Preventive Effects of Dehydroepiandrosterone Acetate on the Fatty Liver Induced by Orotic Acid in Male Rats

Hirohiko GOTO1,2, Shuji YAMASHITA2, and Takashi MAKITA1

1The United Graduate School of Veterinary Science, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, and 2Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kaawauchi-cho, Tokushima 771-0192, Japan

Abstract: Preventive effects of dehydroepiandrosterone acetate (DHEA-A) and clofibrate (positive control substance) on the fatty liver induced by orotic acid (OA) were examined on the male Sprague-Dawley rats fed a high sucrose based diet containing 1% OA and this diet further mixed with 0.5% DHEA-A or 0.5% clofibrate for 2 weeks. Numerous lipid droplets were observed in the hepatocytes of the rats treated with OA alone, but not in those treated with DHEA-A or clofibrate. In comparison to the group with OA alone, the DHEA-A or clofibrate treated rats showed a larger relative liver weight (to body weight) which was accompanied by increased peroxisomes in the hepatocytes. These results indicate that DHEA-A, as well as clofibrate, may prevent OA-induced fatty liver.

Key words: dehydroepiandrosterone acetate, fatty liver, peroxisome proliferator

Dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one, DHEA) is one of the steroid hormones which is known to be an intermediate in the synthesis of testosterone and estrogen [18]. DHEA is secreted by zona reticularis in the adrenal cortex in humans [20]. In rodents, however, DHEA is not detectable in the adrenal glands due to the lack of 17-hydroxylase [21] and also has been shown to induce peroxisomes in the liver [16, 17, 24]. We have previously reported the preventive effects of DHEA and/or phenobarbital on the fatty liver induction by orotic acid (OA) [4]. Since the preventive effects of DHEA was considered to be attributed to the peroxisome induction, we have undertaken to examine the prevention of OA-induced fatty liver by concomitant treatment with OA and DHEA or clofibrate. In the present study, DHEA acetate (DHEA-A), an acetate form of DHEA, was used because of its recommendation to improve the solubility and absorption of DHEA [9, 19]. Clofibrate, a typical peroxisome proliferator, was used as a positive control substance which has been reported to prevent and reverse the fatty liver induced by OA [3, 12].

A total of eighteen 6-week-old male Crl:CD (SD) rats (Charles River Japan Inc.) weighing 166 to 198 g were randomly divided into three different treatment groups. One group were fed a based diet which was a mixture of standard powdered laboratory chow (Oriental Yeast Co., Ltd., Japan) with 50% sucrose and 1% OA (Wako Pure Chemical Co., Ltd.). The other two groups were maintained on the based diet further mixed

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Address corresponding: H. Goto, Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kaawauchi-cho, Tokushima 771-0192, Japan
Table 1. Changes in body weight gain and food consumption

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Body weight gain</th>
<th>Food consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 1–8 (g)</td>
<td>Days 1–15 (g)</td>
</tr>
<tr>
<td>OA alone</td>
<td>44.2 ± 6.8</td>
<td>94.3 ± 11.4</td>
</tr>
<tr>
<td>OA + DHEA-A</td>
<td>10.5 ± 6.7**</td>
<td>62.3 ± 8.0**</td>
</tr>
<tr>
<td>OA + Clofibrate</td>
<td>2.7 ± 10.0**</td>
<td>35.2 ± 14.2**</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. for 6 rats in each group. Body weight gain means the change in body weight from the first day of treatment (day 1). Food consumption was calculated from the means for each measurement interval. *, ** Significantly different from the group treated with OA alone (*P<0.05, **P<0.01).

Table 2. Final body weight and liver weight

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Final body weight (g)</th>
<th>Liver weight Absolute (g)</th>
<th>Liver weight Relative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA alone</td>
<td>246.7 ± 13.9</td>
<td>11.70 ± 1.43</td>
<td>4.75 ± 0.44</td>
</tr>
<tr>
<td>OA + DHEA-A</td>
<td>217.7 ± 10.9**</td>
<td>12.77 ± 1.62</td>
<td>5.83 ± 0.57**</td>
</tr>
<tr>
<td>OA + Clofibrate</td>
<td>204.7 ± 14.3**</td>
<td>11.17 ± 1.11</td>
<td>5.47 ± 0.50*</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. for 6 rats in each group. Relative liver weight means liver weight as a percentage of body weight. *, ** Significantly different from the group treated with OA alone (*P<0.05, **P<0.01).

with 0.5% DHEA-A or 0.5% clofibrate (Sigma Chemical Co.). The rats were individually housed in cages, and were given free access to water and each test diet for 2 weeks. The body weight and food consumption were measured every 7 days. At the end of the treatment, the final body weight after fasting for 16 hr was measured to calculate the relative liver weight (liver/body weight ratio). All rats were euthanized after ether anesthesia and laparotomy. For histological examination, 45-μm-thick liver slices fixed in 2.5% glutaraldehyde were reacted for 60 min at 37°C in alkaline 3, 3'-diaminobenzidine medium for catalase reaction [14] and post fixed in 1% O₃O₄. They were then embedded in epoxy resin, and semi-thin sections were stained with toluidine blue. Body weight gain, food consumption, and organ weight were statistically analyzed by t-test.

In comparison with the OA group, significant decreases in body weight gain and food consumption were observed in both DHEA-A and clofibrate groups from the beginning of the experiment (Table 1). These decreases were more prominent in the clofibrate group than in the DHEA-A group. In comparison with the liver weight in the OA group, the relative liver weight was significantly increased in the DHEA-A and clofibrate groups, although there were no significant differences in the absolute liver weight (Table 2). Peroxisomes were visualized by the catalase reaction among the lipid droplets in the OA group (Fig. 1). There were few lipid droplets but numerous peroxisomes in the hepatocytes of the DHEA-A and clofibrate groups (Figs. 2 and 3). No apparent differences between the DHEA-A and clofibrate groups were observed in the morphology of hepatocytes. The increased relative liver weight, and light microscopic appearance of the hepatocytes (including peroxisomes with catalase reaction) in the present study were almost comparable to those of the DHEA group in a previous report [4]. No apparent evidence suggesting better solubility or absorption was observed in the DHEA-A treated group.

OA, a precursor in pyrimidine nucleotide biosynthesis, is known to induce a specific defect in the secretion of very low density lipoprotein specifically in rodents [22, 23]. This defect results in the formation of fatty liver by accumulating triglyceride, and this change is
facilitated by a high sucrose diet [13]. The accumulation of lipid droplets in the hepatocytes results in the hepatomegaly accompanied by an increase in liver weight [12]. DHEA has been reported to reduce the body fat in man [5, 11] and rats [8, 10]. Inhibition of lipogenesis associated with DHEA treatment has been attributed to the inhibition of glucose-6-phosphate dehydrogenase (G6PDH) activity and a decrease in reducing equivalents (i.e., NADPH) for triglyceride synthesis [2, 15, 26]. In the present study, clofibrate almost completely eliminated the accumulation of lipid droplets, consistent with previous reports [3, 12]. Prevention of fatty liver by clofibrate has been implicated in the induction of peroxisome proliferation and the activation of β-oxidation activity [7, 25]. Increased activity of peroxisomal enzymes caused by the induction of peroxisomes shifts the balance of fatty acid metabolism away from esterification, resulting in a decrease in triglyceride synthesis [1, 5]. Because the number and size of catalase reactions in the DHEA-A group were similar to those in the clofibrate group, the prevention of the OA-induced fatty liver by DHEA-A in the present study might be associated with either the decrease in the lipogenesis or increase in lipolysis.

References