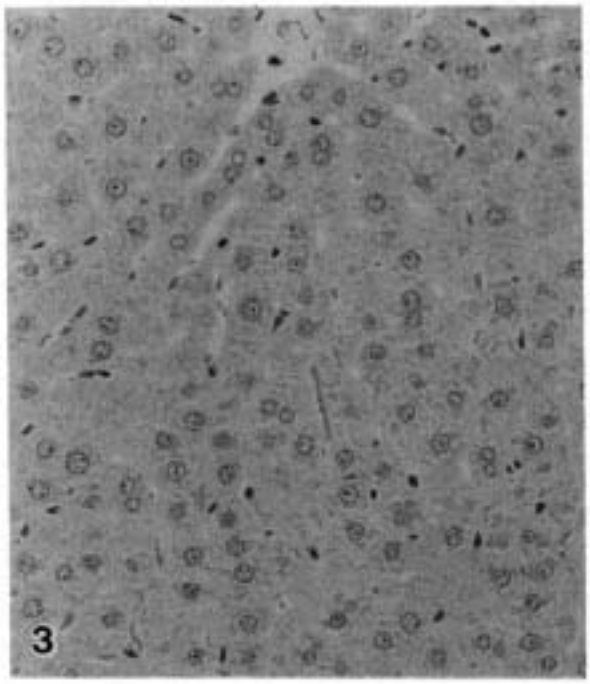

Fig. 3. Hepatocytes in the DHEA group. Little number of lipid droplets are observed in the hepatocytes. H.E. staining. × 80.

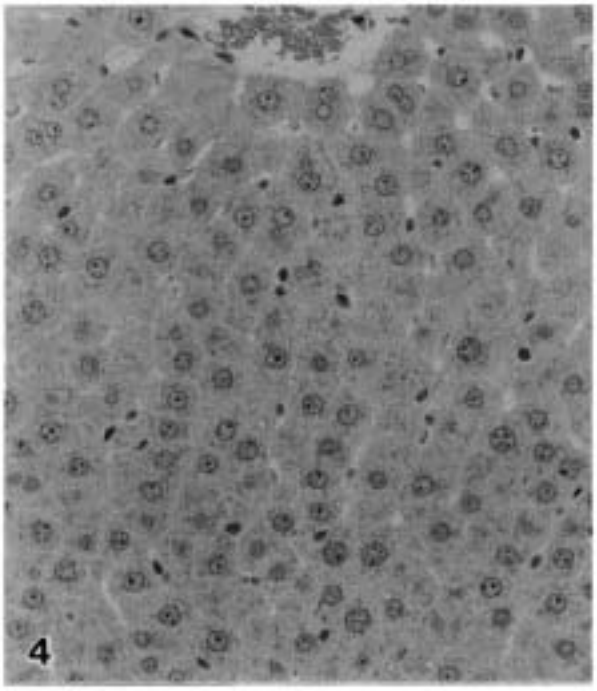

Fig. 4. Hepatocytes in the DHEA + PB group. Little number of lipid droplets are observed in the hepatocytes. H.E. staining. × 80.

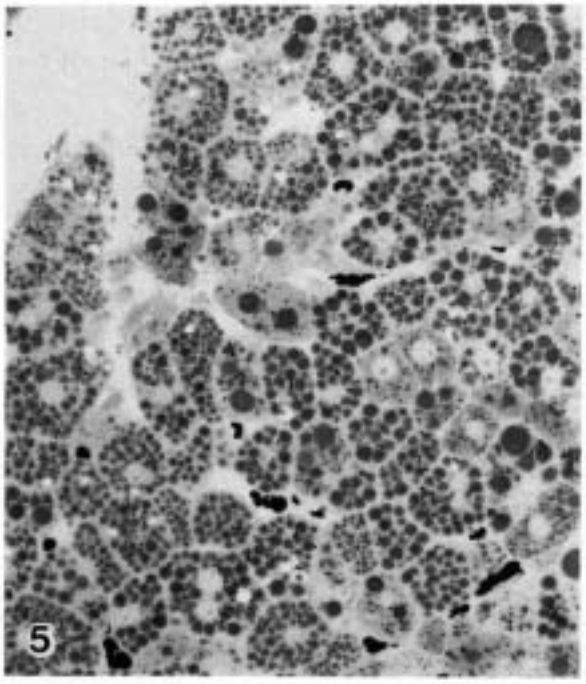

Fig. 5. Fatty liver in the control group. Peroxisomes are visualized by catalase reaction with DAB. Semi-thin section stained with toluidine blue. × 100.

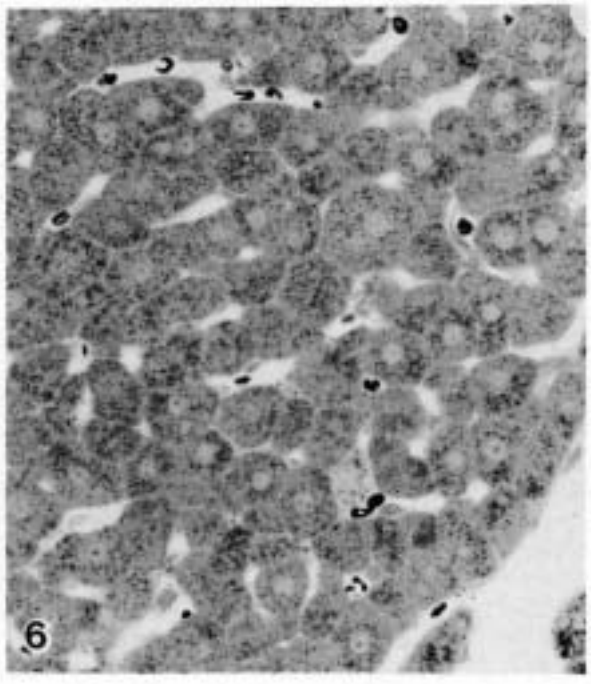

Fig. 6. Hepatocytes in the DHEA + PB group. Peroxisomes are visualized by catalase reaction with DAB. Semi-thin section stained with toluidine blue. × 100.

the hepatocytes of either the DHEA or the DHEA + PB group (Figs. 3 and 4). The centrilobular hepatocytes were larger in the DHEA + PB group than in the PB group, and the sinus was narrowed in the DHEA + PB group. Peroxisomes were visualized by catalase reaction among the lipid droplets in the control group (Fig. 5). In the DHEA

+ PB group, numerous peroxisomes were observed in the hepatocytes (Fig. 6).

DISCUSSION

It is well known that orotic acid, a precursor in pyrimidine nucleotide biosynthesis, induces the fatty liver in rodents by causing triglyceride accumulation in the liver. The increase in triglycerides in the liver is the result of a specific defect in the release of hepatic very low density lipoprotein [29, 30]. The induction of fatty liver by orotic acid is facilitated by a high-sucrose diet [21]. The lipid droplets in the hepatocytes increase in number and volume as the fatty liver progresses [11, 18, 26]. This increase results in the hepatomegaly accompanied by increase in the liver weight [19]. In this study, a number of lipid droplets were observed in the control liver (Figs. 1 and 5) as in the previous reports. Since clofibrate, a representative peroxisome proliferator, was shown to prevent and reverse the fatty liver induced by orotic acid [6, 19], DHEA, another peroxisome proliferator, was expected to prevent the orotic-acid-induced fatty liver. DHEA was reported to decrease triglycerides, obesity and body weight gain without any decrease in food consumption [3, 14]. DHEA, as same as clofibrate, increased peroxisomes in hepatocytes and markedly activated the β-oxidation activity in the peroxisomes [14, 32]. On the other hand, PB, a hypolipidemic agent, inhibits the early synthesis of cholesterol and fatty acid, because it does not convert the plasma fat into other biological substances and inhibits enzymes, such as ATP-dependent citrate lyase and acetyl-CoA synthetase, controling the process of fatty synthesis in the liver [10]. The combined administration of PB and clofibrate was reported to induce the greater hypertrophy of the liver in rodents than either administration of one of these agents alone [4]. The combined administration of DHEA and PB was therefore expected to be more effective in prevention of the fatty liver. In fact, the administration of PB alone decreased the number of lipid droplets but did not eliminate them completely, whereas the combined administration of DHEA and PB completely eradicated them. Induction of cytochrome P-450 in rodents was more prominent after the administration of orotic acid plus PB than PB alone [8]. Therefore, the PB treatment in this study might increase cytochrome P-450 synthesis in the liver and suppress the synthesis of cholesterol and fatty acid. Despite the increase in the liver weight and liver cell size, the combined administration of DHEA and PB was able to eliminate the lipid droplets in the orotic-acid-induced fatty liver. As shown in Fig. 6, the increase in peroxisomes demonstrated that DHEA is a peroxisome proliferator. The proliferated peroxisomes may be responsible for the increase in the weight of the liver and the size of the hepatocytes. The functional mechanism of DHEA and PB to eliminate intracellular lipid droplets remains to be investigated.

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